Effect of Compressed Bovine Lipid Extract Surfactant Films on Oxygen Transfer

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In the lungs, oxygen transfer from the inspired air to the capillary blood needs to cross the surfactant lining layer of the alveoli. Therefore, the gas transfer characteristics of lung surfactant film are of fundamental physiological interest. However, previous in vitro studies—most relied on the Langmuir-type balance—fail to cover the low surface tension range (i.e., less than the equilibrium surface tension of approximately 25 mJ/m^2) due to film leakage. We have recently developed a novel in vitro experimental strategy, the combination of axisymmetric drop shape analysis and captive bubble technique (ADSA-CB), in studying the effect of surfactant films on interfacial gas transfer (*Langmuir* **2005**, *21*, 5446). In the present work, ADSA-CB is used as a micro-film-balance to study the effect of compressed bovine lipid extract surfactant (BLES) films on oxygen transfer. A low surface tension ranging from approximately 25 mJ/m^2 to 2 mJ/m^2 is studied. The experimental results suggest that lung surfactant films at a low surface tension near 2 mJ/m^2 provide resistance to oxygen transfer, as indicated by a decrease of 30−50% in the mass transfer coefficient (k_L) of oxygen in BLES suspensions with respect to water. At higher surface tension (i.e., >6 mJ/m^2), the resistance to oxygen transfer is only modest, i.e., the decrease in k_L is less than 20% compared to water. The experimental results suggest that lung surfactant plays a role in oxygen transfer in the pulmonary system.

1. Introduction

Lung surfactant is a complicated mixture of approximately 90% lipids and 10% proteins.[1] It is synthesized and secreted by type II alveolar epithelial cells and forms a thin film on the surface of alveoli. The main function of the lung surfactant film is to reduce the surface tension of the alveolar surface so that the energy expended to inflate the lungs during inspiration is minimized and the likelihood of lung collapse during expiration is also reduced.[2]

As part of the alveolar−capillary diffusion barrier,[3] the influence of the lung surfactant film on pulmonary gas exchange is of interest to both physiologists and chemical engineers. On the one hand, from the traditional physiological point of view, the lung surfactant film does not provide resistance to gas diffusion between the air space and the capillary blood.[4,5] On the other hand, from the chemical engineering point of view, it has been well-documented that monolayers of certain insoluble surfactants, such as long chain fatty acids, alcohols, and phospholipids, generally show a significant resistance to gas transfer,[6−9] and the resistance is dependent on the molecular structure (e.g., the polar headgroup and the chain length) of the film-forming molecules and the physicochemical characteristics of the films, such as surface tension and film compressibility.[6−9]

The possible effect of lung surfactant films on oxygen transfer has been explored previously. Ladanyi[10] has developed an electrochemical method based on dc and ac polarography to indirectly study the effect of lung surfactant films on oxygen transfer. Using this method, Ladanyi and co-workers qualitatively reported that both dipalmitoyl phosphatidylcholine (DPPC) films (spread at surface tension of 42 and 27 mJ/m^2, respectively)[11] and lung surfactant extracts[10] presented resistance to oxygen transfer and that the resistance increased with deceasing surface tension.[11]

More recent studies of the influence of phospholipid films on interfacial oxygen transfer relied on Langmuir-type balances, in which a spread phospholipid monolayer is compressed by a moving barrier and the surface tension is measured by a Wilhelmy plate.[12−15] Sosnowski et al.[12] studied the resistance of spread DPPC films to oxygen transfer. They found that DPPC films showed no resistance to oxygen transfer at a surface tension of 37 mJ/m^2 at room temperature. However, significant resistance (30% decrease in oxygen uptake) was found at a surface tension

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of 22 mJ/m^2. Ivanov et al.[13] studied the effect of DPPC and rat lung surfactant monolayers on oxygen transfer at room temperature. They found that the resistance to oxygen transfer due to these lipid monolayers was generally 3 or 4 orders of magnitude higher than those traditionally considered. In addition, when the surface tension of the rat surfactant was decreased from 68 to 54 mJ/m^2, the monolayer resistance to oxygen transfer was increased by 1 order of magnitude.[13] Sández Macho et al.[14] studied the effect of dipalmitoyl phosphatidylethanolamine (DPPE) films on oxygen transfer using a photophysical method in which the amount of oxygen transfer was detected by quenching oxygen-sensitive fluorescent dyes. They found that oxygen transfer was significantly hindered by a tightly packed DPPE film, indicated by a surface tension of approximately 20 mJ/m^2. Using scanning electrochemical microscopy (SECM), Zhang et al.[15] also reported that oxygen transfer across a distearoyl phosphatodylcholine (DSPC) film was hindered as surface tension was decreased.

In spite of the above accumulating evidence, the effect of lung surfactant films on oxygen transfer still remains uncertain, due to several limitations in previous studies: (1) Monomolecular film spread at the air−water interface of a Langmuir balance is commonly used in vitro for studying the surface activity of lung surfactant.[2,16] However, this model may not be ideal to mimic the gas transfer in the lungs, as the surfactant film in vivo is formed by adsorption and most likely possesses a complicated multilayer structure, i.e., a number of extra lipid layers closely associated with the interfacial monolayer.[17,18] (2) Most of the previous studies focus on films formed by a single phospholipid component; however, lung surfactant per se is a much more complicated mixture of lipids and proteins. (3) Previous studies using a Langmuir balance do not cover low surface tensions. Surface tension in the lungs may still be disputable but apparently in a range of low values, in order to maintain a large gas exchange area.[19] Direct measurement in situ has established that the surface tension in the lungs ranges from approximately 20 mJ/m^2 at 85% total lung capacity (TLC) to less than 1 mJ/m^2 at 40% TLC (approximately equivalent to the functional residual capacity, FRC).[20,21] However, other researchers believe that the extremely low surface tension near or below 1 mJ/m^2 only occurs under extreme conditions, such as coughing or intense laughing.[22] In spite of this conflict, it is generally agreed that under normal physiological conditions surface tension in the lungs should be near or lower than the equilibrium value of a predominantly phospholipid film, i.e., ∼25 mJ/m^2.[2,19,23] However, it is difficult to use a Langmuir balance to cover this low surface tension range due to the problem of film leakage.[2,16,24] Film leakage occurs when a surfactant film is compressed to a surface tension lower than the surface free energy of the solid in contact with the film, i.e., the walls and the barriers of the balance. For a balance made of Teflon, which has a surface free energy of approximately 18 mJ/m^2, the surfactant film tends to spread onto the solid surface when the film is compressed to a surface tension less than this value. Although different techniques have been developed to minimize film leakage, e.g., priming the solid surface with long chain saturated phospholipid,[25] it is generally accepted that none of the existing methods is able to completely remove film leakage from a trough apparatus.[2,16] Even though a transient low surface tension can be achieved by a carefully primed balance upon extremely high compression,[25] holding this low surface tension for sufficiently long time (i.e., maintaining the metastability of the film) to allow the study of gas transfer is almost impossible,[9] unless for transient measurements.[15] Hence, film leakage limits the minimum stable surface tension that can be studied in a Langmuir balance. Apparently, none of the previous studies concerning the effect of lung surfactant films on oxygen transfer have been conducted at a surface tension less than 20 mJ/m^2. Simply extrapolating the experimental data to low surface tension range may be erroneous due to the complicated phase behavior of lung surfactant films at different surface tensions.[26]

We have recently developed a novel in vitro experimental strategy capable of simultaneously measuring surface tension and interfacial gas transfer.[27] In that method, axisymmetric drop shape analysis in conjunction with the captive bubble technique (ADSA-CB) was used as a micro-film-balance.[27] The interfacial gas transfer, quantified by a steady-state mass transfer coefficient, was determined by photographically analyzing the shrinkage of a CB due to gas diffusion across the surfactant film adsorbed on the bubble surface and out of the bubble. Meanwhile, the surface tension was continuously measured by analyzing the shape of the bubble. The following are the other advantages of this method over the traditional Langmuir trough: (1) Only a small amount of liquid sample, i.e., a few milliliters in comparison with a minimum 50 mL in a Langmuir balance,[2,16] is required (this allows the study of adsorbed lung surfactant films at a reasonable cost). (2) A surface tension well below the equilibrium value can be easily obtained and maintained for a prolonged period.[24,28,29] To our knowledge, so far, this extreme metastability of lung surfactant films can be only achieved in vitro by a CB arrangement owing to its leakage-proof capacity.[24,28,29] (3) No equilibrium in gas transfer is required; hence, the measurements can be completed within a relatively short period, i.e., 5 min. In this paper, we use ADSA-CB to study the effect of bovine lipid extract surfactant (BLES) on interfacial oxygen transfer, covering the low surface tension range, i.e., from the equilibrium surface tension (i.e., ∼25 mJ/m^2) to a value less than 5 mJ/m^2. As a result, we are able to shed light on the effect of lung surfactant film on oxygen transfer in a surface tension range that has not been explored before.

2. Materials and Methods

2.1. Materials. BLES (BLES Biochemicals Inc, London, ON, Canada) is a therapeutic lung surfactant commercially available. It is prepared by organic extraction from bronchopulmonary bovine lung lavage. BLES contains about 98% phospholipids and 2% proteins. The phospholipid components are approximately 45% DPPC, 35% unsaturated phosphatidylcholines (PCs), 12% phosphatidylglycerol (PG), 2% phosphatidylethanolamine (PE), 1% phosphatidylinositol (PI), 1% lysophosphatidylcholine (LPC), and 2% sphingomyelin (SPM). The protein components in BLES are only low molecular weight hydrophobic proteins, SP-B and SP-C.

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High molecular weight hydrophilic proteins, SP-A and SP-D, as well as the neutral lipids (primarily cholesterol), have been removed during extraction with organic solvents. BLES is stored frozen in sterilized vials with an initial concentration of 27 mg/mL. It is diluted to 0.5 mg/mL by a salt solution of 0.6% NaCl and 1.5 mM $CaCl_2$ on the day of the experiment. This phospholipid concentration is much lower than that of the surfactant in the lungs. However, it has been found that in a captive bubble arrangement such a low concentration is adequate to reproduce the physiological and biophysical function of lung surfactants in vivo.[17−19,24,30] Moreover, the maximum phospholipid concentration that can be studied in the captive bubble method is restricted to no more than 1−2 mg/mL.[24,30] This restriction arises from optical limitations, because lung surfactant suspensions become murky and possibly opaque at higher concentrations. The water used in the experiments is demineralized and glass distilled. The final diluted BLES preparation has a pH of 5.6. The oxygen used in this study has a minimum purity of 99.06% (BOC).

2.2. Experimental Methods. A detailed description of the experimental setup and protocol has been given previously.[27] Briefly, a captive bubble chamber was filled with approximately 1.0 mL of BLES suspension (or distilled water tested as a baseline) and thermostatically maintained at 37 ± 0.2 °C by a water bath. The test liquid was presaturated with air, i.e., having an oxygen partial pressure of approximately 21%, measured by a dissolved oxygen (DO) probe (Mini Clark style electrode, #730, Diamond General, MI) in conjunction with a polarographic amplifier (Model 1900, A-M Systems, WA).

The air saturation protocol eliminated the potential changes in gas compositions due to liquid handling procedures. While this procedure introduces nitrogen into the aqueous solution, this systematic deviation can be taken into account in the equation of mass transfer, as described in the Appendix. Furthermore, the interpretation of the changes in mass transfer coefficients with surface tension of BLES suspensions is based on their ratio with respect to the mass transfer coefficient in water-only systems compressed in a similar manner. This mass transfer ratio eliminates not only the systematic deviation produced by nitrogen cross diffusion, but also the systematic deviations produced by differences in hydrodynamic conditions associated with the method of film compression.

After completing the air saturation protocol and introducing the liquid in the captive bubble chamber, a pure oxygen bubble (i.e., ∼100% oxygen partial pressure) with a volume of approximately 20 μL (∼ 3 mm in diameter) was injected into the chamber by a microsyringe (50 μL, #1705, Gastight, Hamilton Corp., Reno, NY). After injection, the bubble immediately rested against the ceiling of the chamber (but separated from the ceiling by a thin wetting film) and formed a Laplacian shape. Within seconds, and most likely also during the formation of the bubble, a lung surfactant film was formed on the surface of the bubble by adsorption of surfactant aggregates. This rapid adsorption was indicated by a surface tension of less than 30 mJ/m^2, recorded immediately after the bubble injection.[27,31]

Subsequently, the bubble was compressed by applying a predetermined pressure to the aqueous subphase. To do so, a motorized syringe (5 mL, #1005, Gastight, Hamilton Corp.) was used to add test liquid into the chamber. The time to pressurize the system was less than 2 s, precisely controlled by a programmable motor controller (18705/6, Oriel Instru, Stratford, CT). The gauge pressures applied to the subphase were 2, 4, 6, 8, and 10 psi, respectively, thus producing various amounts of film compression and different surface tensions. After pressurization, the bubble was left undisturbed for 5 min to allow oxygen diffusion across the lung surfactant film and out of the bubble due to the partial pressure difference between the bubble and the aqueous subphase. During this period, cross-diffusion of dissolved nitrogen from the liquid sample to the bubble also occurs. The effect of this cross-diffusion will be assessed by assuming that

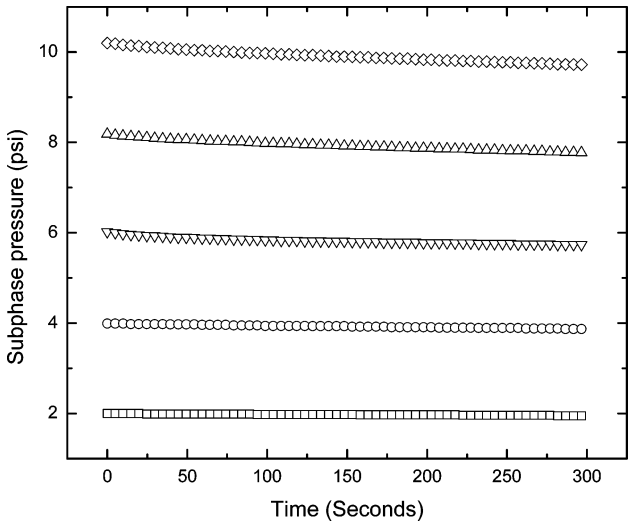

Figure 1. The subphase gauge pressure recorded by the pressure transducer during oxygen transfer after the system was pressurized to 2, 4, 6, 8, and 10 psi, respectively.

the liquid phase controls the overall rate of mass transfer; see the Appendix.[32,33] A CCD camera (Model 4815−5000, Cohu Corp., Poway, CA) was used to acquire the images of the bubble after pressurizing at a rate of 10 images per second. The acquired images were processed by a digital video processor (Snapper-8, Active Silicon LTD, Uxbridge, UK) and stored in a workstation (Sun Blade 1500, Sun Microsystems Inc, Santa Clara, CA) for further analysis by ADSA.

It is most important in these experiments to maintain the subphase pressure because the resulting mass transfer coefficient could be underestimated if the subphase pressure would drop with time but without consideration. To solve this problem, the subphase pressure was continuously measured by a pressure transducer (DP15, Validyne Eng Corp., Northridge, CA) and monitored via a universal data acquisition card (UPC601-U, Validyne). Figure 1 shows the pressure signals recorded during the experiments. It is noted that, although the chamber was tightly assembled, there is a slight decrease in the system pressure within the 5 min observation, especially at moderate and high pressures (i.e., 6, 8, and 10 psi). Hence, in the calculation of the mass transfer coefficient, the real-time system pressure recorded by the pressure transducer was used.

2.3. Calculation of Surface Tension and Mass Transfer Coefficient from CB Images. ADSA is a surface tension measurement methodology based on analyzing the shape of a drop or bubble.[34] It was first used to analyze CB images by Prokop et al.[35] However, its accuracy is unsatisfactory due to the lack of a sophisticated image analysis procedure.[36,37] A new version of ADSA-CB has been recently developed, in which a new image analysis scheme that consists of the Canny edge detector and a novel edge smoothing technique, axisymmetric lipid fluid interface-smoothing (ALFI-S), was utilized.[36] This new version of ADSA-CB was found to be highly automatic and robust against optical noise,[36] thus facilitating precise edge detection from a noisy image, usually the case for a CB in lung surfactant. Consequently, the new ADSA-CB promotes accurate measurement of surface tension (γ), bubble area (A), and volume (V).[37] In addition, it is also capable of outputting detailed parameters pertinent to bubble geometry, such as the lateral area (A_l, i.e., the

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Laplacian area at the bubble−bulk liquid interface), the contact area (A_c, i.e., the flat area at the bubble−wetting film interface), the height of the bubble (H_B), and the radius of curvature at the bubble apex (R_0).[27] As will be seen later, these parameters are important for the calculation of the mass transfer coefficient.

On the basis of the output of ADSA-CB, we have developed a steady-state gas transfer model, which allows the evaluation of mass transfer coefficient by monitoring bubble shrinkage in an aqueous subphase.[27] In principle, for a problem involving interfacial mass transfer, the mass transfer coefficients in the gas phase and the liquid phase should both be considered. However, for sparingly soluble gases, such as oxygen and nitrogen (whose diffusivity in water is up to 5 orders of magnitude lower than the diffusivity in air), it is therefore expected that the interfacial mass transfer is "liquid-phase controlled",[33] i.e., the rate-limiting step is the transfer of gas molecules through the liquid phase. Furthermore, because the bubble initially contains pure oxygen, the oxygen mass transfer is expected to be dominant.

The mass transfer coefficient is defined as the ratio between the flux of mass transferred across the interface divided by the difference between the concentration of the solute at the interface (assumed in equilibrium with the coexisting phase) and its concentration in the bulk phase.[33] The flux (molar basis) is obtained experimentally using the bubble volume and surface area output of ADSA (see ref 27 and the Appendix). For the case of more than one solute (e.g., oxygen and nitrogen), the experimentally obtained flux corresponds to the net molar flux (sum of the molar flux of the individual solutes). The Appendix presents a detailed description of the mass transfer equations involved in the simultaneous transfer of oxygen and nitrogen across the air/water interface. From this equation, the mass transfer coefficient of oxygen through the aqueous phase (k_{LO_2}) is

$$k_{LO_2} = \left[\frac{1}{N}\sum_{i=1}^{N}\frac{\Delta\left(\frac{P^g_i V_i}{-RT}\right)}{A_{li}\Delta t_i}\right] \times \frac{1}{\left(C^*_{O_2} - C^0_{O_2} - \frac{D_{N_2}}{D_{O_2}}C^0_{N_2}\right)} \qquad (1)$$

where $C^*_{O_2}$ is the concentration of oxygen at the interface (assumed in equilibrium with the gas phase), C^0 is the concentration (of oxygen and nitrogen) in the bulk liquid phase. P^g_i is the pressure of the gas phase at a time step "i", V_i is the bubble volume, and R is the gas constant. A_{li} is the lateral area, N is the number of time steps evaluated, Δt_i is the time interval of each step. D_{N_2} and D_{O_2} are the diffusivities of nitrogen and oxygen in water.

The term in brackets in eq 1 is the net flux of gas across the interface and is determined experimentally. The term in parentheses in eq 1 represents the net concentration difference between the liquid side of the interface and the bulk phase. It should be noted that the presence of nitrogen in the aqueous phase is reflected in the calculation of the concentration gradient. Another important observation regarding eq 1 is that the concentration difference only depends on the initial concentration of oxygen and nitrogen and the concentration of oxygen on the liquid side of the interface (which depends on the temperature and pressure of the bubble).

Because of uncertainties associated with the hydrodynamic conditions at which the mass transfer coefficients are obtained, the main conclusions of this work are based on the ratio between the oxygen transfer coefficient determined in water and the oxygen transfer coefficient determined in BLES suspensions compressed under the same conditions. This normalization procedure eliminates the concentration difference term of eq 1.

To simplify the discussion, henceforth k_{LO_2} will be simply referred to as the mass transfer coefficient k_L.

At any time, the gas partial pressure in the bubble is evaluated by Dalton's law and the Laplace equation as follows:

$$P^g_i = P^l_i + \Delta P_i - P^v = P^l_i + \left(\frac{2\gamma_i}{R_{0i}} + \frac{H_{Bi}}{2}\Delta\rho g\right) - P^v \qquad (2)$$

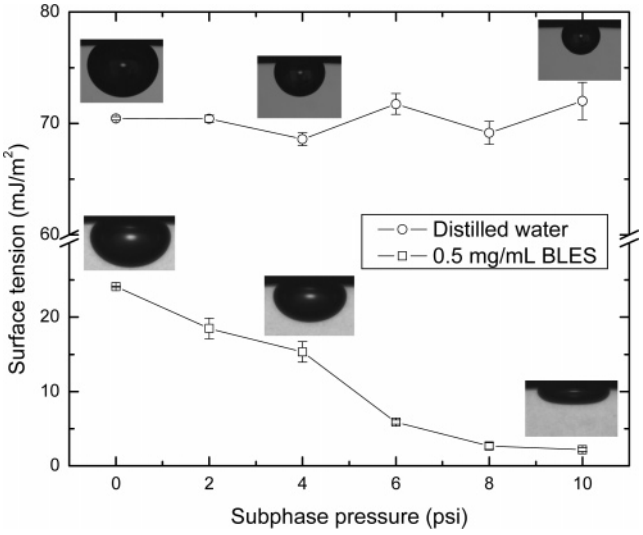

Figure 2. The surface tensions of water and 0.5 mg/mL BLES as a function of the subphase gauge pressure, respectively. The error bar shows the standard deviation (SD) of each measurement. Insets are images of captive bubbles in water and in BLES at the corresponding subphase pressure.

where P^l is the pressure of the liquid subphase, which was continuously measured by the pressure transducer (Validyne); ΔP is the average Laplace pressure in the bubble, which is equal to the sum of the Laplace pressure at the bubble apex and the averaged hydraulic pressure increasing with bubble height; and P^v is the partial pressure of water vapor, which is assumed to be constant and equal to the saturation pressure at the experimental temperature.

There are two points regarding eq 1 that should be kept in mind. First, the mass transfer coefficient calculated from eq 1 is a system parameter only determined by the hydrodynamic conditions of the system and the physicochemical properties of the air−water interface. In principle, it is independent of the system pressure (considered in Henry's law), gas transfer area, size of the bubble, and time of measurement. However, as will be shown later, the calculated mass transfer coefficient does show a dependence on the system pressure, most likely due to the fact that changing the subphase pressure affects the hydrodynamic conditions of the system. This problem can be circumvented by normalizing the mass transfer coefficient measured in surfactant to that measured in water (used as a baseline) at the same controlled subphase pressure. Second, eq 1 is a steady-state mass transfer equation, in which the compositions of the liquid and gas phases, averaged over a specific region and also with respect to any temporal fluctuations, are assumed to be statistically constant. Therefore, the mass transfer coefficient (k_L) calculated from eq 1 is also an average value over the period of observation. (In eq 1, N is the number of discrete ADSA results. N is dependent on the rate of image acquisition.) The validity of the steady-state mass transfer model has been established in numerous experimental studies, provided that certain conditions are met. The key ones are that the temperature and diffusivity at the surface should not be very different from those in the bulk of the liquid and that no chemical reaction occurs, so all molecules of dissolved gas are under the same condition.[40] More detailed validation of this gas transfer model according to the experimental setup and protocol can be found elsewhere.[27]

2.4. Statistical Analysis. The surface tension value (Figure 2) is shown as the mean ± standard deviation (SD). The SD indicates the scatter of surface tensions measured in each experimental run. The mass transfer coefficient (Figure 3) is shown as the mean ± standard error of the mean (SEM). Each mean value was calculated from at least three experimental runs, unless otherwise indicated. The SEM

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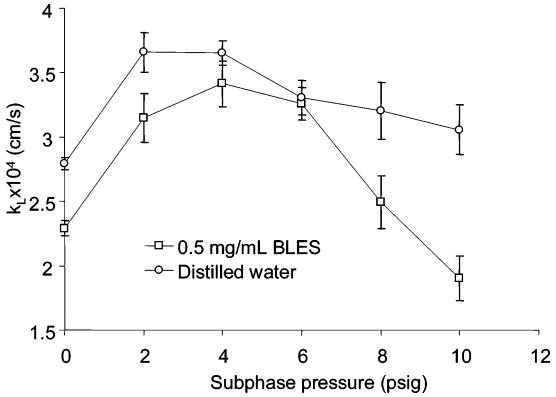

Figure 3. The mass transfer coefficients (k_L) in water and in 0.5 mg/mL BLES as a function of the subphase gauge pressure, respectively. The error bar shows the standard error of the mean (SEM) of each measurement.

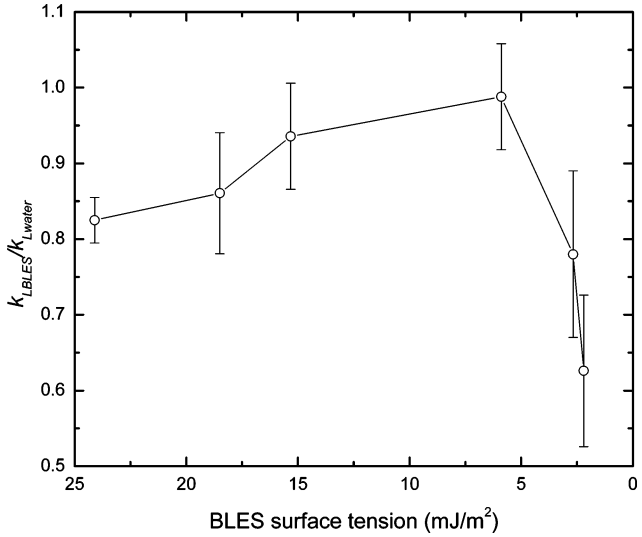

Figure 4. The ratio of the normalized mass transfer coefficient in 0.5 mg/mL BLES to that in water (k_{LBLES}/k_{Lwater}) as a function of BLES surface tension.

indicates the reproducibility of these different experiments. The normalized mass transfer coefficient (Figure 4) is shown with the maximum error propagated from the SEM of k_L in BLES and in water, calculated using the quotient rule.[41]

3. Results

3.1. Measurement of Surface Tension. Figure 2 shows the surface tension of water and 0.5 mg/mL BLES as a function of subphase gauge pressure, where a pressure equal to zero refers to the case of no compression. It should be noted that the dependence of surface tension on the subphase pressure is different for water and for BLES. The measured surface tension of water is close to the literature value of 70 mJ/m^2 [42] and relatively unchanged within the range of pressure variation. This suggests that the surface tension of water is relatively independent of the system pressure, which is in line with previous studies.[43,44]

In contrast to water, the surface tension of BLES decreases with increasing subphase pressure. This is due to the lateral compression of the lung surfactant film adsorbed on the bubble surface. With increasing subphase pressure, the film is compressed to an increasing extent, thus increasing the surface density of the lung surfactant film and decreasing surface tension (see the CB images inserted in Figure 2). It should be noted that at moderate and high subphase pressures (i.e., 6, 8, and 10 psi) surface tensions less than 10 mJ/m^2 are recorded. Such low surface tensions are difficult, if not impossible, to be reached using a conventional Langmuir trough method, because of film leakage.

It should also be noted that, at 10 psi, the surface tension of water is found to deviate from the literature value by more than 2 mJ/m^2 and is associated with a larger error bar than those at lower pressures. This is not real but is due to a limitation of ADSA. As shown in the insert of Figure 2, the bubble in water at 10 psi exhibits a nearly spherical shape, which compromises the accuracy of ADSA, since any drop shape method for measuring surface tension depends on a well-deformed shape.[45,46] Nevertheless, the artifact due to the near-spherical shape is only serious to the surface tension measurement but less important to the determination of bubble geometry.[46] Therefore, these data still can be used for the subsequent calculation of mass transfer coefficients.

3.2. Measurement of Mass Transfer Coefficient. Figure 3 shows the mass transfer coefficients in water and in 0.5 mg/mL BLES as a function of the subphase pressure. At low and moderate system pressures, i.e., 0−6 psi, k_L values in water and in BLES change in parallel as the system pressure increases. That is, the mass transfer coefficient increases as the system pressure increases from 0 to 4 psi and levels off when the pressure is increased to 6 psi. At the high-pressure range, i.e., 6−10 psi, k_L values in water and in BLES significantly diverge from each other: k_L in BLES decreases substantially as the system pressure increases from 6 to 10 psi; in contrast, k_L in water is relatively unchanged in the same range of pressure increase.

The initial increase of k_L in both water and BLES upon compression may indicate a pressure dependence of the interfacial gas transfer. As a result, k_L measured at different system pressures may not be directly comparable, since its dependence on system pressure is still unknown and seems to be controversial.[27] To circumvent this problem, k_L in BLES at different subphase pressures is first normalized to k_L in water (used as a baseline) at the same controlled pressure and then compared.

Figure 4 shows the normalized mass transfer coefficient of oxygen in BLES (i.e., k_{LBLES}/k_{Lwater}) as a function of surface tension. Despite the relatively large errors associated with these normalized results after error propagation, it is evident that, within the limitation of the maximum errors, decreasing the surface tension of a BLES film from the equilibrium value of approximately 24 mJ/m^2 (likely occurring near the TLC[20]) to a low value of approximately 2 mJ/m^2 (likely occurring near the FRC[21]) causes an approximately 25% decrease in oxygen transfer.

4. Discussion

The primary function of the lungs is to exchange oxygen and carbon dioxide between alveoli and capillaries and hence to maintain homeostasis of these two gases in the arterial blood.[3] Passive diffusion is an important transport mechanism involved in the pulmonary gas exchange. Together with the alveolar membrane, capillary endothelium, and blood plasma, the surfactant lining layer of alveoli constitutes part of the alveolar−

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capillary diffusion barrier.[3] Therefore, the study of oxygen transfer properties of lung surfactant films is of fundamental physiological interest.

4.1. Physicochemical Effects of Lung Surfactant Films on Oxygen Transfer. The effect of surfactant on interfacial gas transfer can be attributed to two different mechanisms, physicochemical and hydrodynamic effects, as reviewed in ref 27. Using ADSA-CB, the physicochemical effects can be studied by minimizing the hydrodynamic effects.[27] The physicochemical effects of insoluble surfactants on gas transfer have been treated as an extra energy barrier that is dependent on the structure of the film molecules and the properties pertinent to the conformation of the film, such as surface tension, a measurement of the film compactness.[6,47]

Taking advantage of the leakage-proof environment offered by the captive bubble technique, it is possible to reproduce the low surface tensions occurring in the lungs and simultaneously to examine the dependence of interfacial oxygen transfer on lung surfactant films at these low surface tensions. As shown in Figure 4, the experimental results suggest that the adsorbed BLES films play a role in resisting oxygen transfer, and this resistance is surface-tension-dependent. The BLES films present only limited resistance to oxygen transfer at surface tensions above approximately 6 mJ/m^2; i.e., the decrease in k_L is less than 20% compared to water. At surface tensions of approximately 2 mJ/m^2, however, the BLES films present a significant resistance to oxygen transfer, as indicated by a decrease in k_L by 30−50%. Lung surfactant films in vivo undergo periodical compression and expansion during deflation and inflation of the lungs. The surface tension in the lungs likely varies from a value close to 1 mJ/m^2 at the FRC to approximately 20−30 mJ/m^2 at the TLC.[20,21] Therefore, our experimental results cover the physiological surface tension range during normal tidal breathing. Hence, the results clearly suggest that highly compressed lung surfactant films cause a resistance to oxygen transfer.

It should also be noted that the resistance to oxygen transfer due to BLES films is significantly smaller than that of single-component phospholipid films reported previously.[12−15] The DPPC films, for example, impede oxygen transfer by 30% at a surface tension of 22 mJ/m^2.[12] In contrast, BLES films decrease oxygen transfer by less than 20% at similar surface tensions. This difference in interfacial resistance is likely due to different film configurations between BLES and pure phospholipids. Blank[47,48] found that the basic requirement for high transport resistance is an ordered, tightly packed, and uniform film with a high degree of homogeneity. This kind of film configuration is particularly prevalent in a homogeneous liquid condensed (LC) phase. Films of short chain and/or unsaturated phospholipids do not meet these requirements and hence present no or only modest resistance to gas transfer.[47,48] Using the combination of microelectrode and fluorescence microscopy, Pu et al.[49] have convincingly shown that the oxygen permeability of lipid monolayer shells coated on microbubbles is affected by the microstructure of the monolayers. They found that oxygen permeability increased linearly with domain boundary density, indicating that permeation occurs readily through the accessible area of the disordered interdomain regions.[49] Platikanov and co-workers[50] have developed a diminishing bubble method, in which gas permeability is quantified from the shrinkage of a microbubble (80−200 μm in radius) covered by a phospholipid Newton black film. They also found that the gas permeability was strongly dependent on the physicochemical properties of the black foam films: interference of small solvent molecules with the black foam film of a disaturated phospholipid increases the gas permeability.[50]

As a mixture of saturated and unsaturated phospholipids and surface associated proteins (SP-B and SP-C), BLES does not form as uniform a film as pure phospholipids do. Recent evidence from direct film imaging also suggests that lung surfactant film is composed of coexisting LC and liquid expanded (LE) domains even at a surface pressure approaching 70 mJ/m^2 (i.e., near-zero surface tension).[51] These LC domains are mainly constituted by DPPC and other disaturated phospholipids and take a molecular area fraction of no more than 30−40% of the film.[51] Most likely, the considerable fraction of LE domains, containing mostly the unsaturated phospholipids and hydrophobic proteins, and the LC/LE domain boundaries contribute to the film permeability just like “pores” and “fissures”.

4.2. Experimental Limitations. In spite of the fact that ADSA-CB avoids limitations of the Langmuir balance for low surface tension measurement, it also has the following limitations.

First, only the physicochemical characteristics of lung surfactant films can be studied using ADSA-CB. However, the lung surfactant films in vivo are under periodic dynamic cycling. Hence, hydrodynamic effects, due to movement of the interface, may also play a role in the interfacial gas transfer. Sosnowski et al.[12] found that oxygen transfer was actually enhanced by a spread DPPC film under dynamic cycling. This enhancement was attributed to convection in the subphase near the interface driven by the Marangoni effect, which outweighed the interfacial resistance due to the DPPC film.

Second, the mass transfer coefficient determined in ADSA-CB is an indirect measurement, since the gas transfer is estimated from bubble shrinkage instead of a direct measurement using a gas sensor. Real-time measurement on the increase of DO concentration due to oxygen transfer from a bubble (~20 μL) to the liquid subphase is prevented by the unsatisfactory sensitivity and response time of the DO probes currently available in the market. Another limitation of ADSA-CB as a micro-film-balance is that film compression is correlated with the increase of gas pressure in the bubble. This is not physiologically relevant, as normal tidal breathing only involves very slight variation of the alveolar pressure.[52] The change in the gas-phase pressure also causes the calculated mass transfer coefficients at different system pressures not directly comparable. This problem can be circumvented by normalizing the mass transfer coefficient measured in surfactant suspension to that measured in water (used as a baseline) at the same controlled subphase pressure.

In summary, using a novel experimental methodology, ADSA-CB, we have conducted the first simultaneous measurement of surface tension and interfacial oxygen transfer through adsorbed bovine liquid extract surfactant films in the low surface tension range, i.e., surface tensions less than the equilibrium of approximately 25 mJ/m^2. This range of surface tension may be physiologically relevant and it has never been covered in previous studies. The experimental results suggest that the BLES films at surface tensions higher than 6 mJ/m^2 render only limited resistance to oxygen transfer; however, the resistance increases when further decreasing the surface tension to approximately 2 mJ/m^2. Hence, the experimental results indicate that the surfactant lining layer of the alveoli may contribute to the normal pulmonary

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physiology by playing a role in oxygen transfer. Further validation of this hypothesis requires the study of other lung surfactant preparations with different lipid and protein compositions.

Appendix

Equation for Mass Transfer of Oxygen and Nitrogen across the Interface. The system under consideration has three main components: water, oxygen, and nitrogen. Initially water is saturated with air, which leaves approximately 8.7 mg/L of oxygen and 13.4 mg/L of nitrogen in water (calculated based on Henry's law, as explained later). Once the air-saturated water is placed in the captive bubble cell and a bubble of oxygen is injected, it is assumed that water quickly evaporates and saturates the bubble. Even if complete water saturation does not take place, the maximum error associated with this assumption is 6%,[27] due to the low vapor pressure of water at 37 °C. Therefore, the change in bubble volume is a result of the change in the number of moles in the gas phase (oxygen molecules leaving the gas phase and nitrogen molecules entering the gas phase). Assuming that the gas phase behaves as an ideal gas, the net flux of gas transferred (F_{gas}) has been established by Zuo et al. as[27]

$$F_{gas} = \frac{1}{N}\sum_{i=1}^{N}\frac{\Delta\left(\frac{P_i^{g}V_i}{-RT}\right)}{A_{li}\Delta t_i} \tag{A1}$$

where P^{g}_i is the pressure of the gas phase at a time step "i", V_i is the volume of the gas phase, R is the gas constant, and A_{li} is the lateral area (A_l), which is used as the mass transfer area instead of the entire bubble area (A) because of the extremely small thickness of the wetting film (ranging from 100-500 nm[27]) in contact with the bubble. This thin aqueous layer is most likely to be saturated with oxygen within seconds, i.e., right after bubble formation, thus preventing further gas transfer through this region.[27] Δt_i is the interval of time associated with the time step i. N is the number of time steps evaluated during each run.

The net flux of gas has two contributions, the flux of oxygen from the bubble into the aqueous phase and the flux of nitrogen from the aqueous phase into the bubble. Assuming that the flux of each gas is relatively slow such that the mass transfers of oxygen and nitrogen occur independently of each other, the net flux is simply the sum of each flux: $F_{gas} = F_{oxygen} + F_{nitrogen}$.[33]

In general, the flux across the gas/liquid interface results from balancing the flux on the gas and liquid side of the interface. However, for poorly soluble gases in liquid phases, it has been confirmed that the transfer through the liquid phase is the rate-limiting step, in such case $F = k_L(C^* - C^0)$,[33] where k_L is the mass transfer coefficient trough the liquid phase, C^* is the concentration of the solute in the liquid side of the interface, and C^0 is the concentration of the solute in the bulk liquid phase. Introducing these concepts, into eq A1 yields

$$k_{L,O_2}(C^*_{O_2} - C^0_{O_2}) + k_{L,N_2}(C^*_{N_2} - C^0_{N_2}) = F_{gas} = \frac{1}{N}\sum_{i=1}^{N}\frac{\Delta\left(\frac{P_i^{g}V_i}{-RT}\right)}{A_{li}\Delta t_i} \tag{A2}$$

where k_{LO_2} and k_{LN_2} are the oxygen and nitrogen mass transfer coefficient in the aqueous phase, C^* is the concentration (of oxygen or nitrogen) at the interface (assumed in equilibrium with the gas phase), and C^0 is the concentration (of oxygen and nitrogen) in the bulk liquid phase.

The concentrations of nitrogen and oxygen can be calculated by using Henry's law

$$C_{gas} = H' P_{total} \tag{A3}$$

where $H'_{O_2} = 0.0013$ mol/L bar and $H'_{N_2} = 0.0006$ mol/L bar.[38,39] Using these constants, the initial concentration of oxygen in water ($C^0_{O_2}$) is 8.7 mg/L (2.7×10^{-4} mol/L), and the initial concentration in nitrogen ($C^0_{N_2}$) is 13.4 mg/L (4.8×10^{-4} mol/L). Considering that the gas bubble contains pure oxygen, $C^*_{O_2} = 1.3 \times 10^{-3}(1 + P_{psig}/14.7)$ mol/L and $C^*_{N_2} = 0$ mol/L (negligible concentration of nitrogen in the bubble). The concentration of the dissolved oxygen and nitrogen in the bulk liquid is assumed to be unchanged throughout the observation. This assumption is valid only when the liquid phase is much larger than the gas phase and the amount of gas transfer is small; i.e., both the time and area of contact should be limited. To ensure that this assumption is satisfied, each gas transfer experiment was restricted to 5 min and the liquid subphase was replaced after each experimental run. Measurement after the experiments found no increase of DO in the liquid subphase.[27]

To solve for the mass transfer coefficient using eq A2, it is necessary to obtain an additional equation relating the mass transfer coefficients of nitrogen and oxygen. To obtain such equation, the Chilton−Colburn relations for mass transfer coefficient for flow around a sphere can be applied[33]

$$k_{LA} = \left[2 + 0.6(\mathrm{Re})^{1/2}\left(\frac{\mu}{\rho D_{Af}}\right)_f^{1/3}\right] \times \frac{D_{Af}}{D} \tag{A4}$$

where "A" is the solute (nitrogen or oxygen), "f" is the solvent (aqueous phase), Re is the Reynolds number, μ and ρ are the viscosity and density of the solvent, D_{Af} is the diffusivity of the solute in the solvent, and D is the diameter of the sphere. Because oxygen and nitrogen are exposed to the same flow conditions and geometry, the Reynolds number (Re) and the diameter of the bubble (D) are the same for both gases. The cubic root of the Schmidt number ($[\mu/\rho D_{Af}]^{1/3}$) for oxygen is 9.7 and for nitrogen is 10 (using $\mu_{water} = 7 \times 10^{-4}$ kg/m s, ρ_{water}=1000 kg/m^3, and diffusivities indicated below). On the basis of these considerations, the term in brackets in eq A4 is approximately the same for nitrogen and oxygen. According to eq A4, the ratio between k_{LN_2} and k_{LO_2} is

$$\frac{k_{LN_2}}{k_{LO_2}} = \frac{D_{N_2}}{D_{O_2}} \tag{A5}$$

and hence k_{LN_2} (required to solve eq A2) is

$$k_{LN_2} = k_{LO_2}\frac{D_{N_2}}{D_{O_2}} \tag{A6}$$

Equation A6 can be introduced into eq A2, and considering that $C^*_{N_2} = 0$ mg/L, the following equation to calculate oxygen mass

transfer coefficient is obtained:

$$k_{LO_2} = \left[\frac{1}{N}\sum_{i=1}^{N}\frac{\Delta\left(\frac{P_i^g V_i}{-RT}\right)}{A_{li}\Delta t_i}\right] \times \frac{1}{\left(C^*_{O_2} - C^0_{O_2} - \frac{D_{N_2}}{D_{O_2}}C^0_{N_2}\right)} \quad \text{(A7)}$$

Equation A7 can be used in determining the mass transfer coefficient of oxygen, without disregarding the effect of nitrogen in the aqueous phase. The diffusion coefficients of oxygen (D_{O_2}) and nitrogen (D_{N_2}) in water at 37 °C are 3.1×10^{-5} cm^2/s and 2.7×10^{-5} cm^2/s.[32]

Finally, it is cautioned that the values of mass transfer coefficients are dependent on the hydrodynamic conditions of the experiment (see eq A4), and the use of these values in other experimental conditions is not advised. In this paper we have used a normalization method based on the mass transfer coefficient obtained for a water-only system in order to eliminate or reduce the uncertainties associated with hydrodynamic effects.

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