Surfactant Driven Post-Deposition Spreading of Aerosols on Complex Aqueous Subphases. 2: Low Deposition Flux Representative of Aerosol Delivery to Small Airways

Ramankur Sharma, MS,^{1,2} Amsul Khanal, PhD,^{1,3} Timothy E. Corcoran, PhD,^{3,4} Stephen Garoff, PhD,^{1,5} Todd M. Przybycien, PhD,^{1–3} and Robert D. Tilton, PhD^{1–3}

Abstract

Background: Cystic fibrosis (CF) is associated with the accumulation of dehydrated mucus in the pulmonary airways. This alters ventilation and aerosol deposition patterns in ways that limit drug delivery to peripheral lung regions. We investigated the use of surfactant-based, self-dispersing aerosol carriers that produce surface tension gradients to drive two-dimensional transport of aerosolized medications via Marangoni flows after deposition on the airway surface liquid (ASL). We considered the post-deposition spreading of individual aerosol droplets and two-dimensional expansion of a field of aerosol droplets, when deposited at low fluxes that are representative of aerosol deposition in the small airways.

Methods: We used physically entangled aqueous solutions of poly(acrylamide) or porcine gastric mucin as simple ASL mimics that adequately capture the full miscibility but slow penetration of entangled macromolecular chains of the ASL into the deposited drop. Surfactant formulations were prepared with aqueous solutions of nonionic tyloxapol or FS-3100 fluorosurfactant. Fluorescein dye served as a model “drug” tracer and to visualize the extent of post-deposition spreading.

Results: The surfactants not only enhanced post-deposition spreading of individual aerosol droplets due to localized Marangoni stresses, as previously observed with macroscopic drops, but they also produced large-scale Marangoni stresses that caused the deposited aerosol fields to expand into initially unexposed regions of the subphase. We show that the latter is the main mechanism for spreading drug over large distances when aerosol is deposited at low fluxes representative of the small airways. The large scale convective expansion of the aerosol field drives the tracer (drug mimic) over areas that would cover an entire airway generation or more, in peripheral airways, where sub-monolayer droplet deposition is expected during aerosol inhalation.

Conclusions: The results suggest that aerosolized surfactant formulations may provide the means to maximize deposited drug uniformity in and access to small airways.

Key words: aerosol, Marangoni spreading, pulmonary drug delivery, surfactant transport

Introduction

CYSTIC FIBROSIS (CF) IS AN INHERITED autosomal recessive disease that affects 70,000 people worldwide. It is associated with a malfunction in the transport of chloride and other ions across epithelial cell membranes that results in the

production of abnormally viscoelastic mucus in the lungs, amongst other pathophysiological changes.^(1,2) Accumulated mucus in the lungs of CF patients obstructs the airways and harbors chronic infections.⁽³⁾ Aerosolized antibiotics are used to treat and prevent these infections since they can achieve locally high concentrations while avoiding the significant

¹Center for Complex Fluids Engineering, and Departments of ²Chemical Engineering, ³Biomedical Engineering, and ⁵Physics, Carnegie Mellon University, Pittsburgh, Pennsylvania.

⁴Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

side effects associated with systemic delivery.^(4,5) Yet, a major challenge in drug delivery to obstructed lungs is the non-uniform aerosol deposition caused by altered ventilation patterns.⁽⁶⁾ High local drug concentrations are produced in the central airways with little drug reaching the peripheral airways.⁽⁷⁻⁹⁾ This hinders full eradication of infections and may lead to antibiotic resistance.⁽¹⁰⁻¹²⁾

We propose the use of surfactants in inhaled drug formulations to improve dosing homogeneity in the lungs by driving surface flows that enhance the post-deposition spreading of medications along the airway surface liquid (ASL). Such a mechanism may enhance drug distribution in partially obstructed airways where ventilation is limited, but transport of drug over the ASL surface may still be possible. Reopening of fully obstructed airways with low surface tension liquids has been considered⁽¹³⁾ but is beyond the scope of the current work.

Currently, most medications for nebulization are simple aqueous solutions or suspensions,^(14,15) and their distribution in the airways is determined by aerodynamics alone. Our prior *in vitro* studies on human bronchial epithelial cell cultures and other ASL mimic subphases demonstrated that surfactant addition to aqueous aerosol formulations can significantly increase the final spread area of deposited aerosols or microliter drops as compared to the surfactant-free controls.⁽¹⁶⁻¹⁹⁾

Microliter-scale surfactant-laden drops can have dramatically increased spreading extents on liquid subphases. A general picture of the spreading mechanism for a surfactant-laden drop on a liquid subphase is presented in a companion article by Khanal et al. (hereafter referred to as Part 1). The final spreading state of a drop placed on a liquid subphase is controlled by the capillary balance dictated by the three surface tensions $\gamma_{\text{subphase/vapor}}$, $\gamma_{\text{drop/vapor}}$, $\gamma_{\text{drop/subphase}}$, the densities of the liquids, and the volume of the drop. A key aspect of aqueous surfactant solution drop spreading on a miscible subphase is the rapid lateral transport of surfactant out of the deposited drop and over the surrounding subphase surface. This escaped surfactant may travel distances that are significantly larger than the final diameter of the spread drop.^(18,20,21)

It is uncertain how well microliter-scale drop spreading behavior translates to sub-picoliter scale aerosol droplets. While the basic spreading mechanism of an individual sub- μL aerosol droplet after deposition should be similar to that of a microliter drop, the much larger surface-to-volume ratio of aerosol droplets suggests that surfactant depletion from the small aerosol droplet should occur much more rapidly, perhaps defeating the spreading enhancement. What is fundamentally different, however, is that aerosol droplets are not deposited in isolation: inter-droplet interactions on the subphase may affect the overall lateral spreading extent of the aerosol field. The consequences of surfactant molecules escaping from multiple neighboring droplets and creating heterogeneous surface tension distributions among the droplets are expected to play an important role in the overall transport of droplet contents, particularly in the small airways of the lung where aerosol deposition fluxes are small. An understanding of the spreading mechanism of the field of deposited aerosol droplets is required to engineer effective surfactant-based self-dispersing aerosol drug carriers for pulmonary drug delivery.

In Part 1, we performed calculations to estimate the aerosol deposition flux (deposited volume per area per time)

in different airway generations during a nebulizer treatment. Here we report the post-deposition spreading behavior of aerosol droplets at the lower deposition fluxes typical in small lung airways. The fluxes are low enough that individual droplet spreading and lateral droplet interaction behaviors can be clearly resolved. The fact that individual droplets retain their distinct identities after deposition at low fluxes is the main factor that distinguishes low flux from high flux conditions. If sub-picoliter droplets behave like the previously studied microliter drops, surfactant will escape from each deposited aerosol droplet and transport across the subphase surface. This will establish surface tension gradients around each individual droplet before they have an opportunity to coalesce with other droplets. It is not yet known how these surfactant monolayers escaping from multiple, distinct droplets interact with one another and affect either the extent of individual droplet spreading or the expansion of the field of deposited droplets.

Based on our estimates, the deposition fluxes in these experiments would be similar to those demonstrated in generation 12 and deeper airways during a nebulizer treatment (discussed below). Part 1 addresses spreading at the high deposition fluxes noted at zones of impaction within the large airways.

We performed aerosol spreading experiments on entangled aqueous polymer solutions that mimic key features of the ASL. Pulmonary mucus is a 95% w/w, aqueous, viscoelastic solution of mucin, glycoproteins, DNA, and other solutes,⁽²²⁾ with the composition varying depending on lung health and the particular location in the lung.⁽²³⁾ The principal component of pulmonary mucus is an entangled network of mucin glycoproteins⁽²⁴⁾ with molecular weights ranging from 1–10 MDa.⁽²⁵⁾ We used chemically simple solutions of entangled 1% w/w poly(acrylamide) (PA) solutions or biologically more relevant solutions of entangled 5% w/w porcine gastric mucin (PGM) as ASL mimics. Like ASL, these aqueous solutions are fully miscible with aqueous surfactant solutions, but entanglement severely retards intermixing of the drop and subphase contents.⁽¹⁸⁾ A key determinant of the spreading behavior of a drop on the ASL is $\gamma_{\text{ASL/vapor}}$. The presence of endogenous surfactant has been reported in the airways, especially the peripheral airways, which would lower surface tension and make surfactant driven flows more difficult to effect.⁽²⁶⁾ Values of ASL surface tension reported in the literature vary significantly potentially due to differences in endogenous surfactant distribution within the airway tree, the different measurement techniques used, and the possibility of altering the ASL during sampling.⁽²⁷⁻²⁹⁾ The surface tensions of the PA and PGM solutions used here roughly bracket the high and low limits, respectively, of those reported for the ASL.

Correspondingly, two surfactants, tyloxapol and Capstone FS-3100, with low and extremely low surface tensions at their critical micelle concentrations, $\gamma_{\text{droplet/vapor}(\text{cmc})}$, were used. Tyloxapol was a component of Exosurf, a now discontinued synthetic surfactant-replacement therapy that was delivered as a bolus to treat the lungs of premature neonates.⁽³⁰⁾ FS-3100 is a fluorosurfactant, which as a class, are well known to have extremely low surface tensions.⁽³¹⁾ While the *in vivo* suitability of FS-3100 is unknown, perfluorocarbons have been studied as liquid ventilation agents.⁽³²⁾ Finally, fluorescein was used both as a tracer

species to visualize the extent of deposited aerosol spreading and as a drug mimic.

In the current work, we find that surfactants significantly enhance individual, sub-picoliter-scale aerosol droplet spreading on ASL mimics relative to surfactant-free controls. More importantly, surfactants also produce Marangoni stresses across the subphase, driving a post-deposition convective expansion of the entire deposited aerosol field into previously unexposed subphase regions. The areas spanned by the droplet field expansion are comparable to entire airway generations in the small airways where low deposition fluxes are anticipated.

Materials and Methods

Materials

All the glassware used was cleaned sequentially using ethanol, acetone, and de-ionized (DI) water several times and then dried under a nitrogen stream. DI water with a resistivity of 18 M Ω cm was obtained from a Milli-Q Academic unit (Millipore Corporation, Billerica, MA). The DI water had a surface tension of 71.5 ± 0.4 mN/m (where the quoted uncertainty here and elsewhere in this article is the standard error of the mean unless otherwise noted) and a total organic carbon content < 10 ppb. DI water was used to prepare all solutions. Pyrex glass petri dishes of radius 3 cm and depth 1 cm were used to contain the liquid subphase. All the experiments were conducted at a room temperature of $23 \pm 1^\circ\text{C}$.

Aqueous poly(acrylamide) (PA) solutions at a concentration of 1% w/w and pH 9 (required to ensure the bright fluorescence of the fluorescein dye tracer) were used as one of the ASL mimics. PA (molecular mass 5–6 MDa, CAS# 9003-05-8) was purchased from Polysciences, Inc. (Warrington, PA). The PA solutions are above the entanglement concentration⁽¹⁸⁾ and were prepared as described in Part 1. The surface tension of the 1% w/w aqueous PA solution immediately after pouring a fresh sample into the petri dish was 70.8 ± 0.5 mN/m. The surface tension subsequently decreased by ~ 1 –2 mN/m over the course of 5 min.⁽¹⁸⁾

Porcine gastric mucin (PGM; Type II, bound sialic acid $\sim 1\%$ CAS# 84082-64-4) was purchased in dehydrated form from Sigma Aldrich. A 5% w/w solution of PGM was prepared in DI water as described in Part 1. At 5% w/w, the concentration is above the entanglement concentration of PGM in water.⁽¹⁷⁾ The surface tension of the 5% w/w PGM solution was 38.3 ± 3.5 mN/m (where the uncertainty represents the standard deviation among many samples and experiments). The surface tension of a PGM solution typically decreased by 2–3 mN/m over 5 min after pouring a fresh sample. This decrease in surface tension is likely due to two factors acting individually or in combination 1) slow adsorption of polymer chains at the PGM subphase/vapor interface, and 2) a localized increase in polymer concentration at subphase/vapor interface due to water evaporation.^(18,33) There were batch-to-batch and within-batch variations in both the initial measured surface tension and in the ultimate surface tension achieved after ~ 5 min of pouring for the PGM solutions. This was a source of variability in spreading results when surfactant surface tensions were close to that of the PGM solution (discussed later).

Fluorescein dye (Laser Grade 99%, CAS# 2321-07-5) was purchased from Fisher Scientific and used as received. A 10 mM fluorescein dye stock solution was prepared in DI water and adjusted to pH 9 by aliquots of 1 M NaOH in order to maximize its fluorescence intensity for reliable detection. This molecular dye was incorporated into the surfactant solution to be aerosolized. Experiments also used fluorescent tracer particles to aid in focusing on the subphase surface and to trace the lateral flow of the subphase surface outside of the aerosol deposition zone. These were obtained from Polysciences, Inc. as a 2.5% w/w, surfactant-free aqueous suspension of 3.00 μm diameter fluorescent microspheres. A 0.5–1 μL drop of the microsphere suspension diluted in DI water was pipetted onto the subphase before aerosol administration. The surface tensions of the aqueous (surfactant-free) fluorescein solution and the aqueous microsphere suspension were 71.4 ± 0.5 mN/m and 70.9 ± 0.4 mN/m, respectively.

The surfactants used were tyloxapol (CAS# 25301-02-4), an oligomeric non-ionic surfactant purchased from Sigma Aldrich and a non-ionic fluorosurfactant Capstone FS-3100 (DuPont, Wilmington, DE). The surfactant solutions were prepared in the 10 mM fluorescein stock solution. Two tyloxapol concentrations were used: 0.4% w/w and 1.1% w/w ($\gamma = 37 \pm 1$ mN/m). As shown in Part 1, both of these concentrations were above the critical micelle concentration (CMC = 0.1% w/w) of tyloxapol in the presence of fluorescein at pH 9. Fluorosurfactant FS-3100 was used at 1.4% w/w ($\gamma = 17.5 \pm 0.4$ mN/m), exceeding its CMC of 0.01% w/w in the presence of 10 mM fluorescein at pH 9 as shown in Figure 1. The CMC was estimated from the intersection of the two least-squares regression lines on the semi-log surface tension isotherm plot.

Methods

Aerosol delivery system. An Aeroneb Solo micropump nebulizer (Aerogen, Galway, Ireland) with an Aerogen Pro-X controller was used to produce the liquid aerosols. This nebulizer aerosolizes liquids via a gravity-fed, ultrasonically-vibrated mesh. Concentration changes associated with nebulization were considered by comparing absorbance spectra of collected aerosolized samples and the pre-aerosolized sample that are indicative of concentration. Fluorescein has a strong absorbance peak at 492 nm. Tyloxapol and FS-3100 have a strong absorbance at 280 nm and 227 nm, respectively. No significant changes in concentration were noted by the *t*-test for fluorescein ($p = 0.08$), tyloxapol ($p = 0.16$), and FS-3100 ($p = 0.7$). Since the surfactant concentration does not change on aerosolization, the surface tension values of the aerosolized solutions also do not change.

For aerosol deposition experiments, the nebulizer was located ~ 7 cm away from a petri dish containing the subphase and the aerosol was deposited directly as indicated in Figure 2. The nebulizer was switched on for 1–2 sec to generate a short pulse of aerosol. Most of the aerosol droplets that deposited on the subphase arrived within 5 sec of the aerosol generation. Laser diffraction measurements of aerosol size distributions in air were made separately using a Malvern Mastersizer S (Malvern Instruments, Worcester-shire, UK).

FIG. 1. Surface tension isotherm for fluorosurfactant FS-3100 in 10 mM fluorescein solutions at pH 9. The standard deviation of the measured surface tension values is 0.5 mN/m. The low and high concentration data were fitted with the least squares regression lines shown; the intersection, occurring at 0.01% w/w, approximates the CMC of FS-3100 under these conditions.

The aerosol deposition flux (total volume of aerosol droplets deposited per unit time and area), for all experiments, was estimated to be in the range of $(0.02\text{--}13) \times 10^{-5} \mu\text{L}/\text{cm}^2\text{sec}$ by counting the number of droplets deposited in the first 5 sec of delivery. In Part 1, we performed calculations to estimate the

aerosol deposition flux in different airway generations during a nebulizer treatment. The high end of our experimental range corresponds with estimates of deposition flux in generation 12 airways. The lower end of the experimental range would be associated with even smaller, more peripheral airways.

FIG. 2. Schematic of experimental setup for (A) imaging post-deposition spreading of aerosol and (B) monitoring subphase surface tension with simultaneous deposition of aerosol.

Imaging post-deposition spreading of aerosol

Figure 2A shows the experimental setup for imaging post-deposition spreading of aerosol droplets at low deposition flux. The solution subphase was poured into a petri dish to a depth of ~ 0.5 cm. Approximately one-fourth of the dish was covered by a transparent mask placed ~ 0.6 cm above the subphase surface, so that aerosol only deposited onto the uncovered region of the subphase. A vertical barrier projected downward from the edge of the mask, leaving a gap of less than 1 mm between the mask and the subphase surface, in order to block aerosol that might move in the air under the mask. This procedure mimics the situation in an obstructed airway, where aerosol deposits near a mucus obstruction and must spread over the ASL to penetrate further into the airways.

Microscopy and image analysis

Aerosol deposition and spreading was observed from above with a fluorescence microscope operated in epi-illumination mode. A Nikon AZ 100 microscope (Nikon Instruments Inc., Melville, NY) with an AZ-Plan Apo $0.5\times$ objective (NA: 0.05/WD: 54mm) and equipped with fluorescein filter set, Nikon B-1E, was focused directly on the subphase/vapor interface under the barrier so that the field of view was evenly divided between the aerosol deposition zone and the masked region of the subphase. Time-sequenced images were recorded at various frame rates ranging from 5–10 frames/sec by a video camera (DS-QiMc - Nikon) with $0.6\times$ magnification. Before each experiment, a drop of the fluorescent microsphere (surfactant-free) suspension was deposited on the subphase just inside the masked region to aid in focusing on the subphase surface. Video images were later analyzed

using the Nikon NIS-Elements Advanced Research microscope imaging software.

Diameters of individual droplets were measured within 400 msec of deposition onto the subphase using the image analysis auto-detect algorithm, which successfully resolved droplet diameters when droplet edges were distinct. The auto-detect algorithm failed at longer times when the fluorescein dye had diffused to a significant degree into the bulk subphase and image contrast was diminished. For longer time regimes, an intensity line scan was performed manually across the center of a droplet, and the diameter was expressed as the full width at half maximum intensity.

In order to track the migration of the dye into the masked subphase surface region, intensity scans were executed along lines perpendicular to the barrier, across the entire field of view. The intensity of the line scan, integrated along the direction parallel to the barrier, was then plotted as a function of position to track the leading edge of the advancing field of dye spots. The front of the advancing uniform aerosol field was defined as the position where the mean intensity increased by more than one standard deviation from the baseline intensity recorded in the masked region. On the PA solution subphases, the deposited aerosol droplet field spread over large distances into the masked subphase region, creating a distinct front to the advancing field of individual droplets. On the PGM subphases, experiments were performed at higher magnification since distances transported by deposited aerosol droplets were less than on the PA solution subphase (discussed later). At higher magnification, the droplets were simply tracked as individual entities and a uniform advancing aerosol front could not be created. In these cases, the post-deposition transport of individual droplets was tracked by performing

FIG. 3. Number-median size distributions of surfactant-free and tyloxapol-containing aerosol droplets (A) pre-deposition in air and (B) post-deposition on a PA solution subphase. Symbols: $\triangle$, surfactant-free, pre-deposition ($n=5$); $\square$, 0.4% w/w tyloxapol, pre-deposition ($n=5$); $\blacktriangle$, surfactant-free, post-deposition ($n=3$); $\blacksquare$, 0.4% w/w tyloxapol, post-deposition ($n=3$). Estimated ages of all droplets are between 350–400 msec. Bin size in B is 10 μm . Error bars represent standard error of mean.

the intensity scan across each droplet and tracking the position of the intensity maximum with time.

Surface tension measurement

All liquid/vapor surface tensions were measured using a Wilhelmy pin apparatus (Nima Technology Ltd., Coventry, England). The subphase surface tension was monitored in the masked region during and after aerosol deposition to verify surfactant escape and surface transport away from the deposition site. Figure 2B shows the experimental setup. A hole was drilled in the mask ~ 1 cm away from the vertical barrier. The hole was just large enough to allow the pin to pass without touching the edges in order to minimize stray aerosol deposition through the hole. Because the Wilhelmy pin balance obstructed microscope access, it was not possible to image the aerosol field during these surface tension experiments.

Results and Discussion

Post-deposition spreading of individual aerosol droplets on poly(acrylamide) solution subphases

The pre-deposition size distributions of the surfactant-free and tyloxapol-laden aerosols generated by the nebulizer in air are shown in Figure 3A. Before deposition, both the surfactant-free and tyloxapol aerosols have comparable number median diameters in air of $1.6 \pm 0.1 \mu\text{m}$ ($n=5$; n represents number of different experiments) and $1.8 \pm 0.1 \mu\text{m}$ ($n=5$), respectively, and volume median diameters of $5.2 \pm 0.1 \mu\text{m}^3$ ($n=6$), and $5.0 \pm 0.2 \mu\text{m}^3$ ($n=5$), respectively. The comparison between the two distributions using the Kolmogorov-Smirnov (KS) method gives a p value of 1.0, suggesting that the presence of tyloxapol in the nebulized solution has no impact on the size distribution of the aerosol. (Number-median diameters are utilized in this study to allow comparisons between pre- and post-deposition droplet sizes measured by laser diffraction and microscopy, respectively).

Figure 4 shows representative images of surfactant-free and tyloxapol-containing (0.4% w/w) aerosol droplets during deposition onto a PA solution subphase. The aerosol is made visible by the inclusion of the fluorescein dye in solution. The streaks in the images show droplets moving in

the air prior to deposition. The circular spots show deposited droplets with a range of ages (times after deposition) less than or equal to 400 msec. At this magnification, each pixel corresponds to $2.7 \mu\text{m}$, therefore the streak widths may not reflect the actual droplet diameters in air. The size of the deposited dye spot accurately represents the spread size of the aqueous droplet on the subphase, as fluorescein does not cross the contact line and transport outside the droplet on the subphase surface, as demonstrated in prior work.⁽¹⁸⁾

The convective spreading of a surfactant-laden droplet ends within 400 msec of its deposition after which it spreads diffusively (discussed below). Size distributions of approximately 50–60 deposited droplets with and without surfactant at the end of convective spreading are shown in Figure 3B. The post-deposition number median diameters of surfactant-free and tyloxapol-laden droplets are $33.7 \pm 2.3 \mu\text{m}$ ($n=3$ experiments, $m=50$ –60 droplets/experiment) and $62.8 \pm 11.8 \mu\text{m}$ ($n=3$, $m=50$ –60). The p value obtained using the KS test is <0.001 , indicating that the post-deposition size distribution of the droplets with and without the surfactant is significantly different. Thus, 350–400 ms after deposition on a PA solution subphase, individual tyloxapol aerosol droplets convectively spread more than the surfactant-free ones. The enhanced post-deposition spreading of the tyloxapol droplets is consistent with the enhanced final spread area observed for microliter-scale surfactant-laden drops on liquid subphases.^(17,26)

The rates of increase of the droplet diameters immediately after deposition are different from the rates of increase observed a couple of hundred milliseconds later. During the first hundred milliseconds, the rate of increase cannot be well resolved at the video camera frame rate. In these early times after deposition, the spreading is likely controlled by viscosity and capillarity and, in the case of the surfactant-laden droplets, Marangoni stresses.^(26,34) At the later times after deposition (from approximately 400 msec after deposition), the increase in droplet size is dominated by diffusive growth, as shown in Figure 5, where the scaling of the droplet diameter is consistent with the $t^{1/2}$ diffusive scaling. The fitted scaling exponents are 0.47 ± 0.03 ($m=5$ droplets) for the surfactant-free case and 0.46 ± 0.06 ($m=7$) for the tyloxapol-containing droplets at long times. The exponents

FIG. 4. Representative images from aerosol deposition experiments. Deposition of (A) surfactant-free and (B) tyloxapol-containing (0.4% w/w) aerosol droplets on a PA solution subphase. Images were taken within 400 msec after the first aerosol droplet deposited on the subphase.

FIG. 5. Log-log plot showing spreading diameter with time for individual droplets of (A) surfactant-free and (B) tyloxapol-containing (0.4% w/w) aerosol after at least 400 msec of deposition on a PA solution subphase. Each symbol corresponds to an individual droplet. Triangle indicates slope of 0.5 expected for diffusive growth of droplets.

were obtained by averaging the separately fitted growth for different droplets. These results are consistent with a diffusion-dominated spreading regime for deposited droplets of age 400 msec or more, regardless of surfactant content.

The deposited droplet diameter after the convective spreading along with the known volumes of the droplets as they land permits an estimate of the droplet thickness to be made. For a flat disk with a 50 μm diameter, corresponding to the diameter of the youngest observed droplets, the estimated thickness is on the order of a few tens of nanometers. For microliter-scale drops, water remains in the drops on a PA solution subphase for several minutes despite its complete miscibility with the subphase.⁽¹⁸⁾ This long drop lifetime is due to the slow disentanglement of polymers in the subphase, that limits the rate of drop mixing with the subphase. But given a thickness only on the order of tens of nanometers for the spread aerosol droplets (sub-picoliter-scale), persistence of water in the droplet after it lands on the subphase for a very long time is unlikely. This is consistent with the hundred millisecond timeframe observed for the convective spreading to end. Thus, after ~ 400 msec, before the aerosol field transports significantly (see below), the convective spreading of the individual droplets halts; water in the individual droplets moves into the subphase, leaving the dye and surfactant in the footprint of the droplet.

Post-deposition expansion of aerosol droplet field on poly(acrylamide) solution subphases

Figure 6 shows a series of images of a tyloxapol-laden (0.4% w/w) and surfactant-free aerosol droplet fields after deposition on a PA solution subphase. After a few seconds, the aerosol droplets appear blurry due to diffusion of the fluorescein dye across and into the subphase. The tyloxapol-containing droplet aerosol field convects laterally towards the unexposed, masked subphase region after deposition on the uncovered region of the subphase. No such convection of the aerosol field along the subphase was observed in the case of surfactant-free aerosol.

Figure 7 shows the post-deposition distance moved by the surfactant-free and tyloxapol-containing (0.4% w/w) aerosol field front as a function of time after deposition on a PA solution subphase. The tyloxapol-laden aerosol field convects over several millimeters, which is significantly greater than the distances traveled by the surfactant-free aerosol field ($p=0.02$ by t -test). The experiment-to-experiment variability in the distance traversed by the tyloxapol aerosol, represented by the error bars in Figure 7, is due to difficulty in precisely controlling the total volume of surfactant aerosol deposited from run to run.

The large-scale movement of the tyloxapol-laden aerosol field is consistent with the development of macroscopic surface tension gradients between the aerosol exposed region and the unexposed region of the subphase. These surface tension gradients would be expected to induce a Marangoni flow in the subphase from the low surface tension to high surface tension regions causing the aerosol field to expand.

To verify the existence of Marangoni stresses across the subphase, the change in the PA solution subphase surface tension was monitored with a Wilhelmy pin apparatus in the covered region during and after aerosol deposition. Figure 8 shows a representative plot of the decrease in the PA subphase surface tension recorded ~ 1 cm into the masked subphase region after deposition of the tyloxapol-containing (0.4% w/w) aerosol. The dip in surface tension precedes the arrival of the droplet front (based on the known position of the pin and the spreading rate of the droplet field shown in Fig. 7). This indicates that the surface tension decrease is caused by the surfactant that escapes and then moves ahead of the droplets. The surface tension ahead of the droplets decreased abruptly by a mean magnitude of 2.4 ± 1.2 mN/m ($n=3$) and started within 1 sec of aerosol deposition onto the subphase, which again suggests that it can only have been caused by the escaping surfactant. The speed of the surfactant escape across the subphase is greater than 1 cm/sec, in agreement with the surfactant front speeds observed in previous studies of microliter-scale drop spreading.^(17,18,35)

FIG. 6. Post-deposition transport of (A) tyloxapol-containing (0.4% w/w) and (B) surfactant-free aerosol field on a PA solution subphase.

The surfactant layer escaping from deposited droplets cover the subphase surface within 1 sec of deposition, but the aerosol field keeps spreading for ~ 50 sec (Fig. 7), suggesting that the presence of a surfactant layer on the subphase does not fully stop the convection of the aerosol field across the subphase/vapor interface.

Assuming that surfactant is occupying the space in between droplets within the droplet field, the high surface tension before the decrease confirms that Marangoni stresses are established across the subphase by the surfactant-laden aerosol deposition. The surface tension dip caused by the escaped tyloxapol molecules does not recover over time-scales of the experiment, probably due to slow desorption of the oligomeric tyloxapol molecules from the subphase surface into the bulk PA solution.

The escaped surfactant convects across the masked subphase region that was not directly exposed to aerosol deposition. This convection of the escaped surfactant due to Marangoni stresses induces a flow in the PA subphase/vapor interface far from the region of direct aerosol deposition and ahead of the convecting droplets. The flow in the subphase

FIG. 7. Movement of aerosol field front into masked region as a function of time after deposition on a PA solution subphase. Symbols: ● surfactant-free aerosol ($n=3$); ■ tyloxapol-containing (0.4% w/w) aerosol ($n=4$). Error bars represent standard error of mean. Abscissa error bars represent uncertainty in time due to binning of different data sets corresponding to different experiments. Error bars are smaller than the symbol in the surfactant-free aerosol case.

surface remote from the deposition region was verified by observing the movement of fluorescent microspheres that had been deposited on the masked region of the subphase several millimeters away from the barrier. The spheres are sufficiently small that they act as tracers for the surface flow. The $3\text{ }\mu\text{m}$ microspheres were displaced by $1.1 \pm 0.2\text{ mm}$

FIG. 8. Representative plot showing surface tension change in the masked subphase region recorded by Wilhelmy pin on deposition of tyloxapol-containing (0.4% w/w) aerosol on a PA solution subphase. Aerosol deposition was initiated at $t \sim 0$ sec, as indicated by the arrow. After reaching a minimum, the surface tension dip does not recover in the experimental time frame due to slow desorption of the escaped tyloxapol molecules into the bulk PA solution subphase.

($n=4$) in the first 10 sec of deposition of the tyloxapol-containing (0.4% w/w) aerosol (Fig. 9). Microspheres movement commenced within 1 sec of the initiation of aerosol deposition, consistent with the observation of the surface tension dip caused by the escaping surfactant. Although the data in Figure 9 are shown for a single representative microsphere, the displacements of all the microspheres in a given experiment were similar, suggesting that they are all transported along the subphase/vapor interface by the escaping surfactant. Similar movement of solid particles at a liquid surface under the action of Marangoni stresses has been reported before.^(17,20,36) This convection of the subphase surface is responsible for the post-deposition convection of the aerosol droplet field.

No change in the PA solution surface tension was recorded upon deposition of the surfactant-free aerosol, nor was any motion of the fluorescent microspheres detected upon surfactant-free aerosol deposition. Without the induced Marangoni stresses, there was no post-deposition expansion of the surfactant-free aerosol field.

Post-deposition transport of aerosol droplets on porcine gastric mucin solution subphases

Unlike PA solutions, the surface tension of a freshly poured PGM solution is much lower than water. The surface tension of a fresh 5% w/w PGM solution shortly after pouring is 38.3 ± 3.5 mN/m (where the uncertainty represents the standard deviation). It decreases by 2–3 mN/m in ~ 300 sec after pouring, in some cases becoming lower than the surface tension of a micellar tyloxapol solution and resulting in variable spreading results as discussed below.

FIG. 9. Representative plot showing time-dependent position of a $3 \mu\text{m}$ microsphere at a remote location on the PA subphase/vapor surface and of the corresponding aerosol front upon deposition of surfactant-free and tyloxapol-containing (0.4% w/w) aerosol. Solid symbols represent microsphere and corresponding open symbols represents the corresponding aerosol field. Symbols: $\square$, surfactant-free; $\circ$, tyloxapol (0.4% w/w). Error bars are smaller than the symbols and represent standard error of mean on the measurement.

Figure 10 shows the post-deposition transport of individual droplets of aerosol with and without tyloxapol on a PGM solution subphase. The tyloxapol-containing (0.4% w/w) aerosol droplets are convectively transported to distances ranging between 0.01 mm–0.2 mm after deposition on a PGM solution subphase, which is much less than on the PA solution ($p=0.006$). Because of the smaller surface tension difference between the tyloxapol-containing droplets and the PGM solution subphase, the corresponding Marangoni stresses are thereby weakened. Increasing the concentration of tyloxapol from 0.4% w/w to 1.1% w/w and depositing a comparable volume of aerosol had no significant effect on extent of the spreading ($p=0.5$); both concentrations are above the CMC of tyloxapol and thus the difference between the droplet and subphase surface tensions was unchanged.

The Wilhelmy pin measurements were repeated for the tyloxapol-containing aerosol on the PGM solution subphase, but no surface tension reduction outside the background noise (<0.5 mN/m) was observed on the PGM solution subphase outside the deposition region. Further, no significant surfactant-induced motion of the fluorescent microspheres was distinguished upon aerosol deposition. The particles were displaced by 0.09 ± 0.02 mm ($n=2$) or 0.08 ± 0.01 mm ($n=3$) within approximately 30 sec after deposition of the tyloxapol 0.4% w/w or 1.1% w/w aerosol, respectively. A surfactant solution with a significantly lower surface tension is required to drive the aerosol field expansion over large distances on the lower surface tension PGM solution subphase.

FIG. 10. Distance transported by individual aerosol droplets as a function of time after deposition on a PGM solution subphase. Symbols: Δ , surfactant-free droplets ($m=5$; m is total number of tracked droplets), $(1.7 \pm 0.7) \times 10^5 \mu\text{m}^3$ deposited ($n=2$; n is number of experiments); $\diamond$, tyloxapol-containing (0.4% w/w) droplets ($m=6$), $(56.7 \pm 13.0) \times 10^5 \mu\text{m}^3$ deposited ($n=4$); $\circ$, tyloxapol-containing (1.1% w/w) droplets ($m=7$), $(27.8 \pm 7.2) \times 10^5 \mu\text{m}^3$ deposited ($n=6$); $\square$, FS-3100-containing (1.4% w/w) droplets ($m=11$), $(17.8 \pm 2.2) \times 10^4 \mu\text{m}^3$ deposited ($n=4$). Error bars represent standard error on mean. Abscissa error bars are smaller than the symbols. Error bars are smaller than the symbol in the surfactant-free aerosol case.

To provide this lower surface tension for the aerosol droplets, we used fluorosurfactant FS-3100 at a concentration of 1.4% w/w ($\gamma = 17.5 \pm 0.4$ mN/m). Figure 10 shows that the deposited FS-3100 aerosol droplet field convects to a much greater extent on a PGM solution subphase, ~ 0.8 mm, than do the tyloxapol droplets ($p = 0.0001$). The total volume of deposited FS-3100-laden aerosol was comparable to the tyloxapol and surfactant-free cases. Thus, the enhanced droplet field expansion caused by the FS-3100 (1.4% w/w) aerosol droplets can only be due to the greater surface tension difference between the subphase and aerosol.

As in the tyloxapol case on the PA solution subphase, the surface tension of the PGM solution subphase in the masked region and ahead of the spreading field of droplets decreased markedly upon deposition of the FS-3100-containing aerosol. The Wilhelmy pin recorded a mean surface tension decrease of 2.1 ± 0.1 mN/m ($n = 3$) as shown in Figure 11. The recorded surface tension dip confirms that the FS-3100 surfactant molecules escaped the deposited droplets and created Marangoni stresses on macroscopic length scales across the subphase. Unlike the case of the tyloxapol aerosol delivery on a PA solution subphase (Fig. 8), the surface tension dip caused by the FS-3100 aerosol on the PGM subphase recovered. The surface tension recovery is most likely due to the desorption of the escaped FS-3100 molecules from PGM subphase/vapor interface into the bulk PGM subphase. A similar phenomena of surface tension recovery was observed in the case of microliter-scale solution drops of soluble sodium dodecyl sulfate surfactant on PA solution subphases.⁽¹⁸⁾ No surface tension recovery is observed when an insoluble oleic acid drop is deposited on a PA solution subphase. Since the surface tension recovery phenomenon is dictated by the

FIG. 11. Representative plot showing surface tension change in the masked subphase region recorded by Wilhelmy pin after deposition of an FS-3100-containing (1.4% w/w) aerosol on a PGM solution subphase. Aerosol deposition was initiated at $t \sim 0$ sec, as indicated by the arrow. After reaching a minimum, the surface tension dip starts recovering due to desorption of escaped FS-3100 molecules into the bulk PGM solution subphase.

solubility of the surfactant into the subphase, it is likely to be influenced by both the surfactant and subphase properties.

Similarly, fluorescent microsphere surface tracers were displaced in the direction of the droplet field expansion by distances of 0.7 ± 0.2 mm ($n = 4$) in approximately 30 sec on deposition of the FS-3100 (1.4% w/w) aerosol on a PGM subphase (Fig. 12). The large transport distance of a microsphere on deposition of the FS-3100 aerosol further supports the existence of stronger Marangoni flows relative to the tyloxapol aerosol.

Again, as long as the surface tension of the depositing aerosolized solution is lower than the subphase, the deposited droplet field will expand more than it does with a surfactant-free control. As a reference, three experiments performed with the FS-3100 (1.4% w/w) aerosol on the PA solution subphases showed a similar degree of expansion of the aerosol field as the tyloxapol (0.4% w/w) aerosol on the PA solution subphase (data not shown). Similar to its behavior on a PA solution subphase, the surfactant-free aerosol did not transport convectively after deposition on a PGM solution subphase ($p = 0.2$) (Fig. 10). No surface tension change in the PGM solution subphase was detected by the Wilhelmy pin on deposition of the aerosol. Since the surface tension of the surfactant-free aerosol droplets is higher than the PA and PGM subphases, there are no surface tension gradients developed across the subphase surface. Due to absence of surface tension gradients, induced Marangoni stresses were absent so no flow in the subphase surface occurred. This is supported by the observation that there was no motion of the microsphere at the subphase/vapor

FIG. 12. Representative plot showing time-dependent position of a $3 \mu\text{m}$ microsphere at a remote location and of the corresponding deposited droplets on the PGM solution subphase upon deposition of aerosol with and without surfactant. Solid symbols represent microsphere and corresponding open symbols represent corresponding aerosol droplet. Symbols: $\star$, surfactant-free; ∇ , tyloxapol (0.4% w/w); $\square$, tyloxapol (1.1% w/w); $\circ$, FS-3100 (1.4% w/w). Error bars are smaller than the symbols and represent standard error of mean on measurement. Only FS-3100 produces significant microparticle displacement or droplet field convection.

interface upon deposition of the surfactant-free aerosol on a PGM solution subphase (Fig. 12).

Conclusion

Using entangled aqueous polymer solutions that mimic key features of the ASL, we have demonstrated the enhanced spreading of individual aerosolized surfactant solution droplets in the respirable size range after deposition. We showed that deposited surfactant aerosol fields expand on ASL subphase mimics at aerosol deposition fluxes similar to those expected in small lung airways (beyond the twelfth generation) during aerosol therapies. The tyloxapol aerosol field (0.4% w/w) convected to a distance of several millimeters on a PA solution subphase after deposition, owing to a large difference in the surface tension between the droplets and this subphase. On a PGM solution subphase, that has significantly lower surface tension than the PA solution subphase, the FS-3100 (1.4% w/w) aerosol droplet field expanded to a maximum distance of ~ 0.8 mm after deposition. Although it is difficult to predict how these convected distances will translate to the airways, a rough estimate can be made for the twelfth generation by considering the airway as a tube of radius 0.5 mm and equating the experimental spread area of the aerosol in a circular petri dish to the surface area of the tubular airway. The convected distances, converted to incremental increases in droplet field area, covered by the tyloxapol aerosol on a PA solution subphase and by the FS-3100 aerosol on a PGM solution subphase in a petri dish correspond approximately to distances of 64 mm and 7 mm, respectively, in such a tube. These equivalent transported distances are significant since the lengths of the lung airways after the twelfth generation are 1–3 mm.⁽³⁷⁾

No such transport of the aerosol field is observed in the case of surfactant-free aerosol on either PA or PGM solution subphases. We demonstrated that a surface tension gradient developed at macroscopic length scales is responsible for the transport of the surfactant aerosol field along the subphase. Of course the presence of an endogenous surfactant layer on the ASL would reduce its surface tension and alter the spreading extent of the aerosol field. Grothberg et al.⁽³⁸⁾ have reported that the spreading extent of an exogenous pulmonary surfactant film will decrease with an increase in the surface concentration of endogenous pulmonary surfactant already present on the interface, but spreading is not eliminated unless the surface tension of the subphase with endogenous surfactant is equal to that of the added exogenous surfactant film. Though it is difficult to speculate on subphase surface tension values within the lung, we can state that a deposited aerosol field will convect along the subphase/vapor interface as long as its surface tension is lower than that of the subphase.

The surfactants studied here are not examined or advocated as candidates for clinical applications, but to prove that the surface tension difference between the miscible liquid subphase and the deposited aerosol is the dominant factor controlling the post-deposition convective spreading behavior of the aerosol on the subphase surface. Given the difficulties associated with assessing surface tension values at different locations within the airways, further studies with *in vivo* models offer the best opportunity for assessing the efficacy of the surfactant enhanced pulmonary drug delivery.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

References

1. Patient Registry Annual Data Report. Cystic Fibrosis Foundation, 2012.
2. Welsh MJ, and Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell*. 1993; 73:1251–1254.
3. Gershman AJ, Mehta AC, Infeld M, and Budev MM. Cystic fibrosis in adults: An overview for the internist. *Cleveland Clin J Med*. 2006;73:1065–1074.
4. Geller DE. The science of aerosol delivery in cystic fibrosis. *Pediatr Pulmonol*. 2008;43:S5–S17.
5. Labiris NR, and Dolovich MB. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. *Br J Clin Pharmacol*. 2003;56:588–599.
6. Brown JS, Zeman KL, and Bennett WD. Regional deposition of coarse particles and ventilation distribution in healthy subjects and patients with cystic fibrosis. *J Aerosol Med*. 2001;14:443–454.
7. Smaldone GC, and Palmer LB. Aerosolized antibiotics: Current and future. *Respir Care*. 2002;45:667–675.
8. Svartengren K, Lindestad PA, Svartengren M, Bylin G, Philipson K, and Camner P. Deposition of inhaled particles in the mouth and throat of asthmatic subjects. *Eur Respir J*. 1994;7:1467–1473.
9. Corcoran TE. Inhaled delivery of aerosolized cyclosporine. *Adv Drug Deliv Rev*. 2007.
10. Geller DE, Pitlick WH, Nardella PA, Tracewell WG, and Ramsey BW. Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest*. 2002;122: 219–226.
11. Ramsey BW PM, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, Vasiljev-K M, Borowitz D, Bowman CM, Marshall BC, Marshall S, and Smith AL. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med*. 1999;340:23–30.
12. Merlo CA, Boyle MP, Diener-West M, Marshall BC, Goss CH, and Lechtzin N. Incidence and risk factors for multiple antibiotic-resistant *Pseudomonas aeruginosa* in cystic fibrosis. *Chest J*. 2007;132:562–568.
13. Grothberg JB. Respiratory fluid mechanics and transport processes. *Ann Rev Biomed Engineer*. 2001;3:421–457.
14. Drug label for BETHKIS–Tobramycin Inhalation Solution. Food and Drug Administration, 1980.
15. Drug label for CAYSTON–Aztreonam for Inhalation Solution. Food and Drug Administration, 1986.

16. Marcinkowski AL, Garoff S, Tilton RD, Pilewski JM, and Corcoran TE. Postdeposition dispersion of aerosol medications using surfactant carriers. *J Aerosol Med Pulmon Drug Deliv.* 2008;21:361–369.
17. Koch K, Dew B, Corcoran TE, Przybycien TM, Tilton RD, and Garoff S. Surface tension gradient driven spreading on aqueous mucin solutions: A possible route to enhanced pulmonary drug delivery. *Mol Pharmaceut.* 2011;8:387–394.
18. Sharma R, Corcoran TE, Garoff S, Przybycien TM, Swanson ER, and Tilton RD. Quasi-immiscible spreading of aqueous surfactant solutions on entangled aqueous polymer solution subphases. *ACS Appl Mat Interfac.* 2013;5:5542–5549.
19. Corcoran TE, Thomas HM, Garoff S, Tilton RD, Przybycien TM, and Pilewski JM. Imaging the post-deposition dispersion of an inhaled surfactant aerosol. *J Aerosol Med Pulmon Drug Deliv.* 2012;25:290–296.
20. Lee KS, and Starov VM. Spreading of surfactant solutions over thin aqueous layers at low concentrations: Influence of solubility. *J Colloid Interface Sci.* 2009;329:361–365.
21. Sharma R, Kalita R, Swanson ER, Corcoran TE, Garoff S, Przybycien TM, and Tilton RD. Autophobing on liquid subphases driven by the interfacial transport of amphiphilic molecules. *Langmuir.* 2012;28:15212–15221.
22. Hamed R, and Fiegel J. Synthetic tracheal mucus with native rheological and surface tension properties. *J Biomed Mat Res Part A.* 2014;102:1788–1798.
23. Samet JM, and Cheng PW. The role of airway mucus in pulmonary toxicology. *Environ Health Perspect.* 1994;102:89–103.
24. Verdugo P, Tam PY, and Butler J. Conformational structure of respiratory mucus studied by laser correlation spectroscopy. *Biorheology.* 1983;20:223–230.
25. Bansil R, Eugene S, and LaMont JL. Mucin biophysics. *Annu Rev Physiol.* 1995;57:635–657.
26. Karapetsas G, Craster RV, and Matar OK. Surfactant-driven dynamics of liquid lenses. *Phys Fluids.* 2011;122106:23.
27. Albers GM, Tomkiewicz RP, May MK, Ramirez OE, and Rubin BK. Ring distraction technique for measuring surface tension of sputum: Relationship to sputum clearability. *J Appl Physiol.* 1996;81:2690–2695.
28. Im Hof V, Gehr P, Gerber V, Lee MM, and Schürch S. In vivo determination of surface tension in the horse trachea and in vitro model studies. *Respir Physiol.* 1997;109:81–93.
29. Kirkness JP, Christenson HK, Garlick SR, Parikh R, Kairaitis K, Wheatley JR, and Amis TC. Decreased surface tension of upper airway mucosal lining liquid increases upper airway patency in anaesthetised rabbits. *J Physiol.* 2003;547:603–611.
30. Kresch MJ, Lin W, and Thrall R. Surfactant replacement therapy. *Thorax.* 1996;51:1137–1154.
31. Kissa E. *Fluorinated Surfactants: Synthesis, Properties, Applications.* Marcel Dekker, Inc.: NY, 1994.
32. Wolfson MR, and Shaffer TH. Pulmonary applications of perfluorochemical liquids: Ventilation and beyond. *Paediatr Respir Rev.* 2005;6:117–127.
33. Noskov BA, Akentiev AV, Bilibin AY, Zorin IM, and Miller R. Dilational surface viscoelasticity of polymer solutions. *Adv Colloid Interface Sci.* 2003;104:245–271.
34. Pujado PR, and Scriven LE. Sessile lenticular configurations: Translationally and rotationally symmetric lenses. *J Colloid Interface Sci.* 1972;40:82–98.
35. Berg S. Marangoni-driven spreading along liquid-liquid interfaces. *Phys Fluids.* 2009;21:032105.
36. Gaver DP, and Grotberg JB. Droplet spreading on a thin viscous film. *J Fluid Mech.* 1992;235:399–414.
37. Weibel ER, and Gomez DM. Architecture of the human lung. Use of quantitative methods establishes fundamental relations between size and number of lung structures. *Science.* 1962;137:577–585.
38. Grotberg J, Halpern D, and Jensen O. Interaction of exogenous and endogenous surfactant: spreading-rate effects. *J Appl Physiol.* 1995;78:750–750.

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Reviewed by:
Peter Gehr
Jung Soo Suk

Address correspondence to:
Stephen Garoff, PhD
Department of Physics
Carnegie Mellon University
5000 Forbes University
Pittsburgh, PA 15213

E-mail: sg2e@andrew.cmu.edu