The unusual symmetric reopening effect induced by pulmonary surfactant

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Yamaguchi E, Giannetti MJ, Van Houten MJ, Forouzan O, Shevkoplyas SS, Gaver DP 3rd. The unusual symmetric reopening effect induced by pulmonary surfactant. J Appl Physiol 116: 635–644, 2014. First published January 23, 2014; doi:10.1152/japplphysiol.00814.2013.—This study investigates the stability of a finger of air as it propagates into a liquid-filled model of a liquid-filled model of a pulmonary bifurcation. We seek to elucidate the stability characteristics of the reopening of daughter airways, an event that may be important to the treatment of acute lung disease. To do so, we investigated the symmetry of reopening under conditions of nearly constant surface tension with 1) purified H2O or 2) an anionic surfactant (sodium dodecyl sulfate). Dynamic surface tension was investigated using pulmonary surfactant (Infasurf) with and without the presence of albumin. Flow visualization was accomplished using a microparticle image velocimetry (µ-PIV)/shadowgraph system through which we measured 1) the propagation velocity of the finger of air that reopens each daughter branch, and 2) the instantaneous and averaged velocity field of liquid phase surrounding the tip of the propagating bubble. Only pulmonary surfactant demonstrated the ability of maintaining a nearly symmetric propagation in the daughter channels, which is likely to lead to homogeneous airway reopening. In contrast, when pulmonary surfactant was inactivated by albumin or when the system was held at a nearly constant surface tension, reopening occurred asymmetrically. Our analysis suggests that Infasurf’s dynamic surface tension qualities are important to stabilize the removal of liquid obstructions. This demonstrates a new important function of pulmonary surfactant for airway reopening of a multibranch network.

ARDS; biofluid mechanics; pulmonary surfactant; VILI

MECHANICAL VENTILATION is a necessary life-sustaining medical treatment for acute respiratory distress syndrome (ARDS). However, mechanical stresses and strains exerted on the delicate pulmonary tissues may result in ventilation-induced lung injury (VILI) from either overinflation (volutrauma) or repeated opening and closing of airways and alveoli (atelectrauma). These processes can further damage the pulmonary epithelial plasma membrane and increase paracellular permeability through the disruption of the tight junction (TJ) proteins (3, 12, 18, 19, 21, 25, 29, 30).

Protective techniques to minimize VILI include low-volume ventilation and positive end-expiratory pressure with surfactant replacement therapy, which is highly effective for infant respiratory distress syndrome (5) but has not yet been demonstrated as effective for ARDS, possibly due to large amount of protein infiltrate that deactivates surfactant through competitive adsorption (40). Despite protective ventilation strategies, ~200,000 ARDS cases occur annually in the U.S. with a mortality rate of ~30%. The current standard of care relies on the use of very low tidal volumes (6 ml/kg ideal body weight), underscoring the fundamental importance of minimizing tissue stress and strain (35a).

Due to the lung’s physical heterogeneity, it is nearly impossible to eliminate volutrauma by a reduction of tidal volume without also allowing some regions of the lung to become atelectic. This can result in hypoventilation of closed portions of the lung. Recruitment of obstructed regions of the lung can induce atelectrauma that is caused by large mechanical stresses sweeping across the surfaces of epithelial cells when the airways and alveoli are cleared of liquid obstructions (3, 6, 20). Unfortunately, the use of large pressures to recruit atelectic airways and alveoli may also lead to volutrauma of the patent (open) regions of the lung (the “baby lung” phenomenon). In addition to a long history of physiological study of airflow in respiratory networks, modeling analyses of realistic airway bifurcations (23) and experimental studies of multiphase flow transport in a highly branching network have provided insight into difficulties associated with flow control in bifurcating geometries (7, 33).

The potential for asymmetric bubble propagation in a geometrically symmetric bifurcation poses important questions about protective ventilation strategies related to airway recruitment. Prior mathematical modeling and stability analysis coupled with model experiments have demonstrated that the growth or decay of a perturbation of a reopening finger of air in a bifurcating system is highly dependent on the viscous and elastic force balances, as described by Baroud et al. (2). That study suggests that the elastic nature of the system imposed by an elastic end chamber is essential to the synchronous reopening of daughter airways.

Since much of the mechanical behavior of the lung is determined by the dynamic surface tension of the airway and alveolar lining fluid (24), we hypothesize that the addition of surfactant to the obstruction fluid will provide a more realistic scenario and may influence the stability and symmetry of the reopening of the pulmonary bifurcation without the existence of an end elastic chamber.

Based upon prior analyses of interfacial flows, the following two dimensionless parameters are likely to influence the behavior of this system. 1) The first parameter is the Capillary number, Ca = µU/γ, which represents the relative magnitude of surface tension to viscous forces in the region of the interface, where µ is the dynamic viscosity of the fluid, γ is the interfacial tension, and U is the mean flow velocity. In the steady flow of a finger of air, Ca defines the shape of the bubble tip interface and the thickness of liquid deposited in the wake of propagating bubble (4). 2) The second parameter is the Marangoni number, Ma = (dy/dI)γeq/γeq, where γeq and γeq are the equilibrium surface surfactant concentration and surface tension, respectively. This parameter describes the proportional change in surface tension that occurs with a modification in surfactant concentration.

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Surfactant sorption influences the overall quantity of surfactant at a mobile interface, which is affected by the surfactant molecular properties (e.g., diffusion and sorption rates). Likewise, convection influences the system since the local flow field can deposit or deplete regions of surfactant in regions of converging or diverging stagnation points, respectively (16). The resulting physicochemical interactions can vary greatly between different types of surfactant (11). It is therefore likely that surfactant properties (influenced in disease states) may influence the elastic behavior of the system by changing the average surface tension of the propagating interface. Furthermore, the regional variation of surface tension introduces tangential (Marangoni) stresses. This has the effect of rigidifying the interface because the Marangoni stress opposes the local flow (11, 34, 35, 39). As we will show, each of these interactions could affect the elastic properties of the system and influence the stability and symmetry of airway reopening near an airway bifurcation.

Since the convective flow pattern in the liquid sublayer near the interface affects the distribution of lung surfactant and hence the surface tension, the deformation and expansion of the air-liquid interface at the airway bifurcation and the developing flow at juncture between the parent and daughter channels may influence the interfacial pressure drop and the distribution of surfactant. To elucidate this process, we developed tools to visualize the flow in the vicinity of the bifurcation to observe the relationship between the microscale fluid dynamics and physicochemical interactions of anionic and protein-based lung surfactants that may be important in the macroscale system behavior. This may provide guidance toward methods of reducing atelectasis and diminishing ventilation heterogeneity that exists in ARDS.

**METHODS**

**Microfluidic Device**

Schematic and sample images from experiments are shown in Fig. 1. The microfluidic device is a symmetric Y-shaped rectangular cross-section channel having uniform depth, \( D = 150 \, \mu\text{m} \), and width, \( H_p = 208 \, \mu\text{m} \) at the parent branch and \( H_d = 150 \, \mu\text{m} \) at the daughter branch. Daughter branches were connected symmetrically far downstream to apply a uniform downstream pressure with a single syringe pump. The diverging angle is 40°, and 0.7 mm of daughter channel sections were designed to fit the observation window. Sample images in Fig. 1B present regions of observation windows of the flow field measurement near the bifurcating bubble tip (i), and the downstream region used to monitor the flow-rate for bubble propagation monitoring in daughter channels (ii).

The device was fabricated using standard soft lithography methods (36). The channel was printed on a thick layer of poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning, MI) and sealed by a glass microscope slide covered with a thin layer (~20 \( \mu\text{m} \)) of PDMS to maintain uniform surface properties for all perimeters. The microfluidic device was attached to a 25-\( \mu\text{l} \) microsyringe (Gastight Syringe 1707; Hamilton) via a glass capillary tube (Flexible Fused Silica Capillary Tubing; ID = 550 \( \mu\text{m} \), Polymicro Technologies) to minimize the dead space volume (38).

The flow rate was set to \( Q = 1.35 \, \mu\text{l/min} \), providing an average velocity in the daughter channels of \( u_d = 0.5 \, \text{mm/s} \) (Re = \( 10^{-1} \)). This is comparable to the convective velocity in the 17th generation during normal respiration (9), since the bubble tip propagation speed is nearly identical to the average downstream liquid flow velocity due to the miniscule residual film thickness with range of capillary number: \( 7 \times 10^{-8} < \text{Ca} < 4 \times 10^{-5} \).

**Flow Measurement System**

The \( \mu\text{-PIV/shadowgraph} \) simultaneous data-acquisition system developed for the current application is depicted in Fig. 2. Details of the basic optics principles and the system operation are presented in Yamaguchi et al. (37) with only minor optics modifications and the implementation of a constant-flow system. We defined camera A as the CCD camera for the \( \mu\text{-PIV} \) system and camera B for shadowgraph images. LED, light-emitting diode.

**Fig. 1.** Schematic of the microfluidic bifurcation channel design. A: a sample image of the observation window near the bifurcation where the microscopic particle image velocimetry (\( \mu\text{-PIV} \))/shadowgraph measurements of the flow fields surrounding the progressing bubble tip were taken. B: a fluorescent image of the downstream daughter channels where the flow-rate monitoring of the bubble propagation was performed by using \( \mu\text{-PIV} \).

**Fig. 2.** Schematic of the simultaneous \( \mu\text{-PIV/shadowgraph} \) data-acquisition system. The system utilizes two identical monochrome cameras, two lasers having different wavelengths, and two dichroic beam separation filter sets to simultaneously record \( \mu\text{-PIV} \) and shadowgraph images, LED, light-emitting diode.
graphic imaging. For the μ-PIV, the volumetric illumination is provided by a dual-pulse Nd:YAG laser (λ = 532 nm, power = 15 mJ/pulse, duration = 4 ns, New Wave Laser Pulse Solo Mini; New Wave Research-ESI, Fremont, CA). The liquid phase is seeded by fluorescent particles that have excitation/emission peaks at 535/575 nm (Nile Red Fluospheres; Invitrogen). Therefore only the returning emission spectrum from the particles passed the dichroic filter (λ > 550 nm) and reaches to camera A. For the shadowgraph, an LED pulsed red laser (λ = 660 nm, power = 2 nJ/pulse, pulse duration = 15 ns; MPL-III-660, Opto Engine) is selected for the source of the background light illumination. The projected shadowgraph image signal initially shares the same optical path as the μ-PIV signal and passes the first and second dichroic filters through which it is directed to CCD camera B to record the shape of the air-water interface. The camera and laser timing is controlled by a multichannel synchronizer (model 610035; TSI, Minneapolis, MN).

The process control, image display, and postprocessing were coordinated by Insight 3G (TSI). The interrogation of fluorescent images employs a recursive Nyquist grid with a FFT correlation engine and a Gaussian peak algorithm with one 64 × 64 pixel interrogation window size (32 × 32 pixels with 50% overlap) is determined by a combination of pixel resolution and channel diameter. Fluorescent particle images were interrogated and filtered by using the bubble tip and channel wall geometrical information obtained from the corresponding simultaneously taken shadowgraph image.

Test Solutions

The appropriate spherical diameter of fluorescent seeding particle was determined to be d_m = 1.0 μm based on the pixel resolution of the observation window (1). The optimal particle seeding density was determined as 0.04 vol% following Olsen and Adrian (28) and Meinhart et al. (26). Dulbecco’s phosphate-buffered saline 1x (DPBS) (Invitrogen, CA) was used as a base buffer solution. Throughout the current study, the following test solutions were investigated:

- **DPBS**: The DPBS solution was a mixture of the standard DPBS and 0.04 vol% of the fluorescent particle. It contains 0.1 mg/ml CaCl_2, which is necessary for sufficient pulmonary surfactant function. On its own, DPBS maintains a high uniform surface tension.

- **SDS**: The SDS was 1.73 mg/ml (6.00 mmol/l) of sodium dodecyl sulfate solution. This nonphysiological anionic surfactant maintains a nearly constant surface tension during bubble propagation. It is useful for investigating a reduction in surface tension with small Marangoni effects.

**Infasurf**: The Infasurf solution was 1.0 mg/ml of Infasurf (calfactant) (ONY) in DPBS. This is a pulmonary surfactant analog that is used in surfactant replacement therapy. This is used to elucidate the influence of dynamic surface tension effects in the lung.

**Albumin**: For the Albumin solution, 5.0 mg/ml of bovine albumin (Invitrogen, CA) was added to the Infasurf solution to observe the significance of competitive adsorption that might occur during ARDS (40).

The concentration and ratio of albumin/Infasurf was based on in vitro experiments where the surfactant deactivation by albumin had a clear impact on cell damage after an airway reopening event (13). The concentration of SDS was adjusted to create a nearly uniform reduction of surface tension (γ_m = 42 dyn/cm) (11). All testing solutions were kept at 37°C to maintain physiological relevance and in order for the lung surfactant to work properly.

The air-liquid interface area and shape are expected to change quickly when the propagating bubble tip is cleaved into two at the bifurcation, with each segment entering the daughter channels. This leads to an interferential area and a convection pattern change. Since surfactant is adsorbed dynamically, this can modify the regional surface tension. The dynamic surface tension of DPBS measured using a Langmuir trough/Wilhelmy plate with the barrier closing/opening at a constant speed of 136 cm²/s at 37°C shows that the surface tension is constant and maintains γ = 69 dyn/cm, approximately that of pure water. SDS exhibits γ = 42 dyn/cm with an insignificant hysteresis area during oscillation. This can be explained by SDS’s high solubility and rapid surface adsorption/desorption mobility that is characteristic of an anionic surfactant. In contrast, Infasurf demonstrates a significant hysteresis loop (13 < γ < 32 dyn/cm) and indicates a dynamic surface tension that is caused by Infasurf’s slow adsorption/desorption rate (11). Therefore, we expect that the sudden interface expansion at the bubble bifurcation experiment will dynamically impact the surface tension when the liquid obstruction is doped with Infasurf.

When albumin is added to the Infasurf solution, the dynamic surface tension is modified greatly. The Langmuir trough measurements demonstrate that albumin retards the adsorption rate of Infasurf significantly (t_eq = 20 s → 600 s). Evidently, albumin competitively adsorbs to the air-liquid interface and temporarily inactivates Infasurf, which may result in a nearly constant surface tension as the interface propagates through the airway bifurcation (40).

**Simultaneous μ-PIV/Shadowgraph Imaging**

Data acquisition (Fig. 1Bi, for example) was performed to acquire images from at least five usable experimental trials. Trials were accepted when the first image of the bifurcation process was in close proximity to the bifurcation carina and the interface was devoid of deformation. We defined t = 0 as the instant when the bubble first reached the bifurcation. To analyze the flow-field transition in the daughter channels the original coordinate system was transformed using a 6th-degree polynomial interpolation so that y’ > 0 is directed perpendicular to the interior of the bifurcation and x’ is directed distally as shown in Figure 2.

**Downstream Flow-Rate Monitoring**

The instantaneous bubble velocity was obtained by performing a continuous data acquisition of μ-PIV measurements in the far downstream of the daughter channels (Fig. 1Bi). Since the depth of the vector correlation is ~15 μm (28), the focal plane of the μ-PIV measurement was adjusted to the midpoint depth to capture the maximum velocity (u_max) of the daughter channels. The estimation of the flow rate (Q) from the measured u_max can be obtained using the exact solution of fully developed pressure-driven viscous flow in a square channel from which the relationship between the flow rate Q and maximum velocity u_max are approximated as

\[ Q = 2.15 H^2 u_{\text{max}} \]  

By using the downstream flow-rate monitoring we can easily determine the relative velocities of both daughter interfaces simultaneously.

**RESULTS**

In this section we provide the visualization and flow-rate relationships for the experiments described above. We define channel 1 as the left branch in the frame of the propagating bubble when viewed from above (top half of Fig. 1A), and channel 2 is the right branch.

**μ-PIV/Shadowgraph Imaging**

Experimentally obtained flow streamlines are shown in Fig. 3. Here an Infasurf-doped bubble is observed as the interface approaches and passes the carina. These representations are presented in approximately the bubble-tip frame of reference through the subtraction of the average downstream velocity. This provides a close estimate to the bubble tip velocity since
a miniscule layer of liquid is deposited in the wake of the propagating bubble for small Cu flows (8).

The μ-PIV/Shadowgraph system provides details of the velocity vectors in the liquid phase. At the moment the bubble tip reaches the bifurcation point \((t = 0)\), the flow field near the bubble tip is similar to the upper half of the fully developed flow in the parent channel (15). At this point only a single diverging stagnation point \((-)\) is visible, and a converging stagnation point \(+)\) is located adjacent to the carina. As the interface makes a transition to the steady-state profile, over the period \(0.26 \text{ s} < t < 1.04 \text{ s}\) the converging stagnation point migrates to the daughter channel centerline, and a new diverging stagnation point appears in the lower half of the interface to form a symmetric fully developed bubble tip shape at \(t = 1.30 \text{ s}\).

The flow-pattern progression demonstrates that the transition to a fully developed flow field surrounding the bubble tip takes \(>1 \text{ s}\). However, shadowgraphy indicates that the creation of a nearly spherical air-liquid interface takes \(<0.23 \text{ s}\). Apparently, the transition time for the bubble interface shape is very short and occurs almost immediately after the bubble interface detaches from the carina, even though the surrounding flow field is developing.

**Downstream Flow-Rate Monitoring**

Figure 4 demonstrates the signal from the downstream flow-rate measurement as the propagating bubble approaches and splits at the bifurcation in a system that is obstructed with DPBS. These flow-rate data can be used to identify the frame when the bubble tip encounters the bifurcation carina because there is a sudden velocity drop at that instant. This velocity drop occurs when the bubble tip encounters the carina because an additional force is required to split the finger and expand the air-liquid interface into the daughter channels section having a smaller hydraulic diameter. From the law of Laplace the pressure drop increases substantially as the bubble radius of curvature decreases when the interface is split. Since the flow is induced by a downstream suction (using the syringe pump), the increased pressure drop across the bubble tip causes a slight inward deformation of the square-shaped PDMS channel wall that results from the yield pressure (17). This causes a short hesitation in the bubble propagation that reflects the magnitude of the yield pressure that is directly related to the surface tension of the propagating interface. In addition, the measurement of the downstream flows allows us to examine the rate of bubble motion as the interface propagates through the bifurcation. This provides key information about the stability and heterogeneity of bubble propagation in the daughter channels.

Although the bifurcation was designed to be symmetric, we noted that channel 1 demonstrated a constantly higher velocity when the daughter channels were fully filled with liquid. Measurement of the device indicated that although the apparatus had identical daughter channel lengths, a slightly uneven average width existed in the daughter channel sections; these measurements indicated that channel 1 possessed a width \((H)\) that was \(\sim3.5\%\) wider than that of channel 2. Since the daughter channels share the same outlet and inlet, the hydrodynamic relation of the flow through the fully filled daughter channels can be expressed by Eq. 1:

\[
\frac{u_{\text{max}1}}{u_{\text{max}2}} = \left(\frac{D_{h1}}{D_{h2}}\right)^{2}\frac{L_2}{L_1}
\]

where \(D_h\) is hydraulic diameter and \(L\) is total length of daughter channel section. The observation that the velocity in the completely filled channel is 5–7% faster in channel 1 is therefore consistent with the 3.5% greater channel width. Below we will see that this slight asymmetry is useful in identifying physicochemical interactions that exist when surfactant is added to the obstruction fluid.

The measured flow velocities were numerically integrated to provide the instantaneous bubble tip position from the carina.
If each daughter were to reopen symmetrically the bubble tips would follow identical trajectories, and the relative position difference from the carina would be nil (the dashed line in Fig. 5). However, in general this is not true, as can be seen by the trajectory information in Fig. 5.

**DPBS.** As expected from Baroud et al. (2), the constant high constant surface tension of DPBS ($Ca = 7 \times 10^{-3}$) results in an asymmetrical bubble propagation with channel 1 (the low-resistance pathway) opening preferentially. The difference of velocity is progressively larger as bubbles propagate further into the daughter channels. It is apparent that a yield pressure causes an initial slowdown of the propagation speed (and likely a buckling of the microfluidic channel). This abrupt change in pressure is necessary to decrease the bubble tip radius of curvature, which increases the law of Laplace pressure drop. This reduction in curvature occurs as the carina cleaves the bubble tip into two segments which open each of the daughter branches. Since DPBS shows the most significant slow-down effect due to the highest surface tension, the region of yield pressure effect is indicated and the theoretical trajectory was shifted to the right by $0.5$s.

**SDS, and Albumin + Infasurf.** These solutions display the opposite asymmetric propagation to DPBS. Initially, over $0.0 \, s < t < 0.8 \, s$, the bubble propagation is faster in channel 2 even though our studies show that this is the higher resistance pathway in the fluid-filled system. Eventually, the bubble propagation velocity increases in channel 1, and for albumin the displacement of the bubble in channel 1 eventually surpasses that of channel 2. The yield pressure effect exists over duration of approximately 0.2 and 0.4 s for SDS and Infasurf + Albumin, respectively.

**Infasurf.** The asymmetric reopening was not observed with pulmonary surfactant Infasurf. Instead, the interfaces in the daughter channels propagated at almost identical velocities. The yield pressure is significantly reduced, most likely due to the small surface tension at the air-liquid interface. The yield pressure effect is $0.25\, s$ in duration.

It should be noted that the very small deviation of data over the five independent trials indicates each experiment had very similar propagation patterns. Below we put this information in context with the physicochemical interactions that are likely to exist in this system. We will demonstrate that these microscale processes have implications at a much larger scale because of their importance to the uniform recruitment of an atelectic portion of the lung.

**DISCUSSION**

Figure 5 demonstrates that without surfactant, channel 1 opens preferentially, which is consistent with the lower hydraulic resistance along that pathway. In contrast, with the
inclusion of surfactant, reopening is always initiated along channel 2. This effect is most prominent with the anionic surfactant SDS and when bovine serum albumin is used to deactivate Infasurf. In those cases channel 2 opens preferentially over an extended period of time \((t < 0.8 \text{ s})\). It is significant that symmetric reopening was only observed with the inclusion of Infasurf. The addition of albumin to Infasurf destroyed this symmetry; therefore, the unique properties of Infasurf disappeared with competitive adsorption by albumin, and the propagation proceeded in a manner similar to SDS.

Our observations indicate that the effect of surfactant on the reopening process near the bifurcation is not simply due to an overall reduction of the surface tension. Instead, the dynamic surface tension plays an important role in the propagation behavior, and this coupling occurs through physicochemical interactions. Below we explore the phenomena that may provide insight into how surfactant deactivation affects ARDS and inhibits the process of airway recruitment in atelectic airways.

**Theoretical Concepts**

Following Ghadiali and Gaver (11) the bubble pressure drop along the fluid-occluded section of a daughter channel can be subdivided into three main components as described in Fig. 6

\[
P_{\text{Bubble}} = P_{\text{Cap}} + P_{\text{Hyd}} + P_{\text{End}} \tag{3}
\]

where \(P_{\text{Cap}}\), \(P_{\text{Hyd}}\), and \(P_{\text{End}}\) are defined as follows:

1) \(P_{\text{Cap}}\) is the pressure loss that exists over the air-liquid interface at the progressing bubble tip. \(P_{\text{Cap}}\) is related to the nonequilibrium normal stresses (law of Laplace at low \(C_a\), \(\Delta P = 2\gamma/R\), where \(R\) is the interfacial radius of curvature) and the Marangoni stress that creates a tangential stress along the interface due to a nonuniform surfactant distribution that can "rigidify" the interface.

2) \(P_{\text{Hyd}}\) is the hydraulic pressure loss due to viscous flow downstream of the bifurcation, which is approximated by channel Poiseuille flow,

\[
P_{\text{Hyd}} = \frac{12\mu Q}{H^2D}L(t), \tag{4}
\]

where \(\mu\) is the viscosity, \(Q\) is the flow rate, \(H\) is the channel width, \(D\) is the depth, and \(L(t)\) is the length to of the fluid-filled segment.

3) \(P_{\text{End}}\) is the pressure loss from an end reservoir.

Baroud et al. (2) modeled the reopening of a symmetrical Y-shape bifurcation with separate terminal reservoirs with \(P_{\text{End}}\) that represents the resistance from elastic chambers at the ends of the daughter channels. In constant-surface-tension systems they demonstrated that symmetrical propagation was possible only if a disparity in \(P_{\text{End}}\) canceled out the growing differential of the hydraulic pressure drop that would occur if one channel began to reopen faster than the other [causing a difference in \(L(t)\)]. This disparity in \(P_{\text{End}}\) could be due to compliance of the end reservoirs so that preferential flow in one channel would cause a relative increase in \(P_{\text{End}}\) that would serve to reestablish symmetric reopening. However, in our model system the daughter channels share a common end reservoir system, so \(P_{\text{End}}\) cannot provide this type of perturbation, and therefore we define \(P_{\text{End}} = 0\) as a reference pressure.

When the bubble splits to open the daughter channels (channels 1 and 2), a common upstream bubble pressure exists. Thus the pressure drop from the bubble to the end reservoir must balance. Therefore, from Eq. 3,

\[
(P_{\text{Cap}})_1 + (P_{\text{Hyd}})_1 = (P_{\text{Cap}})_2 + (P_{\text{Hyd}})_2 \tag{5}
\]

Therefore the sum of the capillary and hydraulic pressure drops must be equivalent through channels 1 and 2. We will show below that this provides insight into the observed behavior of our system.

**Entrance Flow Effects**

As discussed in results (Downstream Flow-Rate Monitoring), the hydraulic resistance is slightly greater in channel 2 than in channel 1; therefore, without surfactant (i.e., DPBS) channel 1 opens preferentially and asymmetric reopening occurs. In contrast, we observe that the inclusion of surfactant always leads to the early preferential reopening of channel 2 \((Q_2 > Q_1)\) even though channel 1 has a lower hydraulic resistance \((H_1 \geq H_2)\). From Eqs. 4 and 5, the inclusion of surfactant causes a differential in the capillary pressure drop at the entrance to the daughter channels since

\[
\Delta P_{\text{Cap}} = (P_{\text{Cap}})_1 - (P_{\text{Cap}})_2 = \frac{12\mu L_{\text{Tot}}}{D} \left( \frac{Q_2}{H_2^2} - \frac{Q_1}{H_1^2} \right) > 0 \tag{6}
\]

as the interface moves into the daughter channels. Here \(L_{\text{Tot}}\) is the length of the daughter channels since the interface is just entering from the bifurcation. From this analysis it is clear that the inclusion of surfactant causes \(\Delta P_{\text{Cap}} > 0\).

We hypothesize that the capillary pressure differential at the carina is due to alteration in the surfactant distribution between channels 1 and 2 as the bubble progresses toward the bifurcation \((t < 0)\). When the interface is cleaved by the bifurcation at \(t = 0\) this sets up the initial conditions for the interface that initiates the flow fields in the daughter channels. Based on the analysis of Ghadiali and Gaver (11), two conditions provide likely explanations for the positive \(P_{\text{Cap}}\) at the beginning of the bubble bifurcation: 1) either surfactant accumulation exists

Fig. 6. Schematic diagram of flows, pressures and lengths that exist in the bifurcating channel. In our system, \((P_{\text{ma}})_1 = (P_{\text{ma}})_2\).
along the interface of channel 2 that lowers the surface tension and reduces \( \Delta P_{\text{Cap}} \), or 2) a strong Marangoni stress exists along the interface in channel 1 that rigidifies the interface and elevates \( \Delta P_{\text{Cap}} \).

To determine which of these possibilities is most likely, we analyzed the detailed flow field in Fig. 3. Figure 3 illustrates that the flow field as the bubble is cleaved by the carina is substantially different from the steady-state field because the carina splits the nearly symmetric portions of the flow surrounding the bubble tip as it confronts the bifurcation. Figure 3 represents this flow field and stress transition at 1) the beginning of the bubble bifurcation \( t = 0 \), and 2) the fully developed propagating bubble \( t = 1.3 \) s.

The Marangoni stress is directed from the converging stagnation point (+) where surfactant is attracted toward the diverging stagnation point (−) where surfactant is depleted. This stress opposes the convective field in the bulk, rigidifying the interface and causing additional resistance for the bubble propagation.

Because of the slight but consistent flow-rate advantage along channel 1 as the bubble approaches the carina \( t < 0 \), we hypothesize that a slight asymmetry of the concentration field may cause an interfacial concentration maxima to exist in channel 1 when the interface is cleaved \( t = 0 \). This would induce a larger interfacial concentration gradient over the interface in channel 1 that would rigidify that surface to a greater extent than over the bubble tip entering channel 2, hence leading to \( \Delta P_{\text{Cap}} > 0 \). Following Eq. 6 this would preferentially direct flow toward channel 2 \( \left( Q_2 > Q_1 \right) \).

To test this hypothesis, the detailed flow fields from the \( \mu \)-PIV provide a comparison of the convection flow patterns near the air-liquid interface that demonstrate how the stagnation points on the interface are affected during the propagation in the daughter channel section. Figure 7 provides examples of transition of the axial \( x' \) velocity component across the channel \( y' \) near the bubble tip as the interface propagates past the carina \( t = 0 \) to the fully developed profile \( t = 1.30 \) of channel 1 in an Infasurf-doped system. In Fig. 7, \( y'/H = -1 \) represents the inner surface (the surface where the interface is split by the carina). Figure 7 shows that the velocity in this inner region is substantially lower than that of the outer region despite the fact that the inner region had the larger entrance velocity. This is an indication of the Marangoni stress that rigidifies the interface as it is split.

To quantify the relative behavior of the inner and outer regions of the flow, the transition of the velocity field during the propagation process is summarized by calculating the relative flow on the inner and outer sides of the daughter channels, a term called the flow field symmetry index (FFS) in Fig. 8, where

\[
\text{FFS} = \frac{Q_{\text{inner}}}{Q_{\text{outer}}} = \frac{\int_{y'=-1}^{y'=0} u_x(y')dy'}{\int_{y'=-1}^{y'=0} u_x(y')dy'}.
\]

Smaller values of FFS indicate a greater interfacial resistance to a symmetric flow field. In addition, the proportion distance to FFS = 1 represents a measure of the transition length to fully developed flow.

Figure 8A demonstrates that without surfactant, the FFS along the inner surface is greater in channel 1 than it is in channel 2; this behavior may be due to the slight channel width asymmetry that affects the radius of curvature at the entrances of channels 1 and 2. In contrast, Fig. 8B shows that when SDS is incorporated into the system the inner surface (bifurcation-side) flow along channel 1 is substantially reduced compared with channel 2; this result is consistent with the hypothesis that the interior surface of the interface in channel 1 is rigidified to a greater extent by a greater differential of surface tension along the bubble interface, causing \( \Delta P_{\text{Cap}} > 0 \). When Infasurf is introduced into the system (Fig. 8C), we observe a strikingly
different result: the FFS is nearly identical for channels 1 and 2. Evidently, the dynamic surface tension properties of Infasurf cause symmetry in the FFS despite the fact that there is a difference in the hydraulic resistances between channels 1 and 2. However, the transition time is significantly extended compared with the rapidly adsorbing SDS. Finally, when albumin is added to Infasurf the FFS reverts to the asymmetric case observed with SDS (Fig. 8D).

**Theoretical Analysis**

Evidently, the dynamic surface tension of pulmonary surfactant (Infasurf) allows the flow to be roughly symmetric between channels 1 and 2. When this dynamic surface tension behavior is affected either by very rapid rates of adsorption (demonstrated by SDS) or competitive adsorption (albumin + Infasurf), the microscale reopening behavior is destroyed due to interfacial interactions. This change in behavior correlates with the reopening symmetry that is observed only with Infasurf (Fig. 3) and implies that the specific dynamic surfactant qualities of pulmonary surfactant may be critical to uniform airway recruitment.

To formalize this concept, we investigate a mathematical model that combines the capillary pressure drop across the air-liquid interface, $P_{\text{Cap}}$, and the hydraulic pressure drop caused by fluid being displaced by the propagating finger of air, $P_{\text{Hyd}}$. We model the dynamic surface tension by

$$\gamma = \gamma_0 + \beta U$$  \hspace{1cm} (8)

Here $\gamma_0$ is the static equilibrium surface tension, and $\beta U$ represents the velocity dependent increase in surface tension that describes the dynamic behavior of surfactant. In this first-order description, the increase in surface tension would exist due to surface tension gradients that would decrease the surfactant concentration as the bubble propagates and elongates the interface. Slow transport from the bulk to the interface would reduce the surface concentration and increase the surface tension in the region of bubble growth. In a surfactant-free system, $\beta = 0$; if adsorption rates are high (e.g., SDS), then $\beta$ is small, and if adsorption rates are moderate then $\beta U$ may be of the same order of magnitude as $\gamma_0$ so that the bubble velocity will significantly influence $P_{\text{Cap}}$. This representation is conceptually similar to that presented by Ghadiali and Gaver (11) but ignores the complexity of that analysis in favor of maintaining simplicity.

We approximate the capillary pressure drop at the bubble tip by the law of Laplace relationship,

$$(P_{\text{cap}})_i = \frac{2\gamma_i}{R_i} \times \frac{2(\gamma_0 + \beta U_i)}{R_i}$$  \hspace{1cm} (9)

where $R_i$ is the radius of curvature of the interface in branch $i$, and $U_i$ is the associated velocity in that branch. From Eqs. 4, 5, and 9

$$\frac{2(\gamma_0 + \beta U_i)}{R_i} + \frac{12\mu Q_1 L_1}{H^2 D} = \frac{2(\gamma_0 + \beta U_2)}{R_2} + \frac{12\mu Q_2 L_2}{H^2 D}.$$  \hspace{1cm} (10)

If we approximate $U_i = Q_i/(H D)$ and impose the flow-rate constraint $Q_1 + Q_2 = Q_{\text{Total}}$, then

$$\frac{2}{R_1} \left( \gamma_0 + \beta Q_1 \right) + \frac{12\mu Q_1 L_1}{H^2 D} = \frac{2}{R_2} \left( \gamma_0 + \beta (Q_{\text{Total}} - Q_1) \right) + \frac{12\mu (Q_{\text{Total}} - Q_1) L_2}{H^2 D}.$$  \hspace{1cm} (11)

Finally, if we assume a symmetric system so that $R_1 = R_2 = R$, $H_1 = H_2 = H$, we find

$$Q_1 + Q_{\text{Total}} \left( \frac{2\beta}{R H} + \frac{12\mu L_2}{H^2} \right) = \left( \frac{4\beta}{R H} + \frac{12\mu (L_1 + L_2)}{H^2} \right).$$  \hspace{1cm} (12)

This relationship can thus provide a general understanding of the global behavior that will exist as a function of the dynamic surface tension.

**Small $\beta$ behavior.** For a surfactant-free system or one with a very rapidly adsorbing surfactant, $\beta$ is small and the surface tension is nearly constant. From Eq. 12

$$\lim_{\beta \to 0} \frac{Q_1}{Q_{\text{Total}}} = \frac{L_2}{L_1 + L_2}.$$  \hspace{1cm} (13)

Note that $L_i$ is the length of ith airway that remains obstructed by fluid. So, for example, if channel 1 begins to open faster than channel 2 (i.e., $L_1 < L_2$), then

$$\lim_{\beta \to 0} \frac{Q_1}{Q_{\text{Total}}} > \frac{1}{2}$$

and the flow will be directed preferentially toward channel 1. This behavior is reinforced because as channel 1 reopens faster, $L_1/L_2 \to 0$ and $Q_1 \to Q_{\text{Total}}$. Therefore, all of the flow is eventually directed toward channel 1, and this bolsters asymmetric reopening. Likewise, if channel 2 begins to open first, then flow will continue to be directed preferentially to that channel. This implies that when surface tension is constant, viscous interactions will dominate the determination of the reopening and lead to preferential reopening of either the shortest airway or the airway that begins to open first (through either a local or random interaction). This result is consistent with the analysis by Baroud et al. (2) (see Introduction), which predicts that asymmetric reopening will dominate unless a mechanism such as variable elastic recoil can be incorporated that will stabilize the system by redirection of flow from one branch to another. Figure 5 demonstrates this behavior with DPBS and SDS. This response is consistent with the analysis because the substances maintain nearly constant surface tension. However, the preferred branches are different because of entrance-flow differences that affect the initiation of the reopening, as discussed above in *Entrance Flow Effects.*

**Large $\beta$ behavior.** If $\beta$ is large the surface tension will increase significantly as the velocity increases. This behavior will occur when surfactant adsorbs slowly in comparison to the rate of interfacial creation, a concept described in Refs. 16 and 41. In this case, from Eq. 12,

$$\lim_{\beta \to \infty} \frac{Q_1}{Q_{\text{Total}}} = \frac{1}{2}.$$  \hspace{1cm} (14)

This implies that $Q_1 \sim Q_2$ so each channel will open with an equivalent velocity. This response exists because any increase
in velocity along one channel will substantially increase the surface tension. This increases $P_{\text{Cap}}$ for that channel, which in turn reduces $P_{\text{Hyd}}$ (and hence $Q$) for that branch. This response leads to a commensurate increase of velocity for the opposite branch. Therefore, each of the daughter airways will open in an approximately simultaneous manner, although it is plausible that an oscillatory phenomenon could exist that would depend upon the dynamic surface tension properties. Nevertheless, a roughly symmetric reopening is likely for large values of $\beta$. Therefore, dynamic surface tension is expected to lead to symmetric reopening and could result in homogeneous recruitment of atelectic airways. This response is analogous to the elastic resistance force induced by stiff chambers attached to the end of daughter channels suggested by Baroud et al. (2).

The dynamic surface tension of the Infasurf system causes a nearly symmetric reopening as demonstrated in Fig. 5. This is related to its high $\beta$. However, this on its own is not sufficient because rapid surfactant adsorption could cause the interface to have a nearly constant surface tension. The analysis of Ref. 10 helps to elucidate the fact that transport limitations may contribute substantially to the response. That study demonstrates the three parameters that affect transport rates are:

1) $St = k_c CH/U$ the rate of adsorption to interfacial creation;
2) $\Lambda/H = \Gamma_{\text{eq}}/(HC)$ the dimensionless adsorption depth; and
3) $St \cdot Pe = k_a \Gamma^2/DC$ relates adsorptive to diffusive transport rate.

Using $\Gamma = 3 \times 10^{-4}$ mg/cm$^2$, $k_a = 0.7$ cm$^3$(mg-s), and $D = 10^{-8}$ cm$^2$/s (22), we estimate $St \approx 0.2$, $\bar{E}K/H \approx 0.02$, and $Pe-St \approx 10^4$. These estimates suggest that the transport limitations in our study result from slow adsorption and diffusion compared with the creation of an expanding air-liquid interface. This slow surfactant adsorption is consistent with the large transition time to steady-state flow, as shown in Fig. 8C.

The influence of competitive adsorption. Figure 5 demonstrates that when albumin competes with Infasurf, the reopening stability is neither described by the low nor high $\beta$ predictions. Initially the flow is directed toward channel 2, reflecting the surfactant-induced entrance effects described above in Entrance Flow Effects. The reopening process initially follows a trajectory very similar to SDS, and then after 1 s the flow reverses so that channel 1 begins to dominate. This recovery is substantially different from that demonstrated by Infasurf and is not predicted by the analysis above. This behavior suggests that the reopening behavior is due to competition between the adsorption of albumin and Infasurf. We interpret the reopening response in the following manner: 1) the flow is initially directed toward channel 2 due to surfactant-induced entrance effects; 2) a nearly constant high surface tension initially exists that causes viscous interactions to dominate, and this initially induces asymmetric reopening; and 3) differential adsorption between the static (channel 1) and moving (channel 2) interfaces leads to a reduction of $(P_{\text{Cap}})_1$ and an increase in $(P_{\text{Cap}})_2$ and causes channel 1 to begin and continue to open faster than channel 2.

Unfortunately, the inhibition of Infasurf’s stability properties by albumin suggests that regions with plasma infiltration may be more likely to have heterogeneous recruitment, and alternative strategies may be necessary to accomplish uniform airway recruitment. While the interpretation above remains hypothetical, it suggests that the initiation of interfacial cycling could induce a purified Infasurf layer to the interface and reduce the competitive adsorption effects that lead to the initial asymmetric reopening phenomena. This process has already been demonstrated to reduce epithelial cell damage due to dynamic surface tension effects (13, 31, 32). The interactions that exist in the present study are a simple demonstration of the difficulty of replicating the in vivo environment with in vitro experiments.

Limitations. In this study we have conducted carefully controlled investigations that are intended to elucidate the interactions that may exist as an airway bifurcation is opened by a finger of air under a surfactant-doped system. There are a number of limitations to this study. First, we only explored one concentration of each surfactant system. Clearly the surfactant dynamic properties are concentration dependent and therefore the stability behavior is likely to be influenced by concentration. Since the equation of state for Infasurf is steepest for concentrations below 1 mg/ml, we are likely to be influenced by concentration. Second, the PDMS bifurcations were designed to geometrically model bronchial bifurcations; however, the material properties do not mimic tissue in a number of ways, including compliance and contact angle. The compliance is evident by the yield stress behavior that exists when a bubble is cleaved by the bifurcation (see Fig. 4). This response occurs due to the suction pressure that is provided downstream, which is most relevant to conventional breathing. While a yield pressure would exist in positive pressure reopening, it would not lead to a downstream collapse of the bronchial tree. In addition, this analysis ignores the effects of multiple bifurcations. We have simplified this system to focus on local fluid-structure interactions. Clearly the pressure distribution induced by other airways and parenchymal tethering from adjacent airways and alveoli will also have an important influence on the stability of these systems. Finally, this study only investigated a bifurcation that is slightly asymmetric and does not include any gravitational asymmetry, although these issues may be significant (14). We cannot predict the magnitude of asymmetry can be overcome by appropriate surfactant properties.

Conclusions

This study investigated the reopening of nearly symmetrical fluid-filled microfluidic channels as models of obstructed pulmonary airways. The goal of this study was to determine whether surface tension properties at the air-liquid interface of a propagating finger of air would influence the symmetry of the reopening process. Our studies demonstrate that a surfactant-free system will always open asymmetrically, with the propagation proceeding in the direction of the low-hydraulic-resistance path. With a rapidly adsorbing surfactant, asymmetric reopening also occurs but follows the high-hydraulic-resistance pathway. We only observed symmetric reopening when the fluid was doped with the pulmonary surfactant Infasurf. Unfortunately, when albumin was incorporated into the system (as could happen with plasma leakage due to increased vascular permeability from sepsis), symmetric reopening was destroyed. This could lead to incomplete airway recruitment, resulting in regions of the lung that would remain
atelectic and increasing the likelihood of volutrauma even with a reduction of the ventilation tidal volume.

The analysis in the present study demonstrates potentially important protective mechanisms of functional pulmonary surfactant. This study demonstrates that deactivation can lead to deleterious effects related to the loss of dynamic surface tension effects that can stabilize the reopening of obstructed bifurcating airways. Biophysical techniques that can reestablish these important dynamic surface tension qualities may lead to effective approaches for the reduction of VILI.

REFERENCES


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