FAILURE PRESSURE OF BILAYER LIPID MEMBRANES

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ABSTRACT

The motion and growth of plants is the inspiration for a new biomimetic actuator that uses fluid transport across a bilayer lipid membrane (BLM) to create internal pressure and cause displacement in the actuator. In order for the actuator to be viable the BLM must be able to withstand this internal pressure without failing. In this study BLMs are formed over a porous polycarbonate substrate and a hydrostatic pressure is applied to the BLM and gradually increased until it fails. This test is performed over different pore sizes to measure the failure pressure of the BLM as a function of pore radius. A similar test is used for polymer films to compare the failure pressure trends of a BLM to conventional engineering materials. The polymer films and BLMs are modeled as a simply supported circular plate under uniform load, first with the assumption of small deflections and then with the assumption of large deflections. It was found that the large deflection model better represents the trend of failure pressure versus pore radius than the small deflection model.

INTRODUCTION

Movement in plants such as that in leaves or the opening and closing of stomatal pores is caused by fluid transport across cell membranes.1 Changes in turgor pressure in response to chemical stimuli that occur in specific plant cells, the motor cells, produce changes in volume at the cellular level that lead to bulk deformation of the plant. Thus, according to the engineering definition, a plant can be considered an actuator since it converts biochemical fuels such as adenosine triphosphate into mechanical energy.

The cellular processes that activate plant deformation are inspiring the development of an actuator that can be used in engineering applications that require controlled actuation.2 This biologically-inspired actuator concept, shown in Figure 1, uses ion transporter proteins to pump fluid across a planar bilayer lipid membrane (BLM) from a reservoir into an enclosed expansion chamber. The fluid exerts pressure on the enclosed chamber causing its volumetric expansion.3-5

Purified BLMs, which are reconstituted in vitro for engineering the biomimetic actuator, are useful models for cell membranes because they share many of the same chemical and physical properties. BLMs are the primary structural components of cell membranes in living organisms. They are composed of amphiphilic phospholipid molecules that possess hydrophilic head regions and hydrophobic tail regions. Because of this unique property, phospholipid molecules that are dissolved in an aqueous solution will self assemble into a bilayer membrane of ~ 5 nm thickness.6,7

Over the past years, investigators in biomechanics have been interested in studying the mechanical behavior of spherical shells of lipid bilayers known as vesicles. Studying vesicular assemblies of lipid molecules has been preferred to the study of planar BLMs since they better resemble the spherical shape of cell membranes. The micropipet aspiration technique that was pioneered by Mitchison and Swann8 and has been developed extensively by Evans and Needham9 is among the best established methods for measuring the mechanical properties of vesicles. Several other methods have also been adopted to test BLMs such as atomic force microscopy, magnetic twisting cytometry, and cytoindentation.10 All of these methods can be used to determine the mechanical properties of lipid bilayers, however a comparison among the experimental findings is difficult due to the differences in experimental methods such as lipid type, lipid phase, lipid assemblies, applied mechanical stimuli, etcetera.

Characterizing the mechanical properties of planar BLMs is essential for assessing the feasibility of the biomimetic actuator. In this actuator a BLM-coated porous polycarbonate substrate is used to separate the fluid reservoir from the enclosed expansion chamber (See Figure 1). The maximum deformation of the enclosed chamber depends on the fluid pressure that the BLM covering the pores of the substrate can withstand. For this reason the present study focuses on determining the strength of BLMs that are formed over porous substrates.

A custom built fixture was developed to measure the failure pressure of BLMs over porous polycarbonate substrates. Using this fixture, an increasing hydrostatic pressure was applied to the BLM until its failure was detected. Several types of lipids were tested including asolectin, which is a mixture of soybean phospholipids, 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine (POPE), 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine
(SOPC), mixtures of POPE and cholesterol, and mixtures of SOPC and cholesterol. The mixtures of POPE/cholesterol and SOPC/cholesterol were used because previous studies have shown that cholesterol increases the strength of BLMs. Each type of lipid was tested over the polycarbonate substrates with different pore sizes to determine the trend between failure pressure and pore size. A different experimental apparatus was designed to quantify the failure pressure of conventional engineering materials and compare these trends to BLMs. In particular, mylar polyester and low density polyethylene (LDPE) were fixed over plates with a single circular hole of different sizes and subjected to air pressure. In order to evaluate the strength of BLMs and polymer materials, Timoshenko’s theory for a simply supported circular plate under uniform load was applied to both the lipids and the polymer materials. Two models were applied, one based on small deflections and one based on large deflections.

This paper is presented in the following format. Section discusses the materials and methodology that were used to collect experimental data. In Section two models are presented, one for small deflections and one for large deflections, which were used to predict the failure pressure of the BLMs. Section describes the experimental data, regression lines, and model results. Section discusses the experimental results, comparisons between the results from polymer films and BLMs, comparisons between predictions of the small deflection model and the large deflection model, and limitations of the study.

MATERIALS AND METHODS

Experiments were carried out to evaluate the failure pressure of planar BLMs, which are formed by using different kinds of amphiphilic lipids, when subjected to hydrostatic fluid pressure. Also the failure of polymer films was studied by applying hydrostatic air pressure.

Bilayer Lipid Membranes

Asolectin was purchased in powder form from Sigma Aldrich (St. Louis, MO) and SOPC, POPE, and cholesterol were purchased in powder form from Avanti Polar Lipids (Alabaster, AL). The lipid powders were dissolved in n-decane (99% purity, Alfa Aesar, Ward Hill, MA) at a concentration of 40 mg/mL and mixed for 30 minutes using a sonicator (model 50, VWR, West Chester, PA). For some experiments POPE and SOPC were mixed with cholesterol to create stronger BLMs. In particular, 30 mol% and 50 mol% cholesterol were added to POPE and 50 mol% cholesterol was added to SOPC. The BLMs were reconstituted in an aqueous salt solution over Isopore polycarbonate membrane filters acquired from Millipore (Bedford, MA). These porous polycarbonate substrates were chosen for their hydrophilic nature that facilitates the formation of the BLMs. All of these filters have fixed parameters of 20 µm thickness and 5-20% porosity. The parame-
ter that is controlled for the experiments is the pore radius, which was chosen as 0.6 µm, 1.0 µm, 1.5 µm, 2.5 µm, and 4.0 µm. The aqueous salt solution was prepared with 0.1 M NaCl (100.0% purity, Mallinckrodt Baker, Inc., Paris, KY) and deionized water. The test fixture for the hydrostatic pressure testing of the BLMs was made from polycarbonate. As Figure 2 shows, a hollow cylindrical tube with 3.2 mm inner radius and 4.8 mm outer radius was fitted inside a base structure that had a removable aluminum bottom plate with a center hole of radius 3.2 mm. The height of the tube was marked in millimeter divisions. For each experiment a porous polycarbonate substrate was coated with the lipid/n-decane mixture by using a brush and was then clamped into the bottom plate (See Figure 2). The base and bottom plate were kept in a reservoir containing the salt solution. Additional salt solution was steadily poured into the upright cylindrical tube by small increments until the fluid level was observed to drop. The fluid drop was considered to be indicative of the BLM failure.

For each test the electrical resistance across the BLM was measured using a multimeter (model 87 III, Fluke, Everett, WA) and by placing a silver-silver chloride (Ag-AgCl) electrode (model EP2, World Precision Instruments, Inc., Sarasota, FL) in salt solution on either sides of the polycarbonate substrate. An electrical resistance greater than 100 kΩ was considered to be indicative that a lipid bilayer had fully formed over the porous substrate. In this case, the BLM was observed to support a hydrostatic pressure due to the weight of the fluid column before failing. The resistance was measured again after failure occurred and was consistently on the order of 1 kΩ, which is the same order of resistance of the salt solution alone. Six tests were performed for each combination of lipid and substrate pore size.

Polymers Films

The failure pressure of polymer films including 2.5 µm thick mylar polyester (Structure Probe, Inc., West Chester, PA) and 51 µm thick LDPE (McMaster-Carr, Atlanta, GA) were also tested. The apparatus used for testing these films is shown in Figure 2. It was custom made from polycarbonate and consisted of a base structure that had a removable aluminum bottom plate with a center hole of radius 9.5 mm. Steel plates with hole radii of 1.3 mm, 1.9 mm, 2.5 mm, 3.1 mm, and 3.9 mm were covered by a plastic film and clamped into the base of the test fixture. Pressure was applied through the air fitting using compressed nitrogen. It was steadily increased until the plastic film burst. Six tests were performed for each combination of polymer film and hole size.

MODELING

In order to describe the mechanical properties of BLMs and polymer films observed in the experimental studies, both the BLMs and the polymer films are assumed to behave as isotropic linear elastic solids. Because they occupy circular holes and are subjected to fluid and air pressures during the experiments, they are idealized as simply supported circular plates under uniform load as depicted in Figure 3.

Firstly, assume that the deflections of the plate are small deflections. Under this assumption the deflection of the plate is considered small compared to its thickness, there is no deformation of the mid-plane during bending, normals to the mid-plane remain normal after bending, and stresses that are transverse to the plate are ignored. Then, the relationship between the maximum stress, \( \sigma_{\text{max}} \), and the radius of the plate, \( a \), takes the form:

\[
\sigma_{\text{max}} = \frac{3(3 + \nu)qa^2}{8h^2}, \tag{1}
\]

where \( q \) represents the uniform load, \( \nu \) is the Poisson’s ratio, and \( h \) is the thickness of the plate. The maximum stress occurs at the center of the plate. From Equation (1), it readily follows that

\[
q = \left( \frac{h}{a} \right)^2 \frac{8\sigma_{\text{max}}^2}{3(3 + \nu)}, \tag{2}
\]
where $\sigma_{\text{max}}$ is taken to be the ultimate strength of the material. By taking the common logarithm of both sides of Equation 2 one obtains,

$$\log q = -2\log a + \log \left[ \frac{8h^2\sigma_{\text{max}}}{3(3 + \nu)} \right]. \quad (3)$$

On a logarithmic plot of $q$ versus $a$, Equation (3) becomes a straight line with a slope of -2 and a y-intercept that is equal to the second term of the right hand side.

Secondly, assume that the deflections of the plate are large deflections. This implies that the deflection of the plate is no longer small compared to its thickness but is still small compared to its radius. Deformation of the midplane is considered significant and is included in the analysis. In this case the relationship between the maximum stress and the radius of the plate becomes\(^\text{13}\)

$$\sigma_{\text{max}} = \alpha E w_o^2 \frac{a^2}{h^2}, \quad (4)$$

where $\alpha$ is a constant depending on the boundary conditions, $E$ is the Young’s modulus, and $w_o$ is the deflection at the center of the plate given by

$$\frac{w_o}{h} + A \left( \frac{w_o}{h} \right)^3 = B \left( \frac{a}{h} \right)^4, \quad (5)$$

with $A$ and $B$ as constants depending on the boundary conditions. Again, the maximum stress occurs at the center of the plate. From Equations (4) and (5), it follows that the uniform load is

$$q = \frac{1}{B} \left( \frac{h}{a} \right)^3 \left( \frac{E\sigma_{\text{max}}}{\alpha} \right)^{\frac{1}{2}} + A \left( \frac{h}{a} \right) \left( \frac{\sigma_{\text{max}}}{\alpha^3 E} \right)^{\frac{1}{2}}. \quad (6)$$

By taking the common logarithm, Equation (6) reduces to

$$\log q = \log \left[ \frac{1}{B} \left( \frac{h}{a} \right)^3 \left( \frac{E\sigma_{\text{max}}}{\alpha} \right)^{\frac{1}{2}} + A \left( \frac{h}{a} \right) \left( \frac{\sigma_{\text{max}}}{\alpha^3 E} \right)^{\frac{1}{2}} \right]. \quad (7)$$

When $h << a$ the first term of the logarithm argument, which appears on the right hand side of Equation (7), becomes negligible. Then Equation (7) can be approximated by

$$\log q = -\log a + \log \left[ \frac{A}{B} \left( \frac{h^2\sigma_{\text{max}}}{\alpha^3 E} \right)^{\frac{1}{2}} \right]. \quad (8)$$

When implementing the model to describe the experimental data the edge of the circular plate is assumed to be immovable for the plastic films and free to move for the BLMs. The large deflection model parameters for these boundary conditions and for a Poisson’s ratio of $\nu = 0.3$ are summarized in Table 1.

The elastic modulus and tensile strength for a lipid bilayer formed from an SOPC phospholipid and for a lipid bilayer formed from SOPC/cholesterol with 50 mol% cholesterol mixture were determined by Needham and Nunn using micropipet aspiration.\(^\text{11}\) The lipid bilayer thickness was assumed to be 5 nm. The material properties for mylar are from DuPont\(^\text{15}\) and material properties for LDPE are average values from Matweb.\(^\text{16}\) For the large deflection model the maximum stress and deflection are derived assuming a Poisson’s ratio of $\nu = 0.3$ and therefore Poisson’s ratio was assumed to be $\nu = 0.3$ for all materials. Table 2 is a summary of these geometrical and material parameters.

### RESULTS

The results of the experimental findings for mylar and LDPE films together with their regression lines are shown on a logarithmic plot in Figure 4. The slope of the regression lines have been found to be -0.94 for mylar films and -0.85 for LDPE films. Figure 4 also shows the predictions from Equation (2) and Equation (6) that are obtained by using the values of the parameters reported for the polymer films in Table 2. Equation (2) has a slope of -2 and Equation (6) is approximated by a straight line with a slope of -1 since the term containing $(h/a)^3$ is negligible.

<table>
<thead>
<tr>
<th>Boundary condition</th>
<th>$A$</th>
<th>$B$</th>
<th>$\alpha$</th>
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<tr>
<td>Simply supported, with immovable edge</td>
<td>1.852</td>
<td>0.696</td>
<td>0.905</td>
</tr>
<tr>
<td>Simply supported, with edge free to move</td>
<td>0.262</td>
<td>0.696</td>
<td>0.295</td>
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<table>
<thead>
<tr>
<th>Material</th>
<th>$h$</th>
<th>$\nu$</th>
<th>$E$</th>
<th>$\sigma_{\text{max}}$</th>
</tr>
</thead>
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<tr>
<td>SOPC</td>
<td>0.005 *</td>
<td>0.3 *</td>
<td>38.6</td>
<td>1.14</td>
</tr>
<tr>
<td>SOPC/Chol-50 mol%</td>
<td>0.005 *</td>
<td>0.3 *</td>
<td>156</td>
<td>3.94</td>
</tr>
<tr>
<td>Mylar polyester</td>
<td>2.5</td>
<td>0.3 *</td>
<td>4900</td>
<td>200</td>
</tr>
<tr>
<td>LDPE</td>
<td>51</td>
<td>0.3 *</td>
<td>210</td>
<td>11</td>
</tr>
</tbody>
</table>

* denotes an assumed value.

\(h\) is the lipid bilayer thickness was assumed to be 5 nm. The material properties for mylar are from DuPont and material properties for LDPE are average values from Matweb. For the large deflection model the maximum stress and deflection are derived assuming a Poisson’s ratio of $\nu = 0.3$ and therefore Poisson’s ratio was assumed to be $\nu = 0.3$ for all materials. Table 2 is a summary of these geometrical and material parameters.
compared with the term containing \((h/a)\) over the range of pore radii that were tested.

The experimental data that have been collected for BLMs are reported in Figure 5A and Figure 5B. In particular, Figure 5A is a logarithmic plot of failure pressure versus substrate pore radius for BLMs formed from asolectin, POPE, POPE/cholesterol-30 mol% cholesterol, and POPE/cholesterol-50 mol% cholesterol. Figure 5B is a logarithmic plot of failure pressure versus substrate pore radius for BLMs formed from SOPC and SOPC/cholesterol-50 mol% cholesterol. Linear regression lines are shown with a slope of -1.2 for SOPC and a slope of -1.3 for SOPC/cholesterol-50 mol%. The predictions of Equations (2) and (6) with the values of the parameters reported in Table 2 are also presented. Again, Equation (2) becomes a straight line with a slope of -2 and Equation (6) is approximated by a straight line with a slope of -1 on the logarithmic plot.

To obtain a very rough estimate of the failure pressure of a BLM over a substrate with smaller pore sizes, Figure 6 shows the large deflection model of Equation (6) for the SOPC data extrapolated out to submicron pore radii. The large deflection model changes from a slope of -1 in the range of the experimental data to a slope of -3 in the range of very small pore radii. At a pore radius of 0.01 \(\mu\)m the failure pressure of an SOPC BLM is estimated to be

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Figure 4. Failure pressure of mylar and LDPE films covering plates with various hole sizes. Circles are mylar experimental data; the thin solid line is a linear regression for mylar data; the thin dotted line is Equation (2) for mylar; the thin dash-dot line is Equation (6) for mylar; squares are LDPE experimental data; the thick solid line is a linear regression for LDPE data; the thick dotted line is Equation (2) for LDPE; the thick dash-dot line is Equation (6) for LDPE.

Figure 5. Failure pressure of BLMs versus pore radius of the polycarbonate substrate. (A) Circles are asolectin; squares are POPE; diamonds are POPE/cholesterol-30 mol%; triangles are POPE/cholesterol-50 mol%. (B) Circles are SOPC experimental data; the thin solid line is a linear regression for SOPC data; the thin dotted line is Equation (2) for SOPC; the thin dash-dot line is Equation (6) for SOPC; squares are SOPC/cholesterol-50 mol% experimental data; the thick solid line is a linear regression for SOPC/cholesterol-50 mol% data; the thick dotted line is Equation (2) for SOPC/cholesterol-50 mol%; the thick dash-dot line is Equation (6) for SOPC/cholesterol-50 mol%.
2.5 MPa as indicated by the asterisk.

**DISCUSSION**

The main goal of this study was to establish the trend between the failure pressure of planar BLMs and the pore size of their supporting substrate. The experimental findings demonstrated that the failure pressure increases as the pore size decreases. On a logarithmic plot, this was clearly shown by the negative slope of the almost linear relationship between failure pressure and pore size. Timoshenko’s theory of plates was used to model the results of the experimental investigation. It was demonstrated that the failure pressure of a planar BLM as a function of the pore size was better described by assuming that the BLM undergoes a large deflection rather than a small deflection. On a logarithmic scale, a straight line with a slope of -1 approximates the model for large deflections while a straight line with a slope of -2 represented the model for small deflections. There is enough variability that the slope of the experimental data can not be exactly determined but it is obvious that the slope of the data is more accurately approximated by a straight line with a slope of -1. This is reasonable since when pressurizing a membrane to failure the deflection of the membrane will be great enough to violate the assumptions of small deflections.

In the models the BLMs were assumed to be isotropic linear elastic circular plates with simply supported edges. Violations of some of these fundamental assumptions may have affected the accuracy of the models. The BLMs were modeled as being isotropic but based on their geometry they are transversely isotropic. Also it is possible that multiple BLMs were formed instead of a single BLM. In this case, multiple BLMs would have a thickness much greater than 5 nm and would induce errors in the implementation of the models. Also, the Poisson’s ratio was arbitrarily set to 0.3 for all materials. This may be a better assumption for polymer materials than for BLMs. However, to the authors’ knowledge there are no studies in which the Poisson’s ratio of a BLM was measured. In terms of linear elasticity, Needham and Zhelev have shown that the response of a vesicle to micropipet aspiration is linear elastic although it is unclear whether or not this is true for the experimental method presented here.

Although the maximum pressure that the BLMs could withstand was small in these experiments, it is speculated that BLMs that are formed over substrates with pores of submicron size could support large enough pressures for a viable biomimetic actuator. Limitations in the current experimental setup and model do not permit a reliable extrapolation of failure pressures that are obtained with submicron pore sizes. Nevertheless, if a risky extrapolation is made from the large deflection model of the SOPC data then a substrate with 0.01 µm radius pores would have a failure pressure of 2.5 MPa, as shown in Figure 6. In a well-designed actuation scheme, the BLMs with such submicron pore substrates could generate high stress and strain.

Experimental studies were conducted to evaluate how the failure pressure of circular polymer films was affected by the size of their radius. The failure behavior of these polymer films allowed for useful comparisons to the failure of the BLMs. The trend between failure pressure and hole size is essentially the same for the polymer films and BLMs. As with the BLMs, the failure pressure of the polymer films increases with decreasing radius. Again, when using Timoshenko’s theory for plates the model for large deflections was better suited to describe the experimental data than the model for small deflections. The magnitude of failure pressure for the polymer films is much larger than that of BLMs, which is why compressed air was used in these tests instead of a water column. However, the fact that a BLM does not withstand a pressure as high as a polymer primarily depends on its very small thickness since the elastic modulus and strength of an SOPC vesicle is reported to approach that of LDPE (see Table 2).

Experiments were carried out to investigate the effect of cholesterol on the failure behavior of BLMs. The failure pressure of POPE and SOPC BLMs

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with cholesterol was found to be only slightly higher than the failure pressure of plain SOPC and POPE BLMs. Needham and Nunn\textsuperscript{11} have reported a nearly four-fold increase in strength of SOPC/cholesterol-50 mol% over plain SOPC when studying lipid vesicles by the micropipet aspiration technique. This inconsistency could be due to differences in the lipid structures and/or in the experimental methods.

The failure pressure data for BLMs that are shown here are computed after forming them over polycarbonate substrates with pore radii of 0.6 µm, 1.0 µm, 1.5 µm, 2.5 µm, and 4.0 µm. Because the maximum hydrostatic pressure that the test fixture could generate was 12 kPa, BLMs were not tested over substrates with pore radii below 0.6 µm. The failure pressure was also measured for pore radii of 5.0 µm and 6.0 µm. For these pore sizes, the maximum hydrostatic pressure was highly variable and unrepeatable and, therefore, the experimental data are not reported. In many cases the BLMs would not withstand any measurable hydrostatic pressure and had electrical resistance values $< 100 \text{k\Omega}$. The authors believe that planar BLMs cannot be easily formed and stabilized over pores of such sizes.

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