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On the Low Surface Tension of Lung Surfactant

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Abstract

Natural lung surfactant contains less than 40% disaturated phospholipids, mainly dipalmitoylphosphatidylcholine (DPPC). The mechanism by which lung surfactant achieves very low near-zero surface tensions, well below its equilibrium value, is not fully understood. To date, the low surface tension of lung surfactant is usually explained by a squeeze-out model which predicts that upon film compression non-DPPC components are gradually excluded from the air–water interface into a surface-associated surfactant reservoir. However, detailed experimental evidence of the squeeze-out within the physiologically relevant high surface pressure range is still lacking. In the present work, we studied four animal-derived clinical surfactant preparations, including Survanta, Curosurf, Infasurf, and BLES. By comparing compression isotherms and lateral structures of these surfactant films obtained by atomic force microscopy within the physiologically relevant high surface pressure range, we have derived an updated squeeze-out model. Our model suggests that the squeeze-out originates from fluid phases of a phase-separated monolayer. The squeeze-out process follows a nucleation–growth model and only occurs within a narrow surface pressure range around the equilibrium spreading pressure of lung surfactant. After the squeeze-out, three-dimensional nuclei stop growing, thereby resulting in a DPPC-enriched interfacial monolayer to reduce the air–water surface tension to very low values.

Graphical Abstract

1. INTRODUCTION

Although limited, available experimental evidence suggests that surface tension of the alveolar surface falls to near-zero at the end of expiration.^{1,2} Such a low surface tension is physiologically necessary for maintaining a large surface area of the lung for efficient gas exchange.³ It is well-known that lung surfactant, synthesized by alveolar type II epithelial

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cells and adsorbed at the air–water interface of alveoli, is responsible for this very low surface tension in the lung.⁴ However, the detailed mechanism by which lung surfactant attains such a low surface tension is still debated.^{4–8}

Lung surfactant consists of ~80 wt % phospholipids, 5–10% neutral lipids (primarily cholesterol), and 5–10% proteins.^{9,10} Among the phospholipid species, dipalmitoylphosphatidylcholine (DPPC) (16:0/16:0 PC) is the only major component that has a bilayer transition temperature higher than the core body temperature and hence is capable of sustaining a near-zero surface tension, or surface pressure up to 70 mN/m, by fully packing upon quasi-equilibrium compression with a Langmuir balance.¹¹ Nevertheless, the DPPC content in mammalian lung surfactants is usually less than 40%.^{9,10} Other phospholipids in lung surfactants are primarily unsaturated, with an equilibrium spreading pressure around 45 mN/m.^{4,5} Therefore, the surfactant film in the lung must be in a supersaturated metastable state. It maintains an extraordinary metastability, thus preventing lung alveoli from collapse at very low surface tensions.

A classical explanation of the extraordinary stability of lung surfactant films is the so-called “squeeze-out” model which speculates that less stable fluid non-DPPC components are gradually removed from the interfacial monolayer during film compression.^{12–14} This selective squeeze-out process would result in a pure DPPC monolayer in a nearly homogeneous tilted-condensed (TC) phase at very low surface tensions. (Note that we adopt the nomenclature proposed by Kaganer et al.,¹⁵ who suggest the use of TC phase to replace the commonly used liquid-condensed (LC) phase.) Consequently, DPPC is traditionally taken as the only significant component of lung surfactant for reaching very low surface tensions.

In recent years, this classical model has been critically challenged and amended with experimental evidence obtained using new techniques. On the one hand, based on direct film imaging with microscopy techniques, the classical squeeze-out model has been updated with the addition of multilayers closely attached to the interfacial monolayer, as a surface associated “surfactant reservoir”.^{4,7,16–19} On the other hand, using the captive bubble surfactometer, Hall’s group has introduced a novel supercompression model, in which film stability is shown to arise from a quick (nonequilibrium) compression that transforms a fluid phospholipid monolayer into an amorphous structure that can sustain high surface pressures without collapse.^{8,20,21}

Despite these progresses, the detailed biophysical mechanism of surfactant films in reaching low surface tension is still unknown.^{3,4,8,22} This is in part due to the lack of direct experimental observations of natural surfactants at the physiologically relevant low surface tension range. In the present work, we report a comprehensive study of lateral structure of four animal-derived clinical surfactants, i.e., Survanta (Abbott Laboratories, North Chicago, IL), Curosurf (Cornerstone Therapeutics, Cary, NC), Infasurf (ONY, Amherst, NY), and BLES (BLES Biochemicals, London, ON, Canada). For each surfactant preparation, we focus on the physiologically relevant low surface tension range, in exploring the biophysical mechanism by which these surfactant preparations reach low surface tensions.

2. MATERIALS AND METHODS

2.1. Materials

Curosurf, Infasurf, and BLES were donated by the pharmaceutical companies and Survanta was obtained from the Newborn Special Care Unit at Kapi'olani Medical Center for Women and Children. These clinical preparations are designated modified natural surfactants as they undergo organic extraction during the manufacture, which removes the hydrophilic surfactant protein (SP-A) and in some cases reduces the content of hydrophobic proteins (SP-B/C).²³ Survanta and Curosurf are extracted from minced bovine and porcine lung tissues, respectively. Infasurf and BLES are extracted from bovine lung lavage. Additional procedures are involved in the manufacture of Survanta, Curosurf, and BLES to remove/reduce neutral lipids, mainly cholesterol. Survanta is further supplemented with synthetic DPPC, palmitic acid, and tripalmitin. Detailed chemical compositions of these clinical surfactants can be found elsewhere.^{24,25} Specifically, the DPPC content (weight percentage with respect to the total surfactant) in these clinical surfactants approximately ranks in the order of Survanta (50%) > Curosurf (47%) > Infasurf (43%) > BLES (41%).^{24,25} All four clinical surfactants were extracted with chloroform–methanol using a method modified from Bligh and Dyer.²⁶ The chloroform–methanol extracts were dried under a nitrogen stream and redissolved in chloroform to a final concentration of 1 mg/mL. All stock solutions were stored at -20°C until use.

DPPC was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. All solvents used were HPLC grade. The water used was Milli-Q ultrapure water (Millipore, Billerica, MA), which has a resistivity higher than $18\text{ M}\Omega \cdot \text{cm}$ at room temperature.

2.2. Methods

Spreading, compression, and Langmuir–Blodgett (LB) transfer of surfactant films were conducted with a Langmuir–Blodgett (LB) trough (KSV Nima, Coventry, UK) at room temperature ($20 \pm 1^{\circ}\text{C}$). This trough is equipped with two Delrin barriers and has a large surface area ($\sim 300\text{ cm}^2$) that overcomes the pressure restriction imposed by a smaller trough.^{18,27} Detailed experimental procedures can be found elsewhere.²⁵ Briefly, all surfactants were spread on pure water to increase surface pressure to 1–3 mN/m using 10 μL microsyringes and were left undisturbed for 10 min to allow evaporation of solvent. The spread films were compressed at a rate of $20\text{ cm}^2/\text{min}$, with the surface pressure (π)–surface area (A) isotherms recorded. Surfactant films at characteristic π were deposited to freshly peeled mica surfaces at a dipping rate of 1 mm/min using the LB technique.

Topographical images of the LB samples were obtained using an Innova atomic force microscope (AFM) (Bruker, Santa Barbara, CA). Samples were scanned in air at multiple locations with various scan areas to ensure detection of representative structures. Both contact mode and tapping mode were used. The different scan modes gave equivalent results. A silicon nitride cantilever with a spring constant of 0.12 N/m and a nominal tip radius of 2 nm was used in the contact mode, and a silicon probe with a resonance frequency of 300 kHz and a spring constant of 40 N/m was used in the tapping mode. Area-averaged

heights of multilayers were quantified by grain analysis of the AFM images using the Nanoscope software (ver. 7.30). For each surfactant, LB sample preparation was repeated for at least three times at each surface pressure. Multiple AFM images were taken for each sample at each surface pressure. All data are expressed as mean $\pm$ SD ($n > 5$ unless otherwise indicated).

3. RESULTS

3.1. Compression Isotherms of Surfactant Films

The physiologically relevant metastable state of natural surfactant films is confined between the equilibrium spreading pressure (π_e) and the ultimate collapse pressure (π_c) of the films.^{4,5} Figure 1 shows the typical compression isotherms of Survanta, Curosurf, Infasurf, and BLES, at the physiologically relevant π range, i.e., from 40 to 70 mN/m. Also included in Figure 1 is a typical compression isotherm of pure DPPC monolayers, as a reference. The inset in Figure 1 shows the complete compression isotherms of these surfactant films, with the physiologically relevant π range shaded in gray.²⁵

First, around π_e of 40–50 mN/m, all clinical surfactant films undergo a monolayer-to-multilayer transition plateau, in which π only increases slowly with significant film compression. After passing this plateau, compression isotherms shift to the left, approximately in the order of reducing DPPC content in these surfactant preparations, i.e., BLES < Infasurf < Curosurf < Survanta. Second, after passing the plateau region, all surfactant films increase π steeply upon further film compression. The slope of the compression isotherms is comparable to that of pure DPPC monolayers in this π range. Third, all surfactant films but Survanta collapse at 72 mN/m, corresponding to a near-zero surface tension. Survanta collapses at ~62 mN/m. Therefore, the metastable state of Survanta film is confined between approximately 40 and 60 mN/m.

Figure 2 shows the variations of compressibility of these surfactant films at their physiologically relevant π ranges. The film compressibility (Cm) is defined by

$$Cm = - \frac{1}{A} \frac{dA}{d\pi} \quad (1)$$

As shown in Figure 2, all surfactant films after passing the plateau region have a similarly low film compressibility of $\sim 0.01 \text{ (mN/m)}^{-1}$. This value of compressibility is close to that of pure DPPC monolayers in the same π range, i.e., $\sim 0.006 \text{ (mN/m)}^{-1}$. These compressibility data indicate that all clinical surfactant films undergo a film refining process during the plateau region. After film refining, the interfacial monolayers of all surfactant films contain a similar composition of nearly pure DPPC in the fully packed TC phase. These indications are further proved by the following AFM observations.

3.2. Lateral Structures of Surfactant Films

Figures 3 and 4 show AFM topographic images of Survanta, Curosurf, Infasurf, and BLES, each at four physiologically relevant π , i.e., 40, 45, 50, and 60 mN/m. These characteristic

pressures were selected to demonstrate the film lateral structure at the onset, middle, end, and subsequence of the monolayer-to-multilayer transition. All AFM images in Figure 3 have a large scan area of $50 \times 50 \mu\text{m}$, showing the overview of the lateral structure. In contrast, AFM images in Figure 4 have a small scan area of $20 \times 20 \mu\text{m}$, showing the localized film structure. Insets in Figure 4 show high-resolution AFM images with various scan areas to illustrate detailed film structures. For the purpose of comparison, all topographic images at 40 and 45 mN/m have the same z -range of 5 nm, and images at 50 and 60 mN/m have the same z -range of 20 nm.

At 40 mN/m, i.e., at the onset of the plateau region in Figure 1, all surfactant films are in monolayers. Phospholipid phase separation occurs at both microscale and nanoscale, indicated by formation of micrometer-sized and nanometer-sized TC domains. Lateral chemical analysis using time-of-flight-secondary ion mass spectroscopy (ToF-SIMS) has confirmed that these TC domains consist of disaturated phospholipids (mainly DPPC).^{3,28–30} Phospholipids in these domains extend ~ 1 nm higher than the surrounding liquid-expanded (LE) phase that contains mainly unsaturated phospholipids and proteins.^{3,28–30} The specific domain morphology of Survanta, Curosurf, Infasurf, and BLES differs from each other significantly. These differences have been attributed to the different chemical compositions of these clinical surfactants, raised from different animal sources and manufacture procedures.²⁵ As shown in Figure 4, Survanta monolayer displays circular domains. Curosurf monolayer shows large ramified domains with nanodomains lining up in the horizontal direction. Infasurf monolayer shows a unique domain-in-domain structure, which was shown to be due to the cholesterol medicated liquid-ordered (LO) phase. BLES monolayer at 40 mN/m displays a few microdomains and a large number of nanodomains uniformly distributed in the LE phase. Detailed discussion of these domain structures in surfactant monolayers can be found elsewhere.²⁵

At 45 mN/m, i.e., in the middle of the plateau region, multilayered protrusions start to nucleate from the interfacial monolayer. For Survanta and BLES, it appears that protrusions of 3 nm in height (approximately one bilayer thickness) originate from the LE phase of the interfacial monolayer. These protrusions form a matrix structure that encompasses the TC domains in the interfacial monolayer. Consequently, the original relatively higher TC domains (compared to the LE phase) become relatively lower than the surrounding multilayers. Nevertheless, the size and shape of TC domains remain largely unchanged after formation of protrusions (see Figure 4 for the measurement of representative domain sizes for Survanta and BLES at increasing π). Protrusions of 1.5 nm high are also formed in Infasurf. These protrusions appear to nucleate from the LE and LO phases of the interfacial monolayer, as indicated by the dissociation of the LO phase and retention of the TC phase (i.e., the core of the domain-in-domain structure shown in Figure 4). For Curosurf, only isolated protrusions of 2 nm in height appear. These protrusions tend to line up vertically, i.e., perpendicular to the direction of lateral compression, presumably indicating film folding due to the lateral compression.

At 50 mN/m, i.e., at the end of the plateau region, the multilayered protrusions grow in height but not significantly in the lateral dimension, compared to protrusions at 45 mN/m. As shown in Figure 4, multilayers in Survanta and BLES double the height at 45 mN/m, but

the “holes” of TC domains are relatively unchanged in diameter. A similar phenomenon happens to Infasurf: the multilayers significantly increase in height but the holes only shrink slightly. A majority of protrusions in Curosurf remain to be around 2 nm, but a few isolated peaks of 6 nm high appear.

At 60 mN/m, i.e., subsequent to the plateau region, the multilayered protrusions only grow in the lateral dimension (i.e., increasing packing density as a consequence of film compression) but not significantly in height, compared to 50 mN/m.

3.3. Quantitative Analysis of Multilayers

Figure 5 shows quantification results of multilayers for Survanta, Curosurf, Infasurf, and BLES, each at π of 45, 50, and 60 mN/m. The average height used to quantify multilayers is an area-averaged statistical measure of surface topography. It was calculated based on grain analysis of multiple AFM images with various resolutions. As shown in Figure 5, for all surfactant films, the average height at 45 mN/m, i.e., in the middle of the monolayer-to-multilayer transition plateau, is limited to about 1 nm. With increasing π to 50 mN/m, i.e., at the end of the plateau, the average height of Survanta, Infasurf, and BLES increases significantly to about 4 nm, while average height of Curosurf increases moderately to about 1.5 nm. With further increasing π to 60 mN/m, i.e., subsequent to the plateau, the average height only increases slightly compared to that at 50 mN/m for all surfactant films. These quantification results indicate that the squeeze-out of fluid components from the interfacial monolayer is largely completed during the plateau region between 40 and 50 mN/m. These data fit well to a nucleation–growth model of collapse of Langmuir monolayers that will be discussed in detail later.

4. DISCUSSION

Despite being studied for half a century, the detailed biophysical mechanism by which lung surfactant reduces surface tension of the air–water interface to near-zero is still not fully understood.^{3,4,8,22} The classical squeeze-out model in combination with the surface-associated surfactant reservoir appears to be a plausible model in explaining the low surface tension of lung surfactant *in vitro*. This model predicts that the multicomponent lung surfactant film, collectively adsorbed or spread at the air–water interface, is refined during film compression. This refining process is referred to as “squeeze-out”, in which the fluid non-DPPC components are selectively excluded from the air–water interface, thus leading to an interfacial monolayer highly enriched in DPPC with closely attached multilayers (i.e., the “surfactant reservoir”) of non-DPPC components. However, detailed experimental evidence for this squeeze-out model, especially at the physiologically relevant high π range, is still lacking. Consequently, the current squeeze-out model suffers from criticism in some technical details.⁸ For example, since natural surfactant contains less than 40% DPPC, it would theoretically require an ~60% area reduction to exclude all non-DPPC components.^{8,31} However, the maximum variation of alveolar surface area during normal tidal breathing is no more than 20–30%.^{2,4} Moreover, a theoretical and experimental basis of selective exclusion is the solid–fluid (i.e., TC–LE) phase coexistence.^{4–8} However, due to the existence of cholesterol, the fluid–fluid (i.e., LO–LE) phase coexistence might be

predominant in natural surfactant monolayers and membranes.³² How the cholesterol-mediated LO phase contributes to the squeeze-out process is still not clear.

By studying molecular organization and lateral structure of various modified natural surfactant films at the physiologically relevant π range, the present study provides new experimental evidence toward an updated squeeze-out model in explaining the low surface tension of lung surfactants.

First, we found that after the monolayer-to-multilayer transition plateau the compression isotherms of various modified natural surfactants shift to the left, i.e., the direction of reducing surface area, in the order of decreasing DPPC content in these surfactants (Figure 1). This may indicate exclusion of non-DPPC components from the air–water interface to multilayers during the plateau region.

Second, after the plateau region, all surfactants have low film compressibility close to that of pure DPPC monolayers in the same π range (Figure 2). The compressibility was measured to be as low as 0.01 (mN/m)^{-1} , equivalent to that of fully packed DPPC monolayers in a nearly homogeneous TC phase.^{33,34} These results again indicate selective exclusion of non-DPPC components during the plateau region. It should be noted that strictly speaking the calculation of film compressibility is only meaningful to monolayers.³⁵ But surfactant films beyond the plateau region are clearly multilayers. Therefore, the compressibility evaluated in this π range only provides indication on the composition and phase of the interfacial monolayer, which controls the interfacial tension of the film-covered surface.

Third, our AFM observations clearly demonstrated that multilayers originate from the fluid phases (LE and LO) and encompass the solid phase (TC) at the interfacial monolayers (Figures 3 and 4). These AFM observations are consistent with previous lateral chemical characterization of clinical surfactants using near-field scanning optical microscopy (NSOM)³⁶ and ToF-SIMS.³ Sibug-Aga and Dunn showed that Survanta films at $\sim 60 \text{ mN/m}$ consist of dye-containing multilayered matrix that traces out dye-excluded condensed domains.³⁶ Recent combined AFM and ToF-SIMS study showed that BLES films at π of 50 mN/m consist of lower dipalmitate-containing domains surrounded by higher oleate-containing multilayers, thus supporting squeeze-out of non-DPPC components during the plateau region.³

More importantly, we found that multilayers of fluid phospholipids grow in height only during the plateau region (Figure 5). After passing the plateau, multilayers stop growing in height but only increase packing density in the lateral dimension as a consequence of surface area reduction. These AFM observations suggest that squeeze-out of fluid components from the interfacial monolayer follows a nucleation–growth model in supersaturated Langmuir monolayers.^{37–40} Vollhardt et al. proposed that the formation of compression-driven multilayer from an interfacial monolayer above a critical π initiates from a single-step nucleation, followed by growth of the 3D nuclei, and ends with overlapping of the growing nuclei at which the size of the nuclei becomes limited in all directions.^{37–40} Once the growth of the nuclei is restricted, the compression rate outweighs the relaxation rate of the film, thereby rendering the film a high metastability until ultimate film collapse at π_c .

For lung surfactants, the plateau region between 40 and 50 mN/m corresponds to the equilibrium spreading pressure (π_e) of fluid phospholipids, at which fully hydrated phospholipid vesicles reach equilibrium with the phospholipid monolayer at the air–water interface.⁴ The π_e is also the maximum π that can be reached by fully hydrated phospholipid vesicles during adsorption or spreading.^{34,41} Further increasing π , if possible, can be only achieved by lateral film compression. Lateral structure around π_e could represent the surfactant film formed by *de novo* adsorption of endogenous surfactant or by spreading of exogenous clinical surfactants.

Our data therefore may suggest refining of surfactant films during adsorption, which results in an interfacial monolayer enriched in DPPC with attached multilayers of fluid non-DPPC components. The concept of “selective adsorption” was first proposed by Schürch et al. based on isotherm studies using the captive bubble surfactometer.^{16,42} It was found that after adsorption an air bubble required less area reduction to reach very low surface tension than expected from the lipid composition of the surfactant.^{16,42,43} This was thought to be due to selective adsorption of DPPC at the air–liquid interface of the bubble,^{16,42} a process known to require surfactant proteins.⁴⁴ The present study therefore suggests that DPPC may not be selectively adsorbed but enriched at the air–water interface by selectively excluding non-DPPC components from the interface at around π_e . This interpretation is consistent with the autoradiographic study by Yu and Possmayer, who found that the lipid composition of adsorbed natural surfactant films (interfacial monolayer plus attached multilayers) had no difference to that of dispersed surfactant samples.³¹

The present data show that lipid refining around π_e may stem from phospholipid phase separation prior to reaching the π_e . At 40 mN/m, all disaturated phospholipids in lung surfactants (mainly DPPC) are packed into a solid TC phase, which are separated from the non-DPPC components in a fluid LE phase. In the presence of cholesterol, such as in Infasurf, the fluid phase may also contain a LO phase, depending on cholesterol content. Upon increasing π to π_e , 3D nuclei originate from the fluid phases at the interfacial monolayer. The 3D nuclei develop following the nucleation–growth model and cease growing upon reaching 50 mN/m. All these processes are completed within a narrow π range of 40–50 mN/m, corresponding to the π_e of lung surfactants. After this refining process, the interfacial monolayer is enriched in DPPC and hence is capable of being compressed to very low near-zero surface tensions with a small area reduction.

Our comparative study may also suggest the role of cholesterol in phospholipid phase separation and the lipid refining process. Previous studies have showed that cholesterol induces dissociation of large microscale TC domains into small nanoscale TC domains at 40 mN/m.^{18,25,45,46} Nanodomains are evident in Infasurf and BLES, the two cholesterol-containing surfactants, as demonstrated in insets of Figure 4. The dissociation of TC microdomains into nanodomains significantly enhances the uniformity of phase coexistence at the interfacial monolayer, thus promoting the subsequent lipid refining around π_e . In contrast, Curosurf has the lowest amount of neutral lipids in all clinical surfactant preparations. Different from the others, monolayers of Curosurf at 40 mN/m show large domains and hence nonuniform phase coexistence. Therefore, Curosurf intrinsically lacks nucleation sites for film refining, usually occurring at the domain boundaries.^{19,47} Although

the nucleation–growth model still seems to apply to Curosurf, the 3D nuclei are significantly smaller than other surfactant preparations.

It should be noted that the biophysical mechanism derived in this study is based on the *in vitro* model of self-assembled Langmuir monolayers. Although being a commonly used model to study biophysical properties of lung surfactant, an apparent deviation of this model from the physiological condition is the use of room temperature. It is known that temperature has a large influence on the phase behavior of phospholipid monolayers. It was reported that at 37 °C phase separation in a natural surfactant monolayer is insignificant and rather the fluid phase is predominant.⁴⁵ The lack of phase separation at the physiological temperature may present additional challenge to the selective squeeze-out model.

Another experimental limitation is the slow compression rate used in this study. It is well-known that the domain morphology of phospholipid monolayers and membranes is kinetically dependent.^{48,49} Our previous study has demonstrated three different means of enhancing kinetics: faster film compression, direct spreading/adsorption to higher pressure, and addition of SP-A.¹⁸ All three means improved the rate of π increase and led to more and smaller domains.¹⁸ Such domain structures may promote the formation of uniform matrix structure of squeezed out multilayers. In this sense, the present study should not disapprove Hall's supercompression model.^{8,20,21}

Finally, although we do not specifically discuss surfactant proteins, it is well-known that SP-B and SP-C play a crucial role in monolayer-to-multilayer transition upon film compression, adsorption, and readsorption/respreading of surfactant film upon film expansion.^{4,7,44,50–53} Especially, SP-B appears to be vital in sustaining film stability at the most compressed states.⁵⁴ This may explain the inability of Survanta in reaching surface pressure as high as the other preparations since Survanta lacks SP-B.²⁵

In conclusion, by investigating compression isotherms and lateral structures of various animal-derived clinical surfactants within the physiologically relevant π range, the present study provides an updated squeeze-out model in explaining the low surface tension of lung surfactants. Our model supports a selective enrichment of interfacial monolayer with DPPC around the equilibrium spreading pressure of lung surfactants. Lipid refining of the interfacial monolayer appears to result from phospholipid phase separation prior to the squeeze-out and appears to follow a nucleation–growth model. The squeeze-out of 3D nuclei is largely completed within a narrow π range of 40–50 mN/m. The resultant DPPC-enriched monolayer with attached multilayers of fluid non-DPPC components is responsible for the extraordinary metastability of lung surfactant film.

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Figure 1.

Compression isotherms of Survanta, Curosurf, Infasurf, and BLES at room temperature and the physiologically relevant surface pressure range (i.e., 40–70 mN/m). A compression isotherm of pure DPPC is shown as a reference. The inset shows the complete compression isotherms of these surfactant preparations, in which the physiologically relevant surface pressure range is shaded in gray.

Figure 2.

Film compressibility of Survanta, Curosurf, Infasurf, and BLES at room temperature and the physiologically relevant surface pressure range (i.e., 40–70 mN/m). Film compressibility of pure DPPC is shown as a reference.

Figure 3.

AFM topographic images of Survanta, Curosurf, Infasurf, and BLES (in each column) at the physiologically relevant range of surface pressures (π), i.e., 40, 45, 50, and 60 mN/m (in each row). AFM images at these characteristic π represent the lateral structure of surfactant films at the onset, middle, end, and subsequence of the monolayer-to-multilayer transition. The AFM scan area was $50 \times 50 \mu\text{m}$ for all images. The full z -range was set to be 5 nm for all images at π of 40 and 45 mN/m and 20 nm for π of 50 and 60 mN/m, respectively.

Figure 4.

AFM topographic images of Survanta, Curosurf, Infasurf, and BLES (in each column) at the physiologically relevant range of surface pressures (π), i.e., 40, 45, 50, and 60 mN/m (in each row). AFM images at these characteristic π represent the lateral structure of surfactant films at the onset, middle, end, and subsequence of the monolayer-to-multilayer transition. The AFM scan area was $20 \times 20 \mu\text{m}$ for all images. The full z -range was set to be 5 nm for all images at π of 40 and 45 mN/m and 20 nm for π of 50 and 60 mN/m, respectively. Characteristic lateral and altitudinal dimensions are indicated by double-headed and single-headed arrows, respectively. Detailed topographic features are labeled with boxes and shown in insets.

Figure 5. Quantification results of the area-averaged height of multilayer for Survanta, Curosurf, Infasurf, and BLES, each at π of 45, 50, and 60 mN/m. These three characteristic π represent the middle, end, and subsequence of the monolayer-to-multilayer transition.