Aerosolizing Lipid Dispersions Enables Antibiotic Transport Across Mimics of the Lung Airway Surface Even in the Presence of Pre-existing Lipid Monolayers

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Abstract

Background: Secondary lung infections are the primary cause of morbidity associated with cystic fibrosis lung disease. Aerosolized antibiotic inhalation is potentially advantageous but has limited effectiveness due to altered airway aerodynamics and deposition patterns that limit drug access to infected regions. One potential strategy to better reach infected areas is to formulate aerosols with surfactants that induce surface tension gradients and drive postdeposition drug dispersal via Marangoni transport along the airway surface liquid (ASL). Since this relies on surfactant-induced surface tension reduction, the presence of endogenous lipid monolayers may hinder drug dispersal performance.

Methods: Tobramycin solutions were formulated with dipalmitoylphosphatidylcholine (DPPC), a major component of endogenous pulmonary surfactant, to drive postdeposition aerosol dispersal across a model ASL based on a liquid layer or “subphase” of aqueous porcine gastric mucin (PGM) solution with predeposited DPPC monolayers to mimic the endogenous surfactant. *In vitro* subphase samples were collected from regions outside the aerosol deposition zone and assayed for tobramycin concentration using a closed enzyme donor immunoassay. The motion of a tracking bead across the subphase surface and the corresponding decrease in surface tension on aerosol deposition were tracked both with and without a predeposited DPPC monolayer. The surface tension/area isotherm for DPPC on PGM solution subphase was measured to aid in the interpretation of the tobramycin dispersal behavior.

Results and Conclusions: Transport of tobramycin away from the deposition region occurs in aerosols formulated with DPPC whether or not predeposited lipid is present, and tobramycin concentrations are similar in both cases across biologically relevant length scales (~ 8 cm). When DPPC is deposited from an aerosol, it induces ultralow surface tensions (<5 mN/m), which drive Marangoni flows, even in the presence of a dense background layer of DPPC. Therefore, aerosolized phospholipids, such as DPPC, will likely be effective spreading agents in the human lung.

Keywords: cystic fibrosis, DPPC, Marangoni stress, postdeposition spreading

Introduction

CYSTIC FIBROSIS (CF) is a genetic disease affecting 70,000 people worldwide, with around 1000 new cases diagnosed each year.⁽¹⁾ In CF, airway mucus becomes dehydrated and viscous and cannot be cleared effectively.⁽²⁾ Accumulated

mucus becomes a nidus for bacterial infection and mucus plaques may limit local ventilation.^(3–5) Most mortality in CF is caused by bacterial infections, despite prevalent use of inhaled antibiotics.⁽³⁾ Drug distribution with current inhaled therapies is determined entirely by aerodynamics with inhalation flows and aerosols following the path of least resistance

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through the airways.⁽⁵⁾ Disease-associated obstructions may limit drug penetration or cause local aerosol deposition upstream of sites of disease.⁽⁴⁻⁸⁾

Recently, Marangoni stresses have been harnessed to enhance the surface transport of mock drugs deposited from surfactant-laden aerosols onto surfaces that mimic the key features of the airway surface liquid (ASL).⁽⁹⁻¹⁵⁾ Researchers have been studying the use of Marangoni flows to cause surface transport for years.⁽¹⁶⁻¹⁹⁾ Marangoni flows occur when surface stresses are generated by a gradient in surface tension along an interface.

Surface tension lowering agents such as surfactants can locally lower the surface tension, causing the gradient necessary for flow to occur. These Marangoni flows are directed outward, away from the area where the surfactant is deposited. In cases of CF, aerosols tend to deposit preferentially onto the first few branches or obstructions in the lungs, but infections can occur beyond these areas, deeper in the lung.⁽⁴⁾ Delivery of surfactant containing aerosols to the ASL would locally lower the ASL surface tension and generate flows on the surface and within the ASL that could transport medication over the mucus plaques to reach infection sites deeper in the lung.

Our group has been exploring the use of phospholipids as spreading agents.⁽¹⁵⁾ Because the phospholipid dipalmitoylphosphatidylcholine (DPPC) is endogenous to the lung, it is a prime candidate for addition to aerosols as an exogenous surfactant to enhance their spreading. However, its low solubility requires that it be dispersed in water in the form of vesicles before incorporation into aqueous aerosols for deposition in the lung. While macroscopic drops of lipid dispersion do not induce Marangoni stresses after deposition on an aqueous subphase, direct deposition of aerosolized lipid dispersions does induce flow, even on low surface tension and high viscosity subphases such as porcine gastric mucin (PGM) solutions.⁽¹⁵⁾

The aerosolization process breaks the lipid vesicles in the dispersion, populating the air/water interface of the aerosol with monomeric lipid molecules. This may lead to DPPC surface layers with different structures than those achieved by typical laboratory preparation methods, such as depositing drops of DPPC in a chloroform solution, which are discussed later. The aerosolization process allows the lipid monomers to adsorb to the air/liquid interface upon deposition onto the subphase, where they then lower the surface tension and induce Marangoni flow.⁽¹⁵⁾

Furthermore, the deposition of the aerosol droplets allows the surface to reach ultralow surface tensions, <5 mN/m, which are generally only obtainable under extremely slow compression of a lipid monolayer or on area cycling in a Langmuir trough.^(15,20,21) Attaining these ultralow surface tensions in the deposition region means that a surface tension gradient will be generated by the aerosol deposition and flow will occur even on subphases with initially low surface tensions.⁽¹⁵⁾ Marangoni flow is also associated with subsurface flows that provide an additional possible transport path for nonsurface-active drugs included in the aerosol formulation.

Prior work from our group has examined how the aerosol deposition flux affects the postdeposition flow behavior of the aerosol contents.^(13,14) Two different deposition flux regimes were studied. In the high-flux regime, which occurs in the larger airways, aerosol droplets deposit so quickly that

they coalesce into a single, large drop before spreading.⁽¹⁴⁾ This drop's spread area depends only on the mass of surfactant deposited, not independently on the rate of deposition or the concentration of surfactant in the aerosol. In the low-flux regime, occurring in the smaller airways of the lung, each individual aerosol droplet spreads out before the next drop deposits.⁽¹³⁾ This creates a spreading field of droplets that do not coalesce after deposition. The transition between these two regimes occurs at deposition fluxes of $\sim 0.01 \mu\text{L}/(\text{cm}^2 \cdot \text{s})$.⁽¹⁴⁾

A significant question concerning the possible use of aerosols formulated with surfactants for pulmonary delivery is the uncertain impact of endogenous surfactants on the intended spreading behavior. In order for surface transport to occur, the local surface tension induced by newly deposited surfactant must be lower than that of the pre-existing, potentially phospholipid-covered ASL. Phospholipids exist at high concentrations in the lung's alveoli, where a low surface tension is necessary to increase alveolar volume and promote oxygen exchange. As the alveoli expand and contract, their surface tensions cycle between ~ 30 and ~ 5 mN/m.⁽²²⁻²⁵⁾ Phospholipids also exist in the airways further away from the respiratory zone at lower concentrations than in the alveoli; the distribution of phospholipids among the conducting airways is unknown.⁽²⁶⁾

ASL surface tension also depends on the presence of mucus. The surface tension of the mucus plaques must reflect the adsorption of the hydrophobic groups of mucins to the air/water interface, which will lower the mucus surface tension below that of water. Measurements of entangled PGM solutions have shown surface tensions around 40 mN/m. Endogenous lipid on the ASL could lower the mucus layer's surface tension even further,^(27,28) but with the exceptions of the alveolar surface where airway surface areas cycle significantly, ASL surface tensions are unlikely to reach a value lower than ~ 30 mN/m.^(24,25,29,30) Nonetheless, an important step in developing an aerosol capable of reaching obstructed areas throughout the lung will be to show that aerosols can transport antibiotics despite the presence of endogenous surfactant. We do so in this article.

The surface tension versus area per molecule plot (referred to as the surface tension isotherm) of DPPC on water has been well studied.^(15,31) These isotherms are typically presented in terms of the surface pressure, the difference between the bare liquid subphase surface tension and the surface tension with an adsorbed layer. For example, prior work has shown the different types of structures that lipid monolayers can form on the surface and has identified how such layers behave at their collapse pressures (the maximum pressure that a two-dimensional monolayer can sustain without collapsing into the third dimension).⁽³¹⁾

Sook and Franses have shown that the surface tension of aqueous dispersions containing DPPC vesicles can vary widely according to their method of preparation.⁽²¹⁾ These dispersions, when freshly prepared, start out with surface tensions near that of water and then slowly decrease in surface tension as DPPC vesicles diffuse and break open to form monolayers and multilayers at the air/water interface. The minimum surface tensions achieved by any of these dispersions, without any compression or expansion of the interface, are around 26 mN/m. If the surface area is pulsed, the minimum surface tensions achieved decrease to

around 1 mN/m. This indicates that surfaces of aqueous dispersions containing DPPC, as would likely be the case in the less complaint conducting airways, could have surface tensions of order 20 mN/m, whereas more dynamic surfaces, as would likely be found in the alveoli, could be as low as 1 mN/m.

Surface tension isotherms are highly dependent on the concentrations of the various constituents of the monolayer on the surface. However, the behavior of DPPC on the surface of a mucin solution has not been studied and is crucial to the understanding of how Marangoni spreading might behave in the lungs. This article will show that when DPPC is deposited onto a mucin solution surface, the surface tension at high areas per DPPC molecule (dilute monolayer) is significantly smaller on the mucin solution subphase. At small areas per DPPC (dense monolayer), the surface tension isotherm converges with the isotherm on a simple water subphase. This is strong evidence that at high densities, DPPC is forming a monolayer and excluding most if not all mucin hydrophobic groups that might have been adsorbed to the air/water interface before DPPC deposition.

In this work, we measured the extent of spreading of the antibiotic tobramycin across a surface of PGM solution subphase. We used DPPC as the surfactant spreading agent in the aerosol formulation. As this surfactant is endogenous to the lungs, we predeposited DPPC on the mucin solution surface to determine if endogenous lipid would prevent enhanced transport of tobramycin. We found that the lipid aerosol formulation causes surface flow outward from the region of deposition and transports tobramycin over biologically relevant distances across the surface. Furthermore, the presence of predeposited lipid does not significantly affect the spreading of tobramycin across the surface.

Materials and Methods

DPPC was purchased from Avanti Polar Lipids (Alabaster, AL) in lyophilized form (>99% purity). Multilamellar vesicles (MLVs) of DPPC were prepared using a temperature-cycling process similar to that of Sook and Franses.⁽²¹⁾ A 20 mL vial of 10 mg/mL DPPC was prepared in deionized (DI) water (Milli-Q Academic unit, resistivity 18 M Ω ·cm, Millipore, Billerica, MA). The lipid was mixed with a Fisher Scientific Analog Vortex Mixer (Cat. No. 02215365; Thermo Fisher Scientific, Waltham, MA) for 30 seconds, then heated above its fluid/gel transition temperature (41.4°C) for 10 minutes, and vortexed for 30 more seconds. The dispersion was cooled to below its gel transition temperature for 10 minutes and again vortexed for 30 seconds. The dispersion was heated, cooled, and vortexed three times to achieve MLVs of around 10 μ m in diameter.⁽²¹⁾

The subphases used for the experiments were DI water and an aqueous solution of PGM (Type II, ~1% bound salicylic acid; Sigma Aldrich, St. Louis, MO, CAS No. 84082-64-4). While it is not a perfect match to human pulmonary mucin, PGM was chosen because it is similar in its overall structure and is available in large quantities needed for experimentation. Lyophilized PGM was stored between 2°C and 8°C and rehydrated with DI water to produce a 5 wt% mucin solution, which was orbitally mixed for at least 16 hours until visibly homogeneous. At this concentration, the mucin is above its entanglement concentration in water.⁽¹³⁾ The mixed solutions were kept between 2 and 8°C and used within 10 days of preparation.

Tobramycin (>94.0%, CAS No. 32986-56-4; TCI America, Portland, OR) was purchased and used at a concentration of 60 mg/mL in the aerosol formulations. It is an antibiotic commonly used to treat CF-associated infections.^(32,33) Tobramycin is also highly water soluble, allowing it to be incorporated into the aqueous solution of aerosol formulations. It is not dispersed in the MLV bilayers as would be required of a hydrophobic drug. It was added to the lipid dispersions after they were prepared. Control experiments determine if tobramycin alone can drive spreading. For these experiments, the aerosol formulation consisted of only tobramycin and DI water.

An AeroNeb Solo micropump nebulizer (Aerogen, Galway, Ireland) with an Aerogen Pro-X controller was used for the aerosolization experiments. Each nebulizer is made up of a fluid chamber positioned on top of a 5-mm-diameter mesh plate containing ~1000 tapered, 4.8 ± 0.3 μ m holes, vibrated at 1.2×10^5 Hz. A separate nebulizer was used for each aerosol formulation. Control experiments, during which the aerosol formulation consisted of only tobramycin and DI water, were conducted to determine if tobramycin alone could drive spreading. The aerosol droplets were sized using a Malvern Mastersizer S (Malvern Instruments, Malvern, United Kingdom). As shown in Figure 1, droplet size distributions were similar for all fluids used. The nebulizer is designed to deliver a consistent dose of medication when the full reservoir is emptied, however, the rate of deposition varies somewhat between experiments.

We constructed a contained trough apparatus to measure the surface concentration of the tobramycin outside the area of direct aerosol deposition, as shown in Figure 2. The setup consisted of a glass trough 2 cm wide by 2 cm deep by 20 cm

FIG. 1. Size distribution of aerosols from Aeroneb Solo micropump nebulizer. Ten milligram per milliliter DPPC (●), 60 mg/mL tobramycin (■), 10 mg/mL DPPC and 60 mg/mL tobramycin (▲). Standard errors on all diameters are ± 0.1 μ m unless otherwise stated ($n=3$). For aerosols containing tobramycin at a concentration of 60 mg/mL, the volume-weighted median droplet diameter was 5.0 μ m, with volume-weighted 10th and 90th percentile values of 2.0 μ m and 10.7 ± 0.2 μ m. The volume-weighted median droplet diameter for a tobramycin and DPPC dispersion was 5.3 μ m (with volume-weighted 10th and 90th percentile values of 2.2 and 11.7 μ m). The volume-weighted median droplet diameter for a DPPC dispersion was 5.5 ± 0.2 μ m (with volume-weighted 10th and 90th percentile values of 2.1 μ m and 14.2 ± 1.3 μ m).

FIG. 2. Experimental setup. The trough dimensions are $20 \times 2 \times 2$ cm. The direction of aerosol deposition was perpendicular to the length of the trough.

long. The trough is placed halfway inside an aerosolization chamber along with the nebulizer. Then, the trough is filled with 70 mL, ~ 2 mm below the top of the trough, of 5 wt% mucin solution to minimize the gap between the liquid surface and the top of the hole in the aerosolization chamber through which the trough extends. This limits the amount of aerosol that can escape through the air and deposit onto the liquid surface outside the aerosolization chamber.

To further prevent any aerosol from leaking through the hole and onto the outside portion of the trough, air was slowly and constantly pulled in through the hole and out the opposite side of the aerosolization chamber through a filter, into a containment chamber by a small fan. Results discussed below show that these precautions were sufficient to reduce aerosol flux to the trough surface outside the aerosolization chamber to insignificant levels.

A 1-mm-diameter polystyrene tracer bead was placed on the surface of the liquid in the trough to indicate the presence of surface flows. The nebulizer was charged with 4 mL of aerosol formulation and allowed to operate until empty. This entire apparatus was placed in a glove box to prevent tobramycin from leaking into the laboratory environment.

To determine the spatial distribution of tobramycin after aerosol deposition had occurred and spreading had been completed, samples of the liquid surface were taken by touching an array of 11 disposable glass Pasteur pipettes (Cat. No. 7095B-5X; Corning, Inc., Corning, NY) to the mucin surface. Capillary action causes a small amount of liquid, $\sim 10 \mu\text{L}$, to be drawn from the surface region into the pipettes. Independent experiments with fluorescent dye spread across a liquid surface showed that each pipette sampled a region of ~ 0.3 cm diameter. Each sample was deposited into 2-mL polystyrene vials and diluted with $200 \mu\text{L}$ of DI water.

Five surface samples were also taken from inside the region of direct deposition. These samples were assayed for their tobramycin concentration as described below. To determine the average aerosol flux to the surface during the experiment and normalize for differences between experiments, after the surface samples were taken, all the fluid in the trough was thoroughly mixed and a $200 \mu\text{L}$ sample of this mixture was diluted with $200 \mu\text{L}$ of DI water. The resulting bulk sample was assayed for tobramycin content, which was used to determine the total tobramycin dose to

the subphase. Knowing the tobramycin concentration in the aerosol formulation, we calculate the total volume of solution deposited from which we determine the average volumetric deposition flux.

To mimic the impact of endogenous lipid on tobramycin transport, trials were performed where DPPC was deposited from a 10 mg/mL DPPC solution in chloroform via a pipette onto mucin. The chloroform was allowed to evaporate before delivery of tobramycin aerosol. The deposited lipid layer had an area per molecule of $26 \text{ \AA}^2$, determined from the measured surface tension and the surface tension isotherm for DPPC monolayers on mucin solutions, to be discussed later.

For reference, if the deposition had been on a pure water subphase, this would have been in the “liquid condensed” phase.⁽³¹⁾ This surface concentration was chosen because it is the most DPPC that can be deposited onto a mucin solution surface from chloroform before monolayer collapse; again, shown later in the surface tension isotherm. This produces the lowest subphase surface tension, or equivalently, the largest surface tension barrier to Marangoni spreading possible and thus is a stringent test for spreading against any endogenous lipid that might be present on the ASL within a given airway generation.

The tobramycin concentration in samples from the spreading trough was determined by a closed enzyme donor immunoassay (Reference No. 100018, Tobramycin II; Thermo Fisher Scientific). This method has a detection limit of 0.2 ng/mL and a measurement precision of 0.1 ng/mL.⁽³⁴⁾ The samples were compared to a calibration curve comprising assays of tobramycin solutions of known concentrations in water (0.2 ng/L to 6 mg/mL).

Separate experiments were conducted with a Wilhelmy pin apparatus (Nima Technology Limited, Coventry, England) in place, at the far end of the trough, to record the surface tension during spreading. These experiments were not conducted with tobramycin present in the solution because the Wilhelmy pin apparatus could not be contained in the glove box. Surface tension measurements were recorded at a point 8 cm away from the region of deposition. A roughened platinum Wilhelmy pin was used for all experiments. In these experiments, surface flow was observed by imaging a 1-mm-diameter polystyrene particle on the subphase surface. The bead movement was tracked in NIS-

TABLE 1. AVERAGE DOSE, DEPOSITION TIME, AND VOLUMETRIC DEPOSITION FLUX IN AEROSOL DEPOSITION EXPERIMENTS

	<i>Tobramycin dose (mg)</i>	<i>Deposition time (seconds)</i>	<i>Aerosol volumetric deposition flux (nL/cm²·s)</i>
Tobramycin	0.63 ± 0.12	789 ± 69	1.32 ± 0.13
Tobramycin/DPPC	0.50 ± 0.09	447 ± 8	1.88 ± 0.18
Tobramycin/DPPC predeposited DPPC layer	0.51 ± 0.07	469 ± 18	1.81 ± 0.12

Standard error is given in the table ($n=3$).

DPPC, dipalmitoylphosphatidylcholine.

Elements Advanced Research Software (Nikon Instruments, Inc., Melville, NY).

Surface tension versus area per molecule isotherms were created for DPPC on 5 wt% mucin solution and on water. These isotherms were created in a 14.5-cm glass Petri dish using the same technique used in prior studies.⁽¹⁵⁾ The Wilhelmy pin was used to measure the surface tension as 0.5 μ L droplets of a 10 mg/mL solution of DPPC dissolved in chloroform were successively deposited from a glass syringe onto the chosen subphase. The system was allowed to equilibrate for 2 minutes until the chloroform had evaporated and surface tension had stopped changing after each droplet was deposited. The surface area per molecule was calculated from the known amount of DPPC in each drop and the surface area of the subphase.

Results

Tobramycin spreading

In Table 1, the average tobramycin dose, deposition time, and aerosol volumetric deposition flux for each of the experimental conditions are shown. Only a very small fraction of the 240 mg of tobramycin in the formulation charged to the nebulizer was deposited onto the trough. The vast majority deposited elsewhere in the aerosolization chamber or was removed by the suction applied to the aerosolization chamber.

The calculated average aerosol deposition fluxes for all experiments were well within the “low-flux regime” as defined in our previous publication.⁽¹³⁾ Droplet coalescence is not expected in this regime. Instead, postdeposition dispersal of a field of distinct droplets is expected. During a typical nebulizer treatment, low-flux deposition would be anticipated in airway generations 7 and beyond.⁽¹⁴⁾ A one-way ANOVA shows that statistically similar ($p < 0.05$) doses were delivered to the trough both with and without DPPC in the aerosol formulations, and so, the resulting spreading data should be comparable for all conditions.

Figure 3 shows the concentration profiles determined for tobramycin outside of the aerosolization chamber. Each data point represents the mean of three trials and the error bars show the standard error. The surface sample concentrations were normalized by the total concentration of tobramycin in the bulk of the subphase in the trough, after thorough mixing as described above at the end of each trial. This was $\sim 7 \mu\text{g/mL}$.

When no DPPC was present in the aerosol, no tobramycin was detected on the trough outside the region of direct deposition. This indicates that (a) the precautions taken to prevent aerosol deposition onto the trough outside

the aerosolization chamber were sufficient and (b) no spreading occurred when no lipid was present in the aerosol formulation. Although we cannot assay for lipid on the surface, since the size distribution and density of the aerosol droplets, and thus the Stokes numbers, are similar for all formulations, we expect that the spatial deposition behavior of all the aerosols to be similar.

In cases where DPPC was present in the aerosol, the concentration of tobramycin was detectable across the full length of the trough. This spreading of tobramycin was accompanied by movement of a tracer particle across the surface. The outside portion of the trough is 8.5 cm long, and the average concentration of tobramycin across the trough outside the deposition region is around $\sim 10 \mu\text{g/mL}$ when DPPC is present in the aerosol formulation regardless of the presence of a predeposited DPPC layer on the subphase.

The surface sampled concentrations of tobramycin were similar with or without a predeposited DPPC monolayer. At the very end of the trough, there was a small but reproducible decrease in tobramycin concentration for the trials

FIG. 3. The tobramycin concentration on the subphase surface in the region outside the aerosolization chamber. When no DPPC is present in the aerosol (●), no tobramycin escapes the aerosolization chamber. When DPPC is added to the aerosol, spreading occurs and tobramycin can be measured all the way across the trough (□). Spreading is similar when a DPPC monolayer is deposited on the subphase before delivery of the DPPC/tobramycin aerosol (◆). The error bars show the standard error of three trials ($n=3$). The concentration is normalized by the total deposited dose for a given run. A scaled concentration of one corresponds to a tobramycin concentration of $\sim 7 \mu\text{g/mL}$, although it varies slightly for each trial. Average scaled tobramycin concentrations sampled inside the region of deposition were ~ 5 for all trials.

with a predeposited monolayer of DPPC. This could be due to the predeposited DPPC layer acting like a “piston oil,” as the DPPC monolayer is being compressed against the end wall of the trough and stopping further surface transport of the lipid. While this layer could shut down flow on the surface, tobramycin, as a hydrophilic molecule, can continue to be transported by induced subsurface flows. The trough end blocking of surface flow is an artificial limitation of our apparatus.

Lipid isotherm structure and creation of concentration gradients on mucin

Figure 4A shows the surface pressure (surface tension of bare subphase-surface tension of surfactant laden subphase) versus area isotherm for DPPC on water and on a 5 wt% PGM solution. The monolayer on water illustrates important expected features. A low surface pressure gas phase exists at large areas per molecule. A liquid expanded phase appears, followed by a low slope liquid expanded/liquid condensed coexistence region that transitions to a liquid condensed phase at $\sim 55 \text{ \AA}^2/\text{molecule}$. These features all agree well with the literature.⁽³⁵⁾

At very small areas per molecule, the liquid condensed monolayer begins to collapse at a surface pressure of $\sim 40 \text{ mN/m}$, or surface tension of $\sim 32 \text{ mN/m}$. We note here that collapse pressures are highly dependent on the technique used to create and compress the monolayer, so this is the particular collapse pressure for a monolayer created from successively deposited drops of DPPC dissolved in chloroform. However, the collapse surface pressure achieved by this method is the same as that achieved by a concentrated dispersion of DPPC vesicles in water equilibrating with the air/water surface.⁽³⁶⁾

The isotherm on the 5 wt% PGM solution lacks many of these features. First, it should be borne in mind that a surface pressure of zero on the PGM solution subphase corresponds to a surface tension of $\sim 40 \text{ mN/m}$, whereas it would be 72 mN/m on a simple water subphase. Thus, the PGM solution surface is occupied by a significant amount of hydrophobic mucin segments before any lipid is deposited.

Accordingly, the surface pressure begins to increase at much larger areas per DPPC molecule on PGM solution than on water, an indication that the mucin polymers adsorbed to

the air/water interface are interacting with the deposited DPPC. In this range of large areas per molecule, the compressibility of the DPPC layer on PGM solution is significantly less than that on water. At low area per DPPC molecule, the surface pressure of the DPPC layer on PGM solution levels off to an apparent collapse at a value far below the collapse pressure on water. However, the surface tensions on PGM solution and water subphases converge (Fig. 4B). This indicates that the DPPC is able to completely displace the mucins from the surface when DPPC is present at a high density.

Figure 5A shows the results of the simultaneous measurement of surface tension and tracer particle motion during delivery of aerosolized DPPC dispersions onto a PGM solution surface with no predeposited lipid monolayer. The surface tension immediately decreases and the bead begins to move as soon as aerosolization begins, indicated as 0 seconds on the time axis. This is consistent with the shape of the DPPC isotherm on PGM solution: even small changes in surface area per molecule decrease surface tension in the region of direct deposition relative to the lipid-free PGM solution surface external to that region. The tracer particle moves most of the way across the trough before eventually sinking into the subphase due to the low surface tension. The surface tension reaches a minimum of $\sim 5 \text{ mN/m}$.

Figure 5B shows the simultaneous measurement of surface tension and tracer particle motion during aerosolization onto a PGM solution surface with a predeposited DPPC monolayer. Negative times in the figure represent times before the aerosolization begins when the chloroform evaporates from the DPPC deposition solution and the DPPC monolayer equilibrates. The monolayer equilibrates at $\sim 24 \text{ mN/m}$.⁽³⁶⁾

The tracer particles begin to move as aerosolization commences and the surface tension drops. The beads do not always reach the end of the trough. Often they sink before reaching the end due to the low surface tension that develops as DPPC moves across the surface. They do not always reach the end of the trough even when they do not sink. This could be due to the lower surface tension of the predeposited layer, causing the bead to sit lower in the mucin and experience larger drag forces.⁽³⁷⁾ Another possibility is that the DPPC monolayer compresses and keeps the bead from moving all the way across the trough. The minimum surface tension

FIG. 4. Surface pressure (A) and tension (B) versus area isotherms for DPPC on DI water and on 5 wt% PGM solution. Surface pressures were calculated using the surface tension of 72 mN/m for water (●) and the surface tension of 40 mN/m for the mucin solution (■) as the reference surface tensions. At low area per DPPC molecule, the surface tension for both subphases converges to $\sim 29 \text{ mN/m}$. PGM, porcine gastric mucin.

FIG. 5. Surface tension and movement of a tracer bead during representative spreading experiments, (A) without predeposited lipid on PGM solution, (B) with predeposited lipid on PGM solution, and (C) without predeposited lipid on water. The gray line is the surface tension and the black line is the bead position with 0 cm being at the edge of the aerosolization chamber. Nebulization begins at 0 seconds. In (A), the abrupt surface tension dip at about 500 seconds is a reproducible but yet to be explained phenomenon. Negative times (B) represent times before aerosolization begins, when the DPPC is being deposited onto the subphase.

achieved is consistently lower for experiments when DPPC was predeposited on the surface and this is likely due to there simply being more DPPC available to adsorb to the surface.

For both cases with and without a predeposited DPPC layer, the surface tension decreases to below 10 mN/m by

the time aerosolization stops. According to the isotherm in Figure 3, the DPPC delivered by aerosol is achieving a surface tension significantly lower than what can be obtained by pipetted delivery of a chloroform DPPC solution onto the subphase. Previous work in our group has demonstrated similarly low surface tensions when DMPC or DPPC dispersions are aerosolized directly onto a fluid interface.⁽¹⁵⁾ Because the aerosolized lipid achieves lower surface tensions than the predeposited layer, there is still a gradient in surface tension across the PGM solution surface and Marangoni stresses are induced.

We observe that after 5–10 hours elapse, the surface tension recovers to around 27 mN/m as DPPC apparently desorbs from the surface into the bulk (data not shown). This surface tension recovery happens only after the aerosol stops and is slow compared to the timescale of the experiment. The desorption process observed at the end of these experiments should be active during deposition, but the rate of adsorption during nebulization is significantly higher than the desorption rate, meaning that the difference in these rates may be what allows for the formation of transient, ultralow surface tensions.

To demonstrate the impact of the shape of the isotherm on transport, a DPPC dispersion was directly aerosolized onto a DI water surface; results are shown in Figure 5C. This experiment also results in achievement of an ultralow surface tension (~ 1 mN/m), however, it has a longer lag time. There is an ~ 80 -second delay between the nebulizer being turned on and any measurable decrease in surface tension and correlated bead movement. This is consistent with the initial slopes of the isotherms for DPPC on pure water.

Without a predeposited DPPC layer, the lipid layer must build up on the surface in the gaseous phase for some time before reaching the liquid expanded state, where a sufficiently dense monolayer can significantly decrease surface tension. The lag time was not observed on the PGM solution subphase, with or without a predeposited DPPC layer, because the isotherm shows a significant decrease in surface tension at large areas per DPPC molecule, which would have corresponded to the gas phase on a simple water subphase.

Discussion

Concentrations of tobramycin of over $8 \mu\text{g/mL}$ are found across the PGM solution surface as far as 8.5 cm away from the region of deposition when tobramycin was delivered by an aerosol that contained DPPC. For reference, the minimum inhibitory concentration (MIC90) of tobramycin for *Pseudomonas aeruginosa*, a common CF-associated pathogen, is $8 \mu\text{g/mL}$.⁽³³⁾ The control experiment of a tobramycin aerosol delivered onto the substrate without DPPC did not spread into the measurement region.

These results suggest that administering aerosolized solutions of DPPC and tobramycin will transport tobramycin across biologically relevant length scales in the lung. Assuming tobramycin is transported with the Marangoni flow (~ 10 cm/s as measured from the change in surface tension at the pin after the beginning of deposition), the transport is orders of magnitude faster than mucociliary clearance in either the proximal (~ 0.01 cm/s) or distal ($\sim 10^{-5}$ cm/s) regions of a healthy lung.⁽³⁸⁾ Aerosolized dispersions of DPPC, tobramycin, and their combination have comparable aerosol

droplet size, meaning their aerodynamically controlled deposition patterns in the lungs should be similar. DPPC alone nebulized directly onto a fluid interface can induce Marangoni transport across that interface. When DPPC is deposited with the nonsurface-active tobramycin, the DPPC induces Marangoni transport and tobramycin is carried in the induced subsurface flows.

Tobramycin transport is little affected by the presence of predeposited lipid. There is some evidence that the predeposited layer is compressed by the newly deposited lipid forced onto the surface by the aerosolized dispersion, but this compression is an extrinsic effect due to the finite area of our mucin surface and has very little effect on spreading of tobramycin. *In vivo*, where there are much larger air/water interfaces, we expect these effects to be even smaller. Pre-existing lipid is unlikely to inhibit the effectiveness of the lipid transport of tobramycin.

Our work is limited by our ability to completely simulate all the complex characteristics of the ASL layer, including mucociliary clearance, heterogeneous surfaces, and thin liquid films. Future work will consider aerosol spreading on thin liquid layers, which may differ from the deeper liquid pools used here. In addition, experiments should be performed to consider the high aerosol deposition fluxes that would be expected in the large airways. Further experiments are needed to determine the optimal aerosol compositions to facilitate drug delivery and spreading.

The behavior of DPPC on a mucin solution subphase is significantly different than on water. The isotherm shows that DPPC on a mucin solution is significantly less compressible (has a higher slope on the isotherm) at low surface concentrations and significantly more compressible at high surface concentrations than DPPC on water. DPPC on mucin also appears to lack the phase coexistence regions that exist on pure water.

While it is unclear what is happening at a molecular level on the surface, mucins appear to interact with the DPPC at high areas per molecule to lower the surface tension. The simplest explanation is a compression effect: as both polymer segments and lipids share the surface, the area available to each is less and the surface tension decreases.⁽³⁹⁾ While the behavior of the isotherm on these two surfaces is different, the final, equilibrium surface tension achieved is the same for both subphases (~ 29 mN/m). This indicates that it is likely that the DPPC is completely displacing mucin chains from the surface.

The surface tension of mucin was measured while aerosolized DPPC dispersion was deposited onto a predeposited DPPC layer. Once aerosolization begins, the surface tension immediately decreases. This coincides with the movement of a tracer particle across the trough and is expected from the slope of the isotherm at low surface coverage. The surface tension is reduced to below 10 mN/m on clean PGM solution and in the presence of a DPPC monolayer. This difference is likely due to the higher total amount of DPPC on the surface when a layer is predeposited. The surface tension and bead movement data show that DPPC can induce spreading on a model airway surface, even when a liquid condensed monolayer of lipid is already present.

While the actual variation of surface tension throughout the lung remains in question, the ultralow surface tension obtained by DPPC aerosols should be expected to induce Marangoni flow so long as the lung airway's surface tension

is above ~ 10 mN/m, and perhaps even as low as 2 mN/m. This is probable in all but the respiratory zone of the lung.

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