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Permeability of canine vocal fold lamina propria

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Abstract

Objective—Determine the permeability of excised canine vocal fold lamina propria

Study Design—Basic Science

Methods—Vocal folds were excised from canine larynges and mounted within a device to measure the flow of 0.9% saline through the tissue over time. The resultant fluid volume displaced over time was then used in a variation of Darcy's law to calculate the permeability of the tissue. Permeability was found through each anatomical plane of the vocal fold, with 5 samples per plane. Permeability was also found for lamina propria stretched to 10%, 20%, and 30% of initial length to determine the effects of tensile strain on permeability, with 5 samples per level of strain.

Results—Permeability was found to be 1.40×10^{-13} m³/s/kg through the sagittal plane, 1.00×10^{-13} m³/s/kg through the coronal plane, and 4.02×10^{-13} m³/s/kg through the axial plane. It was significantly greater through the axial plane than both the sagittal ($p=0.025$) and coronal ($p=0.009$) planes. Permeability under strain through the sagittal plane was found to be 1.94×10^{-13} m³/s/kg under 10% strain, 3.35×10^{-13} m³/s/kg under 20% strain, and 4.80×10^{-13} m³/s/kg under 30% strain. The permeability significantly increased after 20% strain ($p<0.05$).

Conclusion—Permeability in canine vocal fold lamina propria was found to be increased along the anterior-posterior axis, following the length of the vocal folds. This may influence fluid distribution within the lamina propria during and after vibration. Similarly, permeability increased after 20% strain was imposed on the lamina propria, and may influence vocal fold dynamics during certain phonation tasks.

Keywords

Permeability; vocal folds; lamina propria; strain

Introduction

Vocal fold lamina propria is a biphasic tissue in which mechanical properties are defined by both solid and liquid dynamics. The tissue is characterized by a porous extracellular matrix composed of various proteins that elicit different mechanical properties, such as hyaluronic

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acid, elastin, and collagen¹. Interstitial fluid saturates this solid matrix, creating a fluid-filled porous material. Together, the continuous solid and liquid components elicit a viscoelastic effect characterized by nonlinear material deformation over time. This property allows for the formation of mucosal waves that result in phonation², but also results in time-dependent displacement of solid and liquid during and immediately following vibration relative to the starting positions. It is thought that the sequestered fluid within the extracellular matrix cushions tissue from impacts^{3,4} preventing damage during high vibratory stress loading⁵. Fluid is also responsible for determining local tissue stiffness², which, when altered, increases the risk of injury⁶. As a result, the movement of fluid within the lamina propria matrix influences vocal fold health. Both physical⁷ and computer⁸ models have found that the vibratory patterns of vocal folds may cause internal fluid redistribution relative to the solid matrix. Consequences of local imbalances in fluid hypothesized by these models may therefore lead to increased stress concentrations where fluid accumulates above normal levels, or expose tissue to greater impact forces where fluid is depleted. The resistance of the tissue to fluid movement within the matrix can be quantified by the permeability of the solid matrix, and is an essential parameter for properly understanding the etiology of vocal fold injuries.

Computer models of vocal fold tissue have recently attempted to describe mechanical effects from both the solid and liquid phases^{9,10}, but have relied on permeability values determined from articular cartilage, where permeability has been extensively measured¹¹⁻¹⁴. Although both vocal fold lamina propria and cartilage are considered viscoelastic and fluid-saturated porous materials, their solid structures differ dramatically. In fact, some reported permeability figures were collected under pressure conditions that are an order of magnitude different from physiological conditions experienced by vocal folds¹¹. Permeability and its effects on vocal fold physiology have remained unstudied, and are likely to be different from cartilage due to a differing extracellular matrix structure and stiffness.

Xu and Chan, 2008 measured the permeability of a decellularized vocal fold in a single direction in order to characterize its properties for use in tissue regeneration¹⁵. However, vocal folds have traditionally been treated as transversely isotropic¹⁶ due to the arrangement of collagen fibers in the extracellular matrix, which led previous biphasic models to assume higher permeability in the anterior-posterior direction^{9,17}. Nevertheless, the permeability of unmodified vocal fold lamina propria remains unknown, and the directional dependence of permeability in the vocal folds has never been physiologically validated. We therefore hypothesized that permeability is significantly different along different directions in the tissue. Similarly, permeability has been assumed to be constant in all previous models. However, the vocal folds can experience up to 20% strain during vibration¹⁸, which in turn could affect permeability. Variable permeability under tissue strain would affect interstitial liquid movement during phonation, possibly resulting in different stress concentrations than previously predicted. We therefore hypothesized that permeability of vocal fold tissues changes when a strain load is applied.

Methods

Vocal folds were harvested from canines euthanized for non-research purposes. Larynges were excised from the canines and stored in 0.9% saline solution at -12°C , similar to the process described by Jiang and Titze (1993)¹⁹. They were subsequently thawed immediately prior to use, as determined by Chan and Titze (2003)²⁰ to preserve their integrity. The larynges were cut along the sagittal at the midline into two halves, and the vocal folds were removed from the surrounding tissue as described by Hanson (2011)²¹. The thyroarytenoid muscle was dissected away, leaving the lamina propria and epithelial cell layer. The tissue samples were subsequently placed in 0.9% saline to stay hydrated before being mounted on the permeability measuring apparatus.

Intrinsic permeability was determined by measuring the flow of 0.9% saline through the tissue sample under a known near-constant hydrostatic differential pressure. The method and apparatus used for the flow measurements was similar to ones previously described^{13,15} (Figure 1). In order to measure permeability through the sagittal plane, lamina propria was laid flat and secured between two rubber rings attached to a metal clamp that was continuous with the water column. The rubber rings left 8.04 mm^2 of the lamina propria surface exposed to the water column and sealed the circumference of the tissue once clamped together. A second ring was inserted around the tissue in order to prevent losses in pressure from possible radial leakage through the tissue. A stiff, porous filter with permeability much greater than that of the tissue was placed beneath the sample to minimize viscoelastic deformation during pressure loading. Below the tissue and in line with the top water column, the clamp was attached to a capillary water column that provided measurements of water volume displaced through the tissue over time with milliliter resolution. The volume of fluid accumulated in the capillary was calculated by using the known column diameter and change in water level measured with a caliper. Flow Q was calculated by dividing the measured displaced volume in the capillary by the time elapsed. Permeability was then calculated using a variation of Darcy's Law¹³,

$$k = \frac{Qh}{A\Delta p} \quad (\text{Equation 1})$$

where k is intrinsic permeability of a substance, A is the cross-sectional area of the substance exposed to the fluid, h is the thickness of the substance, and p is the pressure drop across the substance. Small changes in differential pressure due to fluid displacement through the tissue samples during the experiment were regarded as negligible compared to the overall large difference in pressure.

Differential pressures of 5 kPa ($n=5$) and 20 kPa ($n=5$) were first tested on vocal fold lamina propria samples through the sagittal plane in order to compare the effect of pressure on permeability. These pressures were selected because they represent typical (5 kPa) and extreme (20 kPa) pressures that the lamina propria might experience during phonation²². No significant differences in permeability were found between the two pressure groups ($p=0.098$). As a result, a 20 kPa pressure differential was used for the remaining experiments in order to decrease experiment time. Similarly, the 20 kPa pressure differential required a

taller water column that minimized error caused by minor losses in height due to saline flow through the tissue samples.

In order to measure permeability in the coronal (n=5) and axial (n=5) planes (shown in Figure 3), the excised samples were first cemented between 2 rubber blocks using Loctite adhesive cement (Henkel Corporation, Westlake, Ohio) (Figure 2a, 2b). This cement had previously been determined to be non-permeable by replacing a tissue sample with the adhesive and measuring fluid flow through the dried material. Upon setting, excess tissue extending from the block was removed to expose the cross section of the tissue sample. The values of A and h were measured with a caliper, and the block was placed in the apparatus between the clamps with the cross section of the tissue aligned with the water column. Q and k were subsequently calculated as described above at a differential pressure of 20 kPa.

The permeability of excised vocal fold lamina propria was also measured under strain loads through the sagittal plane. The most significant strain is applied via vocal fold elongation in the sagittal direction, so we chose to study changes in permeability with strain applied in this plane. Vocal folds were again excised, however, this time pieces of thyroid and arytenoid cartilages were left intact on the samples where the vocal folds inserted on the cartilage tissue. Three sutures were then inserted in both the excess thyroid and arytenoid cartilages, allowing the cartilage to anchor the sutures and distribute strain to the lamina propria. Suturing through the cartilage also ensured there was no mucosal damage due to suture placement. Samples with sutures were then mounted on a custom device that allowed a tensile strain to be applied to the samples (Figure 2c). The lengths of the samples prior to elongation were measured with a caliper and were subsequently used to calculate a strain deformation of 10%, 20%, or 30%. Samples were then manually stretched with the device to the desired strain length and inserted between the clamps of the permeability apparatus while held at the new length.

Mean permeability values ($\pm$ standard deviation) calculated from Equation 1 were found for each group of samples, and these means were then compared between groups using t-tests.

Results

The mean ($\pm$ standard deviation) intrinsic permeability values were measured for each anatomical plane, and were found to be $1.40 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 3.98 \times 10^{-14}$) through the sagittal plane, $1.00 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 3.89 \times 10^{-14}$) through the coronal plane, and $4.02 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 2.22 \times 10^{-14}$) through the axial plane (Figure 4). Although the permeabilities through the sagittal and coronal planes were not found to be significantly different, the permeability through the axial plane was found to be significantly higher than both the sagittal ($p=0.025$) and coronal ($p=0.009$) planes. The means and standard deviations for each plane are shown in Table 1. These results therefore indicated a direction-dependency of permeability to fluid flow through the tissue.

Similarly, permeability of lamina propria was measured under several strain loads through the sagittal plane. Mean ($\pm$ standard deviation) intrinsic permeability values were found to be $1.94 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 6.52 \times 10^{-14}$) under 10% strain, $3.35 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 1.60 \times 10^{-13}$)

under 20% strain, and $4.80 \times 10^{-13} \text{ m}^3/\text{s/kg}$ ($\pm 1.21 \times 10^{-13}$) under 30% strain (Figure 5). Although mean permeability generally increased with increasing strain, only tissues strained by 20% had significantly higher permeability than non-strained tissues ($p=0.05$), and tissues strained by 30% had higher permeability than those strained by 10% ($p=0.004$), and unstrained tissue ($p<0.001$). The means and standard deviation at each strain level are reported in Table 2.

Discussion

Vocal fold lamina propria is a fluid-filled porous solid in which interstitial fluid is able to displace relative to the surrounding solid extracellular matrix. The permeability of this tissue is a fundamental determinant of fluid-solid interactions that are consequential to overall mechanics and tissue health. The intrinsic permeability of canine vocal fold lamina propria was found to be dependent on direction within the tissue, with almost a 2-times greater permeability in the anterior-posterior direction. This anisotropy of the permeability through the canine lamina propria may be created by a complex, layered arrangement of extracellular collagen deposition similar to that found in humans²³. Although it is likely that the vibration patterns of vocal folds during phonation are the major contributor to the displacement patterns of fluid within the lamina propria, the permeability may play a role in the degree of fluid displacement that occurs in each axis. Furthermore, the permeability likely plays a role in the redistribution and exchange of interstitial fluid immediately upon cessation of phonation, with a preference for the anterior-posterior axis. It is unknown exactly what role this plays in vocal fold health, but the transversely anisotropic permeability found may be influential in the etiology of certain lesions and progression of edema through the vocal fold structure. Studies investigating the advancement of edema in the vocal folds still must take this into consideration.

Lamina propria also significantly increased in permeability upon undergoing strain loading, indicating that permeability changes occur with just a 20% change in tissue deformation. This suggests that the greatest deformations physiologically possible alter the permeability of the lamina propria. It is therefore necessary to consider the amount of deformation that occurs within the tissue when investigating the movement of fluid through the lamina propria during phonation. Straining the vocal folds could increase the amount of fluid displacing through the tissue during phonation, thereby affecting the stress placed on the local tissue. On the other hand, typical deformation during normal phonation is unlikely to affect the permeability. Small deformations were not found to significantly affect the permeability, with no incremental 10% increase in strain significantly increasing the permeability (permeability required a 20% increase in strain before increasing). As a result, the permeability of lamina propria can be treated as constant within a certain degree of deformation.

The trends and values reported in this manuscript can be generalized to human tissue for the purposes of modeling and understanding tissue biomechanics. However, there were several limitations to this study. First, the viscoelastic nature of vocal folds could have possibly altered the lamina propria structure over time during the experiments, particularly since the edges of the tissue samples were clamped to seal the tissue within the water column.

However, fluid accumulation was observed to linearly increase in all of our experiments, indicating that any viscoelastic influences were minimized by the overall level of permeability. Similarly, the high viscoelasticity of the lamina propria minimized possible deformation to the bulk of the lamina propria caused by edge deformations, a property found with stiffer cartilage¹³. It is also possible that the preparations of the samples altered their permeability values. Despite the likeliness that removal of the muscle layer did affect lamina propria, the majority of lamina propria below the affected surface was not exposed to lacerations and remained intact, therefore minimizing effects of preparation on overall permeability. The small sample size for each group may mask any differences in permeability that do exist. Finally, future work might look at how a trilayered tissues, such as human or leporine, might differ from canine tissue in terms of permeability.

It is also important to note that the epithelial layer could not be removed from the lamina propria samples measured through the sagittal plane. Although it is difficult to determine what effect this had on the measured fluid flow, the results remained on the same order of magnitude as the other directions. Furthermore, we expected the permeability to be different through the sagittal plane due to the layered arrangement of lamina propria from superficial to deep levels²³. Fluid through the coronal and axial plane could traverse such layers simultaneously, whereas fluid through the sagittal plane must traverse many layers one at a time. As a result, these results should be interpreted cautiously, and future studies should focus on this using a larger sample size.

Conclusion

Vocal fold lamina propria was found to be relatively elevated along the anterior-posterior direction compared to the other axes. As a result, the ability for fluid to move within the interstitial space of the lamina propria is dependent on direction. Similarly, physiologically high levels of deformation of the lamina propria also increase permeability. Permeability of vocal fold lamina propria is therefore non-uniform and dependent on the level of deformation applied to the tissue, which should be considered by future studies involving the influences of fluid within the lamina propria on vocal fold lesions and edema.

Acknowledgments

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Figure 1.

A diagram of the full apparatus used to measure permeability. The water reservoir maintains near constant pressure across the tissue sample. A capillary is used to measure the volume of flow through the tissue over time. A cross section of the tissue mounting apparatus is enlarged to show the tissue, rubber ring, and material of known high porosity placed below the sample.

Figure 2.

Diagrams of the mounting apparatus for the tissue. (A) The mounting apparatus for tissue in the sagittal and coronal directions. (B) The mounting apparatus for tissue in the axial (longitudinal) direction. Tissue is mounted between two halves of a rubber disk with a hole in the center. Locite liquid cement is used to create a watertight seal and tissue that protrudes from the disk is cut away. (C) The mounting apparatus for tissue under strain. Sutures are placed in the cartilage to allow the tissue to be stretched to strains of 10%, 20% and 30% by attaching the sutures to the screws and tightening to the desired strain level. A rubber ring is used in all three set-ups to create a watertight seal and prevent radial leakage.

Figure 3.

A schematic of the planes through which permeability was measured: A) sagittal plane; B) coronal plane; C) axial plane.

Figure 4.

A box plot of the permeability results for each plane. The bold middle line in the middle of the box denotes the medians, with the 25th and 75th percentiles shown by the top and bottom of the box. Whiskers show the 5th to 95th percentile range and any points outside that range shown with a circle.

Permeability by Strain Level

Figure 5.

A box plot of permeability results for each strain level. The bold middle line in the middle of the box denotes the medians, with the 25th and 75th percentiles shown by the top and bottom of the box. Whiskers show the 5th to 95th percentile range and any points outside that range shown with a circle.

Table 1

Permeability results for each plane. Mean and standard deviation are reported.

Plane	Mean	Standard Deviation
Coronal	9.99391E-14	3.88898E-14
Frontal	4.02027E-13	2.22149E-13
Sagittal	1.40498E-13	3.9755E-14

Table 2

Permeability results for each strain level. Mean and standard deviation are reported.

Strain	Mean	Standard Deviation
0%	1.40498E-13	3.9755E-14
10%	1.93826E-13	6.51785E-14
20%	3.35033E-13	1.59906E-13
30%	4.7936E-13	1.20898E-13