The Laryngoscope

Development of the Ex Vivo Laryngeal Model of Phonation

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Objectives/Hypothesis: The direct study of human phonation is limited by the invasive and painful nature of human laryngeal neuromuscular manipulation. As a platform for the study of human phonation, indirect models have been utilized for decades such as animal, cadaveric, and computational. We sought to develop a research method allowing direct scientific control of virtually living larynges to expand our ability to understand human phonation.

Study Design: Canine and porcine models.

Methods: Nineteen canine larynges were surgically removed and reperfused with progressively adapting methodologies to create ex vivo phonation.

Results: Full neuromuscular stimulation and phonation were ultimately achieved in the ex vivo larynx. As compared with alternative perfusate solutions, heparinized whole blood was found to result in the most robust neuromuscular response. Modification of the reperfusion technique from a continuous flow to a pulsatile pump system resulted in dramatic increases in neuromuscular response and longevity of the organ. The experimental findings were repeated to demonstrate reliability of the ex vivo model.

Conclusions: The ex vivo larynx model is demonstrated to be a repeatable platform for phonatory research. The process of development has been comprehensively described in the present report. Although the described experimental model was designed for phonatory research, this model can be readily adapted for investigations of organ transplant preservation techniques, effects of organ ischemia, and neuromuscular reinnervation capabilities.

Key Words: Human, canine, larynx, perfusion, physiology, voice.

Level of Evidence: NA

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INTRODUCTION

The study of voice production is critical for our understanding of the numerous pathological conditions impacting effective communication. Over the history of phonatory research, many strategies have been employed, beginning with simple observational study of the human larynx in situ. These laryngoscopic examinations were insufficient to comprehensively describe the complexities of voice production. However, the alternative to the observation of the larynx, that of direct scientific manipulation of the human laryngeal physiology, was unjustifiable secondary to the invasive nature.

Therefore, throughout the greater portion of the 20th century, alternative scientific methodologies were designed and employed, which have resulted in the substantial growth of voice science as a field. The methods of phonatory investigations have included animal models, in particular, the study of in vivo animal phonation. The advantage of the in vivo animal models was the ability to provide physiologic activation of the intrinsic laryngeal musculature.[1,2] Other investigations have also utilized excised animal larynges that could be positioned mechanically to produce phonation.[3,4] However, although the numerous suggested animal models hold anatomic similarities to the human larynx, there universally exists substantial variation from the human larynx from both anatomic and histologic considerations[5,6] that ultimately limit the application to human phonation.

Excised human laryngeal modeling has also been employed in phonatory research, specifically to circumvent the phylogenetic differences of animal models.[7,8] Cadaveric human specimens provided gross anatomic conservation in human phonation; however, the effects of intrinsic laryngeal muscle activation were unable to be modeled, in particular the effect of thyroarytenoid activation toward body layer stiffness.[9] To address the stiffness differential limitations of excised larynges, physical models have been designed to allow for estimated control of the cover-body tension ratios.[10,11] Yet physical models were still limited by less-than-accurate human-specific tissue properties of the physical constructs. Last, computational phonation modeling has been utilized successfully to identify many properties of

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phonation including, for instance, the mechanics of vocal fold self-oscillation.[12,13] However, the mathematical variables utilized in the computational models were defined, based on a large part, from the results of the aforementioned phonation models with their inherent limitations.

As a whole, these methodological strategies have provided critical advancement of voice science, but each one maintains important limitations for direct application to human voice production. To address these limitations, it was hypothesized that human larynges could be kept physiologically alive outside of the body if the appropriate physiologic environment could be provided. If true, physiologic neuromuscular activation could be selectively applied to human larynges resulting in direct human phonatory study. This hypothesis was termed *ex vivo phonation*. The present laboratory sought to evaluate this hypothesis utilizing animal larynges in the ultimate goal of applying the developed techniques to human larynges. Initial work toward ex vivo phonation was performed using canine larynges perfused with a gravity-dependent system that enabled a small increase in muscular contractibility compared with excised and nonperfused larynges but did not provide an adequate environment for phonation sufficient for human application.[14] With these encouraging initial results, we aimed to adjust and improve the techniques of animal ex vivo phonation to achieve prolonged physiologic neuromuscular activation outside of the living body to apply the learned methodology toward producing human ex vivo phonation.

MATERIALS AND METHODS

The Chancellor's Animal Research Committee (ARC) of UCLA, protocol number 2011-151-01, approved the described research. All experiments were performed in accordance with federal and state legislation regarding the protection of animals.

Animal Surgery

In all described experiments, male mongrel canines with approximate weights of 25 kg and age ranging from 2 to 6 years were utilized. All experiments were terminal, and therefore each canine larynx was used only once. The administration of general anesthesia was accomplished via inhaled halothane and intravenous pentobarbital to a level of blockage of the reflexive response to corneal stimulation. The animal was maintained on mechanical ventilation with inhaled halothane via oral intubation until tracheostomy was performed, following which the distal trachea was intubated with a fresh endotracheal tube. To ensure animal safety and pain control, the animal was continuously monitored by respiration rate, heart rate, pulse oximetry, rectal core body temperature, complete muscle tone relaxation, absent response to toe pinch, corneal reflex, and physiologic color of mucosal membranes. Animal temperature was maintained by water-circulating heating pads. Animal hydration was administered by continuous intravenous (IV) hydration with D5 one-half–normal saline solution at a rate of 5 mL/kg/hr. Bladder catheterization was not performed. After the experiment, the canine was humanely killed with IV Eutha-6.

To prepare the ex vivo larynx, the attachments of the larynx were divided in a stepwise manner until they were completely separated from the body under extracorporeal perfusion. The recurrent and external laryngeal nerves were identified first, but were not dissected off the tissue bed. The arterial and venous supply were dissected next, but were divided only after the mucosal attachments were transected. The arterial blood supply to the canine larynx comes from the superior thyroid artery, which is a branch of the common carotid artery. The venous drainage is via a hyoid venous arch that drains bilaterally into the internal jugular veins. Preservation of the hyoid venous arch is essential and requires that the pharyngeal mucosal cuts be performed above the level of the hyoid bone. The mucosal attachments of the larynx were divided starting with division of suprahyoid muscles from the hyoid bone, followed by division of the pharynx circumferentially above the level of the hyoid bone. The mucosal edges were tied with sutures before cutting; this important step prevented leakage of blood and perfusate through the mucosal cuts during the experiment. Inferiorly, the trachea was transected at the level of the sternum, allowing adequate length to place an endotracheal tube directed rostrally to provide airflow for phonation. The esophagus was divided completely at the same level, and the proximal end was suture-tied to prevent fluid leakage. After these mucosal cuts, the larynx remained attached to the body only by the blood vessels and laryngeal nerves.

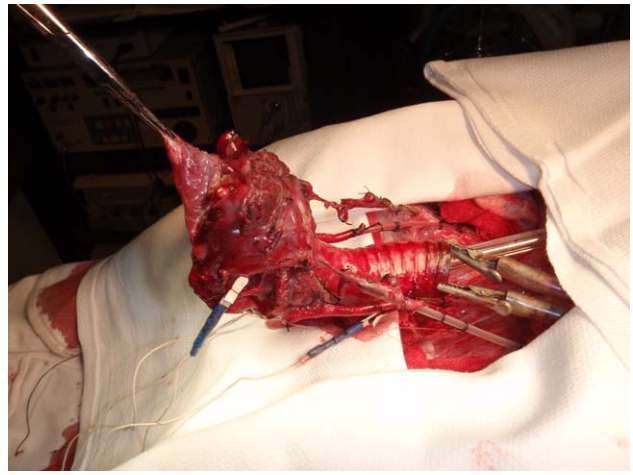

Fig. 1. Explanted canine larynx. Shown is the dissected larynx just prior to explantation and ex vivo phonation. Clip electrodes are seen attached to the superior and recurrent laryngeal nerves. Arterial cannulae are inserted to the vascular supply of the larynx. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

The internal jugular veins were then cannulated bilaterally at a level below the larynx, and all venous attachments were divided except the connection to the larynx via the jugular veins and the hyoid arch. At this point, the two internal jugular vein cannulas were combined into a single cannula. The arterial connections were then sequentially divided, and the procedure was repeated on each side. First, the common carotid artery was divided distal to the superior thyroid artery branch and then cannulated proximal to the superior thyroid artery. The procedure was repeated on the opposite side. The vascular cannulas were sutured to the external trachea to prevent inadvertent kinking of the vessels during transportation and experimental setup. Finally, the nerves were divided as proximally as possible, and the ex vivo perfused larynx was now prepared for further experimental setup as shown in Figure 1.

Laryngeal Perfusion

The composition of the perfusate solutions and methods of perfusion delivery were adapted throughout the study and are displayed Table I. The perfusate solutions were administered at

TABLE I.
Summary of Ex Vivo Laryngeal Perfusion Methodology With Muscular Contractibility and Phonation Endpoints.

Experiment	Perfusion Technique	Perfusion Solution	Neuromuscular Activity (Duration)	Phonation	Comments
1	Gravity dependent	Modified Krebs-Henseleit solution	Intermittent (4 hours)	None	Edema at 1 hour
2	Gravity dependent	Modified Krebs-Henseleit solution with 50 mL per 1 L of 25% mannitol	Intermittent (2.5 hours)	Minimal	Improved edema
3	Gravity dependent	Modified Krebs-Henseleit solution with 100 mL per 1 L of 25% mannitol	Intermittent (30 minutes)	Minimal	
4	Gravity dependent	Modified Krebs-Henseleit solution with 100 mL per 1 L of 25% mannitol with 50 mg glucose	None	None	
5	Gravity dependent	Modified Krebs-Henseleit solution with 100 mL per 1 L of 25% mannitol with 50 mg glucose	Intermittent (1 hour)	None	Large persistent posterior gap
6	Gravity dependent	Modified Krebs-Henseleit solution	Intermittent (15 minutes)	Strong, but limited in duration (<1 minutes)	
7	Gravity dependent	Plasma-Lyte A diluted autologous whole blood (1:1)	None	None	
8	Hand pump	5% dextrose in water solution diluted autologous whole blood (2:3)	Intermittent (1 hour)	Strong, duration of 5 minutes then none	
9	Hand pump	5% dextrose in water solution diluted autologous whole blood (2:3)	Intermittent (30 minutes)	Minimal	
10	Hand pump alternating with physiologic blood flow	5% dextrose in water solution diluted autologous whole blood (2:3)	None	None	
11	Hand pump alternating with physiologic blood flow	Autologous whole blood	Strong (10–20 minutes), needed physiologic flow restoration	Strong initially following physiologic blood flow	
12	Autotransplantation to femoral artery	Autologous whole blood	Strong (>3 hours)	Strong (>3 hours)	
13	Xenotransplantation to porcine femoral artery	Xenotranfusion whole blood (porcine)	Intermittent (15 minutes)	None	Edema at 10 minutes
14	Xenotransplantation to porcine femoral artery	Xenotranfusion whole blood (porcine) with immunosuppression	Intermittent (30 minutes)	None	Edema at 15 minutes
15	Autotransplantation to femoral artery	Autologous whole blood	Strong (>4 hours)	Strong (>4 hours)	
16	Pulsatile pump	Autologous whole blood	Strong (>4 hours)	Strong (>4 hours)	
17	Pulsatile pump	Allogenic packed red blood cells (citrated) diluted in normal saline (1:1)	None	None	Unable to restore physiologic calcium levels
18	Pulsatile pump	Autologous whole blood (larynx pretreated with Wisconsin solution)	Reduced, strength moderately improved with exchange transfusions	Reduced, strength moderately improved with exchange transfusions	
19	Pulsatile pump	Autologous whole blood	>4 hours	>4 hours	

room temperature throughout the trials. The experiments were first designed to utilize perfusate solution (modified Krebs-Henseleit solution) to allow for organ oxygenation. Resulting tissue edema lead to the inclusion of varying mannitol concentrations within the perfusate solutions of experiments 2 through 6. The perfusate solution was subsequently replaced with blood to improve the oxygen-carrying capacity from experiment 7 onward. Experiments 8 onward incorporated some form of pulsatile flow to replace the constant flow of the earlier conditions. Experiments 8 through 11 utilized a hand pump to provide the pulsatile perfusion flow. This hand pump was comprised of the IV bag that held the perfusate solution within an arterial line pressure bag. The pressure bag was sequentially inflated to provide pulsatile flow from the IV bag to the arterial cannulae. Experiments 10 and 11 tested the efficacy of the hand-pump system compared with physiologic cardiac output. These two experiments were carried out with a custom-designed flow switch connected in a circuit to the arterial cannulae. The flow

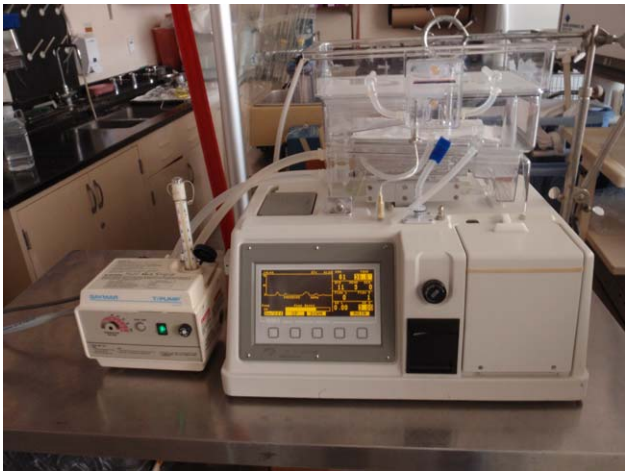

Fig. 2. RM3 pulsatile perfusion unit. The RM3 unit is displayed prior to priming with blood products. The circuit warmer is seen just to the left of the RM3, which maintains the perfusate at physiological temperatures. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

switch is similar in design to a three-way IV tubing stopcock that can rapidly alternate between flow sources. This comparison to physiologic cardiac output flow was confirmed with the autotransplantation of the larynx to the femoral artery in experiments 12 and 15. To evaluate the theory of human laryngeal ex vivo phonation maintained with animal cardiac output, xenotransplantation between canine and porcine was attempted in experiments 13 and 14. Autotransplantation trials were replaced with mechanical pulsatile of the RM3 perfusion system (RM3 Renal Preservation System, Walters Medical Systems, Rochester, MN) in experiments 16 through 19. The RM3 perfusion pump provided pulsating intra-arterial blood pressure and flow, based on clinical use for extended renal preservation. The RM3 has integrated temperature, pressure, and volume flow rate–monitoring capability with calculated arterial resistance as shown in Figure 2. Modifications made to the RM3 included an additional outflow track from the perfusate collection tank used to remove and replace the perfusate or flow additional oxygen through the perfusate to increase oxygen concentration. Intermittent blood analysis was used to guide changes in perfusate electrolytes and oxygenation to maximize phonation potential. The perfusate solution was also adjusted in a stepwise fashion through experiments 16 through 19, aimed to identify the technique most capable in providing sustained ex-vivo phonation.

Ex Vivo Stimulation and Phonation

Following nerve transection involved in the removal of the larynx, custom-designed clip electrodes applied to both the recurrent laryngeal nerve (RLN) and superior laryngeal nerve (SLN) bilaterally at a distance of approximately 2 to 3 cm from their muscle insertions. The electrodes were connected to a constant current nerve stimulator (model 2SLH, WR Medical Electronics Co., St Paul, MN). The nerves were stimulated at 80 Hz with a 0- to 3.0-mA current and 1.5-ms pulse duration. A cuffed endotracheal tube (inner diameter 7.0 mm, outer diameter 9.3 mm) placed within the transected trachea was directed rostrally and positioned with the tip at a distance of 3 cm inferior to the level of the glottis. The endotracheal tube was inflated fully to ensure glottal directed airflow. The air was first passed through humidified chamber with temperature control, and the airflow was regulated between 500 and 700 mL/min adjusted as required to achieve phonation. Bilateral SLN and RLN stimulation was achieved, and simultaneous rostrally directed airflow resulted in phonation with the vibration of the adducted vocal folds. Phonation attempts were performed every 5 minutes. The overall goal of the described experiment was development of the methodology; therefore, neuromuscular activity and phonation were measured and graded perceptually.

RESULTS

Table I reviews the outcomes for both neuromuscular activation as well as ex vivo phonation. Ultimately, long-term ex vivo phonation was achieved by reperfusing the excised organ with whole (noncitrated) blood delivered in a pulsatile flow.

DISCUSSION

The stepwise modification of the development of the ex vivo phonation model is discussed. The a priori hypothesis was that physiologic laryngeal neuromuscular activation could be maintained outside a living body to provide a scientific platform in which to investigate human voice production. Our hypothesis was based on the encouraging experience of ex vivo models for the study of muscular contractibility.[15,16] However, these, among many other ex vivo models, studied isolated tissue types (e.g., skeletal muscle, smooth muscle, liver cells), whereas the larynx is a complex composite tissue organ composed of cartilage, muscle, nerves, and mucosa. From the work with laryngeal transplantation,[17] it was known that ex vivo perfusion of the larynx would be feasible; however, the requirement to reestablish neuromuscular activation, specifically to the level required for phonation, was unknown.

The initial experiments within this described trial were designed based on reports with laboratory ex vivo models, such as those previously referenced,[15,16] namely utilizing a reperfusion solution (Krebs-Henseleit) delivered intravascularly in a constant flow manner. Although this design allowed for intermittent neuromuscular activation, no phonation was achieved, and the organ was quickly marred by mucosal edema during experiment 1. Experiments 2 thorough 6 were subsequently designed to limit the mucosal edema by increasing the dose of infused mannitol, which serves to increase intravascular osmotic pressure. Although these alterations were met with short bursts of phonatory capability, demonstrating the theoretical potential of ex vivo phonation, the experimental design needed significant improvement to provide reliability and repeatability.

With the advancement to experiment 7 and onward, the reperfusion solution was replaced in favor of the oxygen-carrying capacity of blood. Unfortunately, the delivery of blood to the larynx in a constant-flow gravity-dependent nature did not result in restoration of neuromuscular activity to any significant level. The decision was then made to replace the mode of blood delivery from a constant flow to a pulsatile flow to mimic physiologic cardiac output. The combination of blood

reperfusion and pulsatile flow restored neuromuscular activation and achieved the longest duration of strong ex vivo phonation that had been seen to date. However, the repeatability was questioned with experiment 10, when no phonation could be achieved.

In response, the experimental design was adjusted to allow for alternating physiologic cardiac-driven perfusion with the ex vivo perfusion of autologous pulsatile blood flow. The aim of this creative design was to uncover whether the limitation in neuromuscular activation and phonation was due to permanent injury to the organ or due to limitation in the current experimental design. If phonation could be achieved during physiologic perfusion and not during ex vivo perfusion, then it would be presumed that undetermined variables were preventing successful ex vivo phonation. In experiment 11, we found that, in fact, full phonation could be achieved during physiologic blood flow, yet the administration of hand-pump flow resulted in only a short duration of phonatory capacity. Using the custom-designed flow switch, which allowed for rapid conversion from physiologic to hand-pumped flow, demonstrated the true importance for producing phonation. In particular, although the hand-pump could administer an acceptable level of systolic flow pressure, there was no afterload to provide a diastolic pressure level. In comparison, when physiologic cardiac output was restored to the larynx via the flow switch, strong and reliable phonation was readily achieved.

To further investigate the role of diastolic pressure theory of perfusion requirements, the larynx was explanted from the neck in experiment 12 and reimplanted to the femoral artery and vein from the same animal. The presence of strong neuromuscular activation and phonation under reimplantation was replicated in experiment 15. The success of reimplantation underscored the importance of providing physiologic perfusion factors, in particular, the importance of providing diastolic pressure and physiologic appropriate pulse pressure. Based on this finding, the shortcomings of the current ex vivo reperfusion design were reinforced.

Without a readily available platform in which to provide these perfusion requirements, it was suggested that using the combination of animal models with human larynges, physiologic perfusion might be provided through xenotransplantation. In experiments 13 and 14, the xenotransplantation theory was investigated, but ultimately was substantially affected by acute tissue rejection, which was not reversed with systemic high-dose immunosuppression. Immediate and substantial tissue change prevented further investigation in this avenue as a potential platform for human ex vivo phonation.

At this time, effort was placed into identifying an improved design for providing artificial ex vivo perfusion and resulted in identification of the RM3 perfusion unit. Initially designed to prolong kidney viability during long transport times in organ donation, the unit provides adjustable pulsatile flow with real-time monitoring of hemodynamics. Experiments 16 and 19 demonstrated the feasibility and repeatability of prolonged ex vivo phonation utilizing autologous whole blood in a pulsatile fashion. The use of donated packed red blood cells in experiment 17 was unsuccessful, likely due to the inability to correctly equilibrate physiologic calcium levels with the blood preservative, citrate. Citrate prevents clotting within the stored blood products by chelating and binding calcium. Within the closed system as designed, the addition of calcium to the perfusing blood was either insufficient or grossly overdosed the system.

Ultimately, the experimental progression led to the design and establishment of ex vivo phonation. The described methodology allowed for application of this technique to fresh human larynges, which has been recently reported.[18] The ex vivo phonation system has since undergone multiple design improvements as described in those reports, such as the addition of an in-line dialysis filter and inclusion of intravascular flow monitors. Human ex vivo phonation contains additional layers of complexity that present scientific challenges requiring further adjustment as the scientific model continues development. Although animal models can be arranged for consistent size, gender, and age, human larynges need to be obtained from a wide spectrum of body sizes, gender, and ages, which challenges consistent measurements across phonation production. Variations in transportation time from site of laryngeal acquisition to the laboratory also add complexity beyond what was present during animal modeling and will require additional refinement of the described methodologies.

The ex vivo perfusion design described can also show research applications apart from phonatory study. In particular, as a novel platform in which to assess the viability of composite tissue, transplantation research laboratories may utilize this platform to improve tissue preservation techniques. This may be readily accomplished using the ex vivo neuromuscular stimulation methodologies described to maintain scientific consistency while evaluating particular variables of organ perfusion techniques. For example, in the application of limb transplantation, recovery techniques including effects of warm ischemia time and novel pharmaceutical treatments for tissue preservations can be objectively measured for production and maintenance of forearm neuromuscular activity. In line with limb preservation techniques, the ex vivo perfusion platform could also be adapted to improve organ preservation techniques during battlefield amputation injuries. Laryngeal ex vivo perfusion specifically could be applied not only toward phonatory research but toward the refinement of laryngeal transplantation. Clinical trials of laryngeal transplantation have been limited by the absence of neuromuscular function recovery. It is unclear as to the underlying cause of this limitation in light of successful animal model neuromuscular recovery following transplantation.[17] The ex vivo perfusion model can be utilized to specifically analyze the current limitation in neuromuscular regeneration and allow for subsequent improvement toward the ultimate achievement of functional laryngeal transplantation.

CONCLUSION

The ex vivo larynx model is demonstrated to be a repeatable platform for which to study phonation. The

process of development has been comprehensively described in the present report. Although the described experimental model was designed for phonatory research, this model can be readily adapted toward investigations of organ transplant preservation techniques, effects of organ ischemia, and neuromuscular reinnervation capabilities.

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BIBLIOGRAPHY

1. Koyama T, Kawasaki M, Ogura JH. Mechanics of voice production. *I. Regulation of vocal intensity. Laryngoscope* 1969;79:337–354.
2. Berke GS, Moore DM, Gerratt BR, Hanson DG, Bell TS, Natividad M. The effect of recurrent laryngeal nerve stimulation on phonation in an in vivo canine model. *Laryngoscope* 1989;99:977–982.
3. Scherer RC, Cooper D, Alipour-Haghighi F, Titze IR. Contact pressure between the vocal processes of an excised bovine larynx. In: Titze IR, Scherer R.C. *Vocal Fold Physiology: Biomechanics, Acoustics and Phonatory Control*. Denver, CO: The Denver Center for the Performing Arts; 1985:292–303.
4. Alipour F, Jaiswal S, Finnegan E. Aerodynamic and acoustic effects of false vocal folds and epiglottis in excised larynx models. *Ann Otol Rhinol Laryngol* 2007;116:135–144.
5. Kim MJ, Hunter EJ, Titze IR. Comparison of human, canine, and ovine laryngeal dimensions. *Ann Otol Rhinol Laryngol* 2004;113:60–68.
6. Hahn MS, Kobler JB, Zeitels SM, Langer R. Midmembranous vocal fold lamina propria proteoglycans across selected species. *Ann Otol Rhinol Laryngol* 2005;114:451–462.
7. Berry DA, Herzel H, Titze IR, Story BH. Bifurcations in excised larynx experiments. *J Voice* 1996;10:129–138.
8. Švec JG, Schutte HK, Miller DG. On pitch jumps between chest and falsetto registers in voice: data from living and excised human larynges. *J Acoust Soc Am* 1999;106:1523–1531.
9. Choi HS, Berke GS, Ye M, Kreiman J. Function of the thyroarytenoid muscle in a canine laryngeal model. *Ann Otol Rhinol Laryngol* 1993; 102:769–776.
10. Zhang Z, Neubauer J, Berry DA. The influence of subglottal acoustics on laboratory models of phonation. *J Acoust Soc Am* 2006;120:1558–1569.
11. Mendelsohn AH, Zhang Z. Phonation threshold pressure and onset frequency in a two-layer physical model of the vocal folds. *J Acoust Soc Am* 2011;130:2961–2968.
12. Lucero JC. Optimal glottal configuration for ease of phonation. *J Voice* 1998;12:151–158.
13. Smith SL, Thomson SL. Effect of inferior surface angle on the self-oscillation of a computational vocal fold model. *J Acoust Soc Am* 2012; 131:4062–4075.
14. Berke GS, Neubauer J, Berry DA, Ye M, Chhetri DK. Ex vivo perfused larynx model of phonation: preliminary study. *Ann Otol Rhinol Laryngol* 2007;116:866–870.
15. Kushmerick MJ, Meyer RA, Brown TR. Regulation of oxygen consumption in fast- and slow-twitch muscle. *Am J Physiol* 1992;263(3 pt 1):C598–C606.
16. Gambillara V, Thacher T, Silacci P, Stergiopulos N. Effects of reduced cyclic stretch on vascular smooth muscle cell function of pig carotids perfused ex vivo. *Am J Hypertens* 2008;21:425–431.
17. Berke GS, Ye M, Block RM, Sloan S, Sercarz J. Orthotopic laryngeal transplantation: is it time? *Laryngoscope* 1993;103:857–864.
18. Berke G, Mendelsohn AH, Howard NS, Zhang Z. Neuromuscular induced phonation in a human ex vivo perfused larynx preparation. *J Acoust Soc Am* 2013;133:EL114–E117.