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Multi-scale Finite Element Modeling of Eustachian Tube Function: Influence of Mucosal Adhesion

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Abstract

The inability to open the collapsible Eustachian tube (ET) leads to the development of chronic Otitis Media (OM). Although mucosal inflammation during OM leads to increased mucin gene expression and elevated adhesion forces within the ET lumen, it is not known how changes in mucosal adhesion alter the biomechanical mechanisms of ET function. In this study, we developed a novel multi-scale finite element model of ET function in adults that utilizes adhesion spring elements to simulate changes in mucosal adhesion. Models were created for six adult subjects and dynamic patterns in muscle contraction were used to simulate the wave-like opening of the ET that occurs during swallowing. Results indicate that ET opening is highly sensitive to the level of mucosal adhesion and that exceeding a critical value of adhesion leads to rapid ET dysfunction. Parameter variation studies and sensitivity analysis indicate that increased mucosal adhesion alters the relative importance of several tissue biomechanical properties. For example, increases in mucosal adhesion reduced the sensitivity of ET function to tensor veli palatini muscle forces but did not alter the insensitivity of ET function to levator veli palatini muscle forces. Interestingly, although changes in cartilage stiffness did not significantly influence ET opening under low adhesion conditions, ET opening was highly sensitive to changes in cartilage stiffness under high adhesion conditions. Therefore, our multi-scale computational models indicate that changes in mucosal adhesion as would occur during inflammatory OM alter the biomechanical mechanisms of ET function.

Keywords

Biomechanics; Airway opening; Tissue mechanics; Multi-scale Modeling; Mucus

1. Introduction

Otitis Media (OM) is the most common disease for which children receive medical treatment in the United States¹ and the over-prescription of antibiotics to treat OM has led to increased treatment failure rates². Although bacteria/viral infections are responsible for the acute onset of OM, Eustachian Tube (ET) dysfunction is responsible for the development of chronic OM³. The ET is a collapsible upper respiratory airway that connects the Middle Ear (ME) to the Nasopharynx (NP) and is normally closed to protect the ME from nasopharyngeal pathogens. Periodic openings of the ET is critical to maintaining a sterile, fluid-free ME at ambient pressures. However, inflammation of the ME-ET system during OM results in the up-regulation of mucus glycoproteins^{4,5} which increases adhesive forces within the ET lumen and may lead to ET dysfunction. Therefore, an ability to open the ET during conditions of increased adhesion is critical in preventing chronic OM. In this study, we seek to develop novel, multi-scale computational models that can quantify how changes in mucosal adhesion influence ET function.

As shown in Fig 1A, the ET is a complex, 3D structure^{6,7} that contains a lumen surrounded by cartilage and periluminal mucosal tissue (PMT). Several craniofacial muscles, including the tensor veli palatini muscle (TVPM) and levator veli palatini muscle (LVPM), may be attached to the cartilage and PMT and contraction of the TVPM is required for opening of the ET lumen^{8,9}. In addition, changes in tissue mechanics¹⁰⁻¹² and luminal surface tension¹³ may also significantly influence ET function. However, it is not known how changes in mucosal adhesion within the ET lumen influence the biomechanics of ET opening.

Our laboratory previously developed anatomically accurate finite element models of the ET function in different patient populations^{12,14}. Although these models could simulate muscle-assisted ET opening, they could not account for the increased adhesion forces that develop within the ET lumen during inflammatory OM. Specifically, epithelial cells lining the ET lumen up-regulate the apical expression of mucus glycoproteins during inflammation^{4,5} which leads to increased adhesion forces within the lumen. Although these adhesion forces may alter the biomechanical mechanisms of ET function, a detailed analysis of how changes in adhesion influence ET function has not been conducted.

The goal of this study is to develop a multi-scale computational model of ET function that accounts for mucosal adhesion. We also seek to use these models to investigate how changes in adhesion during inflammation influence the functional relationships between tissue mechanics and ET opening. We hypothesize that elevated adhesion forces will alter the relative importance of several tissue mechanical parameters and that ET function is highly sensitive to changes in adhesion levels. To test these hypotheses, we have used multi-scale finite element models of ET function to investigate how changes in mucosal adhesion and tissue biomechanics alters the degree of ET opening during TVPM and LVPM contraction. We have also used a sensitivity analysis to evaluate the relative importance of different tissue biomechanical factors during elevated adhesion conditions. As a result, this study has led to a better understanding of how changes in mucosal adhesion influence the biomechanical mechanisms of ET function.

2. Methods

2.1 Finite Element Modeling

Histological data obtained in n=6 adult patients with no history of ME disease or ET dysfunction (age range 19-76) were used to develop anatomically accurate 3D finite element models of the ET. Histological data was obtained in a de-identified format from the University of Pittsburgh department of Otolaryngology and our analysis of this data was determined exempt by the Ohio State University institutional review board. As described previously¹², sequential outlines of the cartilage and PMT tissue were used to create 3D solid models which were imported into the ADINA finite element program (Watertown, MA). Free-form meshing techniques were used to generate four-node tetrahedral elements within the cartilage and PMT volumes (see green and red elements in Fig 1B). We created finite element models for subjects E32, E37, E41, E63, E82 and R55 and the selected mesh size was based on a mesh independence analysis conducted in our previous study¹². Non-linear solvers within the ADINA software package¹⁵ was used to solve the 3D tissue deformation equations.

$$\frac{\partial \sigma_{ij}}{\partial x_j} = \rho \frac{\partial^2 d_j}{\partial t^2} \text{ where } \sigma_{ij} = \frac{\partial W}{\partial \varepsilon_{ij}} \text{ and } \varepsilon_{ij} = \frac{1}{2} \left(\frac{\partial d_i}{\partial x_j} + \frac{\partial d_j}{\partial x_i} + \frac{\partial d_k}{\partial x_i} \frac{\partial d_k}{\partial x_j} \right) \quad (1)$$

Here σ is the Cauchy stress tensor, $\partial^2 d_j / \partial t^2$ is the acceleration, and W is the strain energy density and ε is the strain. Note that all equations are written in standard tensor notation for a Cartesian coordinate system where $i=1,2,3$ represents the x, y and z directions. Here, the x-axis corresponds to the medial-lateral direction, the y-axis corresponds to the inferior-superior direction and the z-axis corresponds to the distal-proximal direction.

Boundary conditions used to solve Eqn (1) are similar to those used in our previous study¹². Briefly, grounded spring elements were defined on the cartilage's medial surface to simulate attachment to the non-deformable cranial base. The nasopharyngeal and ME ends of the cartilage and PMT were constrained against deformation in the distal-proximal direction. Contraction of the LVPM, which runs inferior to the ET lumen along its distal-proximal axis¹⁶⁻¹⁸, was simulated by applying normally directed pressure forces onto the inferior surfaces of the PMT and cartilage. The magnitude of this pressure load was $P = F_{LVPM} / (A_{LVPM})$ where A_{LVPM} is the total surface area of interaction between the LVPM and PMT/cartilage and F_{LVPM} is the total force exerted by the LVPM on the PMT/cartilage. The TVPM is a flat, ribbon-like muscle that attaches to lateral portions of the cartilage and PMT, descends inferiorly and proximally, and ends in a tendon that winds around the pytergoid hamulus¹⁹. As a result, contraction of the TVPM was simulated by applying nodal forces on the lateral surfaces of the PMT and cartilage. Each force vector was directed towards the pytergoid hamulus (pink arrows in Fig 1B) and "follower load" conditions were used such that each force vector always points towards the fixed hamulus location during tissue deformation. The total force generated by the TVPM was defined as F_{TVPM} .

As described previously⁹, the TVPM and LVPM exhibit an asynchronous muscle contraction sequence where the LVPM typically contracts before the TVPM and exhibits a longer

contraction period than the TVPM. Therefore, we simulated ET opening by applying time-dependent TVPM and LVPM forces as shown in Fig 1B. The maximum force generated by the TVPM and LVPM were based on measurements of velopharyngeal closure force obtained by Kuehn & Moon (i.e. $\sim 0.2 - 0.5\text{N}$)²⁰. First, since the TVPM directly inserts into ET tissues and its primary direction of action is perpendicular to ET surface, the baseline TVPM force was set to $F_{\text{TVPM}} = 0.2\text{N}$. In contrast, the LVPM does not directly insert into ET tissues and only exerts force onto inferior portions of the ET due to out-of-plane muscle movement during contraction. Therefore, the forces exerted by the LVPM on cartilage/PMT tissues was assumed to be lower than the forces generated in the primary muscle direction. Thus, the baseline LVPM force was set to $F_{\text{LVPM}} = 0.05\text{ N}$. Note that these muscle forces values were varied over a wide range ($F_{\text{TVPM}} = 0.02$ to 2.0 N and $F_{\text{LVPM}} = 5\text{e-}3$ to 0.5 N) to capture a range of potential muscle forces.

Following our previous studies^{9,12}, the cartilage was modeled as a hyperelastic neo-Hookean material with a shear modulus (G) and a bulk modulus (κ), which depends on the Young's modulus (E) and the Poison's ratio (ν).

$$G = \frac{E}{2(1+\nu)} \text{ and } \kappa = \frac{E}{3(1-2\nu)} \quad (2)$$

These moduli are then used to define the strain energy density function,

$$W = \frac{G}{2} (J_1 - 3) + \frac{\kappa}{2} (J_3 - 1)^2 \quad (3)$$

where J_1 and J_3 are the reduced strain invariants as defined by Bathe¹⁵. Similar hyperelastic constitutive models have been used to model soft-biological tissues²¹ and more accurately captures the large deformations that might occur during swallowing²². As described previously⁹, the PMT was modeled using a Maxwell viscoelastic material with a time dependent Young's modulus, shear modulus and bulk modulus.

$$E(t) = E_{\text{PMT}} e^{-t/\tau_{\text{PMT}}}, \quad G(t) = \frac{E(t)}{2(1+\nu)} \text{ and } \kappa(t) = \frac{E(t)}{3(1-2\nu)} \quad (4)$$

E_{PMT} and τ_{PMT} are the Young's modulus and relaxation time constant of the PMT. Note that the decay function $G(t)$ and $\kappa(t)$ are readily specified in ADINA and the viscoelastic material model was implemented using convolution integrals relating stress and strain¹⁵.

$$\begin{aligned} s_{ij}(t) &= 2G(0) e_{ij}(t) + 2 \int_0^t e_{ij}(t-\tau) \frac{dG(\tau)}{d\tau} d\tau \\ \sigma_{ij}(t) &= 3\kappa(0) \varepsilon_{kk}(t) + 3 \int_0^t \varepsilon_{ij}(t-\tau) \frac{d\kappa(\tau)}{d\tau} d\tau \end{aligned} \quad (5)$$

Here $s_{ij} = \sigma_{ij} - (1/3)\delta_{ij}\sigma_{kk}$ is the deviatoric stress, $e_{ij} = \varepsilon_{ij} - (1/3)\delta_{ij}\varepsilon_{kk}$ is the deviatoric strain and δ_{ij} is the Kronecker delta. Both the cartilage and PMT are modeled as nearly incompressible,

i.e., $\nu_{\text{cart}} = \nu_{\text{PMT}} = 0.49$. The baseline Young's modulus for the cartilage ($E_{\text{cart}} = 300$ kPa) was based on literature values²³ and the baseline PMT stiffness ($E_{\text{PMT}} = 50$ kPa) was based on previous measurements in glandular breast tissue²⁴. We also assumed a constant $\tau_{\text{PMT}} = 0.25$ sec.

2.3 Adhesion Modeling

During acute OM, inflammation of the ME-ET systems involves increased expression of muco-adhesive glycoproteins including MUC5AC and MUC5B⁵. These glycoproteins are expressed on the apical surface of ME/ET epithelial cells and may dramatically increase adhesion forces within the ET lumen. In this study, we accounted for these mucosal adhesion forces by defining a set of non-linear, rupturable spring elements that connect nodes on opposing sides of the lumen surfaces (see Fig 1B). Specifically, nodes on lateral side of the lumen opening were paired with nodes on the medial side in a 1:1 fashion. Although these springs were specified using a linear force-displacement relationship with spring constant, K_a , they also ruptured at a distance x_r such that the spring force was zero for $x > x_r$. Once the number of spring elements is known (N_s), the total adhesion energy density in the system can be determined by

$$E_{\text{adh}} = \frac{1}{2} K_a x_r^2 N_s / A_s \quad (6)$$

where A_s is the surface area of the lumen. Although direct measurements of adhesion energy in the ET are not known, adhesion energy densities for a single leucocyte rolling on the endothelium have been estimated to be $\sim 1\text{e-}4$ N/m²⁵. However, mucus adhesion in the ET is likely higher and we therefore specified a baseline value of $E_{\text{adh}} = 8.0\text{e-}3$ N/m. However, we also varied E_{adh} over a wide range ($E_{\text{adh}} = 1\text{e-}7$ to 1.0 N/m) to account for both non-inflamed, low adhesion conditions and elevated adhesion during inflammation. The value of E_{adh} was altered by varying K_a and assuming a constant rupture force per spring of $F_r = K_a * x_r = 1.5\text{e-}4$ N.

2.4 Data and Statistical Analysis

In this study, we quantified the degree of ET opening by monitoring the percentage of springs ruptured at maximum TVPM force application. Data regarding ET opening (% spring rupture) as a function of various biomechanical parameters (E_{adh} , F_{TVP} , F_{LVP} , E_{cart} and E_{PMT}) was analyzed with a two-way repeated measures Analysis of Variance (ANOVA) to document statistical difference between groups. Data regarding ET opening vs E_{adh} and F_{TVP} were found to follow a sigmoidal relationship on a semi-log scale. Therefore, a non-linear regression with the following equation was used to analyze data from each subject

$$\Omega = \frac{100\%}{1 + e^{-(x - x_c)/b}} \quad (7)$$

where Ω is the percentage of springs ruptured, x is the logarithm of either E_{adh} or F_{TVP} and x_c represents the critical point where there is 50% ET opening. In this study we report the

inverse logarithms of χ_c as $E_{adh,c}$ or $F_{TVP,c}$ and also use the value of b to calculate the slope of the Ω vs $E_{adh,c}$ or Ω vs $F_{TVP,c}$ functions at the critical point using the following equation

$$m_{adh \text{ or } m_{TVP}} = \frac{1}{4b * 10^{\chi_c}} \quad (8)$$

All critical values and slopes at the critical point were calculated under different conditions and compared using one-way repeated measures ANOVA or one-way repeated measures ANOVA on ranks if data was not found to be normally distributed via a Kolmogorov-Smirnov test.

Finally, a sensitivity analysis was performed to investigate how changes in mucosal adhesion influenced the relative importance of tissue biomechanical parameters on ET function. For this analysis, the biomechanical parameters of interest included F_{TVP} , F_{LVP} , E_{cart} and E_{PMT} and a sensitivity value for each parameter was calculated for three different levels of mucosal adhesion.

$$\text{Sensitivity} = abs\left(\frac{\partial \Omega}{\partial x} \bigg|_{\frac{x_0}{\Omega_0}}\right) \quad (9)$$

Here, Ω is the percentage of springs ruptured, x is the parameter of interest, Ω/x is calculated via a first order derivative approximation, x_0 is the parameter value at the location of the slope calculation and Ω_0 is the percentage of springs ruptured at the location of the slope calculation. Equation (9) represents the maximum slope of the Ω vs x curve in non-dimensionalized form.

3. Results

3.1 Influence of Adhesion Energy Density on ET Function

In previous finite element models of ET function^{12,14}, which did not account for mucosal adhesion, muscle contraction resulted in uniform opening of the ET. However, for a baseline adhesion energy density of 8.0×10^{-3} N/m, muscle contraction in the current multi-scale models of ET function resulted in heterogeneous opening profiles along the length of the ET. Specifically, several subjects (E37, R55, E32) exhibited a “wave-like” opening of the ET lumen (red areas in Fig 2) where adhesion springs initially ruptured in inferior portions of the lumen near the NP at $t=1.237$ and this lumen opening proceeded proximally towards the ME. In other subjects (E82 and E63), the ET initially opened uniformly in inferior portions of the lumen and this opening then proceeded superiorly. Finally, in subject E41, opening occurred uniformly along the length of the ET. Therefore, our multi-scale computational models exhibit spatially heterogeneous and “wave-like” patterns of ET opening that begin distally/inferiorly and proceed proximally/superiorly.

As shown in Fig 3, we investigated how adhesion energy density influences the degree of ET opening by varying E_{adh} over a wide range and monitoring percentage of springs ruptured at maximum TVPM force application. In addition to varying E_{adh} at the baseline TVPM force

(0.2N) we also conducted this parameter variations for larger (0.5N) and smaller (0.08N) TVPM force values. For all F_{TVP} values, the percentage of springs ruptured for all $n=6$ subjects followed an inverse sigmoidal pattern with E_{adh} on a semi-log scale. For baseline TVPM forces (open circles), we observed relatively high amounts of ET opening ($>90\%$) for $E_{adh} < 3e-4$ N/m. However, once a critical E_{adh} value is obtained, ET function, as measured by the amount of opening, declines rapidly and E_{adh} values $> 1e-2$ N/m results in complete ET dysfunction ($<5\%$ opening). Interestingly, increasing the amount of TVP force by 2.5-fold ($F_{TVP} = 0.5N$) resulted in a dramatic rightward shift of the opening vs E_{adh} curve (dark triangles) while decreasing the TVP force by 2.5-fold ($F_{TVP} = 0.08N$) resulted in a less dramatic leftward shift of this curve (dark circles). Statistical analysis indicated that both adhesion energy density and TVPM forces were significant factors ($p<0.001$, two-way repeated measures ANOVA). This data therefore indicates that increasing adhesion energy density beyond a critical value may lead to rapid ET dysfunction and that the magnitude of this critical adhesion energy density depends on the amount of TVPM force generated.

In order to quantify the critical adhesion energy density at each TVPM force, the percentage rupture vs E_{adh} data from each patient was analyzed using equation (7). This non-linear regression was used to calculate $E_{adh,c}$ for each subject under different F_{TVP} conditions. As shown in Fig 4A, the mean $E_{adh,c}$ value under high TVP force conditions ($F_{TVP} = 0.5$ N) was significantly larger than the mean $E_{adh,c}$ value under baseline and low TVP force conditions ($p<0.001$, repeated measure ANOVA). In addition, the mean $E_{adh,c}$ values under baseline and low TVP force conditions was not statistically different. This data indicates that subjects who are able to generate large TVPM forces may be able to open the ET even under elevated adhesion conditions. In addition to quantifying the magnitude of $E_{adh,c}$, we also used equations (7) and (8) to calculate the median value of the slope of the % rupture vs. E_{adh} curves at the critical point. As shown in Fig 4B, the median value of this slope was significantly higher at lower TVP force conditions ($p<0.05$, repeated measure ANOVA on ranks). This data indicates that at low TVP forces, small changes in adhesion can significantly alter the amount of ET opening. Conversely, at high TVP forces, larger changes in adhesion are required alter the amount of ET opening.

3.2 Role of TVP Muscle Forces and Mucosal Adhesion on ET Function

Although previous models^{12,14} indicate that increasing TVP forces leads to better ET function, these models could not evaluate how increased mucosal adhesion influences TVP assisted opening of the ET. As shown in Fig 5, the percentage of springs ruptured for $n=6$ subjects followed a sigmoidal relationship with TVP force on a semi-log scale. Two-way repeated measures ANOVA indicated that both TVP forces and the level of adhesion were statistically significant factors ($p<0.001$). For baseline adhesion energy density (open circles in Fig 5), low muscle forces of $F_{TVP} < 0.08$ N did not result in any opening of the ET lumen while forces of $F_{TVP} > 0.3$ N resulted in nearly complete ET opening ($> 90\%$ spring rupture). Importantly, a 5-fold increase in the level of mucosal adhesion resulted in a dramatic rightward shift of the opening vs F_{TVP} curve (dark triangles) while a 5-fold decrease in mucosal adhesion resulted in significant leftward shift of this curve (dark circles). The percentage rupture vs. F_{TVP} data from each patient was analyzed using equation (7) to determine the critical level of TVP force needed to obtain 50% opening,

$F_{TVP,c}$, under different levels of mucosal adhesion. As shown in Fig 6A, the mean $F_{TVP,c}$ value under high adhesion energy conditions ($E_{adh} = 4e-2$ N/m) is significantly larger than the mean $F_{TVP,c}$ value under baseline and lower adhesion energy conditions ($p < 0.001$, repeated measure ANOVA). In addition, the mean $F_{TVP,c}$ value under baseline and low mucosal adhesion conditions was not statistically different. This data indicates that during conditions of elevated mucosal adhesion, significantly larger F_{TVP} forces are required to open the ET. In addition to $F_{TVP,c}$, we also used equations (7) and (8) to calculate the median value of the % rupture vs. F_{TVP} slope at the critical point. As shown in Fig 6B, the median value of this slope under high adhesion conditions was significantly lower than the slope under baseline or low adhesion conditions ($p < 0.05$, repeated measure ANOVA on ranks). This data indicates that although small changes in F_{TVP} can alter ET opening under low adhesion conditions, ET opening is less sensitive to changes in F_{TVP} under elevated adhesion conditions.

3.3 LVP Muscle Forces do not Alter ET Function

Previous computational studies^{12,14}, which neglected mucosal adhesion, indicate that changes in LVPM forces have little to no effect on the amount of ET opening. Consistent with those result, data in Fig 7A indicate that increasing LVPM forces does not have a significant impact on the amount of ET opening for all three levels of mucosal adhesion. In fact, a two-way repeated measures ANOVA indicated that although adhesion energy density was a statistically signification factor ($p < 0.001$), LVP forces were not a statistically significant factor. Therefore, changes in LVP forces do not alter the amount of ET opening regardless of the level of mucosal adhesion.

3.4 Mucosal Tissue Stiffness Alters ET Function under Low Adhesion Conditions

Previous experimental and computational studies indicate that the compliance of the ET's soft tissue elements, i.e. the perimucosal tissue (PMT) and cartilage, might be important determinates of ET function^{11,12,14,26,27}. In this study, we investigated if increases in mucosal adhesion alter the relative importance of PMT and cartilage mechanics on ET function. As shown in Fig 7B, at moderate and low levels of mucosal adhesion ($E_{adh} = 1.6e-3$ and $8.0e-3$ N/m) changes in EMT stiffness, i.e. E_{pmt} , alters the amount of spring rupture/ET opening. Interestingly, opening as a function of E_{pmt} followed a bell-shaped curve with maximum opening occurring at $E_{pmt} \sim 13$ to 33 kPa. A two-way repeated measures ANOVA indicated that both E_{pmt} and the level of mucosal adhesion were statistically significant factors ($p < 0.01$) where the opening vs E_{pmt} curves at moderate and low adhesion was statistically different than the curve at high adhesion ($E_{adh} = 4.0e-2$ N/m). A one-way repeated measures ANOVA indicated that although varying the magnitude of E_{pmt} under high adhesion conditions did not result in a statistically significant change in opening ($p = 0.8$), varying the magnitude of E_{pmt} under medium or low adhesion conditions does results in a statistically significant change in opening ($p < 0.03$). In addition, for low adhesion conditions, the amount of opening in the range $E_{pmt} = 8.0$ to 50 kPa is significantly different than the amount of opening at $E_{pmt} = 500$ kPa ($p < 0.05$). This data indicates that although changes in mucosal tissue stiffness can alter the amount of ET opening under moderate or low adhesion conditions, mucosal tissue stiffness is not a significant contributor to ET function under elevated adhesion conditions.

3.5 Decreased Cartilage Stiffness Improves ET Function during Elevated Mucosal Adhesion

As shown in Fig 7C, we also investigated how varying cartilage stiffness, i.e. E_{cart} , influenced the degree of ET opening at different levels of adhesion. For all adhesion conditions, decreasing E_{cart} lead to an increase in ET opening and this increased opening was most dramatic under high adhesion conditions. A two-way repeated measures ANOVA indicated that both E_{cart} and the level of mucosal adhesion were statistically significant factors ($p < 0.01$) and that all opening vs E_{cart} curves were statistically different. A one-way repeated measure ANOVA indicated that under high adhesion conditions, reducing E_{cart} below 120 kPa resulted in dramatic increase in ET opening while reducing E_{cart} below ~500kPa under moderate adhesion conditions ($E_{\text{adh}} = 8 \times 10^{-3} \text{ N/m}$) resulted in a more modest increase in ET opening. Therefore, this data indicates that decreasing cartilage stiffness may be an effective way to improve ET function under high adhesion conditions.

3.6 Sensitivity Analysis

Data in Figures 5 and 7 clearly indicate that the relationship between ET opening and tissue biomechanical properties depends on the level of mucosal adhesion. Here, we use a sensitivity analysis to quantify the relative importance of tissue biomechanical properties on ET function at different levels of adhesion. Eqn (9) was used to calculate a non-dimensional sensitivity parameter for each biomechanical property (F_{TVP} , F_{LVP} , E_{cart} and E_{pmt}) in each subject at the three different adhesion levels. Note that higher sensitivity values indicate that a smaller change in the parameter results in more ET opening and thus indicates that ET opening is more sensitive to that parameter. As shown in Fig 8, under low adhesion conditions (black bars), ET opening is highly sensitive to changes in TVPM forces and peri-mucosal tissue (PMT) stiffness and insensitive to changes in LVPM forces and cartilage stiffness. Interestingly, increasing adhesion energy density resulted in a statistically significant decrease in the sensitivity of ET opening to TVPM forces and a statistically significant increase in the sensitivity of ET opening to cartilage stiffness. In addition, although the sensitivity of ET opening to TVP forces was larger than the sensitivity to cartilage stiffness under low adhesion conditions, this trend was reversed under high adhesion conditions where the sensitivity to cartilage stiffness was larger than the sensitivity to TVPM forces. There was also a trend towards decreasing sensitivity to PMT stiffness at high adhesion although this change was not statistically significant ($p = 0.4$). This data clearly demonstrates that increases in mucosal adhesion can significantly alter the sensitivity of ET opening to different biomechanical parameters and thus alters the biomechanical mechanisms of ET function.

4. Discussion

It is well established that an inability to open the collapsible Eustachian tube (ET) leads to middle ear pathologies and can extend the duration and severity of Otitis Media (OM)²⁸. Although previous histological and computational studies^{6,7,9,12,22} have elucidated the anatomical and tissue biomechanical mechanisms of ET function, these studies did not account for the increase in mucosal adhesion within the ET lumen that occurs during inflammatory OM. In this study, we have developed multi-scale finite element models of ET

function that accounts for mucosal adhesion and have used these models to investigate how changes in mucosal adhesion influences the biomechanical mechanisms of ET function.

Unlike our previous finite element models of ET function¹², the current computational models incorporate several physiological and biophysical features relevant to muscle assisted opening of the ET during swallowing. First, we have incorporated a transient TVPM and LVPM contraction sequence into our 3D models to better simulate the muscle contraction patterns that occur in-vivo²⁹. In addition, we incorporated adhesion elements into the ET lumen in order to simulate elevated mucosal adhesion that would occur during increased glycoprotein expression⁵. As shown in Fig 2, these more sophisticated models documented a complex and spatially heterogeneous pattern of ET opening. First, in 3 subjects, opening occurred in a “wave-like” fashion where adhesion springs initially ruptured in inferior aspects of the lumen near the nasopharyngeal orifice and this opening then proceeded superiorly and proximally towards the middle ear. A slightly different pattern of opening was observed in 2 subjects where opening was uniform along the length of the ET but did occur initially in inferior aspects and proceeded superiorly. These heterogeneous patterns of ET opening are consistent with direct endoscopic observations. Specifically, Poe et al³⁰ have used video endoscopy to directly observe the pattern of lumen dilation/opening in adults subjects and these in-vivo studies indicate that the ET opens in a “wave-like” pattern with lumen dilation “beginning distally and inferiorly, then opening proximally and superiorly.”³⁰. Previous computational models of ET opening^{9,10,12,14}, which did not account for mucosal adhesion, were not able to replicate this “wave-like” opening. Therefore, our enhanced multi-scale finite element models more accurately replicate experimental observations regarding the pattern of lumen opening.

Although contraction of the TVPM is required for normal ET function³¹, it is not known how changes in mucosal adhesion might influence the ability of the TVPM to open the ET. As shown in Fig 3, our computational simulations indicate that exceeding a critical value of mucosal adhesion can lead to rapid ET dysfunction and inability to open the ET. Interestingly, even when large TVPM forces are applied to the ET, complete ET dysfunction can occur if the adhesion energy level is high. We also demonstrated that the critical adhesion value depends on the magnitude of TVPM forces (Fig 4A) where lower TVPM forces leads to smaller critical adhesion values. Therefore, the model predicts that subjects with lower TVPM forces may not be able to overcome minor increases in luminal adhesion. In addition, data in Fig 4B indicates that subjects with lower TVPM forces are more sensitive to changes in mucosal adhesion where small increases in adhesion can lead to rapid ET dysfunction. Conversely, subjects with high TVP forces are less sensitive to increases in mucosal adhesion.

As shown in Fig 5, we also quantified the magnitude of TVPM force, F_{TVP} , required to open the ET at a given level of adhesion and demonstrated that ET opening is a sigmoidal function of F_{TVP} on a log-scale. Increases in adhesion resulted in a significant rightward shift of the opening vs F_{TVP} curve and this shift was quantified by calculating the critical TVPM force required to generate 50% opening. As shown in Fig 6A, the critical TVPM force was ~2-3x higher during conditions of elevated mucosal adhesion. However, modest increases in adhesion from $E_{adh}=1.6e-3$ N/m to $E_{adh}=8e-3$ N/m did not significantly

influence the critical TVP force. Therefore, although larger TVPM forces are needed to open the ET during conditions of elevated mucosal adhesion, modest elevations in adhesive forces may not result in significant ET dysfunction in adults. This is consistent with the fact that most adults are able to open the ET and ventilate the middle ear during upper respiratory infections³. However, our models predict that large increases in adhesive forces may lead to ET dysfunction in adults who are not able to generate larger TVP forces. For example, older adult subjects likely have reduced TVPM forces due to atrophy of the TVPM³² and reduced attachments of the TVPM to PMT³³. Our models suggest that these older adults might be more susceptible to developing ET dysfunction during upper respiratory infections. Finally, data in Fig 6B indicates that under low/baseline adhesion conditions, exceeding the critical TVP force value quickly leads to significant ET opening. However, under elevated adhesion conditions, there is a more gradual increase in ET opening as one exceeds the critical TVPM force value. Therefore, although TVPM contraction may be required for proper ET function, our models indicate that increases in mucosal adhesion can alter the sensitivity of ET function to TVPM forces.

In addition to TVPM forces, changes in other tissue biomechanical parameters can also have a significant impact on ET function. For example, tubal compliance has been shown to change with age and decreased compliance in the elderly was associated with stenotic ET³⁴. In addition, computational studies indicate that ET function in adults is equally sensitive to changes in mucosal tissue stiffness and TVP forces¹². In this study we investigated how increased mucosal adhesion alters the sensitivity of ET function to different tissue biomechanical properties including LVPM forces, PMT stiffness and cartilage stiffness (Fig 7). Consistent with previous studies³⁵, changes in LVPM force did not significantly alter the degree of ET opening. Although previous computational studies which neglected mucosal adhesion documented an inverse relationship between ET opening and PMT stiffness^{12,14}, the current multi-scale models of ET function, which account for mucosal adhesion, exhibited a non-monotonic relationship between ET opening and PMT stiffness. For moderate levels of adhesion increasing PMT stiffness initially results in increased ET opening while elevating $E_{pmt} > 40\text{kPa}$ results in decreased ET opening. The initial increase in ET opening with increasing E_{pmt} may be due to the fact that stiffer tissues can more efficiently transmit TVP forces applied on lateral surfaces of the PMT to the luminal surfaces. This increased force transmission would then facilitate spring rupture and thus more ET opening. However, further increases in E_{pmt} would lead to less tissue deformation and this would reduce the number of ruptured springs. Therefore, altering PMT stiffness may have non-monotonic effects on ET opening when adhesion forces are significant.

Consistent with previous studies which neglected mucosal adhesion¹², the current models indicate that under low adhesion conditions, ET opening is highly sensitive to increases in TVPM force and relatively insensitive to LVPM forces or cartilage stiffness (Fig 8). However, under elevated adhesion conditions, ET opening was less sensitive to TVPM forces and more sensitive to cartilage stiffness. This finding might have important clinical implications since it suggests that very large changes in TVPM forces might be needed to restore ET function during inflammatory OM. In addition, data in Fig 7C indicates that when mucosal adhesion is elevated, subjects with increased cartilage stiffness may be more susceptible to ET dysfunction (i.e. 0% opening). Consistent with this finding, previous

experimental studies³⁶ indicate that adults with Otitis Media exhibit decreased tubal compliance (increased tissue stiffness) relative to “normal” adults without Otitis Media. Furthermore, results from this study indicate that reducing cartilage stiffness may be an effective way to restore ET function. Interestingly, a surgical treatment option known as ET tuboplasty which involves laser ablation of mucosal/cartilage tissue, has been reported to improve otologic outcomes in adults³⁷. Since laser ablation would result in decreased tissue stiffness, these results are also consistent with our model predictions. Therefore, changes in mucosal adhesion can significantly alter the biomechanical mechanisms of ET function in adults and alterations in cartilage tissue mechanics may be an effective way to restore ET function during elevated adhesion conditions.

As with all computational studies, several model limitations could be addressed in future studies. First, the finite element models used in this study were based on histological data obtained in healthy adults and thus demonstrate minimal anatomical differences (Fig. 2). As a result, the current models may not directly translate and/or accurately represent ET function in pediatric patients and/or patients with craniofacial abnormalities. Importantly, these patients often exhibit abnormal anatomical and tissue mechanical properties which place them at increased risk for developing ET dysfunction and Otitis Media. Therefore, future studies should develop multi-scale models of ET function in the pediatric and cleft palate populations and use these models to investigate the relative importance of tissue anatomy and mucosal adhesion on ET function/dysfunction. Note that these future models will require both the use of histological images from the pediatric and cleft palate populations and the use of tissue mechanical properties relevant to these populations. Second, in this study the degree of ET opening was characterized by the percentage of springs ruptured during maximal muscle force application. However, previous studies¹² have used computational fluid dynamic (CFD) and fluid-structure interaction (FSI) techniques to calculate the resistance to airflow within the lumen as a measure of ET opening. Although beyond the scope of the current study, incorporating CFD and FSI techniques could yield additional insights into how adhesion dynamics influences ET function. In addition to increases in adhesion due to mucus glycoprotein interactions, modeled as adhesive springs in this study, other biophysical changes including changes in mucosal fluid viscosity and surface tension¹³ may also alter adhesion dynamics. Therefore, future studies could use sophisticated free-surface CFD techniques³⁸ to model air-liquid flows within the ET.

In summary, we have developed a multi-scale model of ET function that accounts for increased mucosal adhesion during inflammatory Otitis Media. These models used adhesion springs to model adhesive glycoprotein interactions and for all subjects a critical value of adhesion, $E_{adh,c}$, was observed where increases in adhesion above $E_{adh,c}$ led to rapid ET dysfunction. Although increasing muscle forces (F_{TVP}) was found to increase $E_{adh,c}$, results also indicate that the sensitivity of ET function to TVPM forces was significantly reduced under elevated adhesion conditions. In addition to altering the relative importance of TVPM forces, our models indicate that increased mucosal adhesion also alters other biomechanical mechanisms of ET function. In particular, although ET opening was relatively insensitive to changes in cartilage stiffness at low levels of adhesion, under elevated adhesion conditions, i.e. infection/inflammation, ET opening was found to be highly sensitive to changes in cartilage stiffness where decreased cartilage stiffness results in improved ET function. This

study has therefore provided new information about how changes in mucosal adhesion during inflammatory OM might alter the biomechanical mechanisms of ET function.

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Figure 1.

(A) Location of ET tissue structures in a cross-sectional histological image. (B) Representative finite element model of ET cartilage (green) and periluminal mucosal tissue (red) with a lumen (blue) containing adhesion springs. Pink arrows indicate location of TVPM and LVPM force application. (C) Time-dependent profile of muscle forces used to simulate ET opening during swallowing.

Figure 2.

Eustachian Tube opening (red) as a function of real time (t) in different subjects. Subjects E37, R55 and E32 exhibit a “wave-like” opening beginning inferiorly near the nasopharynx and progressing into the ET. An inferior to superior pattern of opening was observed in subjects E82 and E63 while a uniform pattern of opening was observed in subject E41.

Figure 3.

Percentage of springs ruptured at maximum TVPM force, F_{TVP} , application as a function of adhesion energy density for different values of F_{TVP} . Data represents mean $\pm$ standard error for $n=6$ subjects. Two-way repeated measures ANOVA indicated that both adhesion energy density and F_{TVP} were statistically significant factors ($p<0.001$).

Figure 4.

A) Average values of the critical adhesion energy density ($E_{adh,c}$) at different TVPM forces. Data represents mean $\pm$ standard error and * indicates statistically significant differences by repeated measures ANOVA ($p < 0.001$). B) Median values of the slope at the critical adhesion point for different TVPM forces. Data represent median $\pm$ 75/25 percentile and * indicates statistically significant differences by repeated measures ANOVA on ranks ($p < 0.05$).

Figure 5. Percentage of springs ruptured as a function of TVPM force for different values of adhesion energy density. Data represents mean $\pm$ standard error for $n=6$ subjects. Two-way repeated measures ANOVA indicated that both TVPM forces and adhesion energy density were statistically significant factors ($p<0.001$).

Figure 6.

A) Average values of the critical TVPM force ($F_{TVPM,c}$) at different levels of mucosal adhesion. Data represent mean $\pm$ standard error and * indicates statistically significant differences by repeated measures ANOVA ($p < 0.001$). B) Median values of the slope at the critical TVPM force for different values of adhesion energy density. Data represent median $\pm$ 75/25 percentile and * indicates statistically significant differences by repeated measures ANOVA on ranks ($p < 0.05$).

Figure 7.

Percentage of springs ruptured as a function of LVPM force (A), mucosal tissue stiffness, E_{pmt} (B) and cartilage stiffness, E_{cart} (C). Data represent mean $\pm$ standard error for $n=6$ subjects for 7B and 7C and $n=5$ subjects for 7A. LVPM force was not a statistically significant factor (A) but E_{pmt} did significantly alter ET opening at low (dark circles) and moderate (open circles) adhesion (B; $p<0.01$). * indicates statistically significant difference with respect to % rupture at $E_{pmt}=500$ kPa. (C) Variations in E_{cart} significantly alter ET opening at all levels of adhesion ($p<0.01$) and ^ indicates statistically significant difference ($p<0.001$) with respect to % rupture at $E_{cart}=3000$ kPa.

Figure 8.

Sensitivity values for different tissue biomechanical parameters as a function of adhesion energy density. Data represents mean $\pm$ standard error for $n=6$ subjects (TVP, Ecart and Epmt) or $n=5$ subjects (LVP). * indicates statistically significant differences within a given tissue biomechanical parameter ($p < 0.05$) and ** indicates statistically significant differences between tissue biomechanical parameters at different adhesion levels ($p < 0.05$).