EVALUATING THE FAILURE PRESSURE OF BILAYER LIPID MEMBRANES USING AN AUTOMATED PRESSURIZATION TEST APPARATUS

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ABSTRACT

A new methodology has been developed to measure the mechanical integrity of a bilayer lipid membrane (BLM) formed over porous substrates. A custom test fixture was fabricated in which a stepper motor linear actuator drives a piston in order to apply pressure to a BLM in very fine increments. The pressure, monitored with a pressure transducer, is observed to increase until the BLM reaches its failure pressure, and then drop. This experiment was performed on 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (SOPC) lipid bilayers formed over porous polycarbonate substrates with various pore sizes ranging from 0.05 – 10 µm in diameter. A trend of increasing failure pressure with decreasing pore size was observed. The same set of experiments was repeated for BLMs that were formed from a mixture of SOPC and cholesterol (CHOL) at a cholesterol concentration of 50 mol%. The presence of cholesterol was found to increase the failure pressure of the BLMs by 1.5 times on average. A model of the characteristic pressure curve from this experiment was developed based on an initially closed fluid system in which pressure increases as it is loaded by a moving piston, and which upon reaching a critical failure pressure allows pressure to decrease as fluid escapes through a porous medium. Since the BLM is formed over many pores, this model assumes that the failure pressure for each micro-BLM follows a normal distribution over all pores. The model is able to accurately predict the major trends in the pressurization curves by curve-fitting a few statistical parameters.

INTRODUCTION

Bilayer lipid membranes (BLMs) are formed from phospholipid molecules which self-assemble into a lipid bilayer with 4 – 8 nm thickness when submerged in an aqueous solution due to their amphiphilic nature.1,2 They are the primary structural component of cell membranes in living organisms and therefore are useful for modeling the properties of cells since they share many of the same chemical and physical properties. Researchers are showing an increasing interest in integrating biological or biologically-inspired components into engineering materials and systems. One such example is an effort by researchers at Virginia Tech to develop a biomimetic micro-hydraulic actuator.3,4 The actuator, shown schematically in Figure 1, uses ion transporter proteins to pump fluid across a BLM from a reservoir into an enclosed expansion chamber. The buildup of turgor pressure in the enclosed chamber generates displacement in the cover plate of the actuator. This actuator has been reported to achieve displacement of up to 30 µm and force of up to 0.5 mN. It is believed that one limitation of these performance characteristics is the amount of pressure that can be withstood by the BLM formed on a porous substrate before it bursts, which is the motivation for this study.

In order to successfully integrate a BLM into an engineering application it is necessary to quantify the mechanical properties of the membrane. Investigators in biomechanics have long been interested in studying the mechanical behavior of spherical shells of lipid bilayers, known as vesicles, because they resemble the spherical shape of cell membranes. The micropipet aspiration technique that was pioneered by Mitchison and Swann5 and has been developed extensively by Evans and Needham6 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.7

The purpose of this work was to measure the maximum pressure that a lipid bilayer can withstand when it is formed over a rigid substrate containing an array of circular pores. Using a porous substrate as a support structure for the formation of a BLM has been used as a technique to enhance its stability.8,9 In a previous study, a custom test fixture...
was developed to measure the failure pressure of a BLM formed on a porous substrate. In this procedure, a porous polycarbonate substrate was coated with a BLM and then clamped between the bottom piece and the base of the test fixture shown in Figure 2. The lipid bilayer was submerged in an aqueous salt solution and then additional salt solution was steadily poured into the upright cylindrical tube positioned above the BLM, which increased the hydrostatic pressure applied to the lipid bilayer. The lipid bilayer was capable of supporting the water column until a critical pressure was reached, at which point the height of the water column was observed to drop. This critical pressure will be referred to as the failure pressure of the BLM.

Several types of lipids were tested including 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (SOPC) and mixtures of SOPC and cholesterol (CHOL). Mixtures of SOPC/CHOL were used because studies have shown that cholesterol increases the strength of BLMs. They have further concluded that cholesterol concentrations of 50 – 60 mol% will maximize the strength of an SOPC/CHOL BLM, which was the basis for the concentration chosen for this study. This test was repeated for different substrate pore radii to produce a plot of the mean failure pressure versus substrate pore radius. The previous results from the water column test fixture are reviewed later in this paper. A trend of increasing failure pressure with decreasing pore size was observed.

The data measured in the previous study was limited to small pressures and therefore substrates with relatively large pore sizes. In an effort to increase the range of pore sizes that could be tested, while also increasing the accuracy and precision of the experiments, a new experimental test fixture was developed. In this test fixture, a stepper motor is used to drive a piston attached to a lead screw. The stepper motor applies pressure in very fine increments (approximately 300 Pa/step but varying with the pressure range) to the internal lower chamber of the test fixture that is sealed with a BLM formed on a porous substrate. The resulting pressure curve is monitored using a pressure transducer, and the maximum pressure achieved before failure of the BLM is recorded as the failure pressure. As with the previous study, this test was performed using various pore sizes to produce a plot of the mean BLM failure pressure versus substrate pore radius.

Finally, the characteristic pressurization curve of a BLM was modeled according to the pressurization and flow of fluid through the porous substrate. Initially, pressure increases as the moving piston creates a load on the fluid in a closed system. Upon reaching the failure pressure of the BLM, pressure decreases as fluid escapes through the porous medium. Since the BLM is formed over many pores, the setup can be thought of as an array of micro-BLMs covering the many pores. Every BLM does not fail at precisely the same pressure, but rather the failure pressure follows a statistical distribution, which in this case was assumed to be a normal distribution.

This paper is presented in the following format. The next section discusses the materials and methodology that were used to collect experimental data. Then, a model is described that was used to predict the pressurization curve of a BLM. Results are presented from the experimental data and the model. Experimental results from the water column and stepper motor test methods are then discussed, as well as the some insight into the behavior of the BLMs that was gained from the model. Finally, some conclusions from this work are provided.

**MATERIALS AND METHODS**

SOPC 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 SOPC was mixed with cholesterol to create stronger BLMs. In particular, 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). The aqueous salt solution was prepared with 0.1 M NaCl (100.0% purity, Mallinckrodt Baker, Inc., Paris, KY) and deionized water. The porous polycarbonate substrates were treated
Table 1. Specifications for porous polycarbonate substrates.

<table>
<thead>
<tr>
<th>Pore Dia. (µm)</th>
<th>Thickness (µm)</th>
<th>Permeability (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>10</td>
<td>2.5 x 10⁻¹⁸</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>2.0 x 10⁻¹⁷</td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>8.0 x 10⁻¹⁷</td>
</tr>
<tr>
<td>0.4</td>
<td>10</td>
<td>2.0 x 10⁻¹⁶</td>
</tr>
<tr>
<td>0.8</td>
<td>9</td>
<td>1.9 x 10⁻¹⁶</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3.0 x 10⁻¹⁶</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>4.2 x 10⁻¹⁵</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1.2 x 10⁻¹⁴</td>
</tr>
</tbody>
</table>

by the manufacturer with a hydrophilic polyvinylpyrrolidone coating which facilitates the formation of a BLM on its surface. Tests were performed for pore diameters of 0.05 µm, 0.1 µm, 0.2 µm, 0.4 µm, 0.8 µm, 2 µm, 5 µm, and 10 µm. For modeling purposes it was also necessary to know the thickness, which was provided by the manufacturer, and the permeability, which was determined experimentally. These values are reported in Table 1. Measuring permeability is further explained in the Results section.

Experiments were carried out to evaluate the failure pressure of planar BLMs. The test fixture for this study was composed of a stepper motor (model L1MGJ-M200XX060, EAD Motors, Dover, NH) used to drive a lead screw in a linear motion. A piston with an outside diameter of 12.2 mm was attached to the end of the lead screw, which had a thread pitch of 1.57 threads/mm. For each step the motor turns 0.9°, which translates into a linear piston motion of 1.59 µm. The piston was used to pressurize a lower fluid chamber inside of the aluminum body of the test fixture, which had an internal volume of 5.0 mL (see Figures 3 and 4). The lower fluid chamber in the aluminum body was connected to a polycarbonate upper chamber via a 3.2 mm diameter hole. The lower and upper chambers were sealed using rubber gaskets. The porous substrate was clamped between the lower and upper fluid chambers so that the lower fluid chamber was under pressure and the upper chamber was open to the atmosphere. A pressure relief valve was connected to the lower fluid chamber and a polycarbonate fluid reservoir. The pressure relief valve remained open while the porous substrate was clamped into place and was then closed before the test was started. This procedure was necessary to prevent an initial pressure buildup during assembly.

Pressure was monitored by two different pressure

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transducers that had different ranges. A pressure transducer with a range of \(0 - 690\) kPa (model PX181B-100G5V, Omega Engineering, Inc., Stamford, CT) was used for pore sizes from \(0.05 - 0.2\) \(\mu\)m and a pressure transducer with a range of \(0 - 41\) kPa (model PX181B-006G5V, Omega Engineering, Inc., Stamford, CT) was used for pore sizes from \(0.4 - 10\) \(\mu\)m. Both pressure transducers are reported by the manufacturer to have an accuracy of 0.3% of full scale. Pressure was recorded using a multifunction data acquisition module (model USB-6009, National Instruments, Austin, TX) and Labview software (National Instruments, Austin, TX).

For each experiment a porous substrate was coated with 20 \(\mu\)L of lipid/n-decane mixture by using a pipettor (model Calibra 822, Socorex, Switzerland) and was then clamped between the lower and upper fluid chambers. Pressure was slowly applied by running the stepper motor at a fixed frequency of 1 Hz for the entire duration of the test. The failure pressure of the BLM was determined at the point when pressure began to decrease in the lower chamber of the test fixture. Ten trials were performed for each combination of lipid mixture and substrate pore size. Control trials were also performed for each pore size. For control trials a porous substrate with no BLM was clamped into the stepper motor test fixture. The stepper motor was run at a frequency of 1 Hz and pressure was recorded.

A silver/silver-chloride (Ag/AgCl) electrode (model EP2, World Precision Instruments, Inc., Sarasota, FL) was mounted on either side of the porous substrate so that it was submerged in the salt solution and the electrical impedance was measured across the BLM using an impedance analyzer (model 4192A, Hewlett Packard, Palo Alto, CA). The impedance was recorded over a frequency range of \(0.01 - 1000\) kHz with a resolution of 50 data points taken with logarithmic spacing. Measurements were taken once at the start of the test before the BLM was pressurized, and once at the end of the test after the BLM had failed. The impedance measurement was used to identify the presence or absence of a BLM on the porous substrate.

**MODELING**

The pressurization curve of a lipid bilayer can be modeled according to the pressurization of fluid and the flow of fluid through the porous substrate. It has been experimentally determined that after all visible air bubbles have been bled from the lower chamber, approximately 1% of the remaining fluid volume is composed of dissolved air. Therefore it is a basic premise of this model that the change in pressure inside the lower chamber is a result of the contraction and expansion of this volume of air according to Boyle’s law, \(PV = k\), where \(P\) is absolute pressure, \(V\) is volume, and \(k\) is a constant of proportionality. The constant \(k\) can be calculated according to the initial test conditions as

\[
k = P_{atm}V_{i,air},
\]

where the initial pressure \(P_{atm}\) is one atmosphere, and the initial volume of air \(V_{i,air}\) is 1% of the internal volume of the lower test chamber.

It is assumed that at the outset of each trial a BLM covers the substrate pores, effectively sealing the lower chamber of the test fixture. As the stepper motor pushes the piston forward, it reduces the internal volume of the lower chamber by the amount \(\Delta V_{piston}\). This change in volume compresses the air that is dissolved in the aqueous fluid, causing the pressure inside the lower chamber to increase. When the BLM fails, fluid is free to flow out through the porous substrate. For each volume increment of fluid that flows out of the lower chamber, the air within the lower chamber is free to expand by that amount, \(\Delta V_{pores}\), thereby decreasing the pressure inside the chamber. This relationship can be summarized as

\[
P = \frac{k}{V_{i,air} - \Delta V_{piston} + \Delta V_{pores}}.
\]

In these experiments the stepper motor was operated at a fixed frequency, \(f_{stepper}\), of 1 Hz, creating a linear piston motion, \(\Delta x_{stepper}\), of 1.59 \(\mu\)m per step. Therefore the volume that has been displaced by the motion of the piston can be calculated by

\[
\Delta V_{piston} = A_{piston}\Delta x_{stepper}f_{stepper}\Delta t,
\]

where \(A_{piston}\) is the cross sectional area of the piston and \(\Delta t\) is the amount of time that has elapsed since the start of the experiment.

When the BLM fails, pressure inside of the lower test chamber forces fluid to flow through the porous substrate to the upper chamber of the test fixture, which is open to the atmosphere. The flow rate of fluid exiting the pores can be approximated using Darcy’s law for fluid flow through porous media,

\[
Q = \frac{\kappa A}{\mu L} \Delta P,
\]

where \(Q\) is the flow rate, \(\kappa\) is the permeability of the medium (which is usually an experimentally determined value), \(A\) is the cross sectional area to flow,
μ is the viscosity of the fluid, L is the length of flow through the medium, and ΔP is the pressure drop. Flow rate Q can be equivalently written as the time derivative of volume, Q = ΔV_{pores}/Δt. For this model, κ will be considered the permeability of the porous substrate and κ’ will be considered the combined permeability of the porous substrate and the BLM. The cross sectional area is the area of the substrate that is under pressure, A_{substrate}, which is based on the 3.2 mm diameter of the orifice that connects the lower and upper fluid chambers. The pressure drop ΔP is simply the difference between the pressure inside the lower test chamber and the atmosphere, ΔP = P − P_{atm}. Therefore Equation (4) can be rewritten as

\[
\Delta V_{\text{pores}} = \Delta t \frac{\kappa' A_{\text{substrate}}}{\mu L} (P - P_{\text{atm}}).
\]

These experiments investigate a BLM that is coated over a substrate with many pores. The BLM is presumed to fail where it covers the pores, and therefore this setup can also be thought of as an array of individual micro-BLMs each covering a pore. In this scenario, all BLMs do not fail at the same pressure, but rather they follow a statistical distribution. It is assumed that the failure pressure of the BLMs follows a normal (Gaussian) distribution. As the pressure in the lower test chamber is increased, BLMs will gradually fail causing a small leak of fluid through those pores. If only a few BLMs have failed then the leak will be insignificant. When the pressure approaches the mean failure pressure, μ_f, a large number of BLMs will fail at once, causing a larger leak and a measurable drop in pressure.

The combined permeability of the porous substrate and the BLMs changes as the BLMs begin to fail. When all pores are covered by a BLM, the permeability is zero. If all of the BLMs were to fail, then the combined permeability would be that of the porous substrate alone. Between these two cases, it is assumed that the combined permeability varies proportionally with the fraction of BLMs that have failed. However, at the outset of the test a BLM usually does not fully form over every pore. Do to random variability in the formation of the lipid bilayer, some pores remain open. The small fraction of micro-BLMs that do not initially form over a pore, χ_{i,f}, is used as a curve-fitting parameter. The fraction of micro-BLMs that subsequently fail after the start of the test due to pressurization is χ_f. Therefore the overall combined permeability of the porous substrate and the BLM is

\[
\kappa' = (\chi_{i,f} + \chi_f) \kappa.
\]

\[\chi_f\] can be equivalently written as

\[
\chi_f = (1 - \chi_{i,f}) \Phi(P),
\]

where \(\Phi(P)\) is the cumulative distribution function of a normal distribution. In this case \(\Phi(P)\) represents the fraction of BLMs that fail at a given pressure \(P\), assuming that failure pressure is a random normal variable. \(\Phi(P)\) can be computed by integrating the probability density function of a normal distribution:

\[
\Phi(P) = \frac{1}{\sigma_f \sqrt{2\pi}} \int_{-\infty}^{P} \exp \left( -\frac{(u - \mu_f)^2}{2\sigma_f^2} \right) \, du,
\]

where \(u\) is an integration variable.

**RESULTS**

Figure 5 is a plot of pressure versus piston displacement for the control trials. These traces represent the pressurization of porous substrates without a BLM formed over them. For most pore sizes, no significant pressure buildup was observed, indicating that fluid was free to flow through the porous substrate in the absence of a BLM. For the 0.05 μm and 0.1 μm pore sizes, a measurable back-pressure was generated with the stepper motor operating at a frequency of 1 Hz. However, this back-pressure was still small when compared to pressurization of a BLM formed over the same substrates.

The permeability of each porous substrate was determined experimentally. To do this, a porous substrate without a BLM was clamped into the stepper motor test fixture. The stepper motor was turned on at time \(t_0\) and run at a high frequency to generate back-pressure inside the lower chamber. Then the stepper motor was turned off at time \(t_1\) and the...
BLM was pressurized by running the stepper motor at 5 Hz starting at time \( t_0 \), and then turned off at time \( t_1 \) to allow pressure to decrease. The experimentally measured curve is marked by a solid line and the model based on a curve-fitted permeability is marked by a dotted line.

Back-pressure was allowed to decrease as fluid exited through the pores. The model described in the Modeling section was used to curve-fit the permeability. The model was modified such that \( \Delta V_{\text{piston}} \) was held as a constant value after time \( t_1 \) when the piston stopped moving, and the permeability was held as a constant value for the entire simulation. Figure 6 is an example in which a substrate with 0.2 \( \mu \)m diameter pores was pressurized by running the stepper motor at a frequency of 5 Hz for approximately 35 s. At time \( t_1 = 35 \) s, the stepper motor was turned off and the back-pressure decreased to zero. Ideally the model should provide a good fit of the experimental pressure curve both when stepper motor is turned on and when it is turned off. However, often times two different permeability values are needed to fit these two different regimes. Before \( t_1 \) the modeling of the permeability is complicated by the fact that pressure is increasing due to the piston motion while simultaneously decreasing due to fluid flow through the pores. The model is simplified after \( t_1 \) since the piston is stopped. The experimental response in this regime is also cleaner and more repeatable. Therefore a permeability value was chosen that provided the best curve-fit after time \( t_1 \), when pressure was decreasing. In the example shown in Figure 6 the permeability was estimated as \( 8.0 \times 10^{-17} \) m\(^2\).

Figure 7A is a plot of pressure versus piston displacement for ten pressurization trials under the same test conditions of SOPC BLMs formed over substrates with 0.2 \( \mu \)m diameter pores. Figure 7B is the same plot for substrates with 2 \( \mu \)m diameter pores. These are typical pressurization curves which have a pressure rise, a peak that represents the bulk failure pressure of the BLM, and a subsequent pressure decrease.

The electrical impedance has been measured for each trial before the BLM was pressurized and again after it failed. For BLMs formed on 0.2 \( \mu \)m pore substrates, Figure 8A is a plot of impedance and phase versus frequency for each trial before pressurization, and Figure 8B is the same measurement after the BLM failed. Figures 8C and 8D are the same measurements for 2 \( \mu \)m pore substrates.

The impedance measurements served as a useful way of identifying whether or not a BLM formed over the porous substrate. An electrical impedance measured at the start of the test that reached a maximum of 100 – 1000 k\( \Omega \) indicated that a lipid bilayer had fully formed over the porous substrate. The impedance measured after failure occurred was consistently on the order of 1 – 10 k\( \Omega \). This was the same magnitude of resistance of the salt solution alone, implying that the BLM no longer covered the pores of the substrate. Figure 8 supports these trends and shows a clear and repeatable difference between the impedance signatures associated with the presence or absence of a BLM.

Due to the large amount of data collected during this study, every trial is not presented here. Instead, a representative trial from each pore size was chosen and are plotted together in Figures 9A, 9B, and 9C. The data has been separated into three plots so that each can be scaled appropriately. Each plot shows the experimentally measured pressure curve as well as a model pressure curve that was fitted to the data. In each model curve, the mean failure pressure, \( \mu_f \), the standard deviation of the failure pressure, \( \sigma_f \), and the fraction of BLMs that are initially failed, \( \chi_{i,f} \), were used as curve-fitting parameters. These parameters are reported in Table 2, as well as the total fraction of failed BLMs at the end of the test, \( \chi_{i,f} + \chi_f \), which was determined by the model.

A logarithmic plot of failure pressure versus pore size is shown in Figure 10, where each point represents the mean failure pressure of all trials for that pore size. In this plot data is shown for SOPC and SOPC/CHOL-50 mol\%, as measured using the water column test fixture from the previous study and using the stepper motor test fixture from the current study. Data points from the water column test fixture represent the average of six repeated trials while data points from the stepper motor test fixture are the average of ten repeated trials. The experimental results establish a clear trend between the failure pressure of planar BLMs and the pore size of

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\[ 6 \]
Table 2. Mean failure pressure, standard deviation, the fraction of BLMs that are initially failed, and the total fraction of failed pores at the end of the test.

<table>
<thead>
<tr>
<th>Pore Dia. (µm)</th>
<th>$\mu_f$ (kPa)</th>
<th>$\sigma_f$ (kPa)</th>
<th>$\chi_{i,f}$</th>
<th>$\chi_{i,f} + \chi_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>405</td>
<td>53</td>
<td>0.180</td>
<td>0.337</td>
</tr>
<tr>
<td>0.1</td>
<td>230</td>
<td>3.8</td>
<td>0.027</td>
<td>0.069</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
<td>0.88</td>
<td>0.015</td>
<td>0.046</td>
</tr>
<tr>
<td>0.4</td>
<td>42.0</td>
<td>0.50</td>
<td>0.006</td>
<td>0.051</td>
</tr>
<tr>
<td>0.8</td>
<td>22.6</td>
<td>0.38</td>
<td>0.006</td>
<td>0.078</td>
</tr>
<tr>
<td>2</td>
<td>9.4</td>
<td>0.13</td>
<td>0.005</td>
<td>0.187</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>0.068</td>
<td>0.015</td>
<td>0.062</td>
</tr>
<tr>
<td>10</td>
<td>1.9</td>
<td>0.054</td>
<td>0.008</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Figure 7. Pressurization curves for ten trials of an SOPC BLM formed on a substrate with (A) 0.2 µm diameter pores, and (B) 2 µm diameter pores.

Figure 8. Impedance measurements (A) before pressurization of an SOPC BLM on a substrate with 0.2 µm diameter pores, (B) after pressurization of an SOPC BLM on a substrate with 0.2 µm diameter pores, (C) before pressurization of an SOPC BLM on a substrate with 2 µm diameter pores, and (D) after pressurization of an SOPC BLM on a substrate with 2 µm diameter pores.
and 10 µm diameter pores. (C) 2 µm, 5 µm, and 10 µm diameter pores.

Figure 9. For the pressurization of SOPC, a representative experimental trial from each pore size is marked by a solid line and the predicted response line fitted to each experiment is marked by a dotted line. (A) 0.05 µm, and 0.1 µm diameter pores. (B) 0.2 µm, 0.4 µm, and 0.8 µm diameter pores. (C) 2 µm, 5 µm, and 10 µm diameter pores.

Figure 10. Failure pressure versus pore size. Squares denote SOPC BLMs measured by the stepper motor test fixture; diamonds denote SOPC BLMs measured by the water column test fixture; plus marks denote SOPC/CHOL-50 mol% BLMs measured by the stepper motor test fixture; X marks denote SOPC/CHOL-50 mol% BLMs measured by the water column test fixture.

their supporting substrate, demonstrating that failure pressure increases as pore size decreases. On the logarithmic plot from Figure 10 this relationship was nearly linear.

**DISCUSSION**

The failure pressures measured using the water column test fixture from the previous study were in good agreement with the failure pressures measured using the stepper motor test fixture. The water column test fixture was limited to small pressure measurements, but the stepper motor test fixture greatly expanded the range and resolution of pressures and pore sizes that could be tested. The stepper motor test fixture provided an automated application of pressure to the BLM and reduced the amount of interpretation by the operator in determining the failure pressure of the BLM. Figures 7A and 7B show that the pressurization curves generated using the stepper motor test fixture are very repeatable.

Cholesterol is known to increase the strength of saturated and mono-unsaturated BLMs such as SOPC.\textsuperscript{11, 12} The experimental results in Figure 10 were consistent with this trend. The failure pressure for a mixture of SOPC and 50 mol% cholesterol was on average 1.5 times greater than that of SOPC alone.

The model presented in the Modeling section and Figure 9 was intended to provide a physical interpretation of the pressurization and failure of the BLMs in this test setup. Most of the parameters that were needed for the model were geometrical or other physical properties of the test fixture and porous sub-
substrates. However, the mean failure pressure, standard deviation of failure pressure, and the fraction of initially failed BLMs were left as fitting parameters. Many aspects of the experimental pressure curves were fitted by the model quite well. In most cases, the experimental pressure curve consisted of a pressure rise, a peak, and a steady decay in pressure to some equilibrium value. However, in some instances the pressure did not level off to a constant equilibrium value but rather showed dramatic fluctuations (as seen in Figure 9c for the responses of BLMs formed on substrates with pore diameters of 2 μm and 5 μm). This phenomena could be due to several factors including failed BLMs reforming to block previously open pores, salt from the NaCl solution clogging open pores, and random local pressure fluctuations near the pressure transducer which appear more dramatic for the smaller pressure measurements associated with larger pore sizes. The model can not account for this phenomena because it assumes that the permeability of the substrate is a constant and that BLMs do not reform after they have failed.

Several interesting insights were gained through the fitting of this model. First, it was found that the bulk failure pressure that was measured in these experiments was close to the curve-fitted mean failure pressure of an individual micro-BLM. In most cases, the curve-fitted mean failure pressure was slightly higher than the bulk failure pressure. However, the two values were close enough that the mean failure pressure can be estimated by simply measuring the bulk failure pressure.

Second, a relationship was determined between the standard deviation of the failure pressure and the shape of the pressurization curve. If the failure of the BLMs has a narrow distribution then most of the micro-BLMs fail together near the mean failure pressure, which is seen as a peak pressure followed by a sharp drop in pressure. This behavior was observed in most trials. However, in some instances the pressure did not reach a local peak, but instead steadily leveled off to the equilibrium pressure. (See, for example, the pressure curve for the 0.05 μm pore size in Figure 9a.) The responses of these BLMs have wider distributions, causing a more gradual pressure rise.

Third, the model provided a way to calculate the fraction of BLMs that have failed at the end of the test, \( \chi_{f} \), which was reported in Table 2. In these experiments the final equilibrium pressure was nonzero, indicating that some portion of BLMs had not failed. If all BLMs were to fail during the test then the pressure would fall to zero. Instead, the BLMs that do not fail continue to block the substrate pores creating a back pressure in the lower chamber. In all cases the fraction of failed BLMs in Table 2 was less than 0.5, meaning that the test never reaches the true mean failure pressure, \( \mu_f \). Also, there was a general trend in which the final fraction of failed BLMs decreased with increasing pore size. This is likely due to the fact that a larger pore allows a greater rate of fluid flux. Therefore with substrates that have large pores, less BLMs are needed to fail to create a measurable pressure drop.

CONCLUSIONS

The new experimental setup that uses a stepper motor to pressurize BLMs was able to improve the range and accuracy of previous measurements that were made using a water column pressurization method. The failure pressure was measured for SOPC and SOPC/CHOL-50 mol% BLMs that were formed over porous substrates with pore sizes ranging from 0.05 – 10 μm in diameter. A clear trend of increasing failure pressure with decreasing pore size was established, which agreed with previous measurements made using the water column. The addition of cholesterol to SOPC resulted in an increased failure pressure. A model based on fluid pressurization and flow through a porous medium was able to predict the major trends in the experimental pressure curves. The model also provided insight into the statistical distribution of the failure of BLMs formed over an array of multiple pores.

ACKNOWLEDGMENTS

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