MECHANICAL PROPERTIES OF POLYTETRAFLOUROETHYLENE ELASTOMER MEMBRANE FOR DYNAMIC CELL CULTURE TESTING

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ABSTRACT

A wide body of existing research on cellular injury has been conducted using cell cultures grown on flexible elastomer membranes deformed by a transient pressure pulse. However, there has been little published information on the material properties of these elastic membranes. In order to facilitate the development of a finite element model of cellular injury, the material properties of the underlying membrane must first be known. A series of static tests of an elastomer yielded a set of pressure-deflection data for applied pressures of 2.5, 5.0, 7.5, 10, 12 and 14 PSIG. Using an optimization technique, the material properties for an elastic finite element model were iteratively changed and compared to these experimental results in order to minimize the difference between experiment and simulation. The final material properties were found to be quite different from the initial guess, with a final modulus of 950,000 Pa, a Poisson's ratio of 0.499, and a density of $5.5 \cdot 10^{-4}$ g/mm^3. The comparison between the experimental and finite element models was conducted using a sum of squares difference for each of the six pressures, yielding an average sum of squares difference of 0.271 mm. The average percent error of the deflection measurements was 3.57%, with errors measured at each pressure ranging between 0% and 12%. Parameter sensitivity was examined and the most influential property was the modulus of elasticity. The least influential parameter was the density, having almost no effect on the maximum deflection.

Keywords: finite element, material model, elastomer, mechanical properties

INTRODUCTION

Mechanical injury tolerance testing at the cellular level typically requires the cells of interest to be isolated, either by careful dissection or culturing, and then subjected to a load [1, 2, 3, 4, 5]. The load is usually intended to harm some or all of the cells for the purpose of determining the type, duration and magnitudes of loads that can cause injury. In the case of cell culture testing, the cells are often grown on the same medium that imparts the load; this is often in the form of a flexible membrane [3, 4, 5]. As the cell culture grows, the cells adhere to the membrane. Because of this, any stretch loading imparted to membrane will be directly transmitted to the cell culture.

If the membrane is stretched in-plane, the strain is imparted in that same plane making it possible to optically validate the developed strain [2, 5]. However, if the stretch was induced by causing the membrane to bulge out by the use of pressurized gas, optical tracking is considerably more difficult [3, 4]. This is because the area of interest does not remain in the same plane making it difficult to image.

One possible method that could be used to calculate the strain on a membrane that has been subjected to pressure loading is the use of a finite element model. This has been employed for biaxial stretch cases [2] and out-of-plane stretch cases [6]. However the material properties of the elastomer membrane are unknown. Here we seek to derive optimized material parameters for Collagen I cell culture plates using experimental data and finite element analysis. Specifically we will determine values for the elastic modulus, E, Poisson's ratio, ν, and density, ρ that best fit this material. That data will be useful for developing models of future experiments utilizing pressure-induced injury to cell cultures.

METHODS

To find optimized values for the elastic modulus, Poisson's ratio and density, a two step approach was used. The first step was to measure the static deflection of the flexible growth membrane by pressurizing the well with low pressure air. For this study Collagen I Flex I Culture Plates (Flexcell International Corporation, Hillsborough, NC) were used as shown in Figure 1. The second step was to use the finite element (FE) model previously developed by the authors to simulate the experimental setup [6].

There are six cell wells on each plate; each well has rigid plastic sides and a flexible polytetraflouroethylene elastomer membrane on the bottom. The membrane deforms outward as the pressure inside the well increases. The resulting deformation is measured from the bottom of the culture plate to the bottom of the membrane as shown in Figure 2.

Figure 1: Collagen I culture plate

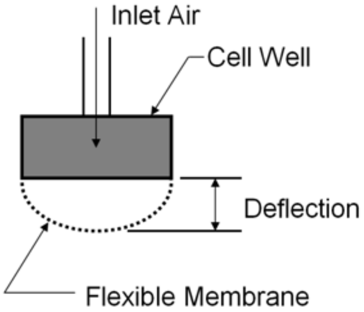

Figure 2: Test rig measurements

A total of six different pressure settings were used ranging from 2.5 to 14 PSIG. Six static deflection measurements were taken for each pressure setting. These samples were averaged to produce a single deflection measurement for each pressure condition. The measurements were made using a custom rig that allowed the cell culture tray to rigidly mount to a base where the flexible membrane was positioned above a linear potentiometer. The linear potentiometer was spring loaded so it would stay in contact with the membrane as it deflected downward. The linear potentiometer reading was then recorded using a data acquisition system to allow analysis of the deflection signal.

Pressure conditions were imposed on the FE model of the cell culture well to simulate the experimental culture wells. Starting values for elastic modulus, Poisson's ratio and density were obtained from previous research [6]. The final deflection of the FE model was compared to the experimental data. Semi-automated iterative changes were made to the elastic modulus, Poisson's ratio and density parameters of the flexible membrane in the FE model and the simulation was run again, comparing the FE and experimental deflections. This process was repeated for all six pressure conditions until the model and experimental data converged.

The finite element model of the culture well was designed as a planar 2D mesh with 1120 elements and a diameter of 20 mm. A top-down view of the "volleyball style" mesh is shown below in Figure 3. The outer edges of the membrane were constrained from all displacement but were allowed to freely rotate to simulate the flexing of the membrane at the wall. Each element was loaded by gravity and a pressure pulse with a varying maximum magnitude. The pressure curve is shown in Figure 4. Although it is not evident from the scale, the pressure curve is sigmoidal in the first 10 ms and flattens out to a constant value of 1 afterward. The finite element model was run for 700 ms, using the LS-DYNA explicit solver, to allow for dynamic oscillations from the rapid loading to damp out of the system. The final deflection of the finite element membrane was compared to the deflection of the real membrane.

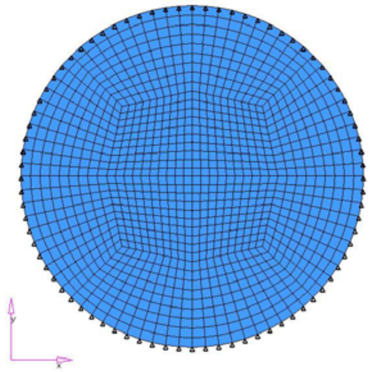

Figure 3: The membrane finite element mesh.

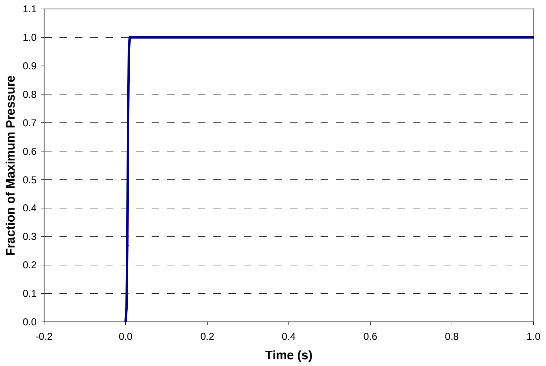

Figure 4: The pressure-time curve.

Because the material of the membrane was an elastomer, the material model was that of an ideal elastic material. The behavior of this material was fully characterized by three properties: density, elastic modulus, and Poisson's ratio. The initial values for these material properties were chosen from the previous research ($\rho = 5.5 \bullet 10^{-4}$ g/mm^3; E = 2,500,000 Pa; $\nu = 0.46$) [6]. The static deflection of the model may depend on some or all of these properties. In order to evaluate the set of properties with the best match to the real experiments, a sum of squares method was employed. For each set of material properties, the sum of squares difference of deflection for each of the six pressure tests was calculated. Then, the properties of the membrane were varied, one property at a time, by a small amount (typically +/-10%). If any of these new sets of properties possessed a lower sum of squares difference, then the model properties were adjusted. This process was repeated iteratively until the variation in the model's material properties failed to produce a better sum of squares fit. At this point, the model was considered to have converged onto the best set of material properties that represent the experimental results.

RESULTS

The typical result of a membrane deflection experiment is shown in Figure 5. Minor oscillation was observed in the beginning, but the deflection reached steady state by 0.4 seconds. For this study only the steady state deflection was of interest, not the dynamic behavior. Oscillations were also observed in the finite element model. As shown in Figure 6, the oscillations tended to lessen but not disappear completely by the time that the termination time was reached. The finite element simulations were terminated before 0.4s because in all experiments the deflection had reached steady state by 0.07s. Because of the oscillating nature of the finite element curves, the maximum deflection values were obtained by averaging the last 50 ms of the curves.

Table 1 presents the experimental data collected. In addition to the deflection measurements from each test the average deflection and standard deviation is also given.

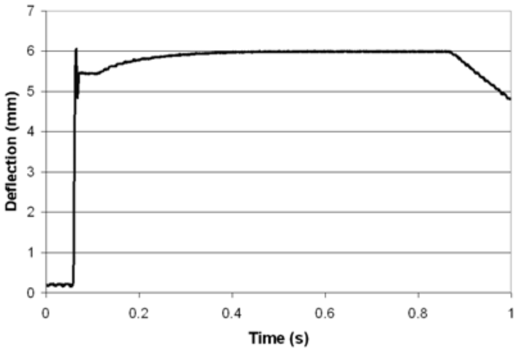

Figure 5: Membrane deflection vs. time curve for 12 PSIG pressure step function.

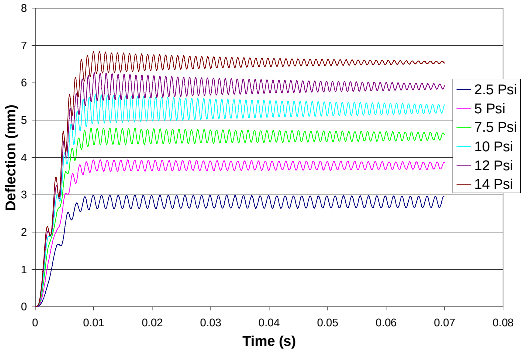

Figure 6: Finite element pressure-time curves for the ideal set of material properties.

Table 1: Experimental Deflection Measurements

Trial	2.5 PSI	5.0 PSI	7.5 PSI	10 PSI	12 PSI	14 PSI
	Static Deflection (mm)					
1	2.72	3.79	4.39	4.91	6.05	7.14
2	2.91	3.81	4.31	4.71	5.84	6.40
3	2.97	3.80	4.65	4.70	5.63	6.78
4	2.83	3.63	4.77	4.67	5.65	6.84
5	2.86	3.54	4.65	4.73	5.53	6.82
6	2.66	3.71	4.66	4.71	5.78	7.12
Mean	2.83	3.71	4.57	4.74	5.75	6.85
Standard Deviation	0.115	0.110	0.178	0.086	0.188	0.270

In Table 2 below, an example of how the iterative process was used to generate the ideal material properties is shown. The iteration shown is the next to last iteration. The row representing a finite element model with the value of ν increased by 10% represents the best set of material properties in which the average sum of squares difference reached a minimum value of 0.073 mm^2, or 0.271 mm. The following iteration (not shown) failed to produce any improvement so the process was stopped. The final set of material properties are shown below in Table 3. The final Poisson's ratio was very close to the limit imposed by the finite element solver and could not be increased any further.

Table 2: The finite element models that lead to the final set of material properties.

Iteration	2.5 PSI	5.0 PSI	7.5 PSI	10 PSI	12 PSI	14 PSI	Avg Sum Sqr Difference
	Static Deflection (mm)						
E + 10%	2.73 mm	3.68 mm	4.42 mm	5.09 mm	5.63 mm	6.18 mm	0.104 mm^2
ν + 10%	2.81 mm	3.79 mm	4.57 mm	5.30 mm	5.90 mm	6.55 mm	0.073 mm^2
Original	2.85 mm	3.84 mm	4.63 mm	5.36 mm	5.95 mm	6.58 mm	0.088 mm^2
ν - 10%	2.91 mm	3.93 mm	4.72 mm	5.45 mm	6.03 mm	6.65 mm	0.119 mm^2
E – 10%	2.98 mm	4.02 mm	4.88 mm	5.68 mm	6.37 mm	7.12 mm	0.261mm^2

Table 3: The original and final material properties for the finite element model

	E (Pa)	ν	P (g/mm^3)
Original	2,500,000	0.46	5.5•10^{-6}
After Convergence	950,000	0.499	5.5•10^{-6}

A comparison of the experimental and finite element results is shown below in Figure 7. The finite element model was very accurate for the lower pressures, but overestimated the deflection at 10 psi and underestimated the deflection at the maximum pressure of 14 psi. The trendlines shown in the figure are also quite similar and reflect a good agreement between the finite element model and the experimental results. A similar numerical comparison is shown in Table 4. The percent error for deflection measurements (calculated as the difference over the experimental deflection) varies greatly but is generally below 5%, except for the deflection at 10 PSI. The average percent error, calculated using the absolute value of the errors, is only 3.57%.

Table 4: Percent difference between finite element model and experimental deflections

	2.5 PSI	**5.0 PSI**	**7.5 PSI**	**10 PSI**	**12 PSI**	**14 PSI**	**Average**
Experiment	2.83 mm	3.71 mm	4.57 mm	4.74 mm	5.75 mm	6.85 mm	
Finite Element	2.81 mm	3.79 mm	4.57 mm	5.30 mm	5.90 mm	6.55 mm	
% Difference	-0.44%	+1.93%	-0.04%	+11.91%	+2.68%	-4.43%	**3.57%**

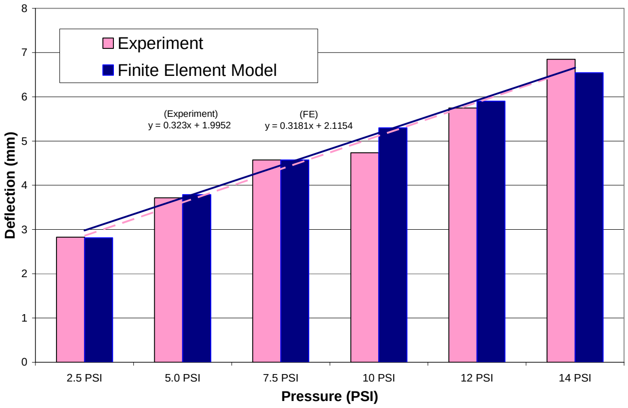

Figure 7: Deflections and linear trendlines for the experimental and finite element data.

DISCUSSION

An excellent agreement was found between the experimental data and the finite element model through the use of the iterative process detailed previously. Although this approach took a significant amount of time to complete, the results were quite accurate. After the final iteration, the average sum of squares difference for all six of the applied pressures was 0.073 mm^2 and the average percent difference was 3.57%. Additionally, the trendlines between the two sets of data were nearly identical for the higher pressures. The deflection at 10 PSI represented the most significant error at nearly 12%; however, approximately 8% is the difference between the deflection measurement and the predicted deflection of the experimental trendline.

In addition to the data presented in the results, a brief study on parameter sensitivity was conducted to determine which parameter had the most influence on the maximum deflection. The resulting parameter sensitivities are shown below in Table 5. As might be expected, the modulus had the most significant effect on the results, with the Poisson's ratio having a smaller effect (about 1/5 of the modulus). The density had almost no effect on the maximum deflection at all. The limited effect of density may be in part due to the static nature of the tests performed. The modulus and Poisson's ratio were also observed to have unique effects on the slope of the trendlines. In particular, increases in the modulus caused a decrease in the slope, while increases in the Poisson's ratio caused an increase in the slope. Thus, it could be said that the modulus decreases the model's sensitivity to pressure and the Poisson's ratio increases the sensitivity.

Table 5: Percent change in maximum deflection as a result of a change in material property.

	E	**ν**	**ρ**
% Change in Max Deflection for - 10% Parameter	-5.97%	-1.74%	-0.09%
% Change in Max Deflection for +10% Parameter	4.90%	1.07%	0.05%

The final material properties, shown in Table 3, represent a significant change from the material properties used in the previous research [6]. The modulus was the most strongly affected parameter, having been reduced by more than 50% to a final value of 950,000 Pascals. The Poisson's ratio was also affected, although not as strongly, increasing to roughly the maximum value (0.499) allowed by the finite element solver. Changes in the density did not affect the deflection significantly. However, the density is the easiest of the three properties to directly measure.

CONCLUSIONS

This study has determined that the material properties of the elastic membrane to be: $E = 950{,}000$ Pa, $\nu = 0.499$, $\rho = 5.5 \cdot 10^{-4}$ g/mm^3. The results showed a good match between the finite element model and experimental results. The average error in the deflection measurements was 3.57%, supporting this conclusion. However, the experimental tests performed were limited to static tests at constant pressures. The next logical step is to perform an evaluation of the finite element model in a more complex situation with a dynamic pressure pulse. Only when the behavior of the membrane is fully validated can the ultimate goal of modeling the response of attached cellular cultures be realized.

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