Equine Laryngeal Model for Training Surgical Residents

Final Report

BME 200/300 Design
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Client: Dr. Kayla Le
Department of Surgical Sciences
UW School of Veterinary Medicine

Advisor: Dr. John Puccinelli
University of Wisconsin-Madison
Department of Biomedical Engineering

Team:
Co-Team Leader: Olivia Jaekle
Co-Team Leader: Kevin Zhang
Communicator: Rebecca Shomaker
BSAC: Bella Zingler-Hoslet
BWIG: Delani Wille
BPAG: Sophia Speece

Abstract

Horses move enormous volumes of air quickly from their nose to their lungs. The larynx is one of the narrowest parts in an equine upper airway, meaning that any abnormality in the larynx can diminish airflow and significantly decrease a horse's athletic ability. A specific treatment for upper airway abnormalities is a Transendoscopic Laser Ventriculocordectomy. One of every 24 horses will need a laser ventriculocordectomy to maintain athletic skills [1]. Currently, cadavers are used to train veterinary residents for this procedure. However, cadavers can be expensive and scarce.

An anatomically accurate equine upper airway model was fabricated to address this problem. It contains both replaceable and static components. The final proposed design is called the Replaceable Laryngeal Disk. The model is almost entirely static except for a disk that is easily replaced after each practice procedure. The disk houses the vocal folds within the laryngeal model, which is disposed of after each use. Two tests were done on the model to ensure accuracy compared to measured values from dissected larynx. This model will improve laser ventriculocordectomy techniques and result in better surgical outcomes by giving residents the expertise to perform this surgery on live patients.

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I. Introduction

Veterinary surgical residents currently need an anatomically accurate model of the equine upper airway. This model would be used for training surgical residents in transendoscopic laser ventriculocordectomy. This procedure treats upper airway abnormalities such as laryngeal hemiplegia, a vocal fold abnormality that causes distress and exercise intolerance in horses [1]. The surgery works by resecting the vocal folds in the horse which allows the airway to open. There are about 3200 veterinary residents every year that need to practice surgeries performed in the equine upper airway [2]. Residents must practice this surgery on models before they perform it on live animals as lack of adequate practice may lead to harming the horse. Currently, residents practice on cadavers which presents two main problems: lack of similarity to the living animal, being that the textures of the vocal folds will be different, and the scarcity of cadavers. Cadavers are, therefore, not very effective in teaching residents about the upper airway and are not sustainable. To help mediate this, the team is looking to create an anatomically correct model of the equine upper airway. There are anatomical models of animals, such as the SynDaver Canine Model shown in Figure 1, but this is a model of a canine and it is focused around using sensors rather than accurate mechanical properties of native tissue [3]. Biosphera shown in Figure 2, has an online anatomical model of the entire equine body [4]. This model allows one to learn by going through the anatomy of the horse layer by layer. This is not a physical model, so it is not helpful to surgical residents for practicing the surgery. As of right now, there are no other equine models on the market that allows surgeons to practice upper airway surgeries. Within the equine model, materials used will replicate exact tissues, textures, sizing, and spacing in the upper airway. The model will also entail replaceable vocal folds that can be reinserted each surgery.

Figure 1: *SynDaver anatomical canine model. This model is focused around using sensors and hard plastic to help surgical residents practice procedures [3].*

Figure 2: *Biosphera anatomical model of the equine body. This model is online and is focused around detailing every layer of the body, which helps to understand the anatomy of the horse [4].*

II. Background

Equine anatomy; Transendoscopic Laser Ventriculocordectomy

Performing the Transendoscopic Laser Ventriculocordectomy includes passing a diode laser through the upper respiratory tract to reach the larynx and removing the vocal cords. As seen in Figure 3, the diode laser enters the respiratory tract through the right nasal passage and is directed through the nasal cavity to the nasopharynx and into the larynx, where the surgery occurs. Incisions are made in the vocal folds to remove them from the larynx. When performing this surgery, it is imperative to avoid cutting within 2-3 mm of the vocal process as it may result in nerve damage [1]. Horses are sedated but not put under anesthesia so vocal folds remain in an open position for removal.

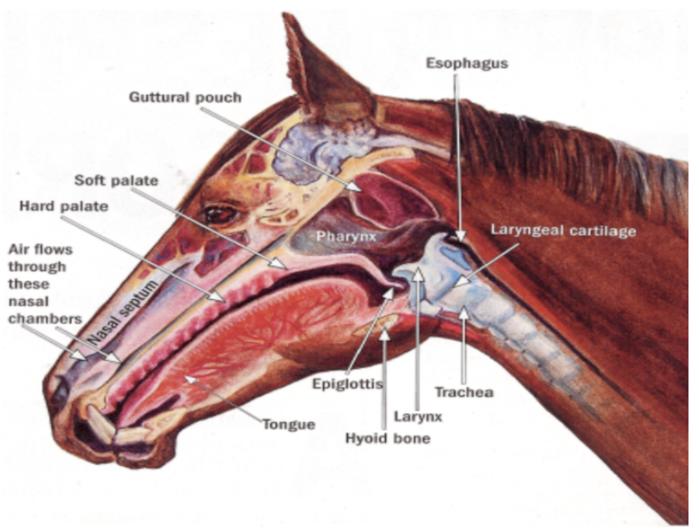


Figure 3*: Equine Upper respiratory tract anatomy[5]*

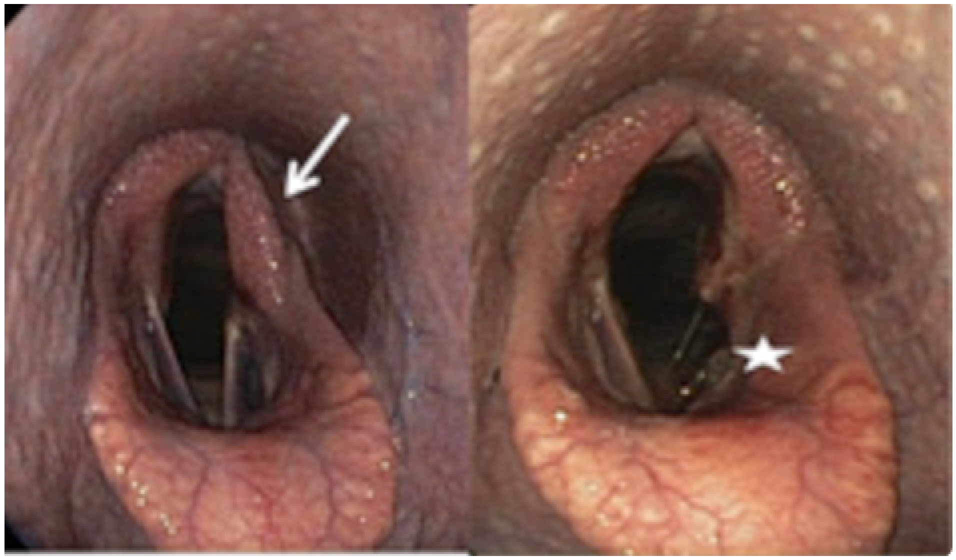


Figure 4*: Equine vocal fold before (left): left sided hemiplegia (arrow points to paralysed arytenoid cartilage) and after* transendoscopic laser ventriculocordectomy surgery *(right): removed vocal fold (star indicates surgical site)* [6]

Importance of Anatomical Surgical Models

3D anatomical models are a great resource for surgeons to reduce the risk of complications during a live surgery [7]. More specifically, 3D anatomical models provide students with hands-on learning opportunities. Not only do 3D anatomical models mimic the in-vivo dimension, size, shape, and mechanical properties of an animal, but 3D anatomical models are also helpful in diagnosing a patient with the correct ailment by comparing the normal anatomical structure to the diseased [7]. The 3D anatomical models help with preoperative planning so the surgeon can come up with a plan of what to do in the operating room. This saves time in the operating room and reduces surgical risks to the animal's body. In addition, a 3D anatomical model will lower the risk of harm to the animal during surgery because the surgery has been practiced before. 3D anatomical models can also help to explain relationships between tissues and structures that cadavers would not be able to discern adequately [8]. These factors make it imperative to create a 3D anatomical model of the equine upper airway.

Materials used for anatomical models

3D printing is a popular fabrication method used for human anatomical models. For example, two BME 200/300 design projects are using 3D printers for fabrication: one team is fabricating a heart and one is fabricating the hip and hind leg of a canine [9][10]. The UW Makerspace houses 4 different types of 3D printers [11]. Out of the four types of 3D printers, Formlabs Form 3s printer allows for the most accurate output in terms of size, shape, and mechanical properties. Formlabs Form 3s printer also provides the most time-efficient and detailed prints. Most importantly, the Formlabs Form 3s is compatible with the resin that best represents the anatomical properties of the larynx. The only disadvantages of using the Formlabs Printer compared to the Ultimaker Printer (also found in the Makerspace) is the higher cost per mL and higher, potentially wasteful, resin usage needed for the Formlabs' detailed print. Since the anatomical accuracy of the Formlabs printer outweighs the drawbacks of the Formlabs printer, the Formlabs Forms 3s printer will be used (Figure 4) to print the equine larynx.

Regarding different resin cartridges for a Formlabs 3D printer, the mechanical properties must match that of cartilage and bone. Flexible 80A resin has been used to simulate tissues in other studies. For example, Flexible 80A was used to manufacture animal blood vessels that imitate real animal blood vessels, so more testing and research frequencies can be increased [12]. As shown in Appendix B, the mechanical properties table for Flexible 80A is within the 5% range of the mechanical properties of the larynx cartilage in a mature equine. Therefore, Flexible 80A resin will be used for all cartilage aspects of the model. Similarly, Biomed White Resin has been used to simulate bones in anatomical structures. For instance, a 3D-printed spine was fabricated with Biomed White resin and the mechanical characterization of the 3D model and a fragment of a human spine have a 15% difference [13]. Appendix C shows that the mechanical properties of Biomed White Resin are within the 10% range of a mature equine's bone mechanical properties. Furthermore, Biomed White Resin will be used to create bones in the nasal cavity.

The Ultimaker (FFF) 3D printer is known for printing large, less detailed parts cheaply, especially with PLA [14]. PLA is a cheap plastic filament that easily 3D prints, is environmentally friendly, safe for medical devices, and comes with a wide range of compost to provide various properties and appearances [14]. Because of its low cost, all other static components of the model will be printed in PLA through an Ultimaker (FFF) 3D printer.

Figure 4: *Formlabs Form 3s printer used to print small, detailed parts of the model [15]*

Client

Dr. Kayla Le is the client for the equine laryngeal surgical model. She is a veterinary sciences professor at School of Veterinary Medicine here at the University of Wisconsin, Madison. The client will be using this product for educational and surgical training purposes.

Product Design Specifications

The device must accurately represent mature equine upper airway anatomy through mechanical properties, texture, dexterity, and dimensions. The model should contain static and replaceable components, with the vocal folds being replaceable. Each resident is expected to practice this procedure weekly to biweekly during the semester, totaling the use for each model to 14 to 28 times per semester. The replaceable vocal folds will be disposed of after each practice procedure. However, the static component must have a shelf life of at least 20 years. The replacement of the vocal fold must take less than 20 minutes due to the schedules of the surgical residents. As for safety, since the model will be interacting with a diode laser, any material used for the fabrication of the model must not produce harmful chemicals when it interacts with the laser. The model should adhere to pertinent codes regarding anatomical models and laser safety guidelines. The current budget for the project is $1000. For specific dimensions, component properties, and further specifications, refer to Appendix A of the PDS.

III. Preliminary Designs and Evaluation

System Design

Design #1: Replaceable Gelatin Vocal Folds

The first design is the Replaceable Gelatin Vocal Folds Design. As seen in Figure 5, this design is based on a current human laryngeal model used for training surgical residents in Endoscopic Phonomicrosurgery [16]. The design utilizes the same concept as the human model, but with tailored dimensions to mimic equine anatomy. In the model, the nasal passage to the top of the larynx is static and made of plastic, while the larynx is removable. The equine larynx would be made of 3D-printed Flexible 80A with modified lateral cavities to house the vocal folds. The laryngeal portion is medially split in order to have access to the cavities for frequent replacement of the vocal folds. The vocal folds would be made of gelatin due to its cost efficiency and biomaterial accuracy of equine vocal cords [17]. In order to achieve semicircle gelatin pieces, a reusable silicone mold is required to fabricate the vocal folds. The advantages of these silicone molds are heat resistance, soft material, and retention of details [18].

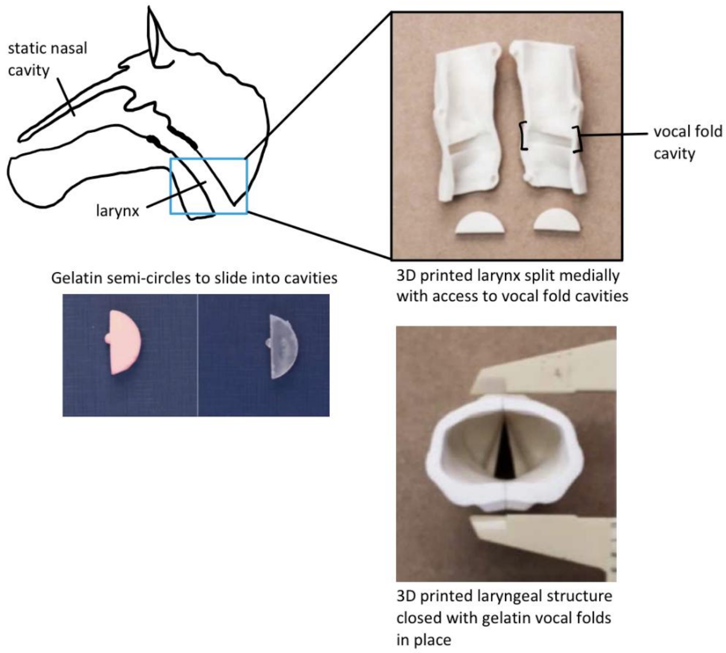


Figure 5: *Open and closed view of 3D printed larynx with modified lateral cavities*

Design #2: Replaceable Laryngeal Disk

Building upon the first design, the Replaceable Laryngeal Disk utilizes the replaceable gelatin vocal folds and simplifies the exchange process. This is simplified by the addition of a removable cartridge that can be inserted into a thin cross-section of the static portion of the model behind the epiglottis and before the trachea (Figure 6). This design maximizes the number of static components, allowing easier fabrication. Instead of the static components excluding the trachea behind the larynx, the trachea, along with the horse's head and the nasal cavity will all be static. The disk itself will be 3D printed from Formlabs Resin. The static components will be fabricated in the same way as the first design. The exterior horse head will be made from PLA plastic with a 20% infill. This makes the design lightweight but strong. The vocal cords are fabricated the same way as in the first design and are clasped between the male and female portions of the disk. The disk itself is then inserted into the model. It is held horizontally between the walls of the model as the cut is extruded inwards but not across. The disk is held in place vertically by a pin mechanism. At the bottom of the disk, there is a small hole that aligns with two L brackets fastened to the very outside of the model. The pin, threaded through the brackets and hole in the disk holds the disk firmly in place. When the disk is inserted into the model it will align perfectly with the rest of the larynx.

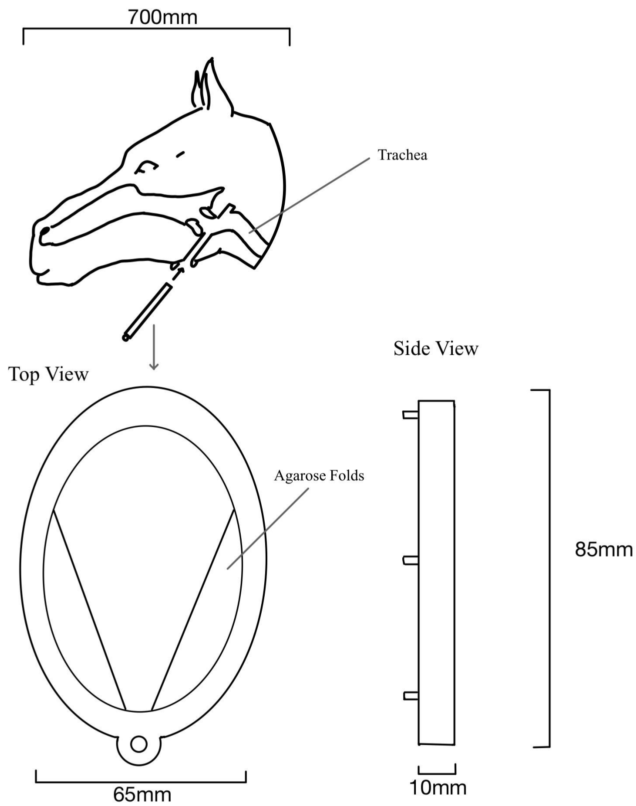


Figure 6: *Front and side view of the replaceable disk cartridge*

Design #3: Replaceable Larynx

The third design is the Replaceable Larynx model. As seen in Figure 7, this model is a simplification of the previous designs as it requires fewer moving parts. This design is a static horse head model representing the equine upper respiratory tract from the nose to the larynx. The static horse head will be 3D printed from PLA with BioMed White Resin representing bones within the structure. The model will have a removable and replaceable larynx with anatomically accurate cartilage and vocal folds. The replaceable larynx will be 3d printed of Flexible 80A Resin to represent cartilage and vocal folds. Laryngeal structure can be removed and replaced through an opening at the bottom of the model. Dimensions of anatomical features will be based on researched dimensions and CT scans of an equine's head. This design is advantageous as it closely mimics equine anatomy and has easy removal and insertion of larynx within the model. The disadvantage of this design is that the larynx will be expensive to replace which is required following each surgery.

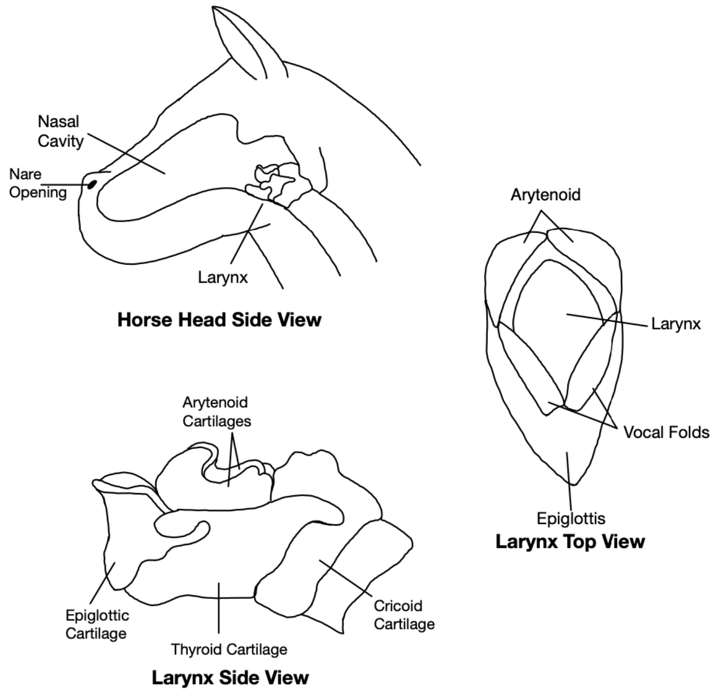
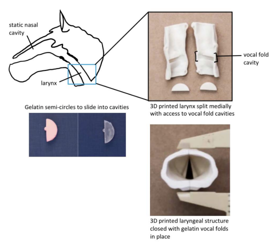
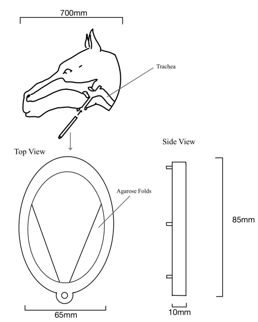
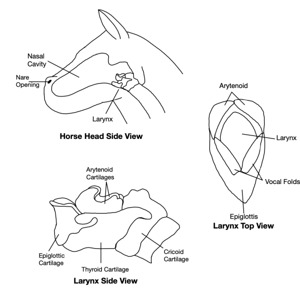


Figure 7: *Side and top view of replaceable laryngeal tissues with cross-section of sensors*

Design Matrix for System Design

Table 1: *Design Matrix with all methods scored on replaceability, ease of fabrication, accuracy and reliability, safety, cost, and life in service.*

							
		Replaceable Gelatin Vocal Folds [17]		**Replaceable Laryngeal Disk**		**Replaceable Larynx**	
Criteria	Weight	Score (5 Max)	Weighted score	Score (5 Max)	Weighted score	Score (5 Max)	Weighted score
Replaceability	30	4	24	3	18	1	6
Ease of Fabrication	20	3	12	4	16	1	4
Accuracy and Reliability	20	4	16	5	20	5	20
Safety	15	4	12	5	15	2	6
Cost	10	5	10	4	8	1	2
Life in Service	5	3	3	4	4	5	5
Total	100	**Total**	77	**Total**	81	**Total**	43

Scoring Criteria:
Replaceability (30%) - The residents should be able to take out the replaceable parts of the model without tampering with the static parts. Additionally, replacing the parts should not be time consuming.

Ease of fabrication (20%)- Ease of fabrication includes efficiency and simplicity to fabricate the replaceable parts since the parts will have to be replaced after every practice operation performed.

Safety (15%) - The product needs to adhere to ISO standard and regulation 13485:2016. Because the model will be interacting with a diode laser, the model should also adhere to IEC-60825. Since the design is an anatomical model, the design should also adhere to ISO/Ts 23541-1:2021 and FDA Code of Federal Regulations Title 21, volume 8. For more information, see Appendix A for the PDS.

Accuracy and Reliability (15%) - The materials chosen for both the replaceable and static parts should represent accurate dimensions and mechanical properties of an equine upper-airway anatomical feature, specifically, from the nostril to the back of the larynx, as found in PDS, Appendix A. Residents should be able to insert a diode laser and a surgical tong into the model and reach the larynx. In addition, residents should be able to practice a laser ventriculocordectomy without any of the static parts being damaged. The accuracy and reliability of the model will be monitored by providing a testing protocol for surgical residents to compare the model to a cadaver.

Cost (10%) - The budget is $1,000, but it is important to consider that the total budget needs to include the static portion of the model and at least 14 replaceable pieces.

Life in Service (5%)- The removable part should last one complete surgical procedure. However, the static portion of the design should be able to last 20 years with at least 14 uses per semester.

Vocal Fold Material

Biomaterial #1: Agarose

Agarose is a frequently used biomaterial to replace and replicate articular cartilage in the equine larynx [19]. This natural material has very similar mechanical properties to cartilage all around the body. This was tested in vivo and vitro to help cell revival and healing of the cartilage [20]. The higher density ratio of agarose to water will increase the mechanical strength of the material. It is important to have the correct ratio in order to correctly replicate the material properties of the equine laryngeal tissue. From literature the failure stress of 1% agarose gel is between 48 ± 8 and 55 ± 3 KPa, and the failure strain is between 0.208 ± 0.025 and 0.43 ± 0.015 [21]. Agarose is about $50 for a small 25g container and the set time is about 45 minutes or less [22].

Biomaterial #2: Gelatin

Gelatin is a common material used in pharmaceuticals, scaffolds and hydrogels in the medical industry [23]. Gelatin is a natural polymer derivative of collagen. Collagen is extremely abundant in the human body specifically in the tendons, ligaments and skin which makes the closely related structure and function of gelatin biocompatible for modeling tissues [24]. The tensile strength of gelatin is 46 MPa with an elongation at break of 3% [25]. Gelatin properties can be tailored to have enhanced mechanical properties and increased biocompatibility through chemical crosslinking. Gelatin is about $40 for a 100g container and the set time is 4-6 hours [26].

Biomaterial #3: Silicone

Silicone is a material used often as a replacement for many different biomaterials in the body [27]. It is used often for tissue membranes, heart valves, finger joints and prosthetics [28]. This means that the mechanical properties of silicone are comparable to the mechanical properties needed for other tissues in the equine larynx. It also shows how silicone can be molded to have different strengths and shapes to be exactly what is needed. The yield strength of silicone is found to be 0.0448-145 MPa [29]. The tensile strength is found to be 0.138-165 MPa [29]. This shows that the biomimicry of silicone is very close to the equine laryngeal tissues the team is looking to replicate. Silicone is about $80 for 25g and takes 21 days or less to set up [30].

Design Matrix for Vocal Fold Material

Table 2: *Design Matrix with all methods scored on biomimicry, duration of fabrication, ease of fabrication, shelf life, and cost.*

		Agarose		Gelatin		Silicone	
Criteria	Weight	Score (5 Max)	Weighted Score	Score (5 Max)	Weighted Score	Score (5 Max)	Weighted Score
Biomimicry	40	4	32	4	32	2	16
Duration of fabrication	20	4	16	3	12	1	4
Ease of fabrication	20	5	20	2	8	1	4
Shelf life	15	2	6	5	15	4	12
Cost	5	4	4	4	4	2	2
Total	100	**Total**	78	**Total**	71	**Total**	38

Biomimicry (40%): The texture and mechanical properties of the biomaterial need to be anatomically similar to the equine vocal fold. More specifically, young's modulus of the vocal fold model should accurately reflect that of the anatomical equine vocal fold. The ultimate strength of the vocal fold should withstand interaction with surgical instruments.

Duration of Fabrication (20%): Time required to complete the fabrication of the vocal fold by following the fabrication protocol. The duration of fabrication should be kept to a minimum as veterinary residents have little time to spare.

Ease of Fabrication (20%): The fabrication protocol for the vocal fold should be as simple as possible. The fewer steps a resident has to take, the fewer possible errors the residents can make, letting the residents have the most accurate practice experience with the model.

Shelf life (15%): The residents are expected to practice on the model twice a week, meaning that a set of vocal folds should have a shelf life of at least one week. However, longer shelf life with lower changes in mechanical properties will be more desirable as extra vocal folds can be stored for future use.

Cost (5%): The material costs should be kept at a minimum. The surgical residents will be creating new vocal folds daily, so the material used to make them will need to be cost effective.

Proposed Final Design

After evaluating the three proposed designs using the design matrix, the team chose to move forward with the second design, the Replaceable Laryngeal Disk. Although the Replaceable Gelatin Vocal Folds design excelled in replicability and cost, the design did not have an efficient way to remove and insert the vocal folds without compromising some of the accuracies of the anatomy in the larynx. The Replaceable Larynx tied with the Replaceable Laryngeal Disk in accuracy and reliability, scored low on safety and ease of fabrication because the replacing the vocal folds would require replacing the the entire larynx structure and could cause more harm to both the replaceable and statics components of the model if hit incorrectly by a diode laser. It would also be very costly to fix this design. In regards to the second design, it scored high in accuracy and reliability because of the mechanical properties, texture, and dexterity of the gelatin folds compared to that of an equine larynx. The third design also scored high accuracy and reliability because the fully replaceable larynx design allows the residents to understand the anatomy and surgical technique impact on tissues surrounding the equine vocal fold when they remove the larynx after each surgery.

Unlike the Replaceable Larynx, the first two designs had higher biocompatibility with the diode laser which caused both designs to score higher in safety. The Replaceable Laryngeal Disk design scored higher than the Replaceable Gelatin Vocal Folds design because the disk components contain the replaceable parts in a firm position that also attach more sturdily to the model with the pin mechanism. Therefore creates less contact between the statics and the replaceable components.

Overall, the Replaceable Laryngeal Disk Design came out on top due to its ease of fabrication, accuracy and reliability, and safety factors. The Replaceable Laryngeal Disk design will proceed to fabrication and testing. For dimensions and specifications on the final disk design, refer to Appendix D.

In regards to the vocal folds, the team decided to move forward with the first material, agarose. Gelatin and agarose were equal in biomimicry showing that they are both acceptable to use for the vocal fold replication. Although gelatin excelled in shelf life compared to the other materials, the setting time was longer compared to that of agarose and the fabrication process was more complex. Agarose outweighed or equaled the other materials for every other category making it the final material choice. This is all seen in Table 2.

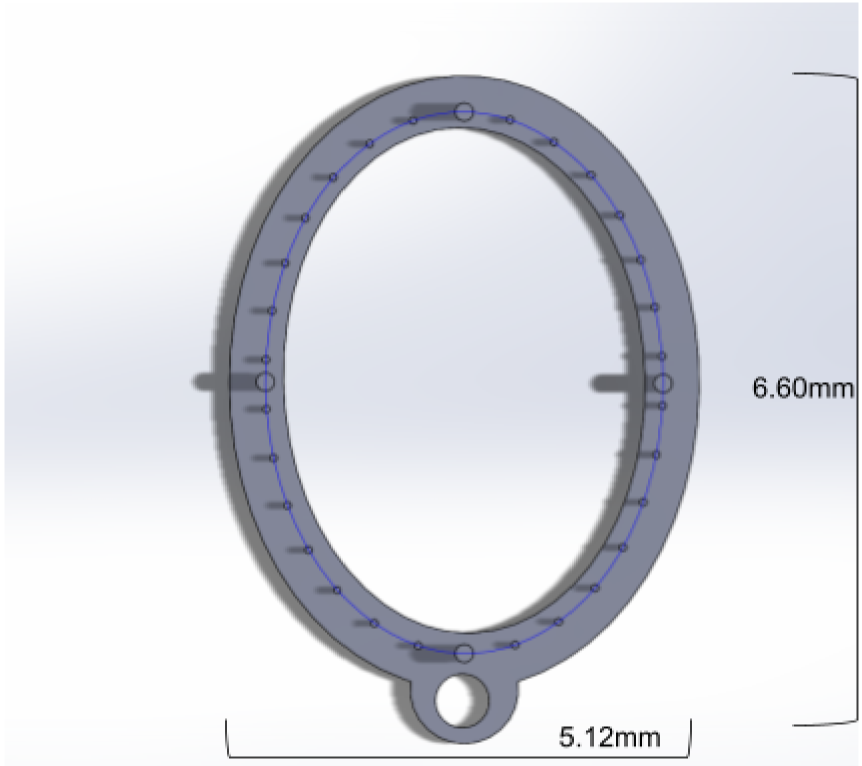


Figure 8: *Final Disk Prototype*

V. Fabrication/Development Process

Materials[1]

<u>Static Components</u>

The static components of this model will include an anatomical horse head that holds a vocal fold disk. The outer equine head model from nose to larynx will be 3D printed from PLA in an Ultimaker. The inner upper respiratory tract anatomy that the diode laser passes through will be made of Flexible 80A Resin to represent cartilage and BioMed White Resin to represent bone. Between the inner respiratory tract and outer horse head, will be filled in with styrofoam.

<u>Replaceable Components</u>

There are three replaceable components to the design: two gelatin folds and one reusable disk. The gelatin folds are molded from a mixture of agarose and water. The mold for the vocal folds and the vocal fold disk cartridge, both outer rim and inner pockets will be fabricated with tough PLA.

[1]See Appendix E for Expense Table

Methods

Fabrication methods began with the acquisition of equine CT scans from the University of Wisconsin School of Veterinary medicine. In Vesalius and Blender, the nasal cavity, larynx, and upper trachea was isolated. Even after isolating the CT scan, the files were too big for the computer to compute into an STL. Therefore, the team moved forward with splitting the frozen larynx, given by the client, and 3D scanning both halves. Then using Blender, the larynx was meshed back together and printed with Flexible 80A resin in a Formlabs 3D printer at the UW Makerspace. See Appendix F for more information about 3D scanning and the fabrication process of the larynx.

The disk portion was designed in SOLIDWORKS where it then is printed with tough PLA in the UW Makerspace. The slot for the cartridge was included in the design of the molds and the 3D print of the outside of the model. Two L brackets and 4 screws were drilled into the outer model. These will provide the holes through which the pin slides and secures the disk into place.

The vocal fold mold was also designed in SOLIDWORKS and then printed from tough PLA in the UW Makerspace. A ratio of 1mg: 10 mL agarose to water was mixed at 150℃ for five to seven minutes. This will create a 1% agarose mixture. This mixture was poured into the vocal fold mold and sat at room temp to harden for 30-45 minutes. See Appendix G for more information about fabrication of the disk and vocal folds.

While the primary focus of this model is on the interior, an outer horse head form will be fabricated from a lightweight material such as PLA with 20% infill. The horse's head will be 3D printed and assembled. It may happen for ease of fabrication that two halves are cut and carved separately, then glued together with a two-part activating glue from the UW Makerspace. The detail in the horse's face will be embellished as time allows.

Final Prototype

The final prototype consists of a static component and a replaceable component, which itself has two parts. The final static component included the larynx from the epiglottis to the trachea. The larynx was 3D printed in Flexible 80A resin and has a height of 83.69 mm, length of 146.70mm, and width of 72.8mm, as seen in Figure 9.

The replaceable component includes a pair of vocal cords and a vocal fold disk that will house the replaceable vocal folds (Figure 10). The disk was printed in Tough PLA and the vocal folds were a 1% mixture of agarose and water. The vocal fold disk has a height of 6.60mm, width of 5.12mm, and thickness of .2mm. The mold for the vocal folds was designed in SOLIDWORKS and printed from PLA on an Ultimaker printer (Figure 11). The design measurements were based on the cartridge disk to get a similar circumferential shape and accurate dimensions. Surgical images of equine vocal folds were used to approximate the angularity of the vocal folds.

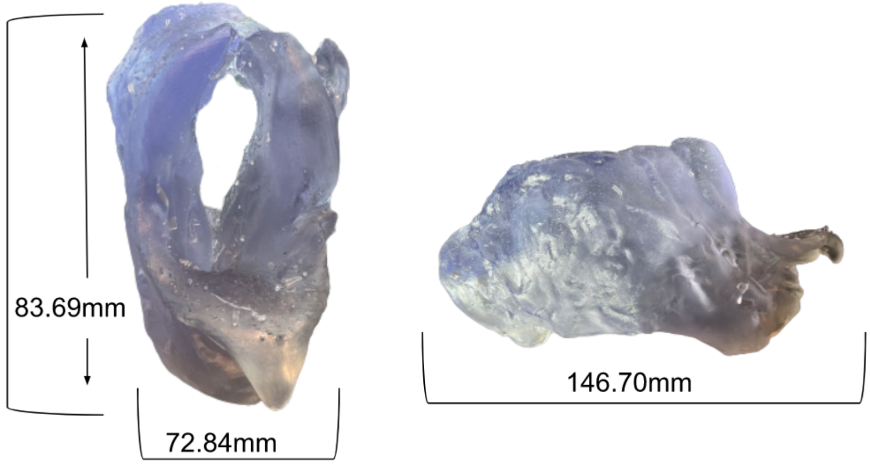


Figure 9: *3D printed larynx*

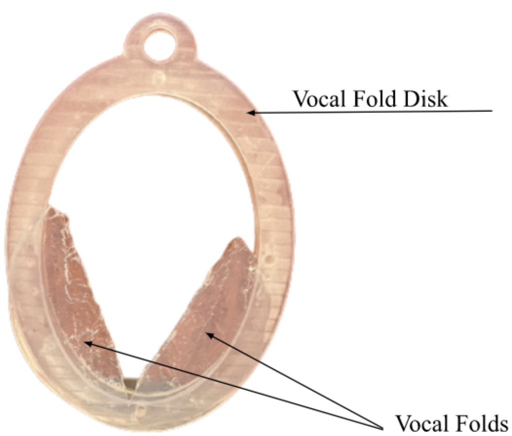


Figure 10*: Vocal Folds and Disk*

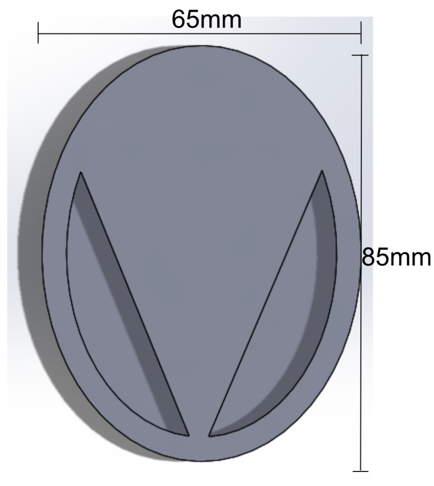


Figure 11: *Agarose Vocal fold Mold*

Testing

The final design will be tested in four aspects: anatomical accuracy, mechanical properties, similarity, and ease of fabrication and replacement. The model's measurements will be compared to the corresponding measurements to ensure anatomical accuracy The MTS machine will be used to assess the mechanical properties of the laryngeal model. The similarity test and ease of fabrication and replacement test will be tested by surgical residents at the UW School of Veterinary Medicine. See Appendix H for testing protocols.

1. *Anatomical Accuracy test:* This test was completed by the team to assess how the structures of the proposed model are replicants of equine anatomical structures. The different variables tested were the larynx's length, width, and height. These measurements were collected from the CT scans, the 3D printed model, the dissected larynx, and the literature values. The test will be passed if the statistically calculated p-value is statistically significant.
2. *Mechanical Properties test:* The mechanical properties of the design will be tested through MTS compression testing. This testing will be completed on the parts of the model that replicate equine vocal cords and equine laryngeal tissues. After completing compression testing on the biomaterials of the design, the goal is to compare the resulting Young's Moduli to the Young's Moduli of the dissected equine laryngeal tissues. The test will be passed if the statistically calculated p-value is statistically significant. The statistical analysis will also require a test for outliers in the samples.
3. *Similarity test:* The model will be tested by 6-8 current surgical residents and/or veterinary surgeons on their personal ranking of the model's biomaterial texture, anatomical shape, relative size, and procedural accuracy. Each category will be ranked on a 1 to 5 scale by the user. A score of 1 indicates zero to no similarity, a score of 2 indicates slight similarity, a score of 3 indicates fair similarity, a score of 4 indicates

considerable similarity, and a score of 5 indicates high similarity. A passed test is defined as a score of 4/5 or higher to keep the percent error similar to that of other tests.

4. *Ease of Fabrication and Replacement test:* The model will be tested by 6-8 current surgical residents and/or veterinary surgeons on their ability to fabricate and replace the vocal folds. This procedure will assess two tests that both have to pass in order for the overall test to be considered passing. For the first part, test 1 is passed if the user is able to create agarose folds using a reusable PLA model in 30 minutes or less [31]. For the second part, test 2 is passed if the user is able to remove the laryngeal disk, remove old vocal folds, replace new agarose vocal folds, and insert the fresh disk back into the model in 10 minutes or less. If test 1 and test 2 are able to be passed, the Ease of Fabrication and Replacement test is passed.

VI. Results

Anatomical Accuracy Test

The anatomical accuracy test was performed on the 3D printed laryngeal model and the dissected equine larynx to evaluate the accuracy of fabricating the model via two 3D scans. Due to the lack of authentic information in literature, the anatomical measurements of the 3D printed model and the dissected larynx were only compared to each other, rather than literature values in the final statistical analysis. The length, width, and height of the two subjects were recorded (Table 3). An ANOVA statistical test was conducted on these measurements and produced a p-value of 0.0468. Therefore, the anatomical measurements were statistically significant, and the test was passed.

Mechanical Properties Test

The mechanical properties test was conducted on three agarose samples and three equine tissue samples. The agarose samples were made using a reusable mold to acquire repeatable and similar dimensions of the samples. A CO_2 laser was used to cut three cylinders of equine tissue to achieve the same diameters as the agarose samples. All diameters and lengths of the six total samples were recorded for MTS input and statistical analysis. After MTS testing results were gathered, stress/strain curves were created, and Young's modulus was derived from the linear region of the curve. Figure 12 depicts the stress/strain curve of agarose and an average Young's Modulus of 0.0976 MPa was calculated with a standard deviation of 0.0197. Figure 13 depicts the stress/strain curve of the equine tissues and an average Young's Modulus of 2.7857 MPa was calculated with a standard deviation of 2.9477 (Table 4). The comparison between agarose and equine tissue stress/strain curves can be seen in Figure 14. A statistical t-test was performed on all six samples to achieve a p-value of 0.2524. Therefore, the mechanical properties were statistically insignificant, and the test was not passed. A Q-test was conducted on the three equine tissue samples to determine if there is an outlier among these samples due to the larger standard deviation. The Q(experimental) value was found to be 1.636. This value was compared to the Q(critical) value of 0.970 for three samples. With Q(experimental) being greater than Q(critical) it was confirmed that there was an outlier in the equine samples. Figure 15 shows the small deviation among the agarose samples compared to the larger deviation among the equine tissue samples inferring that there is an outlier present. This may be a result of one sample having more muscle fibers or cartilage present in the dissected section due to an inability to only dissect the equine vocal cords. All graphs and statistical analysis were created and performed in Matlab, see Appendix I for Matlab code.

Anatomical Accuracy Testing Data

	Length (mm)	**Width (mm)**	**Height (mm)**
3D Printed Model	146.70	72.84	83.69
Actual Equine Larynx	182.88	72.97	81.10

Tabel 3: *Measured Anatomical Values*

Mechanical Testing Data

Material	**Avg. Young's Modulus (MPa)**	**Standard Deviation**
Agarose	0.0976	0.0197
Equine Tissue	2.7857	2.9447

Table 4: *Material Mechanical Properties*

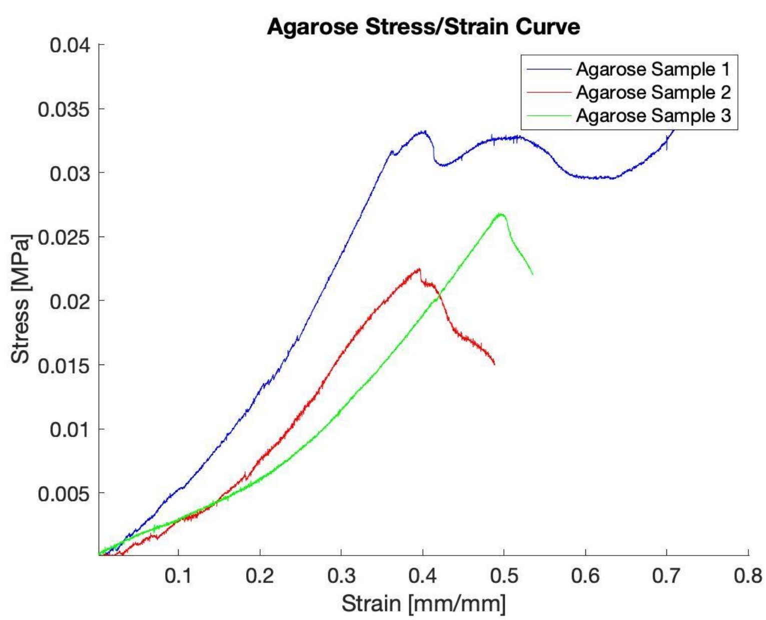


Figure 12: *Stress-Strain curve generated from MTS data for Agarose samples*

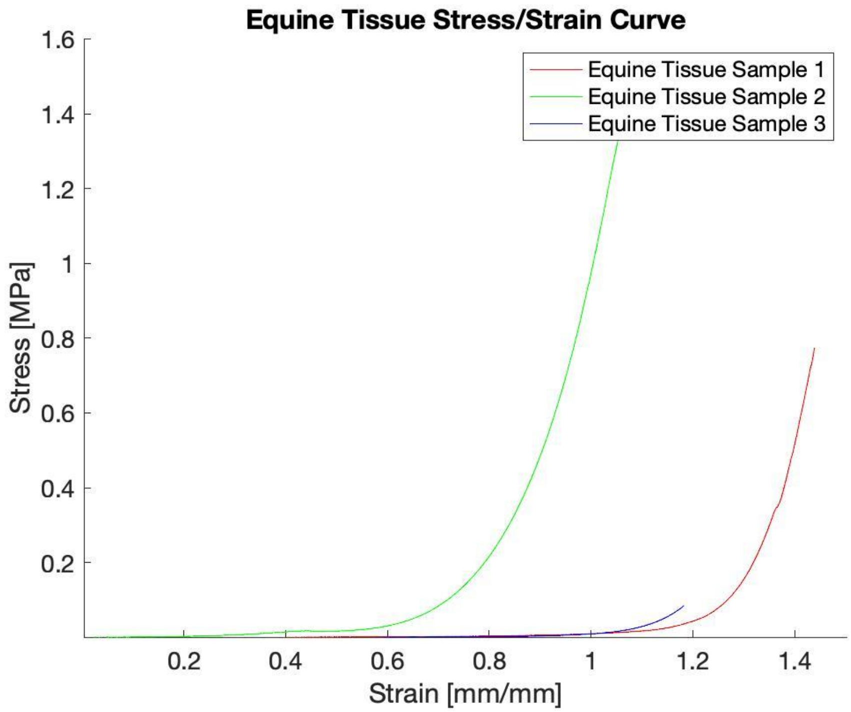


Figure 13: *Stress-Strain curve generated from MTS data for Equine Tissue samples*

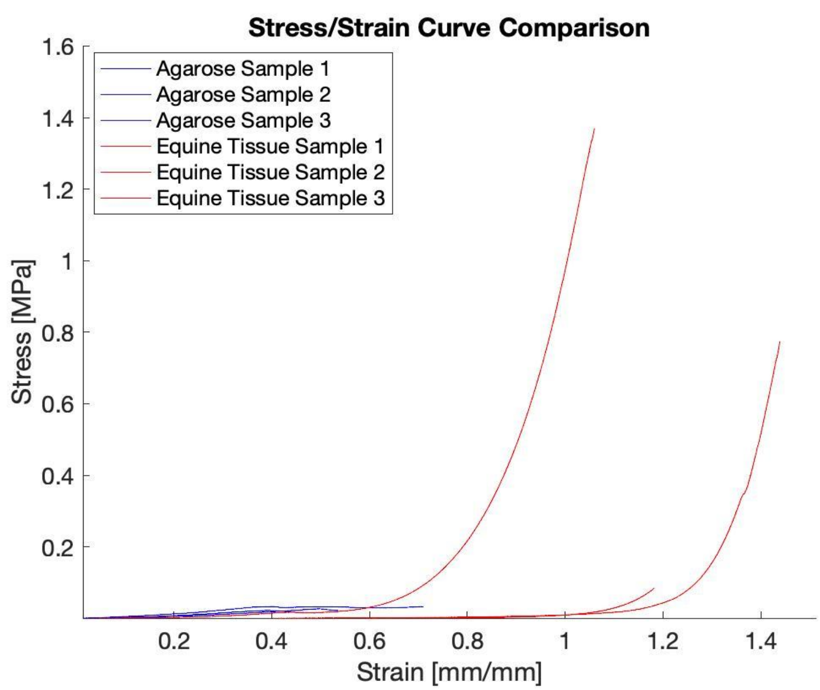


Figure 14: *Stress-Strain curve generated from MTS data for comparing Agarose and Equine Tissue samples*

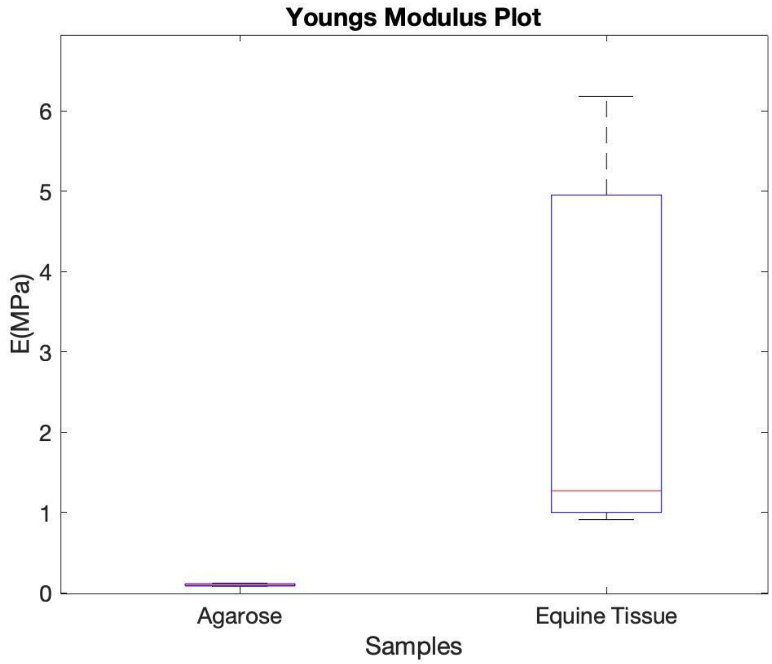


Figure 15: *A box plot comparing the Young's Moduli of both Agarose and Equine Tissue samples*

VII. Discussion

The t-test conducted between the young's modulus of the agarose samples and the tissue samples resulted in a p value of 0.2524. Since the p value is greater than 0.05, the difference between the elastic modulus of the agarose samples and the anatomical tissue samples are not statistically significant. Although the difference is not statistically significant, there is still a 2800% difference between the elastic modulus of the agarose samples and tissue samples. There are three main contributing factors to this result. First, the lack of a robust sample size can contribute to the lack of statistical significance. A sample size of at least 30 is generally needed for a 2 sample t-test while the mechanical test was conducted on three agarose samples and three tissue samples. Second, the samples of the equine tissue may be another contributing factor. The non-uniform cross section of the tissue samples negatively impacts the result of the compression test. Another reason for the question result may be due to non-uniform composition of the tissues. For example, one sample may have more cartilage than muscle compared to the other tissue samples. Although the tissue samples were cut using a CO_2 laser, due to the fibrous nature of the sample, the cross section remained variable throughout. Additionally, the tissue samples used were thawed from a dissected larynx. The use of thawed tissue questions the validity of the testing data as tissue samples from cadavers are not accurate representations of live tissues. Third, the agarose samples were not cross-linked with agents to be tailored to the anatomical mechanical properties.

Furthermore, the anatomical accuracy of the larynx is tested by measuring corresponding measurements from the dissected larynx and the 3D printed model. The test yielded a statistically significant difference between the measurements taken from the model and the dissected larynx. This concludes that the model significantly deviates from the anatomical measurements, rendering the model inaccurate. The original fabrication method for the static portion of the model is to 3D print using the STL file converted from the CT scans. However, due to the large file sizes, the computer could not convert the scans to STL files appropriately. The 3D scanned file had to be heavily patched before being 3D printed, resulting in anatomical inaccuracies. Furthermore, due to the lack of research on the equine larynx, reliable and consistent literature values for adult horses were not found. Thus, literature values were not compared to.

Due to multiple failed printing, a final laryngeal model was not obtained. This did not allow the veterinary residences to work with the prototype and submit a similarity score. The similarity score is imperative to the success of the model, as the model needs to accurately represent the surgical conditions the residents will experience. However, this can be conducted after the final prototype is fabricated. Additionally, the residents were unable to interact with agarose, thus, the time for the residents to fabricate the vocal folds using the agarose hydrogel sample is unknown. This will be addressed when residents are available for testing.

Ethical considerations need to be taken into account as the experience of the model will affect horses suffering from vocal fold deformations. The accuracy of the surgical experience is of the utmost importance. The client plans to use the model to train veterinary surgical residents for equine procedures.

VIII. Future Works

Due to the need for more research on the mechanical properties of the equine laryngeal tissue, the first step is to perform more mechanical testing on anatomical tissues. With this information, the material selection for the model could produce a much more accurate anatomical model. After the CT scan of the equine larynx is converted to an STL file successfully, the converted file will be modified by removing the cross section of the vocal folds so the vocal fold disk can be inserted. The STL file will then be printed by the Formlabs 3D printer using the Flexible 80A resin. In addition to using the CT scan to print the static component of the equine larynx, a more accurate mold of the vocal folds can be fabricated. Different molds for the vocal folds can be fabricated to simulate different surgical complications and conditions for the residents. The different vocal folds will prepare the residents much better for real-time procedures.

Anatomical accuracy is imperative to the application of the equine laryngeal model. So, future testing will consist of consulting residents and veterinary surgeons about the similarity of the experience practicing with the model versus executing the procedure on a living horse. The similarity score obtained from the residents and surgeons will be based on the similarity survey mentioned in the testing protocol. The residents will also carry out the fabrication procedure of the agarose-based vocal folds using the protocol mentioned in section V. If the residents were able to fabricate the agarose-based vocal folds within an average of ten minutes, excluding the setting time for the solution, the fabrication protocol would render as appropriate.

Conclusions

The client is searching for an equine laryngeal model that allows veterinary residents to practice a transendoscopic laser ventriculocordectomy surgery. The model should be anatomically accurate while housing a replaceable vocal fold within an anatomically accurate larynx. The team has proposed, fabricated, and tested a design that includes a 3D printed, static larynx and a 3D printed, removable disk that houses agarose-water mixed vocal folds. The larynx was printed by the Formlabs 3D printer using Flexible 80A resin and the vocal fold disk was printed by the Ultimaker printer using tough PLA. The vocal fold sample made from agarose succeeded in mechanical testing. However, cross-linking agents will be incorporated into the agarose hydrogel sample to increase the mechanical similarity between the model and the actual vocal fold. A more robust sample size should be used to assess the testing results' validity.

On the other hand, the model showed low accuracy in the anatomical accuracy test. The team concluded that using the CT scan to 3D print the model would result in a more accurate model compared to the 3D scanning method of the team. In the future, the overall concepts and designs of the model will continue with the static larynx and removable vocal fold with improvements in the anatomical accuracy of the models and the mechanical properties of the vocal folds.

VIII. References (IEEE)

[1] “Laser Ventriculocordectomy.pdf | Powered by Box.” [Online]. Available: https://uwmadison.app.box.com/s/fywsvuznp0pqtkggz0t8annkwzsh4jij/file/1013021672082. [Accessed: 09-Oct-2022].
[2] K. Copeland, “How Long Does It Take to Become a Veterinarian? (2022 Facts & Statistics),” Pet Keen, Dec. 13, 2021. https://petkeen.com/veterinarian-training-statistics/ (accessed Oct. 12, 2022).
[3] “SynDaver® Surgical Model | SynDaver.” https://syndaver.com/product/syntissue-surgical-model/ (accessed Sep. 27, 2022).
[4] “3D horse anatomy,” Biosphera, 26-Apr-2022. [Online]. Available: https://biosphera3d.com/product/3d-horse-anatomy-software/. [Accessed: 10-Oct-2022].
[5] “Why Is My Horse Struggling to Breathe?” https://flairstrips.com/horse-breathing-problems/ (accessed Oct. 11, 2022).
[6] “Laryngeal Hemiplegia in Horses | American College of Veterinary Surgeons - ACVS.” https://www.acvs.org/large-animal/laryngeal-hemiplegia (accessed Dec. 5, 2022).
[7] J. R. Fredieu, J. Kerbo, M. Herron, R. Klatte, and M. Cooke, “Anatomical Models: a Digital Revolution,” Med.Sci.Educ., vol. 25, no. 2, pp. 183–194, Jun. 2015, doi: 10.1007/s40670-015-0115-9.
[8] “3D Horse Anatomy - Biosphera.” https://biosphera3d.com/product/3d-horse-anatomy-software/ (accessed Oct. 12, 2022).
[9] “Checking the beat: validation of a novel deformable 3D-Printed heart model for radiotherapy applications.” https://bmedesign.engr.wisc.edu/projects/f22/checking_the_beat (accessed Oct. 12, 2022).
[10] “Structural and mechanical functions of bones, muscles and joints by use of 3D models in veterinary medical education.” https://bmedesign.engr.wisc.edu/projects/f22/veterinary_models (accessed Oct. 12, 2022).
[11] “3D Printers,” UW Makerspace. https://making.engr.wisc.edu/3d-printers/ (accessed Oct. 12, 2022).
[12] J. Kuhl, J. Hauschild, and D. Krause, “Comparing friction of additively manufactured materials with animal blood vessels,” Annals of 3D Printed Medicine, vol. 7, p. 100061, Aug. 2022, doi: 10.1016/j.stlm.2022.100061.
[13] J. Hao et al., “Material characterization and selection for 3D-printed spine models,” 3D Print Med, vol. 4, no. 1, p. 8, Oct. 2018, doi: 10.1186/s41205-018-0032-9.
[14] “What is PLA? (Everything You Need To Know).” https://www.twi-global.com/technical-knowledge/faqs/what-is-pla.aspx (accessed Oct. 12, 2022).
[15] “Formlabs Form 3+ Basic Package | Dynamism.” https://www.google.com/imgres (accessed Oct. 12, 2022).
[16]M. K. Caspers, C. D. Bell, and D. M. Tatarniuk, “Transendoscopic Ventriculocordectomy Using Monopolar Electrosurgical Instrumentation for Conjunctive Treatment of Laryngeal

Hemiplegia in Horses: 24 Cases (2017–2019),” Frontiers in Veterinary Science, vol. 8, 2021, Accessed: Oct. 09, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fvets.2021.628410
[17] T. V. Saliba and R. S. M. de Barros, “Development and validation of a 3D laryngeal model in surgical skills training,” Brazilian Journal of Otorhinolaryngology, p. S1808869421001889, Nov. 2021, doi: 10.1016/j.bjorl.2021.09.010.
[18] A. Lucas, A. Ramos, and P. Quadras, “Anatomy models using Silicone Moulds - An innovative method,” National Journal of Clinical Anatomy, vol. 7, no. 4. pp. 209–212, 2018. [Online]. Available: http://www.njca.info/article.asp?issn=2277-4025;year=2018;volume=7;issue=4;spage=209;epage=212;aulast=Lucas
[19] D. F. Duarte Campos, W. Drescher, B. Rath, M. Tingart, and H. Fischer, “Supporting biomaterials for articular cartilage repair,” Cartilage, Jul-2012. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4297118/. [Accessed: 13-Dec-2022].
[20] B. J. DeKosky, N. H. Dormer, G. C. Ingavle, C. H. Roatch, J. Lomakin, M. S. Detamore, and S. H. Gehrke, “Hierarchically designed agarose and poly(ethylene glycol) interpenetrating network hydrogels for cartilage tissue engineering,” Tissue engineering. Part C, Methods, Dec-2010. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2988644/. [Accessed: 13-Dec-2022].
[21] “New insight into agarose gel mechanical properties.” [Online]. Available: https://pubs.acs.org/doi/10.1021/bm005583j. [Accessed: 13-Dec-2022].
[22] “Benchmark agarose Le 25g - organic solvent free,” Universal Medical. [Online]. Available: https://www.universalmedicalinc.com/benchmark-agarose-le-25g-organic-solvent-free.html?campaignid=1028940174&adgroupid=46136329130&adid=243354508307&gclid=Cj0KCQiA4uCcBhDdARIsAH5jyUmYudnq-kHc1_n7DDFot3I6wbT89mHqVLFIo_ngmXkamb4KAjlZtBUaAouwEALw_wcB. [Accessed: 13-Dec-2022].
[23] J. Alipal et al., “A review of gelatin: Properties, sources, process, applications, and commercialisation,” Materials Today: Proceedings, vol. 42, pp. 240–250, Jan. 2021, doi: 10.1016/j.matpr.2020.12.922.
[24] “The Unique Properties Of Gelatin In Tissue Engineering And 3D Bioprinting.” https://www.rousselot.com/biomedical/media/blog/testimonials/the-unique-properties-of-gelatin-in-tissue-engineering-and-3d-bioprinting (accessed Nov 8, 2022).
[25] H. U. Zaman, M. A. Khan, and R. A. Khan, “Studies on the Thermo-Mechanical Properties of Gelatin Based Films Using 2-Hydroxyethyl Methacrylate by Gamma Radiation,” vol. 2012, Jan. 2012, doi: 10.4236/ojcm.2012.21003.
[26] “Gelatin, 250 Bloom, Powder, NF, Spectrum Chemical, Quantity: 500 g | Fisher Scientific.” https://www.fishersci.com/shop/products/gelatin-250-bloom-powder-nf-spectrum-chemical/18602891?gclid=Cj0KCQiA4uCcBhDdARIsAH5jyUl7Hot7ly5v__J0l__iVBXOaDwucgue4H_aSSupOMy_7ImK5WafHMEaAiqWEALw_wcB&ef_id=Cj0KCQiA4uCcBhDdARIsAH5jyUl7Hot7l

y5v__J0l__iVBXOaDwucgue4H_aSSupOMy_7ImK5WafHMEaAiqWEALw_wcB:G:s&ppc_id=PLA_goog_16713066638__18602891___8356276070972442572&ev_chn=shop&s_kwcid=AL!4428!3!!!!x!!18602891 (accessed Dec. 14, 2022).
[27] M. Wang and C. Yu, “Silicone rubber: An alternative for repair of articular cartilage defects - knee surgery, sports traumatology, arthroscopy,” SpringerLink, 31-Jan-2004. [Online]. Available: https://link.springer.com/article/10.1007/s00167-003-0447-7. [Accessed: 13-Dec-2022].
[28] “Wiley Online Library | Scientific Research Articles, journals, books ...” [Online]. Available: https://onlinelibrary.wiley.com/. [Accessed: 13-Dec-2022].
[29] T. Xometry, “7 properties of silicone,” Xometrys RSS, 28-Jul-2022. [Online]. Available: https://www.xometry.com/resources/materials/properties-of-silicone/. [Accessed: 13-Dec-2022].
[30] “Silicon dioxide, powder -40 mesh, 99.998% (trace metals basis), 25g,” eBay. [Online]. Available: https://www.ebay.com/itm/232587203013?chn=ps&mkevt=1&mkcid=28&srsltid=AYJSbAdXAzJyTlz_NCKGvQpC6EZdcz9JKw-uwgYla3j6AAVTdhdnZnOFbHo. [Accessed: 13-Dec-2022].
[31] “Addgene: Protocol - How to Run an Agarose Gel.” https://www.addgene.org/protocols/gel-electrophoresis/ (accessed Oct. 12, 2022).

IX. Appendix

Appendix A: Product Design Specification

Function: The function of the upper airway is crucial to the athletic ability of horses. However, 1 in every 24 horses experiences hemorrhage from the vocal cord site [1]. This condition calls for laser ventriculocordectomy, a common upper airway surgery performed on horses. As of 2020, according to the AAVMC report, there are 14000 total vet students enrolled in vet schools [2]. However, only one or two cadavers are used per year for vet school to learn about equine upper airway procedures [3]. A model must be designed that accurately mimics the anatomy of the equine upper airway for veterinary residents to practice. The device should contain a replaceable vocal fold that will be replaced after every practice procedure. This model can address the lack of cadavers and can provide veterinary surgical residents with more practice for equine upper airway surgeries.

Client requirements:

- The model will be life sized and skeletally reflective of a matured horse at approximately 4 to 6 years of age.
- The model must replicate the entire equine nasal passage to the back of the larynx.
- The model should have removable and replaceable parts so the model can be reused after a single laser practice.
    - The replaceable part will include the two vocal folds of the larynx which will be replaced per laser ventriculocordectomy surgery.
    - All other aspects of the model will be static and will last at least 24 surgeries for one semester
- The materials should be as close in texture and strength as possible to the vocal cords, cartilage, and tissues of a live horse.
    - View the mechanical properties table and dimension table for more information
- Replicating external features of the model is at a lower priority compared to the vocal cords and other inner features of the model
- Materials provided by the client
    - A budget of $1000
    - CT scans of equine larynx
    - A live demonstration of an upper airway endoscopy with a mock surgery
- Timeline: All final deliverables must be completed by December 14th, 2022

Design requirements:

1. Physical and Operational Characteristics

a. ***Performance requirements*****:** The product must imitate the true anatomical size of an adult horse. The model must consist of reusable parts and replaceable parts. Specifically, the replaceable part will be the vocal fold and all other anatomical parts will be static. The portions that will be replaceable are the vocal folds. These replaceable parts must reflect the texture of real tissue. Finally, the model should extend from the head of the horse to the back of the larynx of the horse.

b. ***Safety*****:** The model should not produce toxic products when interacting with the diode laser. The model should also not contain sharp edges and corners due to fabrication. However, sharp edges or corners to imitate the anatomy of the horse is acceptable.

c. ***Accuracy and Reliability*****:** This model will replicate a structurally mature horse, about 4-6 years of age. This will be a true-to-size model of the horse's larynx and throat. The exact dimensions will be taken for a CT scan of a 4-year-old horse to make sure that our error is less than 5%. The texture, dexterity, and durability of the vocal folds and throat will need to be exactly replicated. The strength of the materials in the throat will need to withstand a 5,000-10,000 J carbon dioxide laser [4]. The strength of the vocal folds will need to be able to be cut by this same laser. Refer mechanical properties table, in section j, for specific measurements and error approximations that the model will adhere to.

d. ***Life in Service*****:** The static part of the model should be able to last one semester or 10 years. The replaceable parts in total should be able to last at least 14 surgeries per semester. Likely, there will be about one to two surgeries a week. The durability of the throat material should last the entirety of the semester and not be broken down by usage. The replaceable vocal cord pieces will only last one surgery as they are going to be cut off by the laser.

e. ***Shelf Life*****:** The materials in the model should be able to withstand 20 years of shelf life. The predicted materials used will likely be plastic in nature, so the materials can last between 20-500 years in no sunlight and temperate rooms [5]. The replaceable material will only be able to last about 10 years due to the softer plastic being used.

f. ***Operating Environment*****:** The operating environment would be in a veterinary classroom. However, the model may also be in storage. So the average operating temperature would be around 20 ºC and the average humidity would be around 30% - 50%.

g. ***Ergonomics*****:** This model should not weigh more than 15 pounds so that it is easily transportable to different learning environments. The product should not have any sharp edges and should allow for comfortable surgical positioning [6]. Replacement pieces should be easily accessed and changed.

h. *Size*: The surgical model should be life-size. The model will represent the upper respiratory tract of a skeletally mature horse of 4-6 years of age [7]. The model will start at the nostril and continue to the end of the larynx. In a skeletally mature horse, the larynx should be approximately 7.62 centimeters in diameter [8].The model will mimic the realistic dimensions of a horse with a 63.5 cm nose circumference, 25.4 cm cheek, and 114.3 cm head measurement [9][10]. These measurements will be crossed and evaluated with our CT scans provided by Dr. Le. If the CT scan dimensions are close to the literature values found, our team will move forward using the CT scan dimensions.

Anatomical Feature for a mature horse (4-6 years of age)	Dimensions
Trachea	5.5cm diameter, 70 cm length
Larynx	7.62 centimeters in diameter, height around 5.87 cm
Nose	63.5 cm (circumference)
Head	114.3 cm (circumference)
Distance from cheek to the back of jaw bones	25.4 cm
Nostril	1.9 cm in width

Table 1. Approximate dimensions of anatomical features of the equine head to the back of the larynx. [11] [12] [13] [14]

i. *Weight*: A live adult horse's head weighs approximately 18 kg [15]. This is the maximum allowable weight of the model. Realistically, the model should weigh between 7 and 11kg, because the model will only include the components of a horse head pertaining to the upper respiratory system.

j. *Materials*: The model will have 2 main components in which one is static and the other is a removable and replaceable device. The static portion of the model will replicate the equine nasal cavity and will be made of plastic or 3D-printed material to ensure its hardness as well as flexibility [16]. The removable component will be a replicant of the laryngeal section of the equine throat. No specific material is required for the synthetic vocal cords, cartilage, and tissue substitutes, however, it must be cost-effective, easy to use, and replaceable. Acceptable suitable materials for vocal cords include silicone, gelatin, and rubber [17]. Cartilage can be replicated using a polymer made of nylon in powder form such as polyamide [17].

Anatomical features	Young's Modulus	Yield Stress	Ultimate Strength	Error Approximations
Equine laryngeal cartilage	0.42 ~ 2.51 MPa	x	9.1 MPa	5% error
Bone	16 GPa	110 MPa	226 MPa	10% error
Muscle	1186 KPa	x	x	10% error

Table 2. Mechanical properties of equine laryngeal cartilage, bone, and muscle. [18][19][20]

Since the bones and muscles will remain static in the model, the error for the model will be at 10%. On the other hand, since the equine larynx is the primary target for the surgical residents to practice the procedure, the design must remain anatomically accurate to the native tissue, thus having a smaller 5% error.

k. ***Aesthetics, Appearance, and Finish*****:** The aesthetic of this model should give the same look and feel as a horse head. It should be lighter in weight, but be a size replication of the throat and larynx. This head will be finished both internally and externally. The model will end at the back of the larynx and will be closed off at that point. The model will be able to be taken apart slightly to ease the exchange of the vocal fold removable parts.

2. Production Characteristics

a. ***Quantity*****:** The client has asked for one equine laryngeal model. The model, however, will undergo multiple operations, therefore it is necessary to have many of the replaceable parts. The life in service for the model is one semester where the model will be used up to two times a month by three students each. Therefore the client has requested 24 sets of replaceable parts.

b. ***Target Product Cost*****:** The target product cost for this device is $1000. It will be paid for via UW School of Veterinary Medicine Departmental teaching funds.

3. Miscellaneous

a. ***Standards and Specifications*****:** The model would need to adhere to the ISO 13485:2016 regulation which outlines requirements for regulatory purposes of medical devices. Regarding the equine laryngeal model, this standard specifies that for a technical support device, it must consistently meet customer and applicable regulatory requirements [21]. The model would also need to adhere to ISO/TS 23541-1:2021 regulation which makes sure that all 3D structural representations of humans (in alignment with other animals) are consistent and accurate [22]. In regards to the model being cut with a laser, the model and laser must follow IEC-60825 which provides requirements and specific guidelines for safe operations and maintenance while cutting various materials with a laser [23]. Lastly, The model would also need to follow the FDA's Code of Federal Regulations Title 21, Volume 8 which outlines the requirements for anatomical model devices [24].

b. ***Customer*****:** The client, Dr. Kayla Le, is a graduate professor for the Department of Surgical Sciences in the School of Veterinary Medicine at the University of Wisconsin - Madison. Dr. Le is asking for a life-like surgical equine model that can be used by surgical residents to practice upper-airway surgeries. Having a model that can be reusable, replaceable, and as detailed as possible would result in confidence among the surgical residents and overall better surgery performance.

c. ***Patient-related concerns***: The accuracy of the anatomy, size, shape, and material to imitate an adult equine is of the utmost concern for the client. The model only needs to be from the nostril to the larynx. Additionally, it is also important that the parts within the model can be replaced so that if a resident messes up and defects the model, the model can return to its original shape for the resident to try again. This model is for training surgical residents and gives them a chance to perfect a Transendoscopic Laser Ventriculocordectomy so that when they go into the veterinary field, their procedural success rate will be as high or higher than the average rate of 63% [1]. Therefore if the model is beyond a 5% error in the dimensions or the materials don't withstand the proper mechanical properties, that can lead to improper training.

d. Competition:

I. SynDaver Canine: The biggest competition to this project is the SynDaver Canine. This is an anatomical model of a canine used for surgical practices. This anatomical model is able to simulate not only the normal anatomy of a canine during surgery but also when complications occur. The current purchase price of a SynDaver Canine would be $28,500 and every time the model needs to be refurbished costs $3500 [25].

II. Erler Zimmer Foot of a Horse as Model: This competitor fabricated an anatomical model of a horse's foot from CT and MR co-registered data, making the model incredibly accurate. The model is 3D printed in full color. The structure also includes removable parts. However, this model may not be practical for surgery practice. The model is priced at $2,155.15 [26].

References: (IEEE)

[1] M. K. Caspers, C. D. Bell, and D. M. Tatarniuk, “Transendoscopic Ventriculocordectomy Using Monopolar Electrosurgical Instrumentation for Conjunctive Treatment of Laryngeal Hemiplegia in Horses: 24 Cases (2017–2019),” Frontiers in Veterinary Science, vol. 8, 2021, Accessed: Oct. 09, 2022. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fvets.2021.628410

[2] “Annual Reports,” AAVMC. https://www.aavmc.org/about-aavmc/annual-reports/ (accessed Oct. 09, 2022).

[3] “Animal Use in the DVM Program | Cummings School of Veterinary Medicine.” https://vet.tufts.edu/curriculum/animal-use-dvm-program (accessed Oct. 09, 2022).

[4] C. E. Henderson, K. E. Sullins, and J. A. Brown, “Transendoscopic, laser-assisted ventriculocordectomy for treatment of left laryngeal hemiplegia in horses: 22 cases (1999–2005),” AVMA, 15-Dec-2007. [Online]. Available: https://avmajournals.avma.org/view/journals/javma/231/12/javma.231.12.1868.xml. [Accessed: 14-Sep-2022].

[5] “How Long Does It Take for Plastic to Decompose? | Chariot Energy.” https://chariotenergy.com/blog/how-long-until-plastic-decomposes/ (accessed Sep. 23, 2022).

[6] “Surgery and Ergonomics | Surgery | JAMA Surgery | JAMA Network.” https://jamanetwork.com/journals/jamasurgery/fullarticle/390378 (accessed Sep. 23, 2022).

[7] “Conformation of the horse.” https://extension.umn.edu/horse-care-and-management/conformation-horse (accessed Sep. 23, 2022).

[8] C. W. Rogers, E. K. Gee, and K. E. Dittmer, “Growth and Bone Development in the Horse: When Is a Horse Skeletally Mature?,” Animals, vol. 11, no. 12, p. 3402, Nov. 2021, doi: 10.3390/ani11123402.

[9] C. L. M. Davenport-Goodall and E. J. Parente, “Disorders of the larynx,” Veterinary Clinics of North America: Equine Practice, vol. 19, no. 1, pp. 169–187, Apr. 2003, doi: 10.1016/S0749-0739(02)00072-X.

[10] “Measuring Guide and Size Chart.” https://www.yknotropetack.com/measuring-guide-and-size-chart.html (accessed Sep. 23, 2022).

[11]“Trachea - an overview | ScienceDirect Topics.”
https://www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/trachea (accessed Oct. 07, 2022).

[12]A. Carstens, R. M. Kirberger, R. J. Grimbeek, C. M. B. Donnellan, and M. N. Saulez, “Radiographic quantification of tracheal dimensions of the normal Thoroughbred horse,” Vet Radiol Ultrasound, vol. 50, no. 5, pp. 492–501, Oct. 2009, doi: 10.1111/j.1740-8261.2009.01u570.x.

[13]“Measuring Guide and Size Chart,” Carol Vander Wyst.
http://yknotropetack.com/measuring-guide-and-size-chart.html (accessed Oct. 07, 2022).

[14]T. Liuti, R. Reardon, S. Smith, and P. M. Dixon, “An anatomical study of the dorsal and ventral nasal conchal bullae in normal horses: Computed tomographic anatomical and morphometric findings,” Equine Veterinary Journal, vol. 48, no. 6, pp. 749–755, 2016, doi: 10.1111/evj.12516.

[15] “Comparable Parts No. 3 – Heads or Tales? – Eclectic Horseman.”
https://eclectic-horseman.com/comparable-parts-no-3-heads-or-tales/ (accessed Sep. 23, 2022).

[16] M. L. Smith and J. F. X. Jones, “Dual-extrusion 3D printing of anatomical models for education: Two Materials 3D Printing in Anatomy,” American Association of Anatomists, vol. 11, no. 1, pp. 65–72, Jan. 2018, doi: 10.1002/ase.1730.

[17] T. V. Saliba and R. S. M. de Barros, “Development and validation of a 3D laryngeal model in surgical skills training,” Brazilian Journal of Otorhinolaryngology, p. S1808869421001889, Nov. 2021, doi: 10.1016/j.bjorl.2021.09.010.

[18]S. N. Passman, J. Cheetham, L. J. Bonassar, N. G. Ducharme, and J. J. Rawlinson, “Biomechanical characterisation of equine laryngeal cartilage,” Equine Vet J, vol. 43, no. 5, pp. 592–598, Sep. 2011, doi: 10.1111/j.2042-3306.2010.00315.x.

[19]G. Bigot, A. Bouzidi, C. Rumelhart, and W. Martin-Rosset, “Evolution during growth of the mechanical properties of the cortical bone in equine cannon-bones,” Med Eng Phys, vol. 18, no. 1, pp. 79–87, Jan. 1996, doi: 10.1016/1350-4533(95)00022-4.

[20]M. D. Swanstrom et al., “Passive and active mechanical properties of the superficial and deep digital flexor muscles in the forelimbs of anesthetized Thoroughbred horses,” Journal of Biomechanics, vol. 38, no. 3, pp. 579–586, Mar. 2005, doi: 10.1016/j.jbiomech.2004.03.030.

[21] “ISO - ISO 13485:2016 - Medical devices — Quality management systems — Requirements for regulatory purposes.” https://www.iso.org/standard/59752.html (accessed Sep. 23, 2022).

[22] “ISO/TS 23541-1:2021(en), Health informatics — Categorial structure for representation of 3D human body position system — Part 1: Bones.” https://www.iso.org/obp/ui/#iso:std:iso:ts:23541:-1:ed-1:v1:en (accessed Sep. 23, 2022).

[23] “IEC 60825-1 Ed. 3.0 b:2014 - Safety of laser products - Part 1: Equipment classification and requirements.” https://webstore.ansi.org/Standards/IEC/IEC60825Ed2014?gclid=Cj0KCQjw7KqZBhCBARIsAI-fTKJz9cVLtRo5CAydN1JHtPTCllGma7oKxBkYyUKhd4qa0oIplWW6T94aAqsoEALw_wcB (accessed Sep. 23, 2022).

[24] “CFR - Code of Federal Regulations Title 21,” accessdata.fda.gov, 01-Apr-2020. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=864.2240. [Accessed: 20-Sep-2022].

[25] “Synthetic dogs now used in veterinary education - News - VIN.” https://news.vin.com/default.aspx?pid=210&catId=610&id=8158109 (accessed Sep. 23, 2022).

[26] “Erler Zimmer Foot of a Horse as Model - Model 1.” https://www.medicaldevicedepot.com/Erler-Zimmer-Foot-of-a-Horse-as-Model-Model-1-p/vet4400.htm?dfw_tracker=3918-103941&gclid=CjwKCAjwpqCZBhAbEiwAa7pXeY09-fflw6ffAi5kAQal15WKmJ7Xw40dVvpV9t1r7Py_Cg9QYoEuORoCOBIQAvD_BwE (accessed Sep. 23, 2022).

Appendix B: Flexible 80A Materials Property Data [1]

Flexible 80A Resin Material Properties Data

	METRIC[1]		IMPERIAL[1]		METHOD
	Green	Post-Cured[2]	Green	Post-Cured[2]	
Mechanical Properties					
Ultimate Tensile Strength[3]	3.7 MPa	8.9 MPa	539 psi	1290 psi	ASTM D 412-06 (A)
Stress at 50% Elongation	1.5 MPa	3.1 MPa	218 psi	433 psi	ASTM D 412-06 (A)
Stress at 100% Elongation	3.5 MPa	6.3 MPa	510 psi	909 psi	ASTM D 412-06 (A)
Elongation at Break	100%	120%	100%	120%	ASTM D 412-06 (A)
Shore Hardness	70A	80 A	70A	80 A	ASTM 2240
Compression Set (23 °C for 22 hours)	Not Tested	3%	Not Tested	3%	ASTM D 624-00
Compression Set (70 °C for 22 hours)	Not Tested	5%	Not Tested	5%	ASTM D 395-03 (B)
Tear Strength[4]	11 kN/m	24 kN/m	61 lbf/in	137 lbf/in	ASTM D 395-03 (B)
Ross Flex Fatigue at 23 °C	Not Tested	>200,000 cycles	Not Tested	>200,000 cycles	ASTM D1052, (notched), 60° bending, 100 cycles/minute
Ross Flex Fatigue at -10 °C	Not Tested	>50,000 cycles	Not Tested	>50,000 cycles	ASTM D1052, (notched), 60° bending, 100 cycles/minute
Bayshore Resilience	Not Tested	28%	Not Tested	28%	ASTM D2632
Thermal Properties					
Glass transition temperature (Tg)	Not Tested	27 °C	Not Tested	27 °C	DMA

[1] Material properties can vary with part geometry, print orientation, print settings and temperature.

[2] Data was obtained from parts printed using Form 3, 100 µm, Flexible 80A settings, washed in Form Wash for 10 minutes and postcured with Form Cure at 60 °C for 10 minutes.

[3] Tensile testing was performed after 3+ hours at 23 °C, using a Die C specimen cut from sheets.

[4] Tear testing was performed after 3+ hours at 23 °C, using a Die C tear specimen directly printed.

Solvent Compatibility

Percent weight gain over 24 hours for a printed and post-cured 1 x 1 x 1 cm cube immersed in respective solvent:

Solvent	24 Hour Weight Gain (%)	Solvent	24 Hour Weight Gain (%)
Acetic Acid, 5 %	0.9	Hydrogen Peroxide (3 %)	0.7
Acetone	37.4	Isooctane (aka gasoline)	1.6
Isopropyl Alcohol	11.7	Mineral Oil, light	0.1
Bleach, ~5 % NaOCl	0.6	Mineral Oil, heavy	<0.1
Butyl Acetate	51.4	Salt Water (3.5 % NaCl)	0.5
Diesel	2.3	Sodium hydroxide (0.025 %, pH = 10)	0.6
Diethyl glycol monomethyl ether	19.3	Water	0.7
Hydrolic Oil	1.0	Xylene	64.1
Skydrol 5	10.7	Strong Acid (HCl Conc)	28.6
Tripropylene Glycol Methyl Ether	13.6		

[1] “Flexible 80A Resin,” Formlabs. https://formlabs.com/store/flexible-80a-resin/ (accessed Oct. 12, 2022).

Appendix C: BioMed White Resin Materials Property Data [1]

MATERIAL PROPERTIES DATA **BioMed White Resin**

	METRIC [1]	IMPERIAL [1]	METHOD
	Post-Cured [2]	Post-Cured [2]	
Tensile Properties			
Ultimate Tensile Strength	45.78 MPa	6640 psi	ASTM D 638-14 (Type IV)
Young's Modulus	2020.16 MPa	293 ksi	ASTM D 638-14 (Type IV)
Elongation	10%	10%	ASTM D 638-14 (Type IV)
Flexural Properties			
Flexural Stress at 5% Strain	74.46 MPa	10800 psi	ASTM D 790-15 (Procedure B)
Flexural Modulus	2020.16 MPa	293 ksi	ASTM D 790-15 (Procedure B)
Hardness Properties			
Hardness Shore D	80 D	-	ASTM D2240-15 (Type D)
Impact Properties			
Notched IZOD	15.11 J/m	0.283 ft-lbf/in	ASTM D 256-10 (Method A)
Unnotched IZOD	269.03 J/m	5.04 ft-lbf/in	ASTM D 4812-11
Thermal Properties			
Heat Deflection Temp. @ 1.8 MPa	52.4 °C	-	ASTM D 648-18 (Method B)
Heat Deflection Temp. @ 0.45 MPa	67.0 °C	-	ASTM D 648-18 (Method B)
Coefficient of Thermal Expansion	90.1 µm/m/°C	-	ASTM E 831-13
Other Properties			
Water Absorption	0.40 wt%	-	ASTM D570-98

Sterilization Compatibility

E-beam	35 kGy E-beam radiation
Ethylene Oxide	100% Ethylene oxide at 55 °C for 180 minutes
Gamma	29.4 - 31.2 kGy gamma radiation
Steam Sterilization	Autoclave at 134°C for 20 minutes Autoclave at 121°C for 30 minutes

For more details on sterilization compatibilities, visit formlabs.com/medical

Disinfection Compatibility

Chemical Disinfection	70% Isopropyl Alcohol for 5 minutes

Samples printed with BioMed White Resin have been evaluated in accordance with the following biocompatibility endpoints:

ISO Standard	Description [3]
ISO 10993-5:2009	Not cytotoxic
ISO 10993-10:2010/(R)2014	Not an irritant
ISO 10993-10:2010/(R)2014	Not a sensitizer
ISO 10993-11: 2017	No evidence of acute systemic toxicity
ISO 10993-11: 2017/ USP, General Chapter <151>, Pyrogen Test	Non-pyrogenic

The product was developed and is in compliance with the following ISO Standards:

ISO Standard	Description
EN ISO 13485:2016	Medical Devices – Quality Management Systems – Requirements for Regulatory Purposes
EN ISO 14971:2012	Medical Devices – Application of Risk Management to Medical Devices

[1] Material properties may vary based on part geometry, print orientation, print settings, temperature, and disinfection or sterilization methods used.

[2] Data were measured on post-cured samples printed on a Form3B with 100um BioMed White Resin settings, washed in a Form Wash for 5 minutes in 99% Isopropyl Alcohol, and post-cured at 60°C, 60 minutes in a Form Cure.

[3] BioMed White Resin was tested at NAMSA World Headquarters, OH, USA.

[1] "BioMed White Resin," Formlabs. https://formlabs.com/store/materials/biomed-white-resin/ (accessed Oct. 12, 2022).

Appendix D: Vocal Fold Disk SOLIDWORKS Assembly

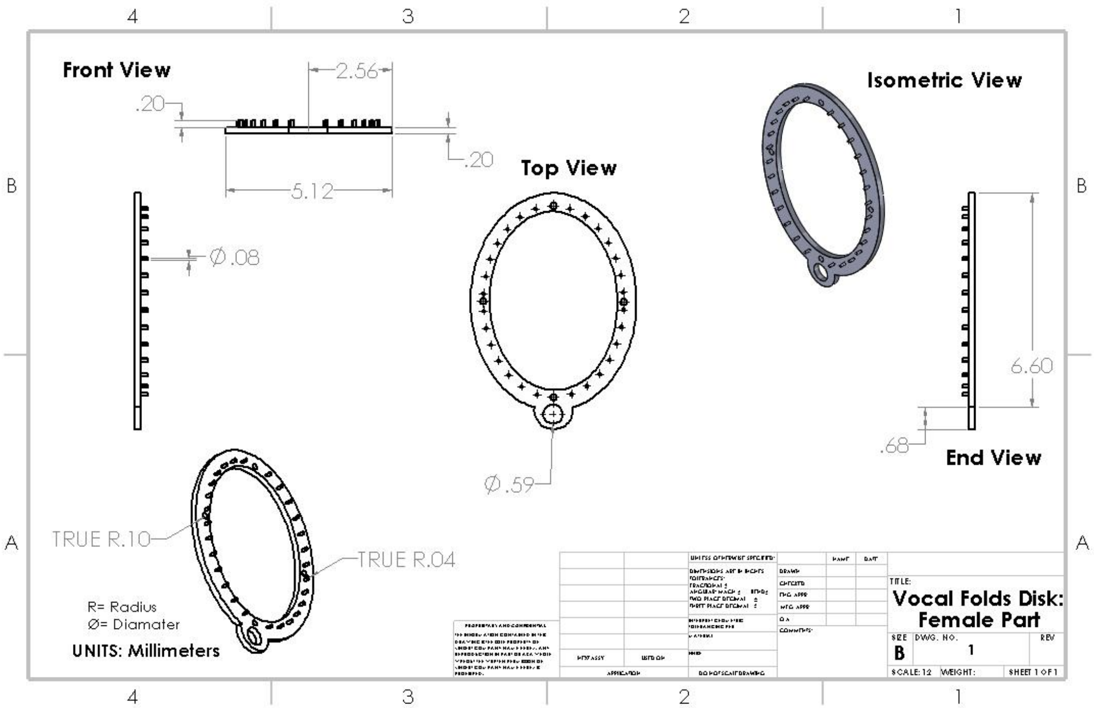


Figure 1: Vocal Folds Disk, Female Part

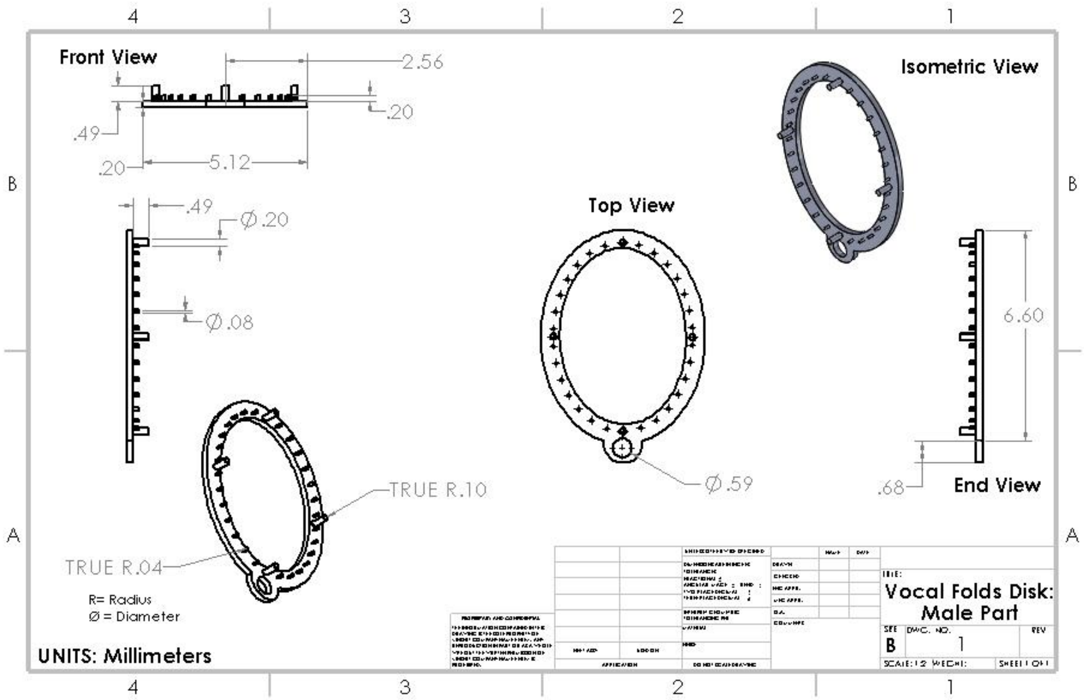


***Figure 2**: Vocal Folds Disk, Male Part*

Appendix E: Materials and Expenses

Table 1: Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Category 1 Vocal Folds and Larynx								
Agarose	lab grade agarose	DOT SCIENTIFIC INC	DS170035	11.5.22	1	$107.64	$107.64	
First Larynx Print	Made of Flexible 80A Resin from Makerspace	Formlabs			1	$105.67	$105.67	
Vocal Fold Mold #1	Elastic 50A online Vocal Fold model printed in Makerspace	Formlabs			1	$78.98	$78.98	
Vocal Fold Mold #2	Tough PLA SOLIDWORKS vocal fold model printed in Makerspace	UltiMaker			1	$4.96	$4.96	
Second	Made of Tough PLA	UltiMaker			1	$15.36	$15.36	

Larynx Print	from Makerspace							
Category 2 Laryngeal Disk								
Crude Laryngeal Disk Prototype	created in SolidWorks, printed in PLA at the MakerSpace	Ultimaker		11.3.22	1	$0.24	$0.24	
Disk Prototype 2	2 part disk prototype	Clear Formlabs Resin		11.7.22	1	$1.59	$1.59	
Another Disk Prototype Print	2 part disk prototype	Black Formlabs Resin		12.6.22		$9.84	$9.84	

TOTAL: $324.28

Appendix F: Larynx Fabrication Process

I. **Larynx Cutting Protocol**

Required Materials:

- Equine Larynx
- Scalpel
- Scissors
- Tissues
- Bucket
- Plastic Bag

Steps:

- Place frozen Larynx in biohazard plastic bag, ensure the plastic bag is pulled through the vocal cavity
- Submerge the larynx inside the plastic bag in a bucket of cold water
- Rinse off larynx with cold water to get rid of ice chunks
- Place thick layer of tissue on table
- Place larynx on tissue
- Mark larynx with cutting route (symmetrically the long way)
    - Insert image
- Start cutting larynx with scissors, if an area is too hard to cut with scissors, switch back to scalpels
- Put cut larynx back into biohazard bag

II. **3D Scanning Protocol**

Required Materials:

- An equine larynx

- Rubber gloves
- Paper towels
- Access to a sink
- Turntable
- Paper plate
- 3D scanning “target” stickers
- The Creaform Handscan 700 3D scanner
- Creaform VX elements software (3D scanning software)

3D scanner information:

- The Creaform Handscan 700 is an amazing engineering tool and will give sub-millimeter resolution scans that are professional grade.
- The Creaform VXElements software is the best tool for post-processing the mesh. It’s super powerful for analyzing the mesh and adding entities like surfaces, lines, planes, points that can be imported into CAD packages. However, the software is only on a couple powerful Makerspace laptops – you can borrow the laptops while in the Makerspace though you’ll need shop manager approval.

3D scanning using the Creaform Handscan 700:

Calibration steps:

1. Calibration steps for the Creaform Handscan 700 3D scanner and Creaform VX software were completed by a Makerspace team representative.

3D scanning steps:

1. While wearing gloves, rinse frozen equine larynx with cold water to remove ice chunks
2. Carefully dry off larynx with paper towels (make sure no paper towel residues remain on larynx)
3. Place larynx on paper plate in upright position with the laryngeal opening face forward with visual of the vocal cords
4. Complete anterior scan of larynx
5. Turn larynx around on the plate so the laryngeal opening faces backwards
6. Complete posterior scan of larynx
7. With the laryngeal opening in the anterior position, rotate the larynx 90 degrees clockwise so it is oriented on its left side (left side down)
8. Complete of larynx orientated as stated in step 7
9. With the laryngeal opening in the anterior position, rotate the larynx 90 degrees counterclockwise so it is oriented on its right side (right side down)
10. Complete of larynx orientated as stated in step 9

11. After the final scan, place the equine larynx back in a biohazard freezer container and dispose of the paper plate and rubber gloves.

Post processing steps

1. Post processing (meshing) of the 3D scans was completed by a Makerspace representative in the 3D scanning department through the Creaform VX elements software.
2. Scans were converted into an STL file

Appendix G: Vocal fold and Disk Fabrication Process

I. Replaceable Disk Fabrication Process

Creating the base

1. Open SOLIDWORKS
2. Create new part
3. Create an ellipse centered at the origin and use smart dimensions to define radii 85mm and 65mm
4. Extrude the ellipse by 5mm
5. Create a second ellipse centered at the origin and use smart dimensions to define the radii as 75mm and 55mm. This will be the inner radius
6. Use this second ellipse to make an extruded cut all the way through the disk.
7. Create a circle with origin located at the bottommost point of the ellipse, make the radius a uniform 25mm
8. Extrude this circle by 5mm so that it is uniform with the rest of the part
9. Create another circle centered at the same point with radius 15mm
10. Extrude a cut all the way through using this second circle. The base of the part is now complete. Save the file

Creating the Male Part

1. Create a new part and import the base from the previous project.
2. Create a curve going around the ellipse 5mm from the outer edges (with radii 80 and 60)
3. Create a circle with radius 1.5 and extrude 12.5mm
4. Use this cylinder to make a curve driven pattern with 4 cylinders on the top, bottom and two sides
5. Create another circle on the curve with radius 1mm. Using the same curve make a curve driven pattern with 30+ iterations to make “spikes”
6. Save the part

Creating the Female Part

1. Repeat steps for male part, except instead of extruding the first circle by 12.5, make an extruded cut of at least 2.5mm into the disk.

2. Save the part

Printing

1. Export the Male and Female parts as STL files to a flash drive or online cloud
2. Go to UW Makerspace, or locate another printer
3. Import the two parts into PreForm and prepare to print. Ensure dimensions are correct and orientation is such that the least amount of support is used.
4. Select the Resin type and begin printing. May need staff assistance.
5. Once print is completed, retrieve from the Makerspace. If there are any rough edges or the parts do not fit together perfectly, use a file or sandpaper to smooth.
6. The disk cartridge is complete.

II. Vocal Fold Mold Process

Creating Vocal Fold Mold

1. Open SOLIDWORKS
2. Create new part
3. Create an ellipse centered at the origin and use smart dimensions to define radii 85mm and 65mm
4. Extrude the ellipse by 10mm
5. Create a second ellipse centered at the origin and use smart dimensions to define the radii as 75mm and 55mm. This will be the inner radius
6. Use this second ellipse to make an extruded cut 8mm into the disk.
7. Use line tool to create a 122mm diagonal line from the bottom center of the ellipse base to the right side.
8. Use line tool to create a line parallel to the previous one off set by 2mm.
9. Use the 3 point arc tool to connect the parallel lines on each side while following the edge of the ellipse to close the shape. Use tangent tool to make the shape flush to ellipse base.
10. Extrude enclosed rectangle by 8mm.
11. Create a construction line down the center of the ellipse. Add symmetry relation so the extruded rectangle is mirrored.
11. Extrude the remaining triangular area between extruded rectangles. The negative mold is now complete. Save the file.

Materials:

50mL DI Water

500mg Agarose

Vocal Fold Mold

Procedure for Agarose-Water Mixture:

1. Add 50mL of DI water into a glass bottle

2. Add the 500mg of Agarose powder to the glass bottle
3. Loosely cap the glass bottle
4. Place the bottle in the microwave for 1 minute, or until the water boils
5. Stir around the agarose and water mixture and reheat as needed until all the crystals of agarose is dissolved
6. Pour the water and agarose mixture into the vocal fold mold
7. Let the mixture cool for about 45 minutes or longer at room temp until solid
8. Refrigerate at 4℃ until use

Appendix H: Testing Protocols

<u>Equine Laryngeal Model Testing Protocols</u>

<u>Anatomical Accuracy test</u>

Introduction:

Name of Tester:

Date of Test Performance:

Site of Test Performance:

Explanation:

This test will be completed by the team to assess how the structures of the proposed model are replicants of equine anatomical structures. The different variables tested will be the trachea's diameter and length, the larynx's diameter and length, and the epiglottis's length and width. These measurements will be collected from the CT scans and the 3D printed model and compared to those of the literature values. The test will be passed if the statistically calculated p-value is statistically significant.

Required Materials:

- Equine Laryngeal Model
- Caliper (mm)

Protocol

1. Place the Equine Laryngeal Model in the standard position of use.
2. Measure, using calipers, the anatomical features of the laryngeal model listed in the chart below (Figure 1).
3. Conduct an ANOVA statistical analysis test to calculate the p-value and determine statistical significance.

Data:

	Length of Larynx	Width of Larynx	Height of Larynx
CT SCAN of Equine Larynx			
3D Printed Laryngeal Model			
Dissected Larynx			
Literature	N/A	76.2 mm	58.7mm

Figure 1. Anatomical Measurements

<u>*Mechanical Properties test*</u>

Introduction:

Name of Tester:
Date of Test Performance:
Site of Test Performance:

Explanation:

The mechanical properties of the design will be tested through compression testing. This testing will be completed by the team and will be done on the parts of the model that replicate equine laryngeal cartilage. After completing compression testing on the biomaterials of the design, the goal is to compare the resulting Young's Moduli to the Young's Moduli of the dissected equine laryngeal tissues. The test will be passed if the statistically calculated p-value is statistically significant. The statistical analysis will also require a test for outliers in the samples.

Required Materials:

- Equine Laryngeal Model
- Experimental Equine Larynx
- MTS Compression Machine
- TWE Software

MTS Machine and TWE Software Set-Up protocol:

- Turn on the computer that connects to the MTS Machine
- Open the TWE Software
- Record the height and diameter of 3D printed material samples
- Insert a sample into the MTS Machine one at a time

- Enter the diameter of a sample into the machine
- The test begins by calibrating the location of the top clevis
- Click the green play button on the computer
- This test stops before the load exceeds 10000 N or when the sample reaches its failure point
- Data for an individual sample is then saved and to be exported to personal computer
- The top clevis is raised
- Repeat process for all samples

Protocol:

1. Follow MTS Machine and TWE Software Set-Up protocol
2. Test the Mechanical Properties of the Thawed Equine Larynx
3. Test the Mechanical Properties of the Agarose Vocal Folds
4. Calculate the average Young's Moduli and standard deviation per sample type
5. Conduct a T-Test on total samples to determine statistical significance
6. Conduct a Q-Test to determine if there are any outliers in the different samples types

Data:

Experimental Mechanical Properties From MTS Compression Testing		
Sample Type	Young's Moduli	Standard Deviation
Flexible 80A Resin		
Agarose		
Dissected Equine Tissue		

Figure 2. Material Mechanical Properties

<u>*Similarity test*</u>

Introduction:

Name of Tester:
Date of Test Performance:
Site of Test Performance:

Explanation:

The model will be tested by 6-8 current surgical residents and/or veterinary surgeons on their personal ranking of the model's biomaterial texture, anatomical shape, relative size, and procedural accuracy. Each category will be ranked on a 1 to 5 scale by the user. A score of 1 indicates zero to no similarity, a score of 2 indicates slight similarity, a score of 3 indicates fair similarity, a score of 4 indicates considerable similarity, and a score of 5 indicates highly similar. A passed test is defined as a score of 4/5 or higher to keep the percent error similar to that of other tests.

Required Materials:

- Similarity test worksheet
- Complete Equine Laryngeal Model

Protocol:

1. Residents perform a practice Transendoscopic Laser Ventriculocordectomy surgery on the design model
2. 6~8 current residents will rate the similarity of the laryngeal model on a scale of 0-5 for biomaterial texture, anatomical shape, relative size, and procedural accuracy.
3. Average the scores for each category.
4. Conduct statistical analysis to determine significance of similarity.

Similarity Survey For Equine Laryngeal Model Rating Scale: - 1: indicates zero to no similarity - 2: slight similarity - 3: fair similarity - 4: considerable similarity - 5: highly similar			
	Rating	Test Result (Pass/Fail)	User Comments
Biomaterial Texture *Description:* *Do the textural properties of the material match that of a real patient?* *Do the surgical tools interact similarly with the model's various materials?*			
Anatomical Shape *Description:* *Does the model contain an accurate representation of the equine laryngeal*			

topography? *Are the vocal folds located and mimicked correctly?*			
Relative Size *Description:* *How does the size of the anatomical parts compare to that of a real patient?* *Does the model portray an accurate opening of the larynx?*			
Procedural Accuracy *Description:* *How accurate is the Equine Laryngeal Model with respect to Transendoscopic Laser Ventriculocordectomy procedures?*			

Figure 3. Similarity Test Worksheet

<u>*Ease of Fabrication and Replacement test:*</u>

Introduction:
Name of Tester:
Date of Test Performance:
Site of Test Performance:

Explanation:

The model will be tested by 6-8 current surgical residents and/or veterinary surgeons at the UW School of Veterinary Medicine on their ability to fabricate and replace the vocal folds. This procedure will assess two tests that both have to pass in order for the overall test to be considered passing. For the first part, test 1 is passed if the user is able to create gelatin folds using a reusable silicone model in 30 minutes or less [19]. For the second part, test 2 is passed if the user is able to remove the laryngeal disk, remove old vocal folds, replace new gelatin vocal folds, and insert the fresh disk back into the model in 10 minutes or less. If test 1 and test 2 are able to be passed, the Ease of Fabrication and Replacement test is passed.

Outline:

1. 6~8 surgical residents will complete the vocal fold fabrication process three times (APPENDIX __)
2. The times will be averaged
3. If the 95 percent of the residents are able to successfully fabricate a agarose based vocal fold, the test is passed

	R1	R2	R3	R4	R5	R6	R7	R8	avg
Trial 1									
Trial 2									
Trial 3									

Figure 4. Time chart for ease of fabrication test

Appendix I: Matlab Code for MTS Testing

```
%% Bug Prevention
clear
close all
clc
%% Data Upload
data1 = load('1DAQ.txt')
data2 = load('2DAQ.txt')
data3 = load('3DAQ.txt')
data4 = load('4DAQ.txt')
data5 = load('5DAQ.txt')
data6 = load('6DAQ.txt')
%% Stress vs Strain for Agarose
figure(1)
hold on
diameter_1 = 15.72
length_1 = 15
crosssecarea_1 = ((diameter_1)^2 / 4) * pi
load_1 = data1(:,2)
crosshead_1 = data1(:,1)
stress_1 = (load_1) / (crosssecarea_1)
strain_1 = (crosshead_1/length_1)
plot(strain_1,stress_1, 'b')
hold on
diameter_2 = 15.72
length_2 = 15
crosssecarea_2 = ((diameter_2)^2 / 4) * pi
load_2 = data2(:,2)
```

```
crosshead_2 = data2(:,1)
stress_2 = (load_2) / (crosssecarea_2)
strain_2 = (crosshead_2/length_2)
plot(strain_2,stress_2, 'r')
hold on
diameter_3 = 15.72
length_3 = 15
crosssecarea_3 = ((diameter_3)^2 / 4) * pi
load_3 = data3(:,2)
crosshead_3 = data3(:,1)
stress_3 = (load_3) / (crosssecarea_3)
strain_3 = (crosshead_3/length_3)
plot(strain_3,stress_3, 'g')
xlabel('Strain [mm/mm]')
ylabel('Stress [MPa]')
title('Agarose Stress/Strain Curve')
legend('Agarose Sample 1', 'Agarose Sample 2', 'Agarose Sample 3')
hold off
%% Stress vs Strain for Equine Tissue
figure(2)
hold on
diameter_4 = 15.72
length_4 = 7.6
crosssecarea_4 = ((diameter_4)^2 / 4) * pi
load_4 = data4(:,2)
crosshead_4 = data4(:,1)
stress_4 = (load_4) / (crosssecarea_4)
strain_4 = (crosshead_4/length_4)
plot(strain_4,stress_4, 'r')
hold on
diameter_5 = 10.08
length_5 = 6.9
crosssecarea_5 = ((diameter_5)^2 / 4) * pi
load_5 = data5(:,2)
crosshead_5 = data5(:,1)
stress_5 = (load_5) / (crosssecarea_5)
strain_5 = (crosshead_5/length_5)
plot(strain_5,stress_5, 'g')
hold on
diameter_6 = 15.72
length_6 = 7.4
crosssecarea_6 = ((diameter_6)^2 / 4) * pi
load_6 = data6(:,2)
crosshead_6 = data6(:,1)
stress_6 = (load_6) / (crosssecarea_6)
strain_6 = (crosshead_6/length_6)
plot(strain_6,stress_6, 'b')
xlabel('Strain [mm/mm]')
```

```
ylabel('Stress [MPa]')
title('Equine Tissue Stress/Strain Curve')
legend('Equine Tissue Sample 1', 'Equine Tissue Sample 2', 'Equine Tissue
Sample 3')
hold off
%% Stress Strain comparison
figure(3)
hold on
diameter_1 = 15.72
length_1 = 15
crosssecarea_1 = ((diameter_1)^2 / 4) * pi
load_1 = data1(:,2)
crosshead_1 = data1(:,1)
stress_1 = (load_1) / (crosssecarea_1)
strain_1 = (crosshead_1/length_1)
plot(strain_1,stress_1, 'b')
hold on
diameter_2 = 15.72
length_2 = 15
crosssecarea_2 = ((diameter_2)^2 / 4) * pi
load_2 = data2(:,2)
crosshead_2 = data2(:,1)
stress_2 = (load_2) / (crosssecarea_2)
strain_2 = (crosshead_2/length_2)
plot(strain_2,stress_2, 'b')
hold on
diameter_3 = 15.72
length_3 = 15
crosssecarea_3 = ((diameter_3)^2 / 4) * pi
load_3 = data3(:,2)
crosshead_3 = data3(:,1)
stress_3 = (load_3) / (crosssecarea_3)
strain_3 = (crosshead_3/length_3)
plot(strain_3,stress_3, 'b')
hold on
diameter_4 = 15.72
length_4 = 7.6
crosssecarea_4 = ((diameter_4)^2 / 4) * pi
load_4 = data4(:,2)
crosshead_4 = data4(:,1)
stress_4 = (load_4) / (crosssecarea_4)
strain_4 = (crosshead_4/length_4)
plot(strain_4,stress_4, 'r')
hold on
diameter_5 = 10.08
length_5 = 6.9
crosssecarea_5 = ((diameter_5)^2 / 4) * pi
load_5 = data5(:,2)
```

```
crosshead_5 = data5(:,1)
stress_5 = (load_5) / (crosssecarea_5)
strain_5 = (crosshead_5/length_5)
plot(strain_5,stress_5, 'r')
hold on
diameter_6 = 15.72
length_6 = 7.4
crosssecarea_6 = ((diameter_6)^2 / 4) * pi
load_6 = data6(:,2)
crosshead_6 = data6(:,1)
stress_6 = (load_6) / (crosssecarea_6)
strain_6 = (crosshead_6/length_6)
plot(strain_6,stress_6, 'r')
xlabel('Strain [mm/mm]')
ylabel('Stress [MPa]')
title('Stress/Strain Curve Comparison')
legend('Agarose Sample 1', 'Agarose Sample 2', 'Agarose Sample 3','Equine
Tissue Sample 1', 'Equine Tissue Sample 2', 'Equine Tissue Sample 3')
hold off
%% Young's Moduli of Agarose and Equine Tissue
as1_slope = (0.027-0.021)/(0.329-0.278)
as2_slope = (0.0168-0.0123)/(0.313-0.265)
as3_slope = (0.0180-0.0136)/(0.389-0.333)
ets1_slope = (0.682-0.441)/(1.426-1.387)
ets2_slope = (1.079-0.782)/(1.016-0.782)
ets3_slope = (0.0731-0.0622)/(1.171-1.159)
as_matrix = [as1_slope ; as2_slope ; as3_slope]
ets_matrix = [ets1_slope ; ets2_slope ; ets3_slope]
figure (4)
hold on
boxplot([as_matrix , ets_matrix])
xlabel('Samples')
ylabel('E(MPa)')
title('Youngs Modulus Agarose Compared to Equine Tissue')
set(gca, 'XTickLabel' , {'Agarose' , 'Equine Tissue'})
hold off
%% Standard Deviation Calculations
stdagarose = std(as_matrix)
stdequine = std(ets_matrix)
%% ttest
%T-Test sample-sample
[h1, p1] = ttest(as_matrix , ets_matrix)
%% qtest
Qexp = (ets1_slope-ets2_slope) / 3
Qcrit = 0.970
%Qexp<Qcrit equine tissue 1 is an outlier
```