Development of Excised Larynx

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Summary

The larynx is a complex organ which has a role in a variety of functions such as phonation, breathing, and swallowing. To research these functions, it is widely accepted that in vivo studies provide more anatomically and physiologically relevant findings. However, invasive procedures are generally needed to measure variables such as subglottal pressure, vocal fold tension and stiffness, and cricothyroid muscle stretch. Performing studies using excised larynges is a useful technique which makes it possible to not only measure phonation parameters but control them as well. Early studies using excised larynges mainly focused on controlling specific parameters and mathematical modeling simulations. The use of these studies has helped further research in laryngeal anatomy, imaging techniques, as well as aerodynamic, acoustic, and biomechanical properties. Here, we describe the progress of this research over the past five years. The number of accepted animal models has increased and ideas from excised larynx studies are starting to be applied to treatment methods for laryngeal disorders. These experiments are only valid for an excised situation and must continue to be combined with animal experimentation and clinical observations

Keywords

Excised larynx; Phonation; Laryngoplasty

INTRODUCTION

The larynx is a complex organ which has a role in phonation, breathing, and swallowing. Many years ago, Leonardo da Vinci found that airflow could animate the vocal folds of a cadaver to produce voice.¹ However, it is difficult to acquire human larynges; thus many

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kinds of excised animal larynges are used to help us understand the mechanisms of phonation. Due to their similarities to the human larynx, it is common to use excised canine and porcine larynges for phonation research. In the past five years alone, the variety of animals included in excised larynx studies has increased. In addition to canine and porcine larynges, recent work has been done with rabbit, sheep, cow, tiger, and elephant larynges.²⁻⁵ Alipour et al. compared six human larynges to other animal species and found that the average oscillation frequency was similar between human and canine larynges, but the human vocal fold had a larger oscillation frequency range which was more similar to those of pig larynges.⁶ In order to properly use different animals as vocal models, some differences must also be accounted for. For example, sheep do not have a defined break between the true and false vocal folds.⁷ The herbivorous cow, sheep, and pig have a larger epiglottis and arytenoids with lateral walls higher than those of the dog and human.⁸ Comparing viscoelastic properties, Alipour et al. found that the tissues of cow, sheep, and pig have different Young's Moduli and that the pig vocal folds offer a good model of phonation.⁹

The differences are important to consider when using these animals as models, because the anatomical and geometric differences can help explain discrepancies in variables of interest. Dollinger et al. summarized applications of human and animal larynges, both excised and in vivo. They depicted a general picture of the excised larynx.¹⁰ Due to the rapid development of excised larynx research, we will mainly contain our discussion to research occurring within the past five years. The main discussion here will be about basic research into the anatomic, acoustic, and aerodynamic properties of the larynx and how those can be used to evaluate clinical treatment options.

Basic research

In order to provide the best clinical treatment of the larynx, it necessary to understand some of its anatomic, acoustic, and aerodynamic properties. These properties are capably researched through imaging techniques such as high-speed video and other methods of determining biomechanical properties. Knowledge of these properties allows clinical testing to determine how they may be affected by various surgical techniques and advanced treatment options. Anatomy

Upon excision of a larynx, it is very common to freeze it. But, does this affect the anatomical structure in any way? Stevens et al. imaged five fresh porcine larynges with a micro-CT scanner, and then rescanned them after they were frozen. They found about 5% geometric distortion in the frozen larynges. They also measured porcine vocal fold medial surface geometry and quantified the 3D geometric data. They found that the geometries of porcine vocal folds were very similar to that of human and canine vocal folds and concluded that porcine larynges are acceptable laryngeal models for study.² Understanding vocal anatomy is at the basis of laryngeal surgery and many researchers use excised larynges to learn more about them. Sperandio et al. studied the intralaryngeal portion of the inferior laryngeal nerve in human excised larynges. They found significant, gender-specific differences in laryngeal dimensions. Within each gender, however, the location where the thyroarytenoid branch of the inferior laryngeal nerve enters the thyroarytenoid muscle has

little variance.¹¹ Mobashir et al. measured the membranous vocal fold (VF) length, cartilaginous VF length, whole VF length, VF width, VF thickness, length of the epiglottis, width of the epiglottis, and height of the larynx both endoscopically and with freshly excised human larynges. They showed that laryngeal dimensions are larger in males than in females. There was a nonsignificant difference between endoscopic measurement and fresh excised laryngeal measurement on the length of membranous vocal folds.¹² An accurate understanding of the position, anatomy, structure, and gender differences of the larynx can increase the value of clinical work.

Acoustic Properties—There are many acoustic properties that can be measured to analyze the vocal folds including fundamental frequency (F_0), jitter, shimmer, sound pressure level (SPL), and signal-to-noise ratio (SNR). F_0 is defined as the lowest frequency of a periodic waveform. While subglottal pressure, tension, stiffness, cricothyroid muscle stretching, mass, and layer composition of the vocal folds all affect F_0 in free-moving animals, it is not easy to account for so many variables with *in vivo* experiments. Garcia et al. used an experimentally-controlled excised larynx setup to measure the minimum fundamental frequency (f_0), body size, and vocal fold length across eleven primate species while controlling subglottal pressure and vocal fold tension. They found that vocal fold length plays a large role in determining f_0 , and that laryngeal size is not always related to the organism's body size.¹³ Pulvermacher and Xue et al. considered acoustic perturbation parameters jitter (frequency instability) and shimmer (amplitude instability) with regards to the symmetry of an anterior glottic web of excise canine larynges. They determined that the symmetry vs. asymmetry of the web had no effect on either parameter.¹⁴ Mills et al. used excised rabbit larynges to show a decreasing trend in SPL at the phonation instability threshold as vocal folds were manually elongated.³ SNR was also successfully measured in excised rabbit larynges by Maytag et al., but no other significant conclusions were made.¹⁵ All of these parameters offer insight into the phonatory function of the larynx.

Aerodynamic Parameters—Aerodynamic properties are also used to describe phonation. Phonation threshold pressure (PTP) and phonation threshold flow (PTF) are defined respectively as the minimum subglottal pressure (SGP) and airflow required to initiate phonation.^{16,17} Jiang et al. developed a noninvasive method of measuring PTP using complete airflow interruption, making it clinically useful.¹⁶ Factors such as vocal tract loading, glottal geometry, and vocal fold tissue properties and behaviors have been shown to affect PTP and PTF.^{17–21} PTF is more sensitive to altering the glottal width than PTP, and thus has more utility in diagnosing vocal fold paresis or paralysis.²² Looking at structures above the glottis, Alipour and Finnegan found that the presence of supraglottic laryngeal structures can increase PTP and SPL levels of the canine larynx.²³ Alipour and Karnell observed high-speed video images, electroglottographs, glottal airflow, SGP, and acoustic signals in seven excised canine larynges with differing ventricular gaps. They found that decreasing the ventricular gap size increases glottal resistance, and that a wider ventricular gap increases F_0 and decreases glottal resistance. SPL was unchanged by the changing ventricular gap size.²⁴ At the level of the glottis, Hou et al. measured the mucosal wave, acoustic signal, and aerodynamic parameters of excised canine larynges with various levels of SGP and differing extents of glottal incompetence (GI). They found that SGP and the

extent of GI played a significant role in affecting specific parameters such as jitter, shimmer, and PTP.²⁵ Oren et al. used a canine laryngeal model to examine how asymmetries of the vocal folds affect acoustic parameters and the intraglottal flow characteristics. They found that asymmetries of vocal fold geometries reduced the strength of intraglottal vortices and decreased glottal efficiency (GE). GE can be calculated using the equation: $GE = SPL / (SGP * Q)$, where Q is the flow rate entering the glottis.²⁶ Devine et al. altered the amount of vocal fold adduction for three excised human male larynges at varying subglottal pressures. They found that the glottal flow resistance and vibrational amplitudes increased with the adduction of vocal folds during sustained phonation.²⁷ This is able to facilitate phonation.²⁸ Finally, considering the anatomy below the glottis, Wang et al. used excised canine larynges to study vocal fold inferior surface hypertrophy (VFISH). They found an increase in VFISH caused an increase in the subglottal convergence angle and F_0 in sustained phonation, which makes phonation difficult.²⁹

Recent research has focused on aerodynamic vortices. Using particle image velocimetry (PIV) with excised larynges, Khosla et al. assessed the glottal flow fields at different SGPs, such as low, medium, and high. With increasing subglottal pressure, flow vortices appeared first unilaterally, and then bilaterally on the upper surface of the vocal cords. The negative pressures generated across the vocal folds during phonation were significantly lower than predicted by theoretical models. They also found that the vortices do not have a large role in phonation at low SGP, but at higher pressures, the vortices help with increasing acoustic intensity.³⁰ Farahani and Zhang used computational models and found that vocal fold vibration was not significantly affected by intraglottal vortices.³¹ But, Oren et al. studied five canine larynges with 2D-PIV to and agreed with earlier research saying that intraglottal vortices can increase acoustic intensity.³² Future studies are needed to identify the mechanisms by which airflow vortices affect initiation and sustenance of vocal fold vibrations.

A relatively newer aerodynamic parameter, phonation threshold power (PTW), is calculated by multiplying PTP and PTF ($PTW = PTP \times PTF$) and is the minimum aerodynamic power necessary to sustain phonation. Vocal fold biomechanics and glottal configuration have a large impact on PTW.³³ Zhuang et al. calculated PTW in human subjects with normal vocal folds, vocal fold immobility, or vocal fold polyps. They found that vocal fold disorders increased PTW significantly, but PTW was not significantly different across the different disorders. Excising vocal fold polyps could lower PTW significantly. They suggested that PTW is more sensitive to the laryngeal pathophysiology because it contained a larger area under the receiver operating characteristic curve than PTF or PTP.³⁴ However, given that PTW is newer than PTP and PTF its current research with the excised method is limited - Regner et al. has done an introductory study and more work on PTW should be considered for future studies.^{33,35} Imaging

High speed video (HSV), videokymography (VKG), and optical coherence tomography (OCT) are commonly used in excised laryngeal imaging. F_0 , periodicity, amplitude of the vocal fold vibration, and interfold and intrafold phase differences are typical parameters measured to assess vocal function. All of these parameters are from traditional linear analysis methods and can be used to evaluate laryngeal health. However, these parameters

are defined from normal vibration. Pathological vocal folds, which are more common in clinical work, vibrate irregularly and are not always suitable for linear analysis. To overcome this, Zhang et al. introduced the nonlinear least squares analysis method for quantifying the subharmonic mucosal waves in digital kymography. This method allowed description of the diseased subharmonic mucosal wave to be more accurate than methods using traditional linear analysis.³⁶ On this basis, Li et al. described four voice types according to vocal fold vibratory patterns with high-speed imaging by using nonlinear dynamic analysis methods. They classified periodic signals as type 1, sub-harmonic signals as type 2, chaotic signals as type 3, and white noise signals as type 4.³⁷ Wang et al. applied 2D scanning VKG to an excised canine larynx to overcome the shortages of digital kymography from high-speed videolaryngoscopy and laryngeal VKG.³⁸ In many previous studies, the excised larynx was most commonly used as a whole, but this set-up only allows the mucosal wave to be viewable from above. Herbst et al. used a modified hemi-laryngeal apparatus, originally used by Jiang and Titze³⁹, with two high-speed cameras recording the 3D vocal fold vibration from superior and lateral directions at the same time. This set-up allows the researcher to see the contact area variation and air flow.⁴⁰ In this way, the imaging of excised larynges has been gradually developing from 2D to 3D.

Biomechanical properties—As high-speed digital imaging and 4-dimensional optical coherence tomography cannot test the mechanical properties of the vocal folds,^{41,42} Bakhshae et al. used digital image correlation to accurately measure the mechanical stress and strain on the surface of excised porcine larynges during phonation.⁴³ On the basis of Jiang's hemi-larynx model, Dion et al. developed a hemilarynx platform with dynamic nanomechanical analysis to quantify the nanomechanical properties of the vocal folds. They found that the complex, storage, and loss moduli increased from the free edge to the inferior end of the porcine vocal folds. This made it possible to assess the biomechanical responses to many kinds of therapies, such as surgeries, drug treatments, or vocal training.⁴⁴ In a separate study, they used a TI950 TriboIndenter (Hysitron, Eden Prairie, MN, USA) to test biomechanical properties of the vocal fold complex of the porcine hemilarynx in different areas. They performed medialization laryngoplasty with Gore-Tex or Silastic block implantation, which can alter storage and loss moduli in different parts of the vocal fold. They commented on the limitation that the laryngoplasty would cause permanent tissue deformations that affect the biomechanical properties of phonation. Overcoming this limitation and helping doctors acquire the best implant effect on phonation is still challenging.⁴⁵

Simulations of Clinical Applications

In recent years, excised larynges have been widely used in research on laryngeal physiology, pathology, surgery, and therapy. Applying the basic research discussed previously to determine the overall effects of different techniques is critical to evaluating potential clinical applications. This way, the pre- and post- treatment outcomes can be compared to decide which treatment options offer the best results.

Ishikawa et al. simulated unilateral and bilateral cricothyroid muscle contraction of excised human larynges and recorded the procedure with videostroboscopy and suggested that

decreasing cricothyroid muscle contraction could decrease F_0 and increase the vibrational amplitude of the vocal fold.⁴⁶ This study deepens our understanding of the physiological functions of cricothyroid muscle.

Dehydration, mechanical injury, chemical irritation, and abnormal structure often cause pathophysiological changes in the larynx, especially in the vocal folds, which will affect the biomechanical properties. Li et al. quantitatively analyzed the effect of dehydration of the vocal folds on phonation using high-speed imaging. They found that F_0 stayed constant, while the amplitude of the mucosal wave decreased as the vocal folds became more dehydrated.⁴⁷ Krausert et al. investigated the effect of micro-scissor injuries to different locations of the vocal folds.

They found that the vibratory amplitude consistently decreased and vocal folds became asymmetric by anterior and medial injuries.⁴⁸ Tse et al. removed the epithelium of the vocal fold from an excised human larynx, and then made it phonate in an excised larynx apparatus. They found that closed quotient decreased and that there was left-right vibration asymmetry.⁴⁹ Xue et al. developed an excised anterior glottic web (AGW) canine model and observed aerodynamic, acoustic, and high-speed parameter variations. Increases in occlusion in cases of clinically symptomatic AGW led to increases in PTP and a more complex vibration pattern that increased the chaos of the system as well.⁵⁰ Then, Pulvermacher and Xue et al. simulated an asymmetric laryngeal glottic web on an excised canine larynx, and they found that PTF, PTP and correlation dimension (of chaos theory) increased, while vocal efficiency decreased significantly. This supports that an asymmetric laryngeal glottic web will decrease the voice quality.¹⁴ When there is damage to the vocal folds, many clinicians try to promote epithelial recovery or repair defects. Cobden et al. applied platelet-rich plasma on wounded rat vocal folds and used the excised larynx set-up to evaluate vascular endothelial growth factor, fibroblast growth factor receptor, and epithelial growth factor receptor (EGFR) immunohistochemical staining. They found that increasing EGFR secretion may accelerate injury rehabilitation.⁵² Since the vocal fold structure of rabbits is very similar to that of humans, they are commonly used in molecular immunology and scar research. Researchers also want to know if rabbit larynges are suitable for excised larynx studies or not. Maytag et al. modified the excised larynx apparatus for measuring electroglottographic, videokymographic, acoustic, and aerodynamic parameters of rabbit larynges. They found that phonation characteristics of rabbit larynges were similar to canine larynges.¹⁵ Mills et al. collected aerodynamic, acoustic, and high-speed video parameters throughout the complete phonatory range of rabbit larynges and found that increasing elongation of the vocal folds and airflow affected F_0 , SGP, and SPL, and the changes were similar to what is observed in humans.³ Mau et al. developed a rabbit vocal fold laser scarring model and used the excised larynx set-up to find that the vibratory amplitude of scarred vocal folds decreased from unscarred vocal folds. They also analyzed the density of collagen content in the injured vocal folds. The results suggested that the amount of type III collagen was unchanged, type I collagen and elastin increased, and hyaluronic acid concentration decreased.⁵² Then, Shiba et al. used the accepted rabbit excised larynx technique to evaluate the phona-fuction of a tissue-engineered mucosa repaired vocal fold. They discovered that their implants, which were based on adipose-derived stem cells, were able to be implanted into the vocal folds with minor scarring.⁵³

Autologous tissue and tissue engineering materials are also commonly used in vocal cords and larynx repair. Hoffman et al. used composite thyroid ala perichondrium flap (CTAP) to repair vocal fold stripping in canines. They showed that CTAP could restore periodic mucosal vibration and glottic closure using an excised larynx test.⁵⁴ Kitamura et al. also used the excised larynx apparatus to test a tissue-engineered, acellular, extracellular matrix scaffold reconstructed from a canine larynx, and showed that it restored normalized glottal gap, normalized mucosal wave amplitude, and nearly normal PTP.⁵⁵

In the past few years, excised larynx studies have been widely used in laryngoplasty research. McCulloch et al. applied arytenoid adduction (AA) through an anterior approach and assessed acoustic and aerodynamic data using an excised larynx model. They showed that anterior AA is easier to perform than traditional AA and produced comparable improvements.⁵⁶ Hoffman et al. evaluated an adjustable balloon implant using an excised canine larynx, and found a much less invasive method of type I thyroplasty.⁵⁷ In a subsequent study, an excised canine larynx was used to evaluate type IIIB thyroplasty combined with bilateral type I thyroplasty for lowering pitch while maintaining the tension of the vocal folds.⁵⁸ Thompson et al. modified the approach of medialization thyroplasty by performing medialization thyroplasty via the subthyroid cartilage approach on an excised canine larynx, showing that it is possible to improve acoustic and aerodynamic parameters without requiring to make a window on thyroid cartilage.⁵⁹ Lagier et al. used ImageJ to measure the mean glottal area of 21 excised human larynges in the situations of no adduction, arytenoid adduction, membranous vocal fold adduction, and both arytenoid and membranous adduction. They found that the combination of arytenoid and membranous adduction was the most effective in reducing the glottal gap. They also determined that the length of the vocal cords increased with decreasing cricothyroid angle.⁶⁰ Adachi et al. performed histopathological assessments on the excised larynx of a patient who had received type I thyroplasty with silicone block implantation. Seven years after implantation, only very mild tissue reactions had occurred. These results suggested that silicone has an affinity for human tissue with a low risk of a tissue reaction.⁶¹ Devine et al. made a hyperadduction excised canine larynx model and performed type II thyroplasty on it. They evaluated the phonatory parameters of the excised larynges and found that PTP increased with hyperadduction, and that 1mm lateralization is best for restoring nearly normal physiology. They also noted the limitation on this small canine larynx model and the best adduction distance is likely different for humans.²⁷ Voice therapy is a treatment option that is common in Europe and the United States. One therapy method is the semi-occluded vocal tract technique which can be tested using an excised larynx model. Conroy et al. used an excised canine larynx to test resonance tubes of different diameter, length, and flow input to investigate the ideal tube size for voice therapy.⁶² Mills et al. tested the feasibility of using a semioccluded face mask phonation therapy method as an alternative to traditional semi-occluded vocal tract methods.⁶³ Future research needs to be conducted in this field of study.

CONCLUSIONS

Excised larynges are commonly used to study laryngeal anatomy, physiology, pathology, histological observation, cell and tissue culture, molecular immunology, surgery, and voice therapy. The most distinctive applications of excised larynx research are to analyze the

acoustic and aerodynamic parameters and perform mucosal wave imaging during phonation to show the characteristics of the vocal organs. However, these observations are only valid for the excised situation, and must be combined with animal experimentation and clinical observational records. Combining all these methods can offer a better physiological and pathological explanations of the research profile and internal mechanisms of phonation.

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