The Effect of Surface Tension and Surfactant Administration on Eustachian Tube Mechanics

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Abstract

The development of Otitis media has been related to abnormal Eustachian tube (ET) mechanics. The ET is a collapsible tube that is periodically opened to regulate middle ear (ME) pressure and clear ME fluid into the nasopharynx. The ability to perform these physiological functions depends on several mechanical properties including the ET’s opening pressure ($P_{\text{open}}$), compliance (ETC), and hysteresis ($\eta$). In this study, a previously developed modified force-response protocol was used to determine ET mechanical properties after experimental manipulation of the mucosal surface condition. Specifically, these properties were measured in the right ear of 6 cynomologous monkeys under baseline conditions, after "washing out" the normal ET mucous layer, and after instillation of a pulmonary surfactant, Infasurf®. Removal of the normal mucosa did not significantly alter $P_{\text{open}}$, but did result in a decrease in ETC and $\eta$ (ANOVA, $p<0.05$). Treatment of the mucosa with Infasurf was effective in reducing $P_{\text{open}}$ and increasing both ETC and $\eta$ to baseline values ($p<0.05$). These results indicate that the mucosa-air surface tension can affect the overall compliance and hysteretic properties of the ET. In addition, this study indicates that surfactant therapy may only be beneficial in patients with rigid or inelastic ETs (large $P_{\text{open}}$ and low ETC and $\eta$).

Key Words: compliance, hysteresis, opening pressure, Otitis media, elasticity
INTRODUCTION

Otitis media (OM) is a common childhood disease that includes inflammation of the middle ear (ME) mucosa and an accumulation of fluid within the ME. By age three, a significant number of children (33%) experience more than 3 episodes of OM (32). The persistence of OM often results in hearing loss with possible effects on language acquisition, speech production, and social and educational development (31). The cost of treating persistent OM by medical and surgical procedures approaches $3 billion annually in the United States (1). Although antibiotic treatment has proven effective in treating acute otitis media (AOM), the overuse of antibiotics has resulted in an increase in the prevalence of antibiotic resistance pathogens (5). In addition, surgical treatments, which include the placement of ventilating tubes in the tympanic membrane (TM), requires general anesthesia and is thus costly and distressing to children and their parents. The development of alternative treatment therapies for OM is therefore one of the most important, unresolved clinical problems in otology.

Although bacterial or viral infections and nasal allergies contribute to the onset of OM, the development of persistent OM is associated with a functional impairment of the Eustachian tube (ET) (2). The structure of ET, which connects the ME with the nasal cavity, is similar to other respiratory airways in that the lumen of the tube is bounded by a fluid layer, the mucosa, and is surrounded by cartilaginous and muscular elements (2). Under normal conditions, the ET exists in a collapsed "closed" configuration which protects the ME from nasopharyngeal secretions. However, the ET is also responsible for maintaining ambient ME pressures and clearing ME fluid into the nasopharynx (2). These pressure regulating and clearance functions require an open ET in which the resistance to air and fluid flow is minimal. ET dysfunction and the resulting disease complications can therefore develop when the tube is excessively patent or if the tube cannot be readily opened.

Filsberg et al. (6) were the first to suggest that the presence of surface-tension lowering substances (surfactants) might influence the function of the ET. Although the role of surfactant in the ET has not been entirely determined, the presence and importance of these surface active substances in the lung have been well documented (33). The components of pulmonary surfactant, which include surface-
active phospholipids and surfactant associated proteins, are synthesized in the type II epithelial cells that line the alveolar walls (33). The presence of these surfactant components has been shown to influence the function and mechanics of the lung (19). Specifically, phospholipid surfactant molecules adsorb to the mucosal air-liquid interface and reduce the interfacial surface tension. This reduction in surface tension, reduces the pressure required to inflate the lung, increases the compliance or flexibility of the lung, and stabilizes the mucosa which prevents airway collapse (3, 14). In addition to these mechanical effects, hydrophilic surfactant proteins have been shown to enhance immunological functions in the lung (20). Premature infants suffering from respiratory distress syndrome (RDS) have not developed a mature surfactant system and must therefore be treated with exogenous surfactant to restore lung function. Several different surfactant systems, including a natural calf lung extract (Infasurf®, ONY Inc.) as well as synthetically modified surfactants, have been developed and used to improve lung function and airway compliance in RDS infants (13).

Several studies have used a variety of biochemical techniques to document the presence of surfactant components in the ME/ET system of both animals and humans (21, 30). Krachev et al. (16) found surfactant-producing cells in the dorsal section of the ET which are morphologically similar to the type II epithelial cells found in the lung. Recently, Paananen et al. (26) measured gene expression for several surfactant proteins in the porcine ET. In addition, Svane-Knudsen et al. (30) demonstrated that the quantity and composition of surfactant components in otologically healthy children is significantly different than the quantity and composition in children with OM. The presence of a sufficient quantity and quality of ET surfactant may therefore be an important determinant of ET function and mechanics.

**EUSTACHIAN TUBE MECHANICS**

The forces required to open the ET, and maintain its patency, will be a function of several mechanical properties including the elasticity of the surrounding tissue and the surface tension of the fluid mucosa layer. When collapsed the ET is generally considered to be a liquid-lined slit-like structure with a non-circular cross-sectional shape (Figure 1). However, histological studies (28) have also demonstrated that this slit-like structure can be buckled into several nearly circular lobes. Once this structure is opened
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to airflow, increases in lumen pressure may result in an opening of the slit-like structure and/or an opening of the circular lobes. To account for either situation, we have depicted a generalized cross-sectional shape in Figure 1, which can be used to represent opening in a slit-like structure when the length/width ratio (L/W) is large or opening in a circular lobe when L/W=1. In either case, the surface tension of the liquid layer is directed towards collapse of the ET lumen. The force required to increase the lumen area (i.e. the translumenal pressure, $\Delta P$), must therefore overcome both surface tension and tissue elastic forces (9, 14, 27). For a static system, the surface tension forces can be related to the pressure drop across the air-liquid interface using a Laplace’s Law relationship, $P_f - P = \gamma \kappa$, where $P$ is the internal lumen pressure, $P_f$ is the fluid pressure within the mucosa, $\gamma$ is the air-liquid surface tension and $\kappa$ is curvature of the mucosa-air interface. The tissue elastic forces can be accounted for with a linear tube-law or pressure-area relationship, $P_f - P_{ext} = \text{E}_{tissue} A$, where $P_{ext}$ is the external pressure, $E_{tissue}$ is the elastic modulus of the tissue and $A$ is the cross-sectional lumen area (9, 10). The translumenal pressure drop, $\Delta P = P - P_{ext}$, will therefore be a function of interfacial and tissue elastic properties, $\Delta P = f(\gamma, E_{tissue})$.

Although $\Delta P$ is related to the inflation of an opened ET, the initial opening process is considerably more complicated. For example, as the ME pressure is increased, a finger of air will penetrate into the ET, separating its walls. At a critical pressure (i.e. the opening pressure, $P_{open}$) the liquid meniscus contained within the lumen will rupture and air will pass freely through an open ET. Although the factors that determine this critical level are complicated, the translumenal pressure at the point of opening can be used to approximate $P_{open} = \gamma \kappa^* + \text{E}_{tissue} A^*$. Here, $\kappa^*$ and $A^*$ are specific values at the instant of opening and can only be determined from a detailed analysis of this opening phenomena (9, 35). Nonetheless, $P_{open}$ will be a function of both interfacial and tissue properties ($\gamma$, $E_{tissue}$). In this study we focus on identifying the importance of interfacial mechanics by introducing surface active agents which reduce $\gamma$ and should therefore reduce the passive opening pressure of the ET, $P_{open}$.

In addition to $P_{open}$, the function of the ET has also been associated with other mechanical properties including the ET compliance or flexibility (15, 22). The compliance of the ET, $E_{TC}$, is defined as...
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in engineering terms as the change in the cross-sectional area of the ET lumen for a given change in the transluminal pressure (ETC = dA/dΔP). Rigid or inelastic ETs (low ETC) are difficult to open and thus might impair the ventilation and clearance functions. Conversely, ETs with high ETC, often described as "floppy" ETs (2), may have impaired protective functions. This lack of stiffness may also affect the ability of the surrounding musculature to actively open the tube during swallowing. The ETC magnitude will be determined by the intrinsic stiffness of the surrounding cartilage and muscular elements and the surface tension forces at the air-liquid interface. Specifically, the applied pressure, ΔP, must overcome surface tension and tissue elastic forces to produce a given change in cross-sectional area, ΔA. An increase in γ or E_{tissue} will result in a larger ΔP and therefore a more rigid ET (small ETC). Conversely, a reduction in E_{tissue} or a reduction in γ due to the presence of surfactants will result in a smaller ΔP and a more compliant/flexible ET (large ETC). Therefore, the ETC will also be a function of both interfacial and tissue elastic properties, ETC = f(γ,E_{tissue}).

Another mechanical property that may influence ET function, but has not previously been considered, is the ET's hysteretic nature. Hysteresis occurs when the forces acting on the ET (i.e. pressure) are dissipated such that they do not produce the same deformation (or cross-sectional area) during inflation and deflation. Fredberg and Stamenovic (8) have quantified hysteretic phenomena in lung tissue using a hysteretic modulus, η. Further, these authors described how surface tension hysteresis at the air-liquid interface due to the presence of pulmonary surfactants, γ_{hv}, and the viscoelasticity of the surrounding tissue, μ_{tissue}, can both influence global hysteretic phenomena. Therefore, η will also be a function of both interfacial and tissue mechanical properties, η = f(γ_{hv}, μ_{tissue}).

The goal of the present study is to selectively alter the mucosal surface condition to determine if interfacial properties can significantly affect global ET mechanical properties. Specifically, we will quantitatively determine how removal of the normal ET mucosal surfactant and the subsequent instillation of a natural pulmonary surfactant extract (Infasurf®) affects the opening pressure, compliance, and hysteresis of the ET. Although a study by Miura et al. (22) attempted to determine the ability of surfactant to modify ET compliance, that study used a non-physiological surfactant and was based on a summary
parameter which is not consistent with the engineering definition of compliance. In addition, ET hysteresis has not been specifically investigated. Therefore, the current study uses a physiological surfactant (Infasurf®) and measures ET mechanical properties with a modified force-response test previously developed by our research group (10). This testing protocol is unique in that ET mechanical properties (ETC and $\eta$) are determined by correlating pressure-flow measurements with a mathematical model of flow in a collapsible tube. The ETC determined by this protocol is therefore consistent with the engineering definition. An accurate determination of how the mucosa-air surface tension and surfactant administration affects ET mechanics may lead to a better understanding of how surfactant therapy may be used clinically to treat OM patients.

**METHODS AND MATERIALS**

For this study, data were obtained from the right ears of six cynomologous monkeys (*Macaca fascicularis*, 2-4 kg). The monkey was chosen as the animal model since previous investigators demonstrated that the operational biomechanics of monkey and human ETs are nearly identical (4). All protocols used in this study were approved by the Children's Hospital of Pittsburgh Animal Research and Care Committee. For each experiment, the monkey was sedated with 30mg Ketamine® and anesthetized with "monkey mix" (10 mg/kg ketamine, 2mg/kg xylazine, and 0.3 mg/kg acepromazine). The external auditory canal was cleaned and normal ME status was verified using tympanometry. Once a myringotomy was performed in the right TM, a probe with an integrated flow sensor and micropressure transducer was hermetically sealed in the right external auditory canal. All pressures were measured relative to the ambient atmospheric pressure. As shown in Figure 2, the probe was connected to a syringe pump which delivered air at specific flow rates. Continuous outputs from the flow and pressure sensors were routed to a microcomputer for visual display in real time, data storage and data processing via a HP VEE data acquisition routine (Agilent Technologies) (10).

After each experimental manipulation described below, data were obtained and analyzed in each animal according to the modified forced response protocol previously developed in our lab (10). In this protocol the syringe pump was used to inflate the ME with ambient air at a flow rate of 5cc/min until the
ET was forced open at the opening pressure, $P_{\text{open}}$, as shown in Figure 3a. After the ET was opened, the syringe pump was programmed to produce a sinusoidal flow rate between 5.0 and 23 cc/min with a period of 72 seconds. The pressure and flow rate were measured simultaneously as a function of time until a steady-state, defined as a <5% change in the maximum and minimum pressure between two successive oscillations, was obtained (Figure 3a). The magnitude of this oscillation cycle is based on the steady-state flow rates used in previous investigations (4) while the oscillation frequency is small enough to ensure that air behaves like an incompressible viscous fluid and large enough to ensure that the test could be conducted in a reasonable amount of time (12). Data for analysis consisted of the pressure and flow rate during the final oscillation period which was plotted as a pressure-flow (P-Q) hysteresis loop (see Figure 3b). This P-Q hysteresis loop was then correlated with a mathematical model of airflow in a collapsible tube (solid line in Figure 3b) to obtain the ET’s compliance, ETC, and the hysteretic modulus, $\eta$ (12). This correlation technique, which is described in the Appendix, is based on a least-squared analysis and resulted in correlation coefficients, $r^2$, that were consistently > 0.95. Note that ETC and $\eta$ are global parameters and may therefore depend on both interfacial as well as tissue mechanical properties. In general, the average slope of the P-Q loop is inversely related to the ETC (i.e. larger slope results in lower ETC) while the area enclosed by the hysteresis loop is directly related to $\eta$ (i.e. larger area results in larger $\eta$). Execution of this protocol therefore results in three primary parameters that describe the mechanics of the ET ($P_{\text{open}}$, ETC, and $\eta$).

These mechanical parameters were determined by performing this modified forced response protocol after various experimental manipulations. First, baseline mechanical parameters were determined in freshly perforated ears before any manipulation (Normal conditions). Second, the ME-ET system was rinsed with 37 °C isotonic saline at 1cc/min for 5min. For this procedure, the animals were placed in a prone position and the ET lumen was washed by injecting saline into the ME via the hermetically sealed probe and collecting the washed fluid at the nasal orifice. Once the ET was rinsed, any residual fluid in the ME was cleared by subsequently introducing airflow at 5cc/min for 2-3 min. Once the normal ET mucosa had been disrupted in this manner, the mechanical parameters were measured via the testing
protocol (Saline conditions). Finally, the ET lumen was treated with a calf lung surfactant extract, Infasurf®, currently used to treat RDS infants. The surfactant solution was injected into the ME/ET system at 1cc/min for 2 min and the residue was collected at the nasal orifice. Following this surfactant installation, residual ME fluid was again cleared with air at 5cc/min for 2-3 min. The mechanical properties of the surfactant treated ET were then measured with the testing protocol (Surfactant conditions). Following these experimental manipulations, the probe was removed and the external ear canal was cleaned with an alcohol solution to reduce the possibility of infection.

The mechanical parameters (P_{open}, ETC, \eta) were determined for six animals under three treatment conditions: normal, saline, and surfactant. A within subjects analysis of variance (ANOVA) was performed to document statistically significant differences among all treatment groups while post-hoc planned comparisons were used to document statistically significant differences between individual treatment groups. Significance for these tests was set at p<0.05.

RESULTS

The opening pressures measured in each animal after a given experimental manipulation is presented in Figure 4. The mean P_{open} values measured under each experimental condition are reported in Table 1. These mean P_{open} values were significantly different (F=27.8, p<0.01). Post-hoc between treatment comparisons indicated that the mean P_{open} measured under normal conditions was not significantly different from the mean P_{open} measured after the ET mucosa had been rinsed in saline (p=0.80). The mean P_{open} measured after surfactant instillation, however, was significantly lower than the mean P_{open} measured under both normal and saline conditions (p<0.01).

The pressure-flow rate (P-Q) hysteresis loops measured in a typical subject during each experimental condition are presented in Figure 5. Only the mathematical correlations are displayed for clarity. The P-Q loop measured after the ET was rinsed with saline had a larger slope and less hysteresis area than the P-Q loop measured under normal conditions. The subsequent administration of surfactant resulted in a slight decrease in the slope of the P-Q loop and an increase in the area of the hysteresis loop. These qualitative observations were quantified by analyzing each loop with the mathematical model
presented in the appendix and described in detail by Ghadiali et al. (12). This analysis resulted in a quantitative measurement of the ETC and \( \eta \) parameters in each animal for a given experimental manipulation. The mean values for these parameters under each experimental condition are reported in Table 1. The variation of ETC between treatment groups is presented in Figure 6a. The mean ETC values were significantly different \((F=4.11, p<0.05)\). Between treatment comparisons indicated that the mean ETC under saline conditions was significantly lower than the mean ETC measured under both normal and surfactant conditions \((p<0.05)\). In addition, the ETC measured under surfactant conditions was not significantly different from the ETC measured under normal conditions \((p=0.48)\). The variation of \( \eta \) between treatment groups is presented in Figure 6b. The mean \( \eta \) values were also significantly different \((F=8.55, p<0.01)\). Between treatment comparisons indicate that the mean \( \eta \) measured under saline conditions was significantly lower than the mean \( \eta \) measured under normal and surfactant conditions \((p<0.05)\). In addition, the mean \( \eta \) measured under surfactant conditions was not significantly different from the mean \( \eta \) measured under normal conditions \((p=0.57)\).

**DISCUSSION**

We measured several ET mechanical properties after flushing the ET mucosa with saline and after the subsequent administration of a natural pulmonary surfactant, Infasurf®. These mechanical properties include the pressure required to open the ET, \( P_{\text{open}} \), the global compliance of the ET, ETC, and the global hysteretic nature of the ET, \( \eta \). These parameters were measured in 6 cynomologous monkeys with the previously developed modified force-response technique. This technique, which is based on an engineering model of airflow in the ET, results in an accurate determination of global ET mechanical properties. Although both interfacial and tissue mechanical properties could affect these global mechanical properties, our goal was to obtain a better understanding of how disruption of the normal mucosal surface condition and treatment of the mucosa with a pulmonary surfactant influences global ET mechanics. Therefore, these experiments were designed to alter interfacial properties without affecting the tissue mechanical properties.
Although washing of the ET mucosa with saline did not result in a significant change in $P_{\text{open}}$, the instillation of Infasurf® into the ET resulted in a reduction in $P_{\text{open}}$ consistent with a reduction in the mucosa-air surface tension. To interpret these results, which are consistent with similar measurements obtained in gerbils (7), we consider the conceptual model for ET opening shown in Figure 1. Here the pressure required to open the ET will be a function of both the mucosa-air surface tension ($\gamma$) and the elasticity of the surrounding tissue ($E_{\text{tissue}}$). Specifically, as discussed in the introduction, $P_{\text{open}} = \gamma \kappa + E_{\text{tissue}} A^*$. The instillation of a pulmonary surfactant that reduces $\gamma$ therefore results in a reduction in $P_{\text{open}}$ (see Figure 4). These results are also consistent with the ability of surfactants to reduce opening or inflation pressures in the lung as documented using both ex-vivo lung models (23) and in-vitro airway models (11). However, the normal vs. saline data in Figure 4 indicates that native surfactant does not significantly contribute to $P_{\text{open}}$ and that under normal conditions $P_{\text{open}}$ is mainly determined by other factors, i.e. tissue elasticity. Even though native surfactant may not influence $P_{\text{open}}$ under normal conditions, the fact that a pulmonary replacement surfactant significantly reduces $P_{\text{open}}$ may have important clinical consequences. For example, patients with OM typically present with an inflamed ME mucosa and consequently have high ET opening pressures due to an elevated $E_{\text{tissue}}$. Under these conditions, surfactant therapy could potentially reduce $\gamma$ and therefore reduce $P_{\text{open}}$ to normal values even if $E_{\text{tissue}}$ were elevated. This paradigm was in fact studied by Nemechek et al. (24) who found that $P_{\text{open}}$ in normal ears was not significantly different from $P_{\text{open}}$ in inflamed ears treated with surfactant.

Although opening pressures may be an important factor, several other mechanical properties, such as ETC and $\eta$, will also be important determinates of ET function. However, the influence of surface tension and surfactant on these properties has not been adequately studied. The current study was therefore designed to provide new information with respect to how surfactants influence the compliance and hysteresis of the ET. Figure 6a demonstrates that the surface tension of the mucosa ($\gamma$) can significantly affect the compliance of the ET. Washing the ET lumen with saline, which potentially removes native surfactant and thus increases $\gamma$, resulted in a decrease in ETC and thus a more rigid ET. Note that although this increase in $\gamma$ is large enough to alter ETC, it is apparently not large enough to alter...
the opening pressure (see Figure 4). In contrast, application of a pulmonary surfactant to the ET lumen, which decreases $\gamma$, resulted in an increase in ETC to pre-washing values and thus a more flexible ET. This inverse relationship between $\gamma$ and ETC can be understood by recalling the definition of ETC as the change in cross-sectional area for a given change in the translumenal pressure (ETC = $dA/d\Delta P$). As discussed in the introduction, the translumenal pressure will be a function of interfacial and tissue mechanical properties: $\Delta P = \gamma \kappa + E_{\text{tissue}} A$. By assuming constant tissue properties ($dE_{\text{tissue}}/dA = 0$) and negligible surface tension hysteresis ($d\gamma/dA \approx 0$), we can express $\text{ETC} = (\gamma d\gamma/dA + E_{\text{tissue}})^{-1}$. Therefore, ETC is inversely related to $\gamma$ when $d\gamma/dA > 0$ (see Figure 1). This inverse relationship is consistent with surface-tension forces directed towards collapse of the ET. Specifically, as $\gamma$ increases, the surface tension collapsing forces increase, requiring a larger applied pressure to maintain lumen area which results in a more rigid ET (lower ETC). This inverse relationship is also consistent with previous studies in the pulmonary system. Specifically, Buchanan et al. (3) demonstrated that airway compliance increases upon surfactant administration (i.e. decrease in $\gamma$).

In contrast to this inverse relationship, Miura et al. (22) reported an opposite behavior in which an index of ET compliance, known as the tubal compliance index (TCI), decreased when $\gamma$ decreased due to the application of a non-physiological surfactant. The TCI was defined as the ratio of the flow resistance, $R_s$, at two different flow rates. Specifically, constant flow in an open ET was established and resistance was calculated as $R_s=P_s/Q_s$, where $P_s$ is the steady-state pressure and $Q_s$ is the applied flow rate. The TCI was then calculated as the resistance at a low flow rate ($Q_s \approx 10 \text{ cm/min}$) over the resistance at a high flow rate ($Q_s \approx 40 \text{ cc/min}$). Since the resistance to airflow in the ET is an inverse function of the lumen area, this parameter essentially measures the relative change in lumen area for a given change in flow rate. However, the compliance or elastic nature of the ET is related to how much force must be applied to produce a given deformation or change in lumen area. The relevant force in this system is the translumenal pressure, not the flow rate. The TCI, therefore, may not be an accurate measure of compliance since it does not relate changes in lumen area (i.e. resistance) with changes in the applied pressure. We believe that the results of the current study, which utilizes an engineering definition of
compliance (i.e. ETC = dA/dΔP) and successfully predicts an increase in compliance with a reduction in surface tension, is a more accurate representation of the influence of surfactant on ET mechanics.

Another potentially important mechanical parameter that can be influenced by the presence of surfactants is the hysteretic nature of the ET as measured by the η parameter. As demonstrated by the arrows in Figure 3b, the pressures obtained during inflation (i.e. increasing flow rate) may be slightly larger than the pressure obtain during deflation, resulting in a P-Q hysteresis loop. These loops are similar to the pressure-volume (P-V) loops observed in the lung. In this study, the area of the P-Q hysteresis loop was quantified using a global hysteresis parameter, η. Fredberg and Stamenovic (8) demonstrated that η may be a function of surfactant induced surface tension hysteresis, γ_h, and viscoelastic tissue properties, μ_{tissue}. In this study we have focused on the influence of γ_h by altering interfacial properties using a pulmonary replacement surfactant. Pulmonary surfactant’s ability to generate a significant hysteresis loop area depends on several complex physical properties, including differences in adsorption and desorption rates (25) and the development of multiple surfactant layers on the air-liquid interface (18).

We have demonstrated that the P-Q hysteresis loop area observed in normal ETs can be eliminated by removing the native ET mucosal surfactant (see Figure 6b). This behavior is consistent with observations in the lung where significant P-V loop area is observed in lungs with a functional surfactant system while the P-V loop area in lungs without a functional surfactant system is negligible (19). Therefore, the chemical components of ET surfactant likely contain the complex physical properties known to exist in native pulmonary surfactant and required to produce significant hysteresis loop area. In addition, the instillation of a pulmonary surfactant into the ET resulted in an increase in hysteresis loop area, as measured by η, to normal values. Krueger and Gaver (18) demonstrated that the pulmonary surfactant used in the current study, Infasurf®, was also capable of producing these hysteresis loops in an in-vitro model of lung alveoli. Therefore, Infasurf® contains the requisite physical properties to maintain P-Q hysteresis in the ET and P-V hysteresis in the lung.

Several recent studies investigated the efficacy of surfactant therapy on the resolution of OM (17, 34). These studies were conducted by inducing AOM experimentally with a bacterial agent,
administering surfactant on a periodic basis, and observing the resolution time. For this experimental model, surfactants were found to be effective in reducing the resolution time. Bacterial infections of the ME cause mucosal inflammation which likely results in increased opening pressures, decreased ETC, and thus a dysfunctional ET. The current study demonstrates that treatment with surfactant restores ET function by reducing the mucosa-air surface tension and thus reducing the opening pressure and increasing the flexibility or compliance of the ET. Although surface active substances may be helpful in resolving AOM due to infection and inflammation, persistent OM can develop due to a variety of pathological conditions related to the structure of the ET. For example, Bluestone (2) demonstrated that persistent OM can develop when the ET is highly compliant or floppy. This hyper-compliance can occur in young children who do not have sufficient quantity of cartilage or in older patients with decreased cartilage cell density or a degraded intracellular matrix. This lack of stiffness may affect the ability of the surrounding musculature to actively open the tube during swallowing. Clearly, the administration of surfactant under these conditions, which would further increase ET compliance, is counter indicated for the resolution of disease conditions. Successful treatment therapies may therefore depend on an accurate understanding of both the specific influence surfactant therapy can have on the mechanics of the ET and the mechanical state of a given patient’s ET. Specifically, surfactant therapy may only be effective in patients with high opening pressures and low ET compliance.

Although we have demonstrated that surfactants and surface tension properties significantly affect global ET mechanical properties, tissue mechanical properties are also expected to play a critical role. Future studies will therefore investigate the potential contributions of tissue properties by measuring P_{open}, ETC and \eta after paralysis of a surrounding muscle (tensor veli palatini). As a result, we will be able to determine how an experimental reduction in tissue elasticity, E_{tissue}, affects the overall mechanics of the ET. Future studies will also focus on developing a delivery system that is more practical than the ME instillation technique used in this study. We will specifically investigate the efficacy of administering a nebulized or aerosolized surfactant via the nasal cavity. In addition, the pulmonary surfactant used in this study, Infasurf®, as well as other surfactants used to treat RDS infants are relatively expensive and
therefore might not be a viable treatment for OM due to financial reasons. Therefore, future studies will also include an investigation of how various non-physiological and synthetic surfactants affect ET mechanics. Finally, as noted above, the use of surfactant as an alternative, non-invasive treatment option for OM will require knowledge of the patient’s ET mechanics. Although the modified force response technique used in this study has also been implemented in a clinical setting, this test requires a perforation of the TM. Since surfactant therapy is potentially an alternative to this surgical procedure, future studies will also investigate other less-invasive means of obtaining the ET mechanical properties investigated in this study, i.e. opening pressure, compliance, and hysteresis.

In summary, we have investigated the influence of the mucosal surface condition and the administration of a pulmonary surfactant on the mechanics of the ET. Removal of the normal mucosal blanket, which potentially removes native surfactant components, did not significantly alter the opening pressure but did significantly decrease both the compliance and hysteretic properties of the ET. Administration of a pulmonary surfactant, Infasurf®, significantly decreased the opening pressure consistent with a reduction in the mucosa-air surface tension. In addition, pulmonary surfactant’s ability to reduce the surface tension resulted in a significant increase in the ET’s compliance and hysteresis to normal values. Knowledge of how surfactant affects these mechanical properties has lead to a better understanding of which patients may benefit from surfactant therapy, i.e. patients with large opening pressures and rigid ETs. With the development of non-invasive testing protocols, effective surfactant delivery methods, and cheaper synthetic surfactants, the use of surfactant therapy may become a practical alternative to standard antibiotic and surgical treatments of OM.

**APPENDIX I - FLUID-STRUCTURE MODEL**

The mechanical parameters (ETC and \( \eta \)) were determine by analyzing experimental pressure-flow rate measurements with the fluid-structure model of airflow in a collapsible tube shown in Figure 7 (12). In this model, a Poiseuille-type relationship is used to describe the pressure drop along the ET in terms of the cross-sectional area, \( A(t) \), and the flow rate, \( Q(t) \),
\[ p(t) - \rho_d = \frac{\mu Q(t)}{A(t)^2} \Gamma_s \quad Q(t) = q_m + q_s \sin(\omega t), \] 

(1)

where \( Q(t) \) is fixed by protocol \((q_m = 14\text{cc/min}, q_a = 9\text{cc/min}, \text{and } \omega = 2\pi/(72\text{sec})) \), \( \mu \) is the viscosity of air, \( L \) is the length of the collapsed segment and \( \Gamma_s \) is a hydraulic-geometric shape factor, which is only a function of the cross-sectional shape. The solid mechanics are describe by a potentially non-linear pressure-area relationship,

\[ p_{\text{mean}} - p_{\text{ext}} = \frac{p(t) + p_d}{2} - p_{\text{ext}} = E_{\text{tube}} A_2^n(t) + \eta \frac{dA_2(t)}{dt} \] 

(2)

Here, \( p_{\text{mean}} \) is the mean pressure in the tube, \( p_{\text{ext}} \) is the external tissue pressure, \( E_{\text{tube}} \) and \( \eta \) represent the global stiffness and hysteretic properties of the ET and \( A_2 \) is the shape independent area defined as \( A_2(t) = A(t)/\Gamma_s^{1/2} \). Note that the mechanical parameters \((E_{\text{tube}} \text{ and } \eta)\) are independent of the cross-sectional shape such that the correlation technique described below does not require a specific \( \Gamma_s \) value. This model also assumes no variations in cross-sectional area along the length of the ET, i.e. \( A = A(t) \) only, as suggested by histological measurements (29).

We seek solutions to these equations for \( p(t) \) as a function of \( E_{\text{tube}}, \eta, \) and \( p_{\text{ext}} \). These solutions, however, require an assumption regarding the downstream pressure, \( p_d \), and the coefficient \( n \). As described in detail by Ghadiali et al. (12), setting \( p_d = 0 \) as the reference downstream pressure and allowing \( n > 1 \) results in a numerical solution scheme. A simpler analytical solution can also be obtained by setting \( p_d = p_{\text{ext}} \) and \( n = 1 \). Note that these assumptions were only made to obtain an analytical solution and may not have any physiological significance. As demonstrated by Ghadiali et al. (12), the choice of models (i.e. numerical or analytical) does not substantially affect the magnitudes of the \( E_{\text{tube}} \) and \( \eta \) obtained when these models are correlated with experimental data. Therefore, the current study has utilized the simpler analytical solution.

Given the \( p_d = p_{\text{ext}} \) and \( n = 1 \) assumptions, Eqs. (1) and (2) can be solved analytically for \( A_2(t) \). With this expression for \( A_2(t) \), Eq. (1) can be used to generate a predicted pressure, \( p_{\text{pred}}(t) \), which will be a function of \( E_{\text{tube}}, \eta, \) and \( p_{\text{ext}} \). For each experimental condition, a least squared regression analysis is performed by varying the three free parameters to obtain the best fit between \( p_{\text{pred}}(t) \) and the
experimentally measured $p(t)$. This regression technique is able to capture the experimentally observed pressure-flow rate hysteresis (see Figure 3b) and consistently results in a correlation coefficient, $r^2 > 0.95$. As a result, we can quantitatively estimate the ET's global elastic and hysteretic properties, $E_{\text{tube}}$ and $\eta$.

Since compliance is defined as $dA/(p-p_{\text{ext}})$, we utilize Eq. (1) to specify the ET's compliance as $ETC=1/E_{\text{tube}}$. Note that this “lumped-parameter” model utilizes global parameters, $ETC$ and $\eta$, that may depend on both interfacial and tissue mechanical properties. Therefore, this model is not able to specifically identify the functional form of these relationships, $E_{\text{tube}} = 1/ETC = f(E_{\text{tissue}}, \gamma)$ and $\eta = f(\mu_{\text{tissue}}, \gamma_h)$. 
ACKNOWLEDGMENTS

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REFERENCES


Table 1: Mean values and standard deviation for the mechanical properties measured under various experimental conditions

<table>
<thead>
<tr>
<th>Mechanical Property</th>
<th>Normal Conditions</th>
<th>Saline Conditions</th>
<th>Surfactant Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{open}}$ (mmH$_2$O)</td>
<td>$471 \pm 72$</td>
<td>$476 \pm 91$</td>
<td>$356 \pm 82$</td>
</tr>
<tr>
<td>ETC ($\times 10^{-7}$ cm$^2$/mmH$_2$O)</td>
<td>$4.12 \pm 0.76$</td>
<td>$2.73 \pm 1.00$</td>
<td>$3.64 \pm 1.49$</td>
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<tr>
<td>$\eta$ ($\times 10^8$ Poise/cm$^2$)</td>
<td>$9.72 \pm 3.78$</td>
<td>$3.62 \pm 2.92$</td>
<td>$8.92 \pm 1.93$</td>
</tr>
</tbody>
</table>
**FIGURE CAPTIONS**

**Figure 1** Cross section of a slit-like ET lumen lined with a fluid/mucosa layer. The surface tension forces of the mucosa ($\gamma_m$) are directed towards collapse of the ET. The transmural pressure, $P-P_{ext}$, will be governed by the surface tension as well as the tissue elasticity, $E_{tissue}$, and tissue viscoelasticity, $\mu_{tissue}$.

**Figure 2** Schematic diagram of the forced-response testing system.

**Figure 3ab** Typical experimental data obtained during a modified force response test. a) pressure and flow rate as a function of time, b) pressure-flow hysteresis loop and the mathematical correlation used to obtain ETC and $\tau$ parameters.

**Figure 4** Changes in ET opening pressure after saline washout of the mucosa and after the application of the pulmonary surfactant (Infasurf®).

**Figure 5** Typical pressure-flow hysteresis loops measured under normal, saline, and surfactant conditions. The P-Q slope increases under saline conditions and decreases upon surfactant administration while the P-Q loop area decreases under saline conditions and increases upon surfactant administration.

**Figure 6ab** Changes in a) the global compliance parameter, ETC and b) the global hysteresis parameter, $\tau$, after saline washout of the mucosa and after the application of the pulmonary surfactant (Infasurf®).

**Figure 7** Engineering model of a collapsible ET where the global parameters ($\tau$ and $E_{tissue}$) will depend on interfacial and tissue mechanical properties.
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