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Nondestructive Measurement of Esophageal Biaxial Mechanical Properties Utilizing Sonometry

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Abstract

Malignant esophageal pathology typically requires resection of the esophagus and reconstruction to restore foregut continuity. Reconstruction options are limited and morbid. The esophagus represents a useful target for tissue engineering strategies based on relative simplicity in comparison to other organs. The ideal tissue engineered conduit would have sufficient and ideally matched mechanical tolerances to native esophageal tissue. Current methods for mechanical testing of esophageal tissues both *in vivo* and *ex vivo* typically are destructive, alter tissue conformation, ignore anisotropy, or are not able to be performed in fluid media. The aim of this study was to investigate biomechanical properties of swine esophageal tissues through nondestructive testing utilizing sonometry *ex vivo*. This method allows for biomechanical determination of tissue properties, particularly longitudinal and circumferential moduli and strain energy functions. The relative contribution of mucosal-submucosal layers and muscular layers are compared to composite esophagi. Swine thoracic esophageal tissues (n=15) were tested by pressure loading using a continuous pressure pump system to generate stress. Preconditioning of tissue was performed by pressure loading with the pump system and pre-straining the tissue to *in vivo* length before data was recorded. Sonometry using piezocrystals was utilized to determine longitudinal and circumferential strain on five composite esophagi. Similarly, five mucosa-submucosal and five muscular layers from thoracic esophagi were tested independently. This work on esophageal tissues is consistent with reported uniaxial and biaxial mechanical testing and reported results using strain energy theory and also provides high resolution displacements, preserves native architectural structure and allows assessment of biomechanical properties in fluid media. This method may be of use to characterize mechanical properties of tissue engineered esophageal constructs.

Keywords

esophagus; biaxial; mechanical testing; sonometry; piezocrystal

1. Introduction

The structure of the esophagus underlies its function of propulsion of food into the stomach. It performs this function through organized peristalsis as a result of synchronization of the neuromuscular components. Importantly, this necessitates mechanical tolerances of the esophagus that are able to withstand repetitive mechanical stress and strains of passage of oral bolus down the gastro intestinal tract; the esophagus must expand from the resting collapsed state to a dilated state to accommodate oral bolus repetitively without rupture or leak. Understanding the biomechanical relationship and varied contributions of the mucosal-submucosal component and the muscular component to composite biomechanical effects is required [1], [2] to elucidate dysmotility syndromes as well for future work to generate suitable constructs for tissue engineered approaches to the esophagus [3], [4] which require replacement to restore foregut continuity after resection of esophageal pathologies such as malignancy,

The impact of esophageal cancer is severe, with dismal outcomes. Esophageal cancer affects approximately a half million new people worldwide yearly [5]. Surgical management requires esophageal resection and subsequent reconstruction. Currently, reconstruction of the native esophagus is impossible given the limited redundancy of tissue and poor collateral vascularization. Therefore, reconstruction typically utilizes autologous tissue, either gastric, small bowel, or colon as a conduit with removal of the esophagus distal to the diseased segment. These treatment modalities are associated with high morbidity and mortality [6]–[9]. Given these limitations in treatment, there is a critical need for a tissue engineered substitute. An esophageal substitute would ideally recapitulate the mechanical properties of the native esophagus. Generated esophageal constructs would ideally be mechanically assessed in a non-destructive method to determine suitability and fitness for implantation.

The zero-stress and unloaded states of the esophagus have been well characterized thus far by Gregersen, Yang, and others [2], [10]–[13]. There is limited data in large animal models of esophageal biomechanics, particularly as assessed biaxially [1], [14], and none to our knowledge have assessed esophageal biomechanics preserving native architecture in a fluid media, or using sonometry as a nondestructive approach. Current methods of uniaxial and biaxial mechanical testing often require tissue sample alteration or destruction, and in order to determine reproducibility, several tests must to be performed which limits the available tissue for subsequent use. For tissues which are easily attainable this may be an acceptable method for biomechanical testing. However, as tissue engineering processes advance, and with increasing complexity involved in the synthesis of bioengineered tissues, there is a need to assess the mechanical suitability and stability of generated constructs biaxially in a preserving native architecture, preferably in a fluid media to preserve the cellularized component of these grafts, which would have important implications for engineered tissues [15].

Current non-destructive approaches such as optical tracking are limited. While biaxial testing and triaxial testing may be performed in a tubular conformation using specialized equipment and significant technical expertise [16], [17], without this equipment the sample has to be effectively brought into a plane conformation, which would alter a tubular tissue conformation from its native architecture of a three-dimensional structure. While maintaining a tubular configuration is possible [16], [17] this would represent challenges with accuracy in determination of out of focal plane and edge displacements. Furthermore, if these challenges were to be surmounted, maintaining tissue engineered constructs requires fluid media. This represents technical challenges for mechanical testing equipment as well as rarefaction for optical approaches, but is ideally suited for sonometry. Using sonometry which also permits displacement tracking to be performed outside line of sight may allow for further *in vivo* applications for determination of deformations under loading and would have significant *in vivo* application for diagnosis of esophageal motility dysfunction and mechanical assessment for pathological or physiological measurements.

We applied a nondestructive method for mechanical assessment using piezoelectric elements and sonometry, previously utilized and validated to assess mechanical properties of thin walled arteries [18], to determine biaxial mechanical properties in *ex vivo* esophagi. To characterize the the mechanical properties of esophageal tissue we tested multiple esophageal muscle and mucosal-submucosal layer samples, and composite esophagi. Biomechanical characterizations were performed with pressure loading and measurements of the radial and longitudinal strain made using piezocrystal sonometry. From the applied pressure and measured strain values, we computed circumferential and longitudinal strain assuming the esophageal structure as a thick walled tube and characterized the response using strain energy function modeling .

2. Methods

2.1 Overview

The composite structure of the esophagus represents a unique opportunity for both mechanical testing and tissue engineering as compared to other tubular structures of the body. There is an easily detached interface between the esophageal mucosa-submucosa and the muscular layers. These separate layers reflect different nonlinear material properties [1], [2], [12]. We performed these tests on an esophageal composite, mucosal-submucosal, and muscular layers independently to determine biaxial stresses and strains and strain energy functions to examine the composite and separate layers of the esophagus.

2.2 Specimen Preparation

Fifteen esophagi were harvested from 60–70 kg domestic swine euthanized according to Institutional Animal Care Use Committee guidelines. The entire esophagus: cervical, thoracic and abdominal portions were mobilized and excised through either a median sternotomy, or right thoracotomy. The fresh tissue was stored in phosphate buffered saline (NaCl 137 mmol/L KCL 2.7 mmol/L Na₂HPO₄ 10mmol/L KH₂PO₄ 1.8 mmol/L) until transfer to –80 °C freezer as would be performed for tissue engineering approaches for generation of scaffolds. Esophageal specimens were gradually rewarmed to atmospheric

temperature, taken to be 22 °C for mechanical testing. Specimens were gently mechanically separated to generate samples (composite n=5, mucosa-submucosa n=5, and muscular n=5 layers) used for testing. Small piezoelectric elements (Sonometrics, London, Ontario, Canada) were then affixed through loops in the piezoelectric elements to the outer wall of the specimen using 8-0 suture in a longitudinal direction to not impede esophageal motion, as fully described below and Fig 1. The specimen was then trimmed so 12 cm of mid-thoracic esophagus was isolated which was then mounted on cannulae of esophageal luminal diameter and fixed with plastic securement ties so that 10 cm of esophageal tissue was exposed. After cannulation, each segment was filled with degassed normal saline until free of air at a baseline pressure of ~5 mmHg and immersed vertically in a bath of degassed normal saline and mounted on a holding frame. One end of the esophagus was fixed with a weight was attached to give a pre-stress to *in vivo* length (~15%). The experimental setup is shown in Fig. 1.

2.3 Sonometry Experiments

The proximal esophageal cannula was then attached to a calibrated pressure transducer. Deformation of the esophagus under a time-varying pressure load (KDS210, Kd Scientific, Holliston, MA) and measured (PX319-015G5V, Omegadyne Inc., Sunbury, OH) during loading and unloading sequences. This generates a biaxial strain with observed hysteresis, allowing the circumferential and longitudinal strain, respectively, to be measured. This method was applied to *ex vivo* native esophagi with (n = 5) loading and unloading cycles for preconditioning. Infusion and withdrawal of 15 mL was performed at a volume at 60 mL/min. The speed of sound in the saline media was measured at 22 °C with five acquisitions using a pulse-echo method [19] and the mean and standard deviation were 1495.3 ± 0.65 m/s. The time-of-flight is calculated from initial distances which were measured between the elements with calipers.

One piezoelectric element was designated for transmission only (Tx). Two other piezoelectric elements were used as receivers (Rx). One was placed at the same level as the Tx piezocrystal but across the diameter (Rx1), and the other piezocrystal (Rx2) was placed along the length of the esophagus on the same side as the Tx piezocrystal about 2 cm away.

The transmitted signal was a ten-cycle burst at 2 MHz (center frequency of the piezoelectric elements) (5 μ s) produced by a signal generator (33120, Agilent, Palo Alto, CA) with a pulse repetition frequency of 20 Hz. The signal was amplified by a custom made 40 dB power amplifier. The received signals on each of the receiving piezocrystals were amplified with custom made amplifiers and filtered with 2 MHz bandpass filters. These signals were digitized at 100 MHz (ATS460, Alazartech, Montreal, QC, Canada). The total length of time for the measurements was 60 seconds.

To extract the motion of the esophagus from the ultrasound signals, a cross-correlation algorithm was used to determine the time delays between consecutive signals. Before the cross-correlation was performed, the samples were up-sampled by a factor of 5 to 500 MHz. The normalized cross-correlation of consecutively acquired signals was performed and the

time delay associated with the peak of the normalized cross-correlation was used to estimate the motion [20]. These time delays were converted to displacement by using [21].

$$\Delta d_n = c \Delta t_n \quad (1)$$

$$d_n = d_0 + \sum_{i=1}^n \Delta d_n \quad (2)$$

where d_n is the incremental displacement estimated between ultrasound signal acquisitions, c is the speed of sound in the saline, t_n is the time delay estimated between ultrasound signal acquisitions, and d_0 is the initial length. With the 500 MHz sampling frequency the displacement resolution is 3.08 μm . For each sample the initial distance between Tx-Rx1 and Tx-Rx2 were measured with a calipers to obtain initial distances, d_0 , for strain calculations.

The stress was calculated from the pressure of the esophagus and the different layers will be considered as thick-walled tubes [2]. All of the ultrasound and pressure measurements were synchronized during the acquisition so that the stress-strain curves could be calculated. We briefly present the relevant theory below.

$$\sigma_L = \frac{P_i r_{i,l}^2 - P_o r_{o,l}^2}{r_{o,l}^2 - r_{i,l}^2} \quad (3)$$

$$\sigma_C = \frac{P_i r_{i,l}^2 - P_o r_{o,l}^2}{r_{o,l}^2 - r_{i,l}^2} - \frac{r_{o,l}^2 r_{i,l}^2 (P_o - P_i)}{r_l (r_{o,l}^2 - r_{i,l}^2)} \quad (4)$$

$$h = r_o - r_i \quad (5)$$

$$\varepsilon_L = \frac{L - L_0}{L_0} \quad (6)$$

$$\varepsilon_C = \frac{r_{o,l} - r_{o,n}}{r_{o,n}} \quad (7)$$

where σ is the stress, r is the radius, $r_{o,l}$ is the outer radius during loading, $r_{i,l}$ is the inner radius during loading, $r_{o,n}$ is the outer radius at rest, P_i is the pressure in the esophagus, P_o is the pressure outside the esophagus which is assumed to be 0, h is the wall thickness, subscripts L and C represent the longitudinal and circumferential directions.

We followed the model set forth in Yang, *et al.*, to compute the circumferential and longitudinal moduli [1]. Yang, *et al.*, presented theory for applying a strain energy function (SEF) for esophagus testing. To use the SEF in this application we present its formulation where in the unloaded state the radius is derived from the diameter

$$r_{o,n} = D_{o,n}/2. \quad (8)$$

The annular area of the esophagus is assumed to be constant and incompressible so the inner radius can be calculated from measurements of the outer radius

$$A_n = \pi r_{o,n}^2 - \pi r_{i,n}^2, \quad (9)$$

$$h_l = r_{o,l} - \sqrt{r_{o,l}^2 - A_n/\pi}. \quad (10)$$

Using these geometric relationships, the longitudinal stretch ratio, λ_{zz} , is given by

$$r_{i,l} = \sqrt{r_{o,l}^2 - \frac{A_n}{\pi \lambda_{zz}}}, \quad (11)$$

$$\lambda_{zz} = \frac{A_n}{\pi (r_{o,l}^2 - r_{i,l}^2)}. \quad (12)$$

The longitudinal Green's strain is

$$E_{zz} = \frac{\lambda_{zz}^2 - 1}{2}. \quad (13)$$

The circumferential stretch ratio, $\lambda_{\theta\theta}$, and the circumferential Green's strain, $E_{\theta\theta}$, are

$$\lambda_{\theta\theta} = \frac{r_{i,l} + r_{o,l}}{r_{i,n} + r_{o,n}}, \quad (14)$$

$$E_{\theta\theta} = \frac{\lambda_{\theta\theta}^2 - 1}{2}. \quad (15)$$

The circumferential Kirchhoff's stress is

$$S_{\theta\theta} = \frac{\Delta P r_{i,l}}{h_l \lambda_{\theta\theta}^2}, \quad (16)$$

where ΔP is the change in pressure between a loaded and unloaded state.

The longitudinal Kirchhoff's stress is

$$S_{zz} = \frac{\pi P r_{i,l}^2}{\lambda_{zz}^2 \pi (r_{o,l}^2 - r_{i,l}^2)}, \quad (17)$$

where $P = P_i$.

Assuming that there is no shear strain, the strain energy function is given as

$$\rho_0 W = \frac{C}{2} \exp(Q), \quad (18)$$

$$Q = a_{11} (E_{\theta\theta}^2 - E_{\theta\theta}^{*2}) + a_{22} (E_{zz}^2 - E_{zz}^{*2}) + 2a_{12} (E_{\theta\theta} E_{zz} - E_{\theta\theta}^* E_{zz}^*), \quad (19)$$

where ρ_0 is the density of the wall, W is the strain energy per unit mass, C , a_{11} , a_{22} , and a_{12} are material constants and $E_{\theta\theta}^*$ and E_{zz}^* are strains associated with arbitrarily selected stresses $S_{\theta\theta}^*$ and S_{zz}^* , which in this work is associated with the start of the data acquisition. The stresses are given as

$$S_{\theta\theta} = \frac{\partial (\rho_0 W)}{\partial E_{\theta\theta}} C \exp(Q) (a_{11} E_{\theta\theta} + a_{12} E_{zz}), \quad (20)$$

$$S_{zz} = \frac{\partial(\rho_0 W)}{\partial E_{zz}} C \exp(Q) (a_{22} E_{zz} + a_{12} E_{\theta\theta}). \quad (21)$$

Using the chain rule for the exponential function, the stress equations can be explicitly simplified as

$$\frac{\partial(\rho_0 W)}{\partial E_{\theta\theta}} = \frac{C}{2} \exp(Q) \frac{\partial Q}{\partial E_{\theta\theta}}, \quad (22)$$

$$\frac{\partial(\rho_0 W)}{\partial E_{zz}} = \frac{C}{2} \exp(Q) \frac{\partial Q}{\partial E_{zz}}, \quad (23)$$

$$S_{\theta\theta} = \frac{C^2}{2} \exp^2(Q) (a_{11} E_{\theta\theta} + a_{12} E_{zz}) (2a_{11} E_{\theta\theta} + 2a_{12} E_{zz}), \quad (24)$$

$$S_{zz} = \frac{C^2}{2} \exp^2(Q) (a_{22} E_{zz} + a_{12} E_{\theta\theta}) (2a_{22} E_{zz} + 2a_{12} E_{\theta\theta}), \quad (25)$$

where

$$\frac{\partial Q}{\partial E_{\theta\theta}} = 2a_{11} E_{\theta\theta} + 2a_{12} E_{zz}, \quad (26)$$

$$\frac{\partial Q}{\partial E_{zz}} = 2a_{22} E_{zz} + 2a_{12} E_{\theta\theta}. \quad (27)$$

Fitting of the strain energy functions was performed in MATLAB (MathWorks, Natick, MA) with the lsqcurvefit function with specified lower and upper bounds for the parameters C , a_{11} , a_{22} , and a_{12} . The root-mean-square (rms) error was computed between the data and fits for $S_{\theta\theta}$ and S_{zz} .

3.0 Results

Figures 2–4 show examples of the measured displacements and pressures and the calculated stress, strains for a composite, mucosa-submucosa, and muscle sample, respectively. The

five curves are from consecutive measurements. The agreement is generally very good between acquisitions. It should be noted that as pressure is applied, the esophagus expands in the circumferential dimension and shortens in the longitudinal direction thereby making the strains positive and negative, respectively.

Figure 5 shows the circumferential Kirchhoff stress versus Green's strain from one acquisition for the five samples for each configuration.

Figure 6 shows examples of the SEF fits and the Kirchhoff stresses through time for the results shown in Figs. 2–4 for two consecutive acquisitions.

Tables 1–3 summarize the parameter values found using the SEF fits along with the rms values to evaluate the goodness of the fits for five different esophagi samples for the composite, mucosa-submucosa, and muscle configurations, respectively. All reported values are mean $\pm$ standard deviation for the repeated acquisitions for each sample.

The C values were much higher in the composite samples compared to the mucosal and muscular samples. We also observed variation among the different samples for a given configuration. One aspect of the fitting that was found is that the a_{22} value was set to a minimum value of 0.1 and often returned that limiting value, which indicated an insensitivity to the E_{zz} values that it modifies. The rms values for the SEF fits were on the same order for the samples with mean values ranging from 0.44–2.66 kPa.

4.0 Discussion

Our results demonstrate that biaxial mechanical properties of *ex vivo* esophagi may be reproducibly determined using piezoelectric elements and sonometry using an approach with preservation of native architecture. Determination of biaxial mechanical properties in a nondestructive manner which does not rely on optical tracking may allow for future *in vivo* approaches for assessment and diagnosis of esophageal motility disorders and quantification of biomechanical aspects of engineered tissues which require a fluid media to maintain the cellular component.

Within a given sample, the results are very repeatable. The longitudinal stresses were shown to be much larger than the circumferential stresses because the esophagus deforms much more readily in the circumferential direction as opposed to the longitudinal direction. This is consistent for all configurations. There was considerable variation among different samples within a configuration as shown in Fig. 5 and the tables. The composite showed a much higher degree of nonlinearity. The muscle samples showed the most consistency among different samples. All configurations showed considerable hysteresis.

The SEF fits were generally good, but some obvious time lags were observed in the fits for the depressurization stage, and in the pressurization stage for the composite and muscle configurations.

A strong advantage of the methods employed in this study is the preservation of native architecture when evaluating the intact esophagus or individual layers. Additional

advantages include the high displacement spatial resolution (3.08 μm at 500 MHz) and temporal resolution (dependent on sampling and digitization) permitted by sonometry. While it may be advantageous to use piezoelectric elements with higher frequency to improve displacement resolution, the degree of displacement resolution realized with the upsampling and normalized cross-correlation analysis with these elements was excellent [22]–[24]. Other mechanical testing approaches are destructive, and only use a small portion of the sample, or alter architecture [3], [25], [26].

While optical tracking may be employed as a nondestructive approach, this method is limited with displacement resolution dependent on field of view, and optical transmission properties of the media and changes in the transmission media such as rarefaction. Temporal resolution in optical tracking is dependent on frame rate as compared to high sampling rates permitted by sonometry. Measurements require the specimen to be visible as opposed to possible *in vivo* methods using sonometry which may be transduced out of line of sight. The results of our nondestructive approach could be used for future modeling of the biomechanics of the esophagus under varied conditions *in vivo* or maintaining tissue engineered constructs in a fluid media and performing biomechanical testing before implantation.

To determine suitability of our technique, we must compare the determined biomechanical properties to previously reported values. It has been previously shown that the mucosa and submucosal layers have higher moduli than the muscular layers in both the longitudinal and circumferential directions [14], [16], [27], [28]. While there are marked and significant differences in small versus large animal esophageal models (particularly in large animal ruminates) which limit our ability to compare to literature, swine are considered to be histologically similar to human. Given varied histology across species, our findings are similar to the work of Fan and colleagues [10] despite differences in modeling approaches and species of esophageal origin. In a study examining pressure-diameter relationships in rat esophagi, where the investigators tested mucosa-submucosal, muscular, and composite layers and modeled based on Green's strain and Kirchhoff's stress, those investigations found similar results with respect to tangential moduli of the independent layers being significantly stiffer with a linear increase in the tangential moduli as pressure increased similar to our study. Fan, *et al.*, proposes this may be due to collagen "uncrimping" gradually, until its maximal length has been reached. While this may explain the individual layers and high moduli at low pressures seen in this study, it does not fully explain why the layers independently have higher moduli than the composite.

We have observed that the SEF of the composite samples determined by curve fit of the composite, muscosa-submucosa and muscular layers that *C* values of composite specimens were higher by an order of magnitude than their isolated counterparts. This is difficult to reconcile with prior experimental data. However, by taking species consideration into account this may resolve differences. Smaller or ruminant mammals, tend to have skeletal muscle throughout the esophageal length. When compared to large non-ruminant mammals, a blend of skeletal and smooth muscle in the mid thoracic esophagus is present, and mucosa and submucosa tend to occupy a larger proportion than small animal counterparts. One could make an analogy that the two layers act as springs in series, and the effective stiffness or

modulus is lower than the two individual layers. This explanation would need further work to be validated either through modeling or additional experimentation.

In summary, our non-destructive biaxial measurements using SEF compare favorably with other investigations of esophageal mechanics despite major species specific differences, variation in testing methodology and with similar modeling approach.

5.0 Limitations

The esophagus is not homogeneous throughout its course through the cervical, thoracic and abdominal regions; we have attempted to remove the possibility of sample to sample variation by isolation of thoracic esophageal specimens. Despite excision by the same operator (JMA) at esophageal isolation it may be possible that there were sample to sample anatomical region differences which may contribute to sample inhomogeneity. Additionally during the mucosa-submucosa and muscular sample preparation, or during fixation of the piezoelectric crystals to the esophageal wall, though not observed by investigators, there may have been minor trauma to either of the layers that may have affected the biomechanical response to testing. While higher frequencies than 2 MHz may be beneficial for improved motion tracking the center frequency used for transmit for the piezoelectric elements used was selected to optimize acoustic output for measurements within the bandwidth of these crystals. While it is possible to test the composite independently from the isolated layers of the mucosa-submucosa and muscular components, the behavior of the composite material is likely not a complete reflection of the layers individually. There is likely a combination of mechanical coupling, and changes in the diameter at baseline of the separated materials which reflects inconsistent mechanical properties of the layers relative to the composite structure. Furthermore, while all samples were preconditioned, and pre-strained to their native *in vivo* length, there may be imprecision in perfect loading to *in vivo* length and pre-straining the diameter as the normal configuration of the esophagus is a collapsed state in the *in vivo* environment. While our method uses local strain to determine mechanical properties, more global estimation of strain using optical tracking, though cumbersome in this testing scenario may have benefit if technical challenges such as sampling rate, field of view, and diffraction in a fluid media could be solved. Furthermore, we made several mathematical assumptions in our analysis, namely a thick walled cylinder which is not the resting esophageal state and may not be reasonable for the composite structure. Additionally, there may be variation between the inner and outer surfaces and shear stress and compression through the cross section which our model neglects as we assumed that cross-sectional thickness was preserved. Furthermore, we used a single loading rate, and in theory a more robust methodology would be to use a varied strain rate or alternative wave-based method [18], [29]–[31] to determine a more full viscoelastic characterization, but this was outside the scope of the present study.

The placement of the crystals was done by hand, and while as precise as achievable by this method, a more uniform approach would also improve the consistency of the data. By inspection, we did not observe that the crystals or their wires impeded or altered the natural motion of the esophagus with applied loading.

We did observe in some samples some twist and bulging of the samples during the pressure loading cycle, so the distances measured may not always have been purely circumferential or longitudinal in nature as is assumed by the model. We did perform five preconditioning cycles for each sample so that we could obtain consistent results, but the degree of preconditioning which occurred may vary based on the sample. It would also be instructive to test more samples in the future to obtain bounds on the natural variation of the esophagi under study.

6.0 Conclusion

Our data support the use, and reproducibility of piezoelectric elements and sonometry to assess the *ex vivo* biaxial mechanical properties of the intact esophagus as a composite structure and mucosa-submucosa and muscular isolated layers. The application of this technique may be of utility in nondestructive biomechanical assessment of tissue engineered esophagi.

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Figure 1.

Experimental setup for sonometric esophageal testing. The esophagus is submerged in degassed saline and pre-stained to in vivo length. The sonometric and pressure transducer signal processing chain is also briefly described in the flow chart.

Figure 2.

Typical example from five repeated measurements from one composite esophageal sample.

(a) Circumferential displacement, (b) longitudinal displacement, (c) pressure, (d), circumferential stress and strain curves, (e) longitudinal stress and strain curves, (f) circumferential Kirchhoff stress and Green's strain.

Figure 3.

Typical example from five repeated measurements from one mucosa-submucosa sample. (a) Circumferential displacement, (b) longitudinal displacement, (c) pressure, (d), circumferential stress and strain curves, (e) longitudinal stress and strain curves, (f) circumferential Kirchhoff stress and Green's strain.

Figure 4.

Typical example from five repeated measurements from one esophageal muscle sample. (a) Circumferential displacement, (b) longitudinal displacement, (c) pressure, (d), circumferential stress and strain curves, (e) longitudinal stress and strain curves, (f) circumferential Kirchoff stress and Green's strain.

Figure 5. Kirchoff stress for multiple samples of the different configurations. (a) Composite samples, (b) mucosa-submucosa samples, (c) muscle samples.

Figure 6.

Comparison of measured Kirchhoff stresses and SEF fits for two acquisitions. All solid lines are the measured data and the dashed lines are the SEF fits. The blue and red colors indicate the first and second acquisitions, respectively. (a) Composite, $S_{\theta\theta}$, (b) Composite, S_{zz} , (c) Mucosa-submucosa, $S_{\theta\theta}$, (d) Mucosa-submucosa, S_{zz} , (e) Muscle, $S_{\theta\theta}$, (f) Muscle, S_{zz} .

Table 1

Summary of SEF fit parameters and rms values for composite esophagus samples.

Sample	C, kPa	a_{11}	a_{22}	a_{12}	rms S_{ϕ} kPa	rms S_{zz} kPa
1	16.72 ± 1.62	3.46 ± 0.12	56.59 ± 35.08	3.71 ± 0.16	1.99 ± 0.12	2.02 ± 0.09
2	22.83 ± 3.76	2.88 ± 0.32	9.05 ± 0.96	2.99 ± 0.31	1.61 ± 0.39	1.49 ± 0.36
3	18.34 ± 3.73	1.31 ± 0.07	0.10 ± 0.00	1.67 ± 0.09	1.89 ± 0.39	2.65 ± 0.54
4	56.06 ± 3.76	0.48 ± 0.03	0.10 ± 0.00	0.66 ± 0.04	0.63 ± 0.07	0.88 ± 0.10
5	72.80 ± 18.03	0.67 ± 0.08	0.10 ± 0.00	0.85 ± 0.11	1.39 ± 0.74	1.79 ± 0.91

Table 2
Summary of SEF fit parameters and rms values for mucosa-submucosa esophagus samples.

Sample	C , kPa	a_{11}	a_{22}	a_{12}	rms $S_{\theta\theta}$ kPa	rms S_{zz} kPa
1	0.59 ± 0.32	0.48 ± 0.33	0.10 ± 0.00	0.41 ± 0.29	0.75 ± 0.02	0.44 ± 0.01
2	0.11 ± 0.01	3.28 ± 0.36	0.10 ± 0.00	2.73 ± 0.31	1.25 ± 0.04	0.74 ± 0.02
3	0.11 ± 0.01	5.97 ± 0.84	0.10 ± 0.00	4.73 ± 0.66	1.56 ± 0.11	0.88 ± 0.06
4	0.49 ± 0.23	3.03 ± 1.34	0.10 ± 0.00	2.28 ± 1.01	1.75 ± 0.07	0.91 ± 0.04
5	0.16 ± 0.01	2.97 ± 0.28	0.10 ± 0.00	2.42 ± 0.22	1.33 ± 0.06	0.77 ± 0.03

Table 3

Summary of SEF fit parameters and rms values for muscle esophagus samples.

Sample	C, kPa	a_{11}	a_{22}	a_{12}	rms $S_{\phi\phi}$ kPa	rms S_{zz} kPa
1	0.21 ± 0.07	1.55 ± 0.47	0.10 ± 0.00	1.26 ± 0.39	1.60 ± 0.08	0.94 ± 0.04
2	0.10 ± 0.02	3.18 ± 0.96	0.10 ± 0.00	2.65 ± 0.79	1.19 ± 0.03	0.71 ± 0.01
3	0.99 ± 0.58	0.47 ± 0.58	0.10 ± 0.00	0.41 ± 0.49	1.22 ± 0.03	0.70 ± 0.01
4	0.71 ± 0.41	2.13 ± 1.71	0.10 ± 0.00	1.64 ± 1.32	1.92 ± 0.08	1.02 ± 0.05
5	0.05 ± 0.02	30.03 ± 2.05	0.86 ± 1.69	24.44 ± 1.66	1.56 ± 0.06	0.91 ± 0.04