Multiaxial mechanical response and constitutive modeling of esophageal tissues: Impact on esophageal tissue engineering

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Abstract

Congenital defects of the esophagus are relatively frequent, with 1 out of 2500 babies suffering from such a defect. A new method of treatment by implanting tissue engineered esophagi into newborns is currently being developed and tested using ovine esophagi. For the reconstruction of the biological function of native tissues with engineered esophagi, their cellular structure as well as their mechanical properties must be considered. Since very limited mechanical and structural data for the esophagus are available, the aim of this study was to investigate the multiaxial mechanical behavior of the ovine esophagus and the underlying microstructure. Therefore, uniaxial tensile, biaxial tensile and extension-inflation tests on esophagi were performed. The underlying microstructure was examined in stained histological sections through standard optical microscopy techniques. Moreover, the uniaxial ultimate tensile strength and residual deformations of the tissue were determined. Both the mucosa-submucosa and the muscle layers showed nonlinear and anisotropic mechanical behavior during uniaxial, biaxial and inflation testing. Cyclical inflation of the intact esophageal tube caused marked softening of the passive esophagi in the circumferential direction. The rupture strength of the mucosa-submucosa layer was much higher than that of the muscle layer. Overall, the ovine esophagus showed a heterogeneous and anisotropic behavior with different mechanical properties for the individual layers. The intact and layer-specific multiaxial properties were characterized using a well-known three-dimensional microstructurally based strain-energy function. This novel and complete set of data serves the basis for a better understanding of tissue remodeling in diseased esophagi and can be used to perform computer simulations of surgical interventions or medical-device applications.

Keywords:
- Ovine esophagus
- Biomechanical behavior
- Uniaxial and biaxial tensile testing
- Extension-inflation testing
- Constitutive equation
- Residual strains

1. Introduction

Esophageal atresia occurs in 1 out of 2500 live births [1]. It can occur in several anatomical variations: the most common form, with a percentage of 87%, is a blind esophageal pouch with a fistula between the trachea and the lower half of the esophagus. Other forms, like an isolated atresia or an isolated tracheoesophageal fistula, occur less often [2]. The standard method to repair esophageal atresia is to connect the esophageal segments by primary anastomosis. However, this method is difficult to perform in cases with large gap atresias. A potentially viable alternative is the implantation of tissue engineered esophagi [3]. At this stage of development, ovine esophageal epithelial cells are seeded onto collagen scaffold sheets. These sheets are then wrapped around a sterile endotracheal tube to get a tubular geometry and subsequently implanted into the omentum of the lamb. After 8–10 weeks, they are removed for histological and morphological evaluations [3]. To ensure that tissue engineered esophagi show the same biomechanical behavior as their naturally grown counterparts, the behavior of the substitutes has to be investigated. Furthermore, the identification of the mechanical properties is a prerequisite for the proper evaluation of transmural stress distributions, which in turn is central to the understanding of esophageal physiology and pathophysiology [4,5]. These properties will also serve as a basis to assess remodeling processes of esophageal tissue in diseased states and to run computer simulations of surgical interventions and medical-device applications [5,6].

In general, the esophageal tube consists of multiple layers, which is typical for the whole gastrointestinal system. The most inner layer is the so-called mucosa, followed by the submucosa. The mucosa is a layer of epithelial cells, forming a continuous and homogeneous sheet. The submucosa is a broad zone of connective
tissue, consisting of collagen and elastic fibers and including a large amount of water, allowing the mucosa to move freely. Attached to the submucosa is the muscle layer, which is the source of peristaltic movements. While humans have both striated and smooth muscle cells, ruminant animals like sheep only have striated muscles and, therefore, can conduct antiperistaltic movements to transport the food back into the mouth for rumination. The muscle layer is surrounded by a layer of connective tissue, the adventitia, which supports the flexibility of the esophagus and its integration in the thorax [7–9]. Hence, from a biomechanical perspective, the esophagus is a multilayered composite structure mainly consisting of the inner mucosa-submucosa layer and two outer muscle layers. This heterogeneity of the material requires that layer-specific properties need to be determined for a sound biomechanical understanding.

The in vivo loading conditions of soft biological tissues are mainly multiaxial, therefore multiaxial mechanical testing (like biaxial tensile or extension-inflation tests) for an appropriate characterization of the mechanical properties of such tissues is required. In contrast to other biomechanical studies of the esophagus reported in the literature, for the first time we provide a complete data set incorporating layer-specific strength values from uniaxial tensile tests, layer-specific multiaxial mechanical responses from biaxial tensile tests and multiaxial mechanical data of the intact esophagus obtained from extension-inflation tests at different axial pre-stretches. The multiaxial properties are characterized by a suitable microstructurally based three-dimensional (3-D) strain-energy function (SEF), proposed for arteries and soft biological tissues by Holzapfel and colleagues [10]. Additionally, the zero-stress states of the esophageal layers are qualitatively assessed.

2. Materials and methods

2.1. Sample preparation

Fresh ovine esophagi (n = 12) were obtained from a local abattoir and stored in phosphate-buffered physiological saline (PBS) at 4 °C. Before mechanical testing, the esophagi were carefully cleaned from surrounding fat and connective tissue with the aid of scissors (Fig. 1(a)). All samples were inspected for potential damage during slaughtering or harvesting. Parts with visible damage could not be used and were discarded. The esophagi were gently rinsed with water to clean the esophageal mucosa from grass and other cud. The specimens for the individual tests were prepared immediately afterwards. All tests were finished within 36 h from the removal. In order to minimize the proteolysis and possible deterioration of the tissue properties, the tissue was submerged in PBS at 4 °C over 36 h.

2.2. Histological and microstructural investigation

For histological investigations, a square sample (10 × 10 mm) was extracted from an axially cut esophagus and embedded in paraffin according to standard histological protocols. The resulting

Fig. 1. Preparation and specimens for the different types of mechanical tests: (a) ovine esophagus after removal from surrounding fat and connective tissue. Specimens prepared for the uniaxial tensile tests displaying the mucosa-submucosa layer (b) and the muscle layer (c) of the esophagus. Similarly, prepared biaxial specimens obtained from the mucosa-submucosa (d) and the muscle layer (e). (f) Prepared tube specimen from the intact esophagus ready for extension-inflation testing. The white bars indicate a length of 10 mm.
paraffin block was cut into two halves; one was utilized to obtain in-plane sections and the other to obtain transverse (cross-) sections of the circumferential-radial plane. All tissue sections were at 5 μm and subsequently stained with Masson’s trichrome stain to highlight collagen and connective tissue, which appear blue, and muscle fibers, which appear red (see Figs. 2 and 3). All images were acquired with a Zeiss Axio Scope.A1 microscope using an AxioCam MRc5 camera in combination with Axiovision 40 software (Carl Zeiss IMT GmbH, Vienna, Austria). Eyepiece magnification was typically set at 2.5× or 5×.

2.3. Uniaxial and biaxial tensile testing

The cleaned esophagus was cut open axially to extract a rectangular piece of tissue. With surgical tools the esophagus was then dissected into a single mucosa-submucosa layer and a single muscle layer (containing both muscle layers). This procedure was relatively easy due to the loose interconnection of the submucosa and the muscle layer.

For the uniaxial tensile tests, two dogbone-shaped specimens, one in the circumferential direction and the other in the axial direction, were prepared from each layer using a punch cutter and a scalpel. Therefore, four individual uniaxial test specimens were obtained from each esophagus specimen. The punch cutter had an overall length of 38 mm, a grip-to-grip length of 20 mm and a gauge width of 4 mm. To provide good and secure clamping in the tensile testing machine and to prevent slippage during testing, small pieces of sandpaper were glued to the ends of the prepared strip specimens with superadhesive gel. For a detailed description of the uniaxial testing setup the reader is referred to Ref. [11] or Ref. [12]. Finally, two small strips (cut from a black ribbon, ~3 × 0.5 mm) were glued transversely and in parallel onto the middle part of the specimens to act as gage markers for the axial deformation measurements via a video-extensometer (see Fig. 1(b) and (c)). The strip specimens were left to equilibrate for approx. 15 min in PBS with ethylene glycol tetraacetic acid (EGTA, 0.1 g 1−1) at 37 °C before subsequent testing.

The specimens were then subjected to different values of first Piola–Kirchhoff (1. PK) stresses, of 25, 50, 100 and 200 kPa for the mucosa-submucosa and of 12.5, 25, 50 and 100 kPa for the muscle layer. The applied stress values for the muscle were half of the values for the mucosa-submucosa, because of the lower rupture strength determined from preliminary tests as well as the higher thicknesses of the muscle layer in comparison to the mucosa-submucosa layer. For each stress value, preconditioning was achieved by executing four loading and unloading cycles at a constant crosshead speed of 5 mm min−1 to obtain repeatable stress–strain curves. Thereafter, the specimen underwent one additional quasi-static cycle with continuous recording of the tensile force, gage length and width for mechanical data evaluation. After all the loading–unloading cycles for each stress level were completed, the load was continuously increased until rupture occurred to obtain the ultimate tensile stretch and strength of the tissues. Before and after testing, the average thickness of the gage region of the strips was determined by means of a video-extensometer (for a detailed description see Ref. [13] or Ref. [11]).

For the biaxial tensile tests, square (cuboid) specimens with dimensions 30 × 30 mm were prepared from the separated mucosa-submucosa and muscle layers. The specimens were prepared with their sides aligned in the circumferential and axial directions of the esophageal tube. Four black markers were attached in the center of the specimen for deformation measurements with the video-extensometer. Finally, five nylon sutures were hooked to each side of the square specimen using fish hooks and mounted in the biaxial tensile testing machine (see Fig. 1(d) and (e)). For a detailed description of the biaxial testing setup the reader is referred to Ref.

Fig. 2. Representative Masson’s trichrome-stained tissue sections of a lamb esophagus. Note that collagen and other connective tissue appear blue and muscle fibers appear red. Panel (a) shows a transverse (cross-) section highlighting the three distinct layers of the esophagus wall, namely, from top (luminal) to bottom: (i) the mucosa (Mu), comprising a layer of epithelial cells; (ii) the submucosa (SMu), consisting of a thin layer of dense connective tissue, followed by a thin layer of muscle fibers and a thick layer of loose connective tissue; (iii) the muscle layer (M), consisting of two distinct layers of muscle fibers separated by a thin layer of connective tissue. Panel (b) shows an in-plane section of the mucosa (red) with the nuclei of the epithelial cells clearly visible (small dark dots), penetrated by evenly distributed (cross-sectional) rete ridges (light blue discs). The inlay in panel (b) features a higher magnification image of the mucosa (scale bar: 10 μm). Panels (c) and (d) display in-plane sections of connective tissue in the submucosa. Note that the horizontal and vertical sides of both images correspond to the axial and the circumferential direction of the esophagus, respectively. While image (c) was obtained adjacent to the mucosa and shows a very dense and isotropic structure, image (d) was taken from a central region in the submucosa and displays a much looser structure compared to (c).
During the biaxial tensile tests, the specimens were submerged in PBS with EGTA (0.1 g l\(^{-1}\)) at 37 °C. The nylon sutures are connected to specially designed carriages that allow for self-equilibrated loads of each suture line. The specimens were quasi-statically stretched at 4 mm min\(^{-1}\) using a stretch-controlled protocol with different stretch ratios between the circumferential and the axial direction (1:1, 1:0.75, 0.75:1, 1:1). Moreover, different maximum stretches, ranging from 1.2 to 1.4 in 0.1 steps, were applied to the mucosa-submucosa layer, and stretches starting at 1.1 up to 1.3 in 0.1 steps were applied to the muscle layer. Each specimen was preconditioned through four loading-unloading cycles. For the Cauchy stress calculations, the thickness of the specimen was accurately measured before and after testing by means of a video-extensometer and a micrometer.

2.4. Extension-inflation testing

The cleaned intact esophagus was shortened to a length of 8–9 cm. The intact esophagus tube segment was then cannulated at both ends with specially designed tube connectors matching the inner diameter of the esophagus and subsequently mounted in the testing machine. To prevent slippage from the tube connectors, the esophagus was glued and additionally fixed with a cord to the canulas. To measure the elongation of the esophageal tube during testing, two small strips of black ribbon, acting as markers, were glued to the middle of the specimen at a distance of 1 cm apart (see Fig. 3(f)). For a detailed description of the insertion procedure and the experimental setup, see Ref. [13] or Ref. [16].

Extension-inflation tests with continuous recording of the inflation pressure \((p)\), axial force \((F_z)\), outer diameter \((d_o)\) and gage length \((L)\) of the esophagus segments were performed at pressures ranging from 0 to 2 kPa at several axial pre-stretches \(\lambda_z = L/L_o\), ranging from 1.1 to 1.4 in increments of 0.1, where \(L\) denotes the gage length (distance of the two markers) in the axially stretched and pressurized tube, and \(L_o\) denotes the corresponding length in the unloaded (reference) state. At each increment of \(\lambda_z\), the intact esophagus was preconditioned axially by performing five axial elongation cycles \((l_e = 1 \text{ mm min}^{-1})\), ranging from the unstretched condition to the desired axial pre-stretch, where it was held after the fifth cycle. At this axial pre-stretch it was then preconditioned circumferentially by performing 10 inflation-deflation cycles, ranging from 0 to 2 kPa \((p = 10 \text{ kPa min}^{-1})\). Finally, the specimen was inflated and deflated one more time to perform the so-called “measurement cycle”, yielding the recorded raw data for further analysis. All inflation tests were conducted in PBS with EGTA (0.1 g l\(^{-1}\)) at 37 °C. To obtain the average thickness of the specimen, the gage region of the tube was cut out and opened radially, and measured by means of a video-extensometer.

2.5. Data analysis and material modeling

2.5.1. Uniaxial and biaxial tensile response

The Cauchy stresses \((\sigma)\) and stretches \((\lambda)\) were computed to quantify the uniaxial and biaxial tensile responses of the tissues. With the assumptions of negligible shear components during biaxial testing and incompressibility of the adipose tissue, the Cauchy stresses in the circumferential and the axial directions can be determined according to

\[
\sigma_{yy} = \lambda_y \frac{f_y}{L_z}, \quad \sigma_{zz} = \lambda_z \frac{f_z}{L_y},
\]

(1)

where \(\lambda_y = x_{y}/X_y\) and \(\lambda_z = x_{z}/X_z\) represent the tissue stretches in each direction based on the marker distances in the loaded \((x_{y}, x_{z})\) and unloaded \((X_y, X_z)\) configurations. The measured forces in each direction are denoted by \(f_y, f_z\), \(T\) is the mean thickness in the unloaded reference configuration, and \(L_z\) and \(L_y\) are the measured lengths in the circumferential and axial directions of the specimen in the undeformed state, respectively, i.e. for the biaxial specimens \(L_z = L_y \approx 30 \text{ mm}\). Note, that for the uniaxial tensile testing response, \(L_z\) and \(L_y\) (in the denominators in Eq. (1)) correspond to the widths of the specimen in the circumferential and axial directions, respectively.
From the uniaxial tensile tests, the mean ultimate tensile strengths and corresponding stretches in the circumferential and axial directions were recorded and statistically compared using a paired Student’s t-test. A p-value < 0.05 was considered statistically significant. All values were expressed as mean ± SD.

2.5.2. Material modeling of the biaxial tensile response

For material modeling of the biaxial mechanical behavior, we used the anisotropic structurally based hyperelastic SEF proposed by Holzapfel and co-workers [10], developed for arterial walls and generally recommended for collagen-reinforced soft biological tissues. The SEF has the form

$$
\Psi = \frac{c}{2} (I_1 - 3) + \frac{k_1}{k_2} \left\{ \exp \left[in \left( \frac{I_4 - 1}{I_4} \right)^2 - 1 \right] \right\},
$$

(2)

with

$$
I_1 = \lambda_0^2 + \lambda_2^2 + \lambda_z^2, \quad I_4 = \lambda_0^2 \cos^2 \varphi + \lambda_z^2 \sin^2 \varphi,
$$

(3)

where \(c > 0\), \(k_1 > 0\) are stress-like parameters and \(k_2 > 0\) is a dimensionless parameter. \(I_1\) and \(I_4\) are invariants [17], where \(\lambda_0, \lambda_2, \lambda_z\) are principal stretches in the circumferential, axial and radial directions, respectively. The collagen fibers, responsible for the characteristic biomechanical tissue response, are assumed to be oriented in the 0z-plane of the specimen. Consequently, the parameter \(\varphi\) in Eq. (3) denotes the angle between the (mean or principal) “collagen fiber” orientation and the circumferential direction, and therefore acts as a geometrical parameter. For a more detailed discussion regarding principal collagen fiber orientations see Refs. [18,19]. The geometrical parameter \(\varphi\) is used as a phenomenological variable in the analysis. In esophageal tissue, the “collagen fibers” comprise the collagen structures in the mucosa-submucosa layer as well as collagen networks surrounding and connecting the muscle fibers in the muscle layer.

Cauchy stresses can be expressed as derivatives of the SEF \(\Psi\) with respect to the work conjugate strain measures [17]. For incompressible hyperelastic materials, this yields the constitutive equations

$$
\sigma_{0a}^\Psi = \frac{\partial \Psi}{\partial \epsilon_{0a}} - p, \quad a = \theta, z, r,
$$

(4)

where \(\sigma_{0a}^\Psi\) denote Cauchy stresses in each direction derived from \(\Psi\), and \(p\) serves as an indeterminate Lagrangian multiplier which can be interpreted as a hydrostatic pressure and can be calculated from boundary conditions [17]. In biaxial tensile tests, \(p\) can be determined from \(\sigma_{0\theta} = 0\). Hence, with Eq. (2) and the incompressibility condition \(\lambda_r = \lambda_0^{-1} \lambda_z^{-1}\), we obtain for the Cauchy stresses

$$
\sigma_{0\theta}^\Psi = 2(\lambda_0^2 - \lambda_0^{-2} \lambda_z^2) \psi_1 + 2 \lambda_0^2 \cos^2 \varphi \psi_4,
$$

(5)

$$
\sigma_{0z}^\Psi = 2(\lambda_z^2 - \lambda_0^{-2} \lambda_z^2) \psi_1 + 2 \lambda_z^2 \sin^2 \varphi \psi_4,
$$

(6)

where the abbreviation \(\psi_i = \partial \Psi/\partial \epsilon_{0i}\) (i = 1, 4) was used. Note that both Eqs. (5) and (6) are only valid by assuming negligible shear deformations during testing.

Since we observed only small variations in the biaxial mechanical response of the esophagus layers for each stretch level, we only considered averaged mechanical data (at different stretch levels) in the biaxial tensile data fitting. Moreover, for the mucosa-submucosa layer, the averaged mechanical data were fitted to the SEF \(\Psi\) (Eq. (2)) using the same set of constitutive parameters for each stretch value (1.2, 1.3 and 1.4). In contrast, fitting the averaged mechanical data of the muscle layer required a different set of constitutive parameters for each tested stretch value (1.1, 1.15, 1.2 and 1.3), due to severe “softening” effects at increased stretch levels.

The data from the three biaxial stretch protocols (\(\lambda_0^2/\lambda_z^2 = 1:1, 1:0.75, 0.75:1\)) associated with the circumferential and axial directions at the above-stated maximum stretch levels were simultaneously fitted to the material model. Therefore, best-fit values of the constitutive parameters were determined by means of the non-linear least-squares regression method using the Mathematica software package (Wolfram Research Inc., IL, USA). In particular, the global minimum of the objective function

$$
\chi^2 = \sum_{i=1}^{n} \left( [\sigma_{0\theta} - \sigma_{0\theta}^\Psi]_i + [\sigma_{0z} - \sigma_{0z}^\Psi]_i \right)
$$

(7)

is determined, where \(n\) is the number of data points considered. Consequently, the four parameters \(c, k_1, k_2\) and \(\varphi\) were obtained for each specimen.

As a measure of the goodness-of-fit the square of the Pearson’s correlation coefficient \(r^2\) was computed for the transverse and longitudinal Cauchy stresses. Additionally, the error measure \(e\), based on \(\chi^2\) from Eq. (7), was calculated to evaluate the goodness-of-fit. This error measure is defined as

$$
e = \frac{100}{\sigma_{ref}} \sqrt{\frac{\chi^2}{n - q}}
$$

(8)

where \(n\) is the number of data points considered and \(q\) is the number of parameters of the SEF, which in our case is five. Hence, \(n - q\) yields the number of degrees of freedom. The scalar value \(\sigma_{ref}\) denotes the sum of the Cauchy stresses over all data points divided by the number of data points considered. Thus, the error measure \(e\) captures the percentage of the root-mean-squared error per statistical degree-of-freedom (RSME) between experimental and related model values, normalized with respect to \(\sigma_{ref}\).

2.5.3. Material modeling of the extension-inflation response

We consider the intact segment of the esophagus to be an incompressible thick-walled cylindrical tube, subjected to extension and inflation under the assumption of no torsion. During extension and inflation the load-free (reference) configuration changes into the current (loaded) configuration. In terms of cylindrical coordinates \((r, \theta, z)\), the current configuration can be described using

$$
r_i \leq r \leq r_o, \quad 0 \leq \theta \leq 2\pi, \quad 0 \leq z \leq l_z,
$$

(9)

where \(r_i\) and \(r_o\) denote the inner and outer radii of the tube, respectively. Using the incompressibility condition, the inner radius \(r_i\) of the loaded tube can be determined from

$$
r_i = \sqrt{r_o^2 - \frac{R_o^2 - R_i^2}{l_z}}
$$

(10)

where \(R_i = l_z/l_z^2\) is the measured axial stretch. Note that the outer radius of the loaded tube may be expressed as \(r_o = R_o l_z\), where \(l_z\) is the (loaded) circumferential stretch and the load-free inner radius is \(R_i = R_o - H\), with \(H\) denoting the (unloaded) wall thickness. Finally, \(r_i\) can be expressed solely as a function of the circumferential and axial stretches (\(\lambda_\theta, \lambda_z\)) according to

$$
r_i = \sqrt{(R_\theta \lambda_\theta)^2 - \frac{2R_\theta H - H^2}{\lambda_z}},
$$

(11)

using the measured constants \(R_\theta\) and \(H\).

From the only non-trivial solution of the equilibrium equation, the internal pressure \(p_i\) in a thick-walled tube can be obtained in the form [10]

$$
p_i = \int_{r_i}^{r_o} (\sigma_{0\theta} - \sigma_{0\theta}^i) \frac{dr}{r},
$$

(12)

where \(\sigma_{0\theta}\) and \(\sigma_{0\theta}^i\) denote the Cauchy stresses in the circumferential and radial directions, respectively. For a static equilibrium, the sum
of the (measured) axial force \( F_z \) and the pressure force \( p_i \pi r_i^2 \) is equal to the integral of the axial stresses over the cross-section of the vessel wall, i.e.,

\[
F_z + p_i \pi r_i^2 = 2 \pi \int_{r_i}^{r_f} \sigma_{zz} r \, dr,
\]

where \( \sigma_{zz} \) denotes the Cauchy stress in the axial direction. With Eq. (13), the measured axial force becomes

\[
F_z = \pi \int_{r_i}^{r_f} (2 \sigma_{zz} - \sigma_{yy} - \sigma_{yy}) r \, dr.
\]

For material modeling, we used the same hyperelastic SEF according to Eq. (2). Analogously, the four constitutive parameters \( c_{ij}, k_i, k_j, \phi \) were obtained by means of a nonlinear least-squares regression using a Levenberg–Marquardt algorithm [20]. Briefly, we minimized the objective function

\[
\chi^2 = \sum_{i=1}^{n} \left\{ w_p (p_i - \bar{p}_i)^2 + w_f (F_{zz} - \bar{F}_{zz})^2 \right\},
\]

where \( n \) is the number of data points considered, and \( w_p \) and \( w_f \) are weighting factors for the internal pressure \( p_i \) and the axial force \( F_{zz} \), respectively, while \( \bar{p}_i \) and \( \bar{F}_{zz} \) are the internal pressure and the axial force, respectively, predicted by the SEF \( \Psi \) for the \( j \)th data record, and \( p_i \) and \( F_{zz} \) are the corresponding experimentally measured internal pressure and axial force, respectively. The mean values of the axial forces \( \bar{F}_{zz} \) and the internal pressures \( \bar{p}_i \) are used as weighting factors, in order to equate contributions of the internal pressure and the axial force terms in the objective function \( \chi^2 \). Because no values for the axial in vivo pre-stretches for the lamb esophagus were available, for one set of constitutive parameters \( c_{ij}, k_i, k_j, \phi \) we considered pre-stretches in the range of 1.2–1.4, based on reported values for various animal species [21, 22–24, 45].

For the fitting process, 18 representative data points were chosen from the final (preconditioned) loading cycle of each experimental data set. Analogous to biaxial data fitting, as a measure for the goodness-of-fit we calculated the mean squared Pearson's correlation coefficient \( \rho \) and the internal pressures \( p_i \) and axial forces \( F_{zz} \), respectively. Thus, the error measure \( \varepsilon \) is the integral of the axial stresses over the cross-section of the vessel wall, i.e.,

\[
E = \left[ \frac{\chi^2}{n-q} \right]^{1/2},
\]

where \( q \) denotes the number of model parameters in the SEF and \( n-q \) is the number of degrees of freedom, while \( p_{\text{fit}} \) and \( F_{zz,\text{fit}} \) are the mean values of the internal pressure and axial forces, respectively. Thus, the error measure \( \varepsilon \) denotes the percentage of the RSME between experimental and related model values, normalized with respect to \( p_{\text{fit}} \) and \( F_{zz,\text{fit}} \).

2.6. Residual stress measurements

Three-dimensional residual deformations were determined for intact esophagus rings and strips, as well as for the separated mucosa-submucosa and muscle layers in the passive state. Therefore, a ring and a strip cut along the axial direction of the esophageal tube were removed from the anterior site of the intact esophagus. Typical specimen dimensions were 15 × 5 mm (diameter × height) for the rings and 30 × 5 mm (length × width) for the axial strips. Moreover, another ring and axial strip were extracted from adjacent tissue, but this time the mucosa-submucosa was separated very carefully from the muscle layer using minimal force and surgical instruments. The specimens were placed in a tissue bath with PBS, which was maintained at a constant temperature of 37 °C, and scaled digital images of all specimens were taken immediately. Next, each ring was cut open radially and images were taken immediately after the cut and subsequently after 1, 2, 3 and 6 h.

3. Results

3.1. Histology

Representative images of Masson's trichrome-stained tissue sections of the lamb esophagus are shown in Figs. 2 and 3. The entire wall can be divided into three main layers, visible in the transverse (cross-) section shown in Fig. 2(a): (i) the mucosa (denoted M in panel (a)), the innermost layer of the wall which forms a continuous and homogeneous sheet consisting of epithelial cells. Fig. 2(b) shows an in-plane section of the mucosa (red) with the nuclei of the epithelial cells clearly visible (small dark dots). The light blue discs (magnified in the inlay in (b); scale bar: 10 μm) are cross-sections of rete ridges which evenly penetrate the lower regions of the mucosa (visible as small ridges in panel (a)) between the border of the mucosa and submucosa; (ii) the submucosa (denoted SMu in panel (a)), consisting of a thin layer of densely packed connective tissue (in-plane section shown in Fig. 2(c)), followed by a thin layer of muscle fibers and a thick layer of loose connective tissue. Sometimes, the collagen fibers in the loose connective tissue displayed a (overall) preferential orientation along the axial direction of the wall (see in-plane section in Fig. 2(d)), which could lead to a slightly stiffer axial mechanical response in some samples. Note that the horizontal and vertical sides in (c) and (d) correspond to the axial and circumferential directions of the esophagus, respectively. This preferred axial orientation could explain the observation of mechanical anisotropy in mechanical tests; and (iii) the muscle layer (denoted M in panel (a)), which itself consists of two distinct layers of muscle fibers that are separated by the adventitia, a thin sheet of connective tissue visible as blue lines surrounding the muscle fiber bundles in panel (a). The adventitia appeared to be a loosely organized, isotropic network of collagenous fibers. Fig. 3(a) shows an image of an esophagus segment, cut open axially, featuring the outermost surface of the muscle layer (\( x \) and \( y \) denote the circumferential and the axial direction of the esophagus, respectively). At closer inspection, an almost sinusoidal change in muscle fiber orientation in the outer muscle layer with respect to the circumferential direction \( x \) becomes evident, illustrated as solid black curves in panel (b). After bisecting both muscle layers, we also noticed a counter-rotating, sinusoidal muscle fiber orientation in the second (inner) muscle layer, illustrated as dashed gray curves in panel (b). Panels (c) and (d) are in-plane micrographs of both layers, showing parallel-aligned muscle fibers with some collagen fibers aligned between them.

3.2. Thickness of the esophagus wall

The measured mean thickness of all mucosa-submucosa specimens was 1.2 ± 0.3 mm. In contrast, the muscle specimens showed average thicknesses of 2.3 ± 0.4 mm, thus being, on average, approx. 1 mm thicker than the mucosa-submucosa layer.

3.3. Uniaxial tensile response

Representative uniaxial Cauchy stress–stretch behavior (five loading–unloading cycles) of the mucosa-submucosa layer at 1. PK stresses of 25, 50, 100 and 200 kPa, and of the muscle layer at 1. PK stresses of 12.5, 25, 50 and 100 kPa for both directions are given in Fig. 4. Both layers revealed nonlinear (exponential) and clearly anisotropic behaviors, with distinctly stiffer behavior for
For each 1. PK stress level, we observed larger circumferential stretches than corresponding axial stretches for the mucosa-submucosa. For the muscle layer, almost twice the circumferential stretches compared to axial stretches were observed (see Table 1 and Fig. 4). In the circumferential direction, both the mucosa-submucosa and the muscle layer showed high extensibilities, with maximum stretch ratios of more than two (see Table 2 or Fig. 4(a) and (b)). For both layers in both directions, the hysteresis of the first cycle was much bigger than during the remaining cycles, indicating that most energy stored in the esophageal tissue was lost after the first imposed loading [24].

**Table 1**

Mean values and standard deviations (n = 11) of stretches and Cauchy stresses (kPa) for the mucosa-submucosa and muscle layers in the circumferential and axial direction during uniaxial tensile testing at different 1. PK stresses (kPa).

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stretch</td>
<td>Cauchy stress</td>
</tr>
<tr>
<td>25</td>
<td>1.34 ± 0.11</td>
</tr>
<tr>
<td>50</td>
<td>1.48 ± 0.12</td>
</tr>
<tr>
<td>100</td>
<td>1.61 ± 0.14</td>
</tr>
<tr>
<td>200</td>
<td>1.77 ± 0.18</td>
</tr>
</tbody>
</table>

**Table 2**

Layer-specific ultimate tensile strength \( \sigma_{ult} \) (in terms of Cauchy stresses in MPa) and corresponding stretch \( \lambda_{ult} \) for all tested ovine esophagus samples I–XII in the circumferential and axial directions obtained from uniaxial tensile tests.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Mucosa-submucosa</th>
<th>Muscle</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>circ. axial</td>
<td>circ. axial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.5</td>
<td>1.66</td>
<td>1.2</td>
<td>1.33</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>—</td>
<td>—</td>
<td>0.9</td>
<td>1.53</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>1.3</td>
<td>2.09</td>
<td>2.2</td>
<td>1.73</td>
<td>0.6</td>
</tr>
<tr>
<td>IV</td>
<td>1.2</td>
<td>1.83</td>
<td>3.4</td>
<td>1.47</td>
<td>0.4</td>
</tr>
<tr>
<td>V</td>
<td>0.8</td>
<td>1.73</td>
<td>2.9</td>
<td>1.61</td>
<td>1.1</td>
</tr>
<tr>
<td>VI</td>
<td>2.2</td>
<td>2.44</td>
<td>2.9</td>
<td>1.65</td>
<td>0.5</td>
</tr>
<tr>
<td>VII</td>
<td>1.4</td>
<td>2.49</td>
<td>3</td>
<td>1.51</td>
<td>0.8</td>
</tr>
<tr>
<td>VIII</td>
<td>1.2</td>
<td>1.97</td>
<td>2.5</td>
<td>1.31</td>
<td>0.4</td>
</tr>
<tr>
<td>IX</td>
<td>1.8</td>
<td>1.9</td>
<td>2.8</td>
<td>1.97</td>
<td>0.6</td>
</tr>
<tr>
<td>X</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.9</td>
</tr>
<tr>
<td>XI</td>
<td>2.4</td>
<td>2.41</td>
<td>1.9</td>
<td>1.58</td>
<td>0.4</td>
</tr>
<tr>
<td>XII</td>
<td>2.7</td>
<td>2.85</td>
<td>4.5</td>
<td>2.03</td>
<td>0.6</td>
</tr>
<tr>
<td>Mean</td>
<td>1.5</td>
<td>2.14</td>
<td>2.6</td>
<td>1.60</td>
<td>0.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.7</td>
<td>0.39</td>
<td>1.0</td>
<td>0.21</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The axially oriented specimens. For each 1. PK stress level, we observed larger circumferential stretches than corresponding axial stretches for the mucosa-submucosa. For the muscle layer, almost twice the circumferential stretches compared to axial stretches were observed (see Table 1 and Fig. 4). In the circumferential direction, both the mucosa-submucosa and the muscle layer showed high extensibilities, with maximum stretch ratios of more than two (see Table 2 or Fig. 4(a) and (b)). For both layers in both directions, the hysteresis of the first cycle was much bigger than during the remaining cycles, indicating that most energy stored in the esophageal tissue was lost after the first imposed loading [24]. In general, the hysteresis and also “softening” were more pronounced in the esophageal muscle compared to the mucosa-submucosa.

Ultimate tensile strength and associated stretch values for all specimens are listed in Table 2. The mean ultimate tensile strength for the mucosa-submucosa layer in the circumferential and axial directions were determined as 1.5 ± 0.7 and 2.6 ± 1.0 MPa, respectively. In contrast, the muscle layer showed significantly lower strength values, with 0.6 ± 0.3 and 0.7 ± 0.3 MPa in the circumferential (\( p = 0.001 \)) and the axial direction (\( p < 0.001 \)), respectively. On average, the axial direction of the mucosa-submucosa was significantly stronger (66%) than the circumferential direction (\( p = 0.001 \)). Also the strength of the axially oriented strip of the
muscle layer was on average higher (16%) than the circumferentially cut muscle layer, though not statistically significant ($p = 0.27$). The average stretches in the circumferential direction, where the uniaxial tensile test specimens ruptured, were determined to $2.14 \pm 0.39$ for the mucosa-submucosa and $2.14 \pm 0.46$ for the muscle layer. For the axial direction, we determined $2.14 \pm 0.39$ for the mucosa-submucosa and $2.14 \pm 0.46$ for the muscle layer. Both layers were significantly more extensible in the circumferential direction than in the axial direction ($p = 0.001$ for the mucosa-submucosa and $p = 0.002$ for the muscle layer).

However, the maximum stretches in a specific orientation (circumferential or axial) were similar for both layers, i.e. there was no significant difference between circumferentially ($p = 0.98$) and axially oriented layers ($p = 0.39$).

### 3.4. Biaxial mechanical response

Representative biaxial stress–stretch behaviors during preconditioning for different stretch levels of both layers are shown in Fig. 5. The mucosa-submucosa layer showed nonlinear exponential behavior but, in contrast to the uniaxial tensile data, only a marginally anisotropic behavior. Interestingly, the mechanical anisotropy becomes more pronounced at increased stretches. Under biaxial loadings, the “passive” muscle layer showed distinctly larger hysteresis during the first cycle than for the remaining cycles. Moreover, the muscle layer showed “softening” during cyclic preconditioning as well as with increasing stretch (see Fig. 5(c) and (d)). An interesting phenomenon was observed for the muscle layer, which displayed a mechanical recovering capability, clearly evident in the Fig. 5(c) and (d) by comparison of the last and first cycles at subsequent increased stretch levels.

![Fig. 5. A typical example of the equibiaxial Cauchy stress–stretch behavior during preconditioning at different stretch levels of the mucosa-submucosa and muscle layer in the circumferential (a) and (c) and axial (b) and (d) directions. (a) and (b) Show four loading–unloading cycles for different stretch levels (1.2, 1.3, 1.4 and 1.5) of the mucosa-submucosa layer, and (c) and (d) show four cycles for the stretch levels (1.1 and 1.15) and ten cycles for the stretch levels (1.2 and 1.3) of the muscle layer.](image)

For the different applied axial pre-stretches ($\lambda_z = 1.1$ to 1.4), the inflation pressure and the axial force were plotted as functions of...
3.6. Zero-stress state

Generally, the observed residual deformations are characteristic for each layer (mucosa-submucosa and muscle layer) and specimen direction. Dissection of the intact esophagus ring by separating the two main layers was associated with a release of compressive loads in the mucosa-submucosa layer and of tensile loads in the muscle layer, clearly visible in Fig. 8(a). Initially, the mucosa-submucosa was folded inside the intact esophagus, whereas in the separated state the mucosa-submucosa appeared to be half as thick, with an increased internal diameter, compared to the intact ring. Contrarily, the internal diameter of the muscle layer decreased compared to the intact ring [4].

After cutting the rings radially, a release of circumferential compressive residual stress at the inner wall and tensile residual stress at the outer surface of the wall caused the ring to spring open, leaving it in a arc-like geometry, as shown in Fig. 8(b). Circumferential strips of the mucosa-submucosa showed a more pronounced opening angle compared to circumferential strips of the muscle layer and the intact esophagus. Note that axial residual stress is probably also released during this process, although this is not apparent from the resulting geometry. After 3 h no further geometrical changes were observed, indicating that at that point most of the residual stresses had been released.

Fig. 8(c) features the resulting deformations of the axial strips after 3 h of equilibration in the tissue bath. Interestingly, we observed a strong coiling of the axial segments of the intact esophagus strips and the muscle layer strips, with a full rotation (360°) around the longitudinal axis of the strips within approximately 18 ± 2 mm (n = 16). We believe that the reason for the strong coiling is the counter rotating, sinusoidal structure of the two adjacent muscle layers, as illustrated and discussed in Fig. 3. The axially cut mucosa-submucosa strips showed pronounced bending (curling) away from the longitudinal axis of the tubular esophagus.

4. Discussion

Esophageal atresia is a birth defect which causes the esophagus to end in a blind-ended pouch rather than connecting normally to the stomach. For large gap atresias, esophagus tissue engineering is seen as a possible solution for tissue replacement. Reliable benchmarks are required for designing and characterizing artificial prosthesis of the esophagus, and our study of the biomechanical behavior and the underlying microstructure of the ovine esophagus yielded such novel and reliable data. To that end, uniaxial-, biaxial- and extension-inflation tests, residual strain measurements as well as histological investigations of the intact wall.
including its individual layers were performed. To quantify the 3-D multiaxial, finite strain, heterogeneous and anisotropic responses of the intact and layer-specific esophagi, constitutive parameters were identified by fitting a hyperelastic strain-energy function to datasets from biaxial tensile tests and at different stretches and extension-inflation tests at different axial pre-stretches.

### 4.1. Thickness of the esophagus

For the mean thickness values of the mucosa-submucosa and muscle layer specimens we obtained 1.2 ± 0.3 and 2.3 ± 0.4 mm, respectively, yielding average thickness ratios of 1:2. Yang and colleagues [25] reported mean thickness values for porcine esophagi mucosa-submucosa layer of 2 mm and for the muscle layer of 3 mm. The only values found for sheep esophagi were obtained by Floyd and Morrison[26], but unfortunately they only reported the thickness of the muscle layer. With 1.5 mm it was thinner than the muscle layer investigated here. For the intact porcine esophagus, Yang et al.[25] found a mean thickness of 3–4 mm, compared to our findings of 3.5 ± 0.5 mm for the entire wall of the ovine esophagi. For rabbit esophagi, Sokolis [5] found that the mucosa-submucosa was 2 and 3 times thinner than the muscle and the intact esophagus, respectively, which is similar to our findings.

For a better comparison of the biomechanical behavior between the ovine and the tissue engineered esophagi, similar thickness ratios between the two would be desirable. At the moment, tissue engineered esophagi show a thickness of 4 mm, which is significantly higher than its “real” counterpart. Furthermore, such
engineered constructs consist only of the mucosa-submucosa (epithelial cells and collagen) and have no additional muscle layer [3].

4.2. Uniaxial tensile behavior

Under uniaxial tensile loadings, both layers showed pronounced nonlinear and anisotropic behavior, with a distinctly stiffer response in the axial compared to the circumferential direction. All curves showed an initially flat region, followed by a kink and a very steep region, which is typical for collagen-rich tissues. This highly nonlinear behavior of the mucosa-submucosa and the “passive” muscle layer might be advantageous to the esophageal function. At low stretches the esophagus is very compliant, which most likely is a useful tissue property for the swallowing act. At high stretches, on the other hand, the mucosa-submucosa layer will increase its resistance rapidly to prevent the esophagus from being overdilated [8], a function also common in the circulatory system where the adventitia prevents overstretching of arteries at high blood pressures [10,27]. For porcine esophagus, Yang et al. [25] also obtained a distinct nonlinear and stiffer behavior in the axial than in the circumferential direction. Similar findings obtained Stavropoulou et al. [28] for porcine esophageal layers. The high anisotropy in the mucosa-submucosa and the higher non-linearity in the axial direction suggest that the fibers are preferably aligned along the axial direction. In order to serve as a comparable alternative to naturally grown tissue, tissue engineered esophagi would need to show the same (or at least similar) anisotropic, heterogeneous and nonlinear biomechanical behavior.

Yang and colleagues [25] found, on average, distinctly higher ultimate tensile strength values for porcine esophageal layers than we have obtained for the ovine esophageal layers. Similar to our findings, the ultimate tensile strength values of porcine esophagi in the axial direction were also higher than in the circumferential direction. Stavropoulou et al. [28] also determined, on average, higher strength values in the axial direction than in the circumferential direction, but with exceptions of the mucosa-submucosa in the cervical region and the inner muscle layer in the abdominal region. Here, the ratio of axial to circumferential ultimate tensile strength was determined to 1.7 ± 1.2 in the mucosa-submucosa and 1.2 ± 0.4 in the muscle layer. The ratio of the ultimate tensile strength between the mucosa-submucosa and the muscle was 3.5 ± 1.0 and 2.5 ± 0.8 in the axial and circumferential direction, respectively. For porcine esophagi, Yang et al. [25] found in average a higher ratio of axial to circumferential ultimate tensile strength of 2.3 in the mucosa-submucosa and a similar ratio of 1.3 in the muscle layer. The reported ratio of the ultimate tensile strength between the porcine mucosa-submucosa and muscle layer was 5.5 in the axial and 3.0 in the circumferential direction, both of which are clearly higher compared to our findings for the ovine esophagi. The differences of the ultimate tensile strength ratios of porcine and ovine esophagi could be a result of the different thickness ratios between the muscle and mucosa-submucosa layers between the two species. The large difference of the ultimate tensile strength between the mucosa-submucosa and the muscle layers can be explained by the increased amount of collagen fibers in the mucosa-submucosa compared to the muscle layer [4].

The ovine esophagi were more extensible in the circumferential than in the axial direction, with a maximum stretch of 2.1 in the circumferential and 1.52–1.6 in the axial direction. Similar findings were obtained for porcine esophagi, except for in the cervical region, where the axially oriented mucosa was most extensible [25]. In contrast, for each region (cervical, thoracic and abdominal) of porcine esophagus, Stavropoulou et al. [28] observed a more extensible mucosa-submucosa layer in the circumferential than in the axial direction, which coincides with our observation. In addition, Stavropoulou et al. [28] also showed notable topographical differences in material and failure parameters between the cervical and abdominal regions. However, their observations contrast with those of Yang et al. [23], who found that the cervical region was the most extensible and that no regional differences existed in strength.

A future study on the ultimate tensile strength of tissue engineered esophagi should reveal similar values and an equally high heterogeneity in order to be a suitable substitution for naturally grown tissue.

4.3. Biaxial tensile behavior

Results for the biaxial tensile behavior also revealed distinct nonlinear behavior but, in contrast to the uniaxial tensile behavior, only marginal anisotropic differences were found, which became more pronounced at increased stretches. Compared to uniaxial tests in two orthogonal directions, multiaxial tests (like biaxial or extension-inflation tests) have several advantages. The biaxial and inflation tests lead to a coupling of the circumferential and axial behavior, resulting in a more physiological deformation during these tests while at the same time avoiding non-physiological fiber rotations which might occur during uniaxial testing [29]. The “passive” muscle layers showed a “softening” behavior in both orientations during preconditioning and with increasing stretches (Fig. 5). Such a high “softening” was not observed during preconditioning of uniaxially tested specimens. The mechanical recovering of the muscle layers between the last and the first
cycle of subsequently increased stretch levels was only observed during biaxial tensile testing. This recovering was probably a result of a short resting period of ~15 s between subsequent loading series, suggesting that “softening” during preconditioning is partly reversible. The loss of stiffness during preconditioning was attributed to two distinct history- and strain-dependent properties [24]: one is the “viscoelastic” loss of stiffness, where the stress depends on the time-history of the strain and the “softening” can be recovered; the other is strain softening (known as the Mullins effect), which is associated with irreversible structural changes. This strain softening appears to be irreversible in the passive state only. It is known that the loss in stiffness in the detrusor smooth muscle appears to be reversible when the muscle is not producing active force but is reversible on full muscle activation [30,24].

Based on the results of the model data in Fig. 6, we demonstrated that it is feasible to use our model to characterize the biaxial mechanical behavior of the esophagus layers very well, confirming our hypothesis that the SEF used, which was originally designed for blood vessels, is also applicable to the esophagus. The phenomenologically determined collagen fiber angles $\phi$ were larger than 45°, which indicates that the collagen fibers show a preferred axial orientation for both the mucosa-submucosa and the muscle layer. This was also reported for the porcine esophagus [29]. With regard to the mucosa-submucosa, this angle was evaluated at 49.2°, which is similar to the range reported for porcine esophagi in the literature [25,31].

4.4. Extension-inflation behavior

The high interspecimen variations of the extension-inflation data made it difficult to interpret the results. As expected, the esophagi showed a strong “softening” in the circumferential direction during the testing protocol, which reached its maximum at an axial pre-stretch level of 30%. With pre-stretch values beyond that, the samples became stiffer in the circumferential and axial directions. The axial stretch of the samples also showed a slight increase during the tests with a much smaller standard deviation compared to the circumferential stretch, which decreased further with higher axial pre-stretches (Table 4).

Yang et al. [29] tested the inflation behavior of the separated layers (muscle and mucosa-submucosa) of porcine esophagi between 0 and 5 kPa at axial pre-stretches of 0, 2.5 and 25%. Their results showed a nonlinear behavior with a significant increase of the radius at low pressures in the mucosa-submucosa, and both layers showed stiffening with increasing pre-stretch values. Similar to our layer-specific uniaxial and biaxial tensile test results, their data indicate that the mucosa-submucosa is more compliant initially and becomes stiffer than the muscle layer at increasing pressures. Stavropoulou et al. [4] found a significant increase of the radius in the muscle layer of rabbit esophagi between 0 and 1.33 kPa at axial stretch ratios of 1.2, 1.3, 1.4 and 1.5, similar to our observation. Zeng and colleagues [32] investigated the circumferential and the axial stress–strain relations of inflated rat esophagi and found that the mucosa-submucosa was stiffer than the muscle layer, both in the circumferential direction, due to inflation, and in the axial direction, due to elongation.

The strong “softening” in the circumferential direction observed here is possibly a result of the passive response of the muscle layer, which stems from the small amount of collagen fibers (confirmed visually from histological sections) and the non-active response of the muscle fibers [24]. Studies in active tissue have shown that the tissue “softening” is adjustable because of its dependence on the muscle activation. We suggest that future studies regarding the strain softening should include experiments with active muscle function to increase our understanding of the underlying physiological drivers behind the mechanical behavior in gastrointestinal tissues.

The model we used was also able to characterize the extension-inflation behavior at different axial pre-stretches of the intact esophagus quite well. The values of the constitutive parameters $c$ and $k$ were small for both the biaxial- and extension-inflation data, indicating “very soft” material components of the esophageal tissue acting in the low loading domain.

4.5. Zero-stress state

The opening angle of the mucosa-submucosa layer in the circumferential direction was larger than that of the muscle layer, which is in accordance with reported observations for other species [33,21]. Interestingly, the (entire) intact wall showed similar small opening angles in the circumferential orientation compared to the muscle layer. The photographs before and after cutting the rings open radially (Fig. 8(b)) reveal that the residual strain at the no-load state of the intact ring is compressive in the inner wall and tensile in the outer wall, which was also found in other studies [34,35]. Extracting a strip of the mucosa-submucosa in the axial direction caused it to curl away from the longitudinal axis, whereas the axially oriented intact esophagus and muscle strips caused a marked coiling around their longitudinal axes (Fig. 8(c)). The muscle layer is composed of an outer and inner layer (see Fig. 2), arranged symmetrically as two counter-rotating muscle fiber families that show an almost sinusoidal change in fiber orientation with respect to the circumferential orientation (Fig. 3). This particular organization is quite different from, for example, arterial tissue, where two fiber families are also present, but with dispersion and without a continuous change in orientation [18,36]. The coiling and bending of the axially cut intact strips and dissected muscle strips is most likely caused by the changing orientations of the two muscle fiber families within the tissue.

4.6. Limitations

To avoid regional regional variations in the tissue properties, we tried to obtain the specimens for the particular tests from similar regions. Unfortunately, this was not always possible, due to superficial cuts in the esophagus wall coming from the extraction processed by the butcher. Hence, the location of the extracted samples along the esophagus varied slightly, and potential changes in the biomechanical behavior along the axial length of the esophagus could occur, but were not considered.

During dissection of the mucosa-submucosa from the muscle layer, it was not possible to avoid the removal of a small part of the connective tissue belonging to the submucosa. Since the submucosa consists mostly of collagen fibrils, this could potentially cause small changes in the mechanical tissue response [25,31].

The results of the inflation tests should be considered cautiously, because not all samples were tested with axial pre-stretches of 10, 20, 30 and 40%; some tests began at 20%, while others did not reach 40% pre-stretch values. This influenced the statistical analysis and is one of the causes for the high standard deviations that occurred in the data.

Both the mucosa-submucosa and the muscle layer were modeled as homogeneous materials, despite the structural subdivision of the mucosa-submucosa into a collagen-rich submucosa, a thin muscle layer and a thin mucosal lining, and of the muscle layer into an inner and outer muscle layer with different orientations of the muscle fibers.

Moreover, the zero-stress states were only qualitatively assessed.
4.7. Conclusions and perspectives

The collected mechanical data, in particular the biaxial tensile and extension-inflation data, were used for the determination of constitutive parameters in the realm of hyperelastic continuum mechanics. The hyperelastic constitutive model of [10] that we used was chosen to represent the mechanical data presented herein. With the constitutive model and corresponding parameters determined in this study, the stress distribution, which cannot be measured directly, can be predicted. For example, finite element simulations of the esophagus during the process of swallowing or during extension following surgery could be conducted, potentially yielding useful insights for clinical studies on the esophageal physiology and related disorders.

Saxena and colleagues [3] have shown that it is possible to form a hollow tubular construct, consisting of a porous collagen scaffold, which can be draped around an endotracheal stent and seeded with esophageal epithelial cells. These early experiments were successful in developing a rudimentary esophageal conduit with a constant thickness of 4 mm. Although these conduits display a certain morphological and macroscopical density, as well as ingrowth of the epithelial cells into the collagen scaffold, histological investigations have revealed the fragility of such segments. Biaxial tensile tests of the collagen scaffolds used by Saxena et al. [3] have revealed a stiffness much higher than that of the native esophageal tissue. At the current stage of development, tissue engineered esophagi are not a suitable replacement for naturally grown esophagi, thus there is a need for continuing research in this promising research area.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1–3 and 8 are difficult to interpret in black and white. The full color images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2013.07.041.

References