Passive Mechanical Response of a Healthy Ovine Aortic Tissue: Experiments and Constitutive Modelling

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Abstract

Aortic tissue exhibits highly nonlinear elastic and anisotropic mechanical behaviour. Those characteristics are provided by its constituents such as collagen and elastin. Yet the mechanisms of deformation of this material are not well understood. This study addressed the passive mechanical behaviour of a healthy ovine aortic tissue. Various biaxial and uniaxial tests were conducted to experimentally characterise the passive mechanical properties of ovine aorta tissue, including different biaxiality ratios, to find the influence of tissue microstructure on its anisotropic response. Furthermore, two versions of the orthotropic eight-chain model, i.e. the freely-jointed chain (FJC) and worm-like chain (WLC), were applied in this study in order to capture the anisotropic response of the tissue. The results showed that ovine aortic tissue demonstrated a nonlinear and anisotropic behaviour under biaxial and uniaxial loads. The circumferential direction was found to be stiffer than the longitudinal one, which resulted from the internal tissue structure formed by collagen and elastin. The response of the tissue was anisotropic even at low stretches, and the anisotropy increased with applied deformations. Moreover, the predictions of the orthotropic models for stresses in two perpendicular directions were in a good agreement with experiments. Also, the validity of the model was confirmed by employing the single set of model parameters obtained from the fitting of biaxial data in order to capture the uniaxial stress-stretch response of the tissue. This clearly suggests that the orthotropic eight-chain model is able to capture the anisotropic hyperelastic mechanical behaviour of ovine aortic tissue under different loading conditions with a single set of parameters.
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Glossary

Nomenclature

$a, b, c$ Orientations of the chains in the unit cell along principal axes for the 8 chain model

$A$ Persistence length in the WLC model

$c_i$ Dimensionless constants in the Fung strain energy model which control the level of orthotropy

$c_f$ Material constant in the Fung strain energy model

$C$ Modified right-Cauchy Green stress tensor

$E_{ij}$ Components of Green’s strain tensor

$f_{\theta}, f_L$ Measured forces in the circumferential and longitudinal direction in the experimental tests

$I_i$ Strain invariants

$I_i^0$ Dimensionless parameters which account for the initial crimping of collagen fibers in the modified HGO model

$J$ Volume ratio

$k$ Boltzmann constant

$k_1, k_2$ Material properties in the HGO strain energy function

$l$ Length of chain

$L$ Contour length in the WLC model

$N$ Constant to describe the finite chain extensibility

$p$ Weighting factor in HGO model which controls anisotropy

$Q$ Exponent in the Fung strain energy function

$r$ chain length
\( t \) Mean thickness of tissue in the experimental test

\( T \) Absolute temperature

\( T_{\theta \theta}, T_{LL} \) Tensile force in the circumferential and longitudinal directions

\( w \) Strain energy function of a single chain

\( w_0 \) Material constant in the Bischoff eight chain model

\( W \) Strain energy function of a material

\( W_{iso}, W_{aniso} \) Isotropic and anisotropic parts of the strain energy function

\( W_{entropy} \) Strain energy due to entropy

\( W_{repulsion} \) Strain energy due to repulsive forces

\( W_{bulk} \) Strain energy due to isotropic response of the interstitial fluid or a ground substance

\( \lambda_i \) Principal stretches

\( \mu \) Material property in the HGO strain energy function

\( \theta \) Mean angle between collagen fibres and the circumferential direction of the arterial layer in the HGO model

\( \rho \) Normalised deformed chain length

\( \sigma_{\theta \theta}, \sigma_{LL} \) Hoop and longitudinal stress

**Abbreviations**

AA Abdominal aorta

AAA Abdominal aortic aneurysm

CVD Cardiovascular diseases

CCD Charge-coupled device
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EL</td>
<td>Elastic lamellae</td>
</tr>
<tr>
<td>ES</td>
<td>Elastin strut</td>
</tr>
<tr>
<td>EP</td>
<td>Elastin pore</td>
</tr>
<tr>
<td>FEA</td>
<td>Finite element analysis</td>
</tr>
<tr>
<td>FJC</td>
<td>Freely jointed chain</td>
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<tr>
<td>HGO</td>
<td>Holzapfel-Gasser-Ogden</td>
</tr>
<tr>
<td>IEF</td>
<td>Interlamellar elastin fibres</td>
</tr>
<tr>
<td>ILT</td>
<td>Intraluminal thrombus</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline (solution)</td>
</tr>
<tr>
<td>SBFSEM</td>
<td>Serial block-face scanning electron microscope</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
</tr>
<tr>
<td>SEF</td>
<td>Strain energy function</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TC</td>
<td>Tropocollagen</td>
</tr>
<tr>
<td>tr</td>
<td>Trace (of a tensor)</td>
</tr>
<tr>
<td>WLC</td>
<td>Worm-like chain</td>
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Chapter 1

Introduction

1.1. Background

Diseases of the heart and circulatory system, generally called cardiovascular diseases (CVD), are the number one cause of death worldwide, especially in the developed countries. A commonly occurring CVD that occurs in the abdominal aorta is an abdominal aortic aneurysm (AAA) which is a progressive enlargement of the abdominal aorta (see Fig. 1), which has a tendency to rupture suddenly, leading to mortality. AAA is characterised by loss of vessel distensibility (increased stiffness of vessel wall) with incremental growth. Most AAA cases are near-fusiform shape, that is widening all around the circumference of the aorta (Humphrey, 2002).

Figure 1. Human abdominal aorta and AAA (Source from www.drugs.com)

In Europe and the US, the total number of AAA cases reaches approximately 10% among those over the age of 65 (Sakalihasan et al., 2005). Most AAA cases are typically asymptomatic and the aneurysm may enlarge until rupture, leading to death in 80–90% of all cases (Wilmink et al., 1999). Approximately 30% estimation of those surviving patients will face moderate to severe disability. The mortality rate due to AAAs is estimated to be 15,000/year in the United States and 8,000/year in the UK (Sakalihasan et al., 2005; Sanfellipo, 2003), overall the 13th most common cause of disease related death (Sanfellipo, 2003).
In current clinical practices, the diameter of the aneurysm is used for rupture risk assessment and thus as an indicator for surgical intervention. Typically, the clinician make a decision to proceed for surgery when a threshold diameter has been attained between 5.0 to 5.5 cm (Humphrey, 2002; Upchurch and Schaub, 2006; Vorp, 2007). At this stage, the risk of rupture is considered to be higher than the risk of dying from intervention related complications. However, about 7 % (34/473 cases) of the patients with an AAA experienced a rupture before reaching 5 cm. Moreover, despite the fact that patients with an AAA of 5 cm or more have a higher risk of rupture, not all of these aneurysms will eventually rupture, as reported from Darling et al (1977). Since the maximum diameter criterion is not a sufficient predictor for AAA rupture, other parameters have been proposed to predict AAA rupture; these inclue the AAA expansion rate, wall stiffness, increase of intraluminal thrombus (ILT) thickness, wall tension, and peak AAA wall stress. While some of the above parameters can be determined clinically by using Computer Tomography scans (e.g. AAA expansion rate), others may not be easily accessible by clinicians (e.g. wall tension, or peak AAA wall stress). Therefore, computational methods using Finite Element Analysis (FEA) are employed to assist clinical inspections and calculate e.g. peak AAA wall stresses.

Recent studies suggest that the peak AAA wall stress is the most promising predictor (Vorp, 2007). However, calculation/prediction of AAA wall stresses requires not only the generation of accurate geometrical models, but also accurate constitutive models, which can capture the deformation behaviour of healthy and diseased tissue. Such a model should reflect the actual experimental data over as wide a range of deformations as possible. In addition, the material parameters involved in the constitutive model should contain the information related to the internal structure of the tissue. However, the situation is complicated by the complex tissue morphology and its evolution with time. For example, structural reorganisation manifested by the change of interconnections of the aorta constituents, which is influenced by the vascular activity and aging process, results in changes in material properties. For instance, in the case of an AAA, the aortic tissue becomes stiffer due to alterations in morphology and the composition of its major structural constituents such as smooth muscle cells (SMCs), elastin fibres and collagen fibres. Fewer numbers of elastin and an increase in the number of collagen fibres were found as a result of aging (Carmo et al., 2002) and, additionally, the stiffening effect was related to an increase of the number of collagen crosslinks which can reduce the degree of tissue extensibility.
Providing an accurate, reliable constitutive model proves to be an extremely challenging task. Attempts have been made to develop constitutive models that contain parameters, which allow for some description of vascular wall structure, and relate it to its mechanical response. In particular, constitutive models developed for polymers, either of phenomenological nature or those based on statistical mechanics, have been employed with some success to capture the mechanical behaviour of aorta tissues. The phenomenological models, e.g. Fung Model and Holzapfel-Gasser-Ogden (HGO) model, are predominantly based on the observation that the soft tissue (e.g. in aorta) follows a nonlinear elastic (hyperelastic), rubber-like behaviour with little or no compressibility. Statistically-based polymer models use the analogy between the network-like structure of polymer chains, and the arrangements of elastin and collagen fibres. The network of cross-linked long chain molecules where the free energy is dominated by the configurational entropy rather than the internal energy is the starting point for these models. One of the most prominent statistical mechanics-based models used for elastomers is the eight-chain model, proposed by Arruda and Boyce (1993), which provides some physical rigour such as finite extensibility characteristics. Later on, Bischoff et al. (2002a, b) extended the eight chain model to capture the orthotropic hyperelastic behaviour of cross-linked polymers. The model was successfully applied to simulate bubble inflation test of rat pulmonary artery by Zhang et al. (2005) and biaxial test of bovine aorta and its elastin network by Zou and Zhang (2009). However, the model was not validated for other aortic tissues such as an ovine tissue, which is the subject of this work.

The accuracy of a constitutive model can be determined by fitting the model with an experimentally obtained stress-strain curve. There are various methods in testing mechanical properties of arterial tissue both in vivo and in vitro, such as ultrasonic Doppler techniques, magnetic resonance imaging, pulse wave velocity, and conventional mechanical testing methods. The conventional mechanical testing such as uniaxial, biaxial and inflation tests are commonly employed to help determining the material parameters (Fung, 1993; Raghavan et al., 1996; Wells et al., 1998; Lally et al., 2004; Delgadillo et al., 2010; Zou and Zhang 2009; Guinea et al. 2010; Rezakhaniha et al., 2011). Uniaxial testing is the simplest mechanical characterisation method that can be performed to acquire the stress-strain response. However, biaxial and the inflation tests are currently more frequently utilised by most researchers in developing constitutive models as these since give a more representative physiological loading (Fung, 1993; Humphrey, 2002).
1.2. Objectives

The primary objective of this study is to characterise experimentally the passive mechanical properties of ovine aorta tissue under uniaxial and biaxial loading conditions and to assess the accuracy of the orthotropic version of the eight-chain model of Bischoff et al. models with freely jointed chain (FJC) and worm-like chain (WLC) approaches. It is expected that the results from this study can be used for further development of a physically-based constitutive model that can thoroughly describe the complex mechanical behaviour of aortic tissues, accounting for tissue morphology.
Chapter 2

Literature Review

2.1. Aorta Structure

The aorta is the largest blood artery which transports oxygenated blood from the heart to the body through the systemic circulation. The aorta consists of an ascending portion, the so-called arch, and the descending thoracic and abdominal segments. The abdominal part starts from the diaphragm level extending down to the level of the fourth lumbar vertebra and branches off into two common iliac arteries.

In general, arterial wall including aorta consists of three primary layers: the tunica intima, the tunica media, and tunica adventitia as shown in fig. 2. The tunica intima (innermost layer) is made up of one layer of endothelial cells and an underlying thin (~80nm) basal lamina (Humphrey, 2002). It also includes a sub-endothelial layer that contains some smooth muscle cells (SMCs), often oriented axially, and connective tissue. The internal elastic lamina separates the intima and media. In healthy arteries, this layer is very thin and its contribution to the mechanical properties of the arterial wall is inconsiderable. The tunica media (middle layer) is the largest layer of the aorta and contains a complex three-dimensional network of SMCs (circumferentially oriented), collagen fibres, elastin, and ground substance matrix. The collagen fibrils, elastic laminae, and smooth muscle cells together constitute a continuous fibrous helix (Faserschraube) which has a small pitch (Holzapfel et al., 2000). This structural arrangement gives the media high strength and resilience. It can also resist loads in both the longitudinal and circumferential directions. Relating to the mechanical properties, this layer is the most significant layer in healthy artery. The tunica adventitia (outermost layer) contains a dense network of type I collagen fibres with admixed elastin, nerves, fibroblasts, and the vasa vasorum. This layer demonstrates high tensile strengths (>1 MPa) and can bear load at higher pressures at which it changes to a stiff ‘jacket-like‘ tube that prevents the smooth muscle from overstretch and rupture (Holzapfel, 2004). However, the adventitia is much less stiff in the unloaded configuration and at low pressures than the media.

The arterial wall is known to be elastic and strong, due to its three main constituents i.e. elastin, collagen, and SMCs shown in fig. 3. In fig. 3, elastin features include elastic lamellae (EL), the dense network of interlamellar elastin fibres (IEF shown with black arrows), elastin struts (ES), and reinforced elastin pores (EP). Collagen fibres (white arrows) are adjacent to
lamellar surfaces, arranged in layers of parallel bundles oriented circumferentially. It is very often that many investigators do not include the contribution of the SMCs in order to simplify the investigation, so the response is solely governed by the two constituents, elastin and collagen. This behaviour is usually called the passive response of the aortic wall. Under the physiological condition, the active response that includes the smooth muscle tone results in a change of aortic wall diameter and thickness, and influences the stress and strain distribution along the wall. Therefore, it is very important to include the SMC, if one investigates the \textit{in vivo} mechanical response of aortic wall.

\textbf{Figure 2.} Detailed structure of aorta wall: the tunica intima, the tunica media, and the tunica adventitia layers (from Holzapfel et al., 2000).

It is believed that elasticity of the arterial wall comes from the elastin fibres which are usually found in vertebrates. Also, some investigators (Zou and Zhang, 2009; Holzapfel, 2001; Humphrey, 2002; Hanuza et al., 2009) have shown that elastin plays a significant role for the initial stiffness of aortic wall structure. Elastin is composed of elastin fibrils which have thickness about 0.1 – 0.2 μm (Ushiki, 2002). These elastin fibrils are present individually or in bundles to form elastin fibres which are arranged to form an elastin ‘meshwork’. Further, the elastin sheet is formed by the elastin meshwork (fig. 4a). The elastic modulus of elastin is about 0.6 MPa (Fung, 1993). Moreover, the long flexible elastin molecules are secreted into the extra-cellular matrix and assemble into elastic fibres. These fibres are cross-linked together by unique amino acids, desmosine and isodesmosine (Eyre, 1984), to form a rubber-like structure (fig. 4b), which may be stretched to about 2.5 times its initial length when unloadings. The source of the elasticity of elastin may be explained by the concept of entropic elasticity. Its elasticity arises via the changes in the configurational entropy of
molecular chains, i.e., decreases the entropy or increases the internal energy (Fung, 1993; Holzapfel, 2001).

**Figure 3.** 3-D illustration of the medial aortic microstructure of rat aorta rendered artistically from serial block-face scanning electron microscope (SBFSEM) volume (from O’Connell et al., 2008).

**Figure 4.** (a) Schematic drawing of elastin components (Ushiki, 2002) (b) Elastin structure under mechanical loading (Alberts et al., 2003).
Collagen is the most common fibrous protein which primarily constitutes the ECM of connective soft tissues. Collagen fibres consist of tropocollagen (TC) molecules which are staggered in arrays to form collagen fibrils and the fibrils arrange to form collagen fibres (fig. 5). Generally, collagen is much less extendable than elastin. A single collagen fibre can only deform less than 10% from its original length when stretched (Humphrey and Holzapfel, 2012). These fibres have a high stiffness and tensile strength (1 GPa), which is due to their cross-linking density (Shadwick, 1999).

Figure 5. Schematic view of hierarchical structures of collagen fibres, ranging from amino acids at nanoscale up to the scale of collagen fibres (Buehler, 2006).

A vascular smooth muscle cell (SMC) is a type of muscle cell usually found within the walls of blood vessels, such as in the tunica media layer of aortic tissue. Structurally, SMC is approximately 100 μm long and 5 μm in diameter in the wall, has a spindle-shape, and consists of a single nucleus and a substantial portion of the volume of the cytoplasm, which are composed by the molecules actin and myosin (Matsumoto and Nagayama, 2012) as shown in fig. 6. Moreover, SMC provide a significant contribution for good performance of vascular wall especially during vessel remodelling in physiological conditions. The contraction or relaxation of SMC changes the luminal diameter, which leads to regulate the local blood vessel tone, blood flow, and blood pressure distribution at arteriolar level. Those activities of SMCs may have a contribution to control the intramural stress distribution along the vascular wall.
2.2. Mechanical Characteristics of Aortic Tissue

It is important to have a good understanding regarding the mechanical characteristics of healthy aorta tissue, which can be used as a reference for comparison, quantification and evaluation of the diseased aorta tissue. It is believed that aorta tissue is highly nonlinear, incompressible, anisotropic and viscoelastic.

2.2.1. Nonlinearity

Healthy aortic tissue demonstrates nonlinear elastic behaviour. When the aortic tissue is stretched, it becomes stiffer as the strain changes and hence the tangent modulus is not constant. This behaviour is mainly due to the combination of the properties of the stiff (collagen) and rubbery (elastin) constituents. Fig. 7 shows a description made by Holzapfel (2001) when the collagen fibres in soft tissue are stretched. At the initial stage (phase I), it is assumed that there is no contribution of the collagen fibres until a certain strain and the collagen fibres are wavy and unaligned. At this stage, the elastin fibres bear the load and the aorta tissue exhibits nearly isotropic response and demonstrates a linear stress-strain relationship, similar to a very soft rubber sheet. At the next stage, the collagen fibres start to align with the load direction and bear the load as the load is increased. In this stage, the collagen fibres slightly straighten and tend to interact with the matrix. In stage III the waviness of collagen fibres is negligible and the fibres become completely taut. The collagen fibres then start to resist extension and the tissue becomes stiff at high stress. In this stage, the stress and strain becomes linear again. If the tissue continues to be extended, they can reach the ultimate strength and start to break.
Figure 7. Schematic of the aorta tissue morphology evolution during loading through alignment of collagen fibres and the corresponding stress-strain curve of soft tissue (Holzapfel, 2001).

2.2.2. Anisotropy

Histology of aorta tissue exhibits that its constituents, such as SMC, collagen, and elastin, have specific orientations (Holzapfel et al., 2000). This structural arrangement leads to different responses when the load is applied in different directions. The elastin fibre tends to be organised into thin concentric sheets (Humphrey, 2002). The SMC tends to be oriented circumferentially, while the collagen fibres are oriented axially. Numerous studies have shown some variations in the degree of anisotropy where the response of large strain in the circumferential direction is stiffer than in the longitudinal direction. Recently, O’Connell et al. (2008) developed a novel technique to extract 3D volumetric information of aortic medial microstructure on rat abdominal aorta. The results suggested that the collagen bundles, SMC and IEF have predominantly circumferential orientation (illustrated in fig. 3), which implies a stiffer behaviour around the circumference. The latter is in a good agreement with the findings of Rezakhaniha et al. (2011) and Zou and Zhang (2009). In particular, Rezakhaniha et al. (2011) performed the inflation-extension test on tissue from the common carotid artery of rabbit and used transmission electron microscopy (TEM) and serial block-face scanning electron microscopy (SBFSEM) to support the microstructural evidence of the elastin anisotropy. Likewise, a study by Zou and Zhang (2009) who examined different components of bovine thoracic aorta using biaxial testing (see Fig. 8) and used histology and scanning electron microscopy (SEM) to verify the separated components of the aorta.
Figure 8. Stress-strain curves of intact bovine thoracic aorta sample and its elastin network under equi-biaxial testing (Zou and Zhang, 2009). C indicates circumferential direction; L indicates longitudinal directions.

The results of those studies (Rezakhaniha et al., 2011; Zou and Zhang, 2009) showed that the elastin fibres have a preferred orientation in the circumferential direction (fig. 8) and thus their contribution to anisotropy should also be considered, in addition to the collagen contribution.

2.2.3. Incompressibility

Most of the studies relating the arterial wall elasticity have assumed that the aorta is incompressible. This assumption is due to the significant hydrostatic stresses, which are larger than shearing stresses in the response of aorta wall. Actually, a typical arterial wall contains significant amounts of intracellular and extracellular water, some 70% and 80% by wet weight. There is water movement due to the presence of shear stress which leads to local changes of aorta density and volume, and thus the artery tissues are not truly incompressible. However, Carew et al. (1968) and Chuong and Fung (1984) have shown that on average the responses of arterial wall have a tendency to undergo nearly isochoric motions. They also suggested that incompressibility is a reasonable assumption for practical purposes.

2.2.4. Viscoelastic and Strain-Rate Dependency

Most biological tissues can be categorized as viscoelastic because, during cyclic loading, they exhibit a hysteresis which is almost independent of the strain rate within several decades of
the variation (Fung, 1993). On the other hand, Bergstrom and Boyce (2001) stated that many soft biological tissues behave similar to rubber-like materials. Bergstrom and Boyce also observed three phenomena including: the stress required to attain a certain strain increases with an increase in strain rate; the stress will relax with time at a given strain until reaching a strain-dependent equilibrium value; and the magnitude of hysteresis loop strongly depends on strain rate. Based on their studies, they proposed a constitutive model to describe these features which showed a good agreement with experimental data on monkey liver tissue (Dokos et al., 2000) and rat septal myocardium tissue (Miller et al., 2000). Moreover, Pioletti et al. (1999) performed traction tests on the bovine anterior cruciate ligament–bone complex at different strain rate and concluded that the overall tangent stiffness of the tissue increases as strain rate increases.

In contrast, some experiments carried out on liver tissue (Hu and Desai, 2004) and pig thoracic aorta (Delgadillo et al., 2010) showed that the tangent stiffness of arteries decreases with an increase of deformation rate. Moreover, the results from Giles et al. (2007) showed that there is a discrepancy in the strain-rate behaviour when the testing is carried out in stretch-control or force-control as shown in fig. 9. A stiffer response is produced as the strain rate increases in the stretch-control mode, while the same stiffening trend is obtained with decreasing strain rate in the force-control mode. The interaction of nonlinear elastic and viscous effect may play a role in this anomalous behaviour, suggesting that the strain rate sensitivity phenomenon still needs to be explored in order to have a robust result.

The mechanical properties of aorta tissue also depend on age since the tissue has an ability to grow and remodel in response to disease, injury and changes in their mechanical environment (Holzapfel, 2005a). Moreover, the aortic tissue is found to decrease its distensibility as a function of age which could be correlated to an increase number of collagen fibres and a decrease of elastin number. A current study of viscoelastic properties such as stress relaxation and creep on bovine aortic tissue has been performed by Zou and Zhang (2011). They assessed the viscoelastic properties of elastin fibres, decellularized Extra Cellular Matrix (ECM), and intact aorta. The result showed that stress relaxation occurs and is linearly dependent on the initial stress levels. Creep response is negligible for the elastin and the intact or decellularized aorta.
2.3. Constitutive Models for Aortic Tissue

Since the aortic tissue demonstrates non-linear elastic behaviour, incompressibility and anisotropy, the theory of linear elasticity is insufficient to describe its behaviour. There are two basic approaches that are used to mathematically model the mechanical behaviour of aortic tissue. The first approach is a physically-based one, based in a molecular structure and using physical descriptions of the interactions on the molecular level in terms of physically meaningful parameters (e.g. elastin cross-link density). The second is phenomenological and it is based directly on the principles of nonlinear continuum mechanics (Holzapfel, 2005a). Most of the hyperelastic models for soft tissues have been built based on the phenomenological approach in order to capture the response near the physiological state and they have been successful in capturing the aorta response, with their parameters fitted to experimental data (Holzapfel, 2004). Such phenomenological models have been developed with the aim of describing, rather than explaining, the material behaviour, which is on the basis of macroscopic experimental data and the use of nonlinear elasticity theory. Fung (1993) developed an exponential strain energy function (SEF) which describes the 3-D deformation of the arterial wall. This model is incompressible and orthotropic.

\[
W(E_{11}, E_{22}, E_{33}) = \frac{c_f}{2} (c_f^0 - 1) \tag{1}
\]

\[
Q = c_1 E_{11}^2 + c_2 E_{22}^2 + c_3 E_{33}^2 + 2c_4 E_{33} E_{22}^2 + 2c_5 E_{11} E_{22}^2 + 2c_6 E_{11}^2 E_{33}^2 \tag{2}
\]

\[
E_{11} = \frac{1}{2}(\lambda_1^2 - 1), \quad E_{22} = \frac{1}{2}(\lambda_2^2 - 1), \quad E_{33} = \frac{1}{2}(\lambda_3^2 - 1) \tag{3}
\]
where $c_f$ is a material parameter with stress dimensions and $c_1$, $c_2$, $c_3$, $c_4$, $c_5$, and $c_6$ are dimensionless. $E_{11}$, $E_{22}$, and $E_{33}$ are the components of Green's strain tensor of the axial, circumferential and radial directions of the artery, respectively. Orthotropy is dependent on the choice of the parameters $c_1$-$c_6$. Shear strains are not included in this model as suggested by Fung (1993) because the three directions (i.e. axial, circumferential and radial) are considered to be principal. Later, Holzapfel et al. (2000) proposed a set of SEFs known as the HGO model that includes isotropic and anisotropic parts. They considered the artery as a composite reinforced by two families of collagen fibres, (shown in fig. 10), which are arranged in symmetrical spirals. The collagen fibres in the arterial wall are not active at low strains as they do not store strain energy. Hence, deformation is associated with the mechanical response of the non-collagenous matrix (elastin), which can be assumed to be isotropic. On the other hand, the collagen will take part as the resistance to stretch at larger strains. Thus, the equation for the SEF is written as

$$W(I_1, I_4, I_6) = W_{iso}(I_1) + W_{aniso}(I_4, I_6)$$ (4)

$$W_{iso}(I_1) = \mu(I_1 - 3)$$ (5)

$$W_{aniso}(I_4, I_6) = \frac{k_1}{2k_2} \sum_{i=4,6} \{\exp[k_2(I_i - 1)^2] - 1\}$$ (6)

$$I_1 = \text{tr}\mathbf{C}, \quad I_4 = \mathbf{C} : (a_{01} \otimes a_{01}), \quad I_6 = \mathbf{C} : (a_{02} \otimes a_{02})$$ (7)

$$a_{01} = [0, \cos\theta, \sin\theta], \quad a_{02} = [0, \cos\theta, -\sin\theta]$$ (8)

where $\mu > 0$, $k_1 > 0$, $k_2 > 0$ are material parameters and $\mathbf{C}$ is the modified right-Cauchy Green stress tensor. Anisotropy arises due to the two invariants $I_4$ and $I_6$. In Eq. 8 $\theta$ is the (mean) angle between the collagen fibres and the circumferential direction of the arterial layer. In the HGO model, it is assumed that fibres only contribute to the strain energy in extension and not in compression. As can be seen, the isotropic part is based on the neo-Hookean model. Holzapfel et al. (2005b) further modified the SEF to incorporate isotropic behaviour as a special case. Hence, the anisotropic SEF in equation (6) can be replaced by

$$W_{aniso}(I_1, I_4, I_6) = \frac{k_1}{2k_2} \frac{1}{\sum_{i=4,6} \{\exp[k_2((1 - p)(I_i - 3)^2 + p(I_i - 1)^2)] - 1\}}$$ (9)

where the scalar parameter $p \in [0, 1]$ may be seen as a weighting factor. If $p = 1$, the model is fully anisotropic, which is an ideal alignment of collagen fibres. On the other hand, the model will be fully isotropic if $p = 0$, similar to eq. (5). Thus, $p$ may be seen as a "switch" parameter.
between isotropy and anisotropy, describing the ‘degree of anisotropy’. Rodriguez et al. (2008) proposed a modified HGO model which accounts for the initial crimping of the collagen fibres on the anisotropic part of the SEF. The SEF can be described as follow

\[ W_{\text{aniso}}(I_1, I_4, I_6) = \frac{k_1}{2k_2} \sum_{i=4,6} \exp[k_2((1 - p)(I_1 - 3)^2 + p(I_1 - I_0^i)^2)] - 1 \]  

where \( I_4^0 > 1 \) and \( I_6^0 > 1 \) are dimensionless parameters which account for the initial crimping of the two families of collagen fibers. The anisotropic terms only contribute when either \( I_4 > I_4^0 \) or \( I_6 > I_6^0 \), or both.

The proposed SEFs are phenomenological in nature and thus they do not incorporate any physically meaningful parameters. By employing the statistical mechanics approach, Bischoff et al. (2002 a, b) have developed microstructurally-based SEFs for orthotropic hyperelastic materials by extending the eight chain model developed by Arruda and Boyce (1993). Later the model was used by Zhang et al. (2005) to capture the elastic behaviour of rat pulmonary arteries. In particular, the model assumes that the artery is constructed by a network which contains eight randomly oriented molecular chains representing the anisotropic behaviour as shown in fig. 11. This SEF consists of three contributions: (1) the strain energy due to the configurational entropy of the unit cell (\( W_{\text{entropy}} \)), (2) the strain energy due to interchain repulsive forces in the unit cell (\( W_{\text{repulsion}} \)) and (3) the strain energy used to capture additional bulk incompressibility (\( W_{\text{bulk}} \)). Both \( W_{\text{entropy}} \) and \( W_{\text{repulsion}} \) can be represented by molecular chain models. These are the freely jointed chain (FJC) and worm-like chain (WLC) models. The FJC model describes a random walk or uncorrelated chain and it is typically used to describe the nature of conformations change in rubber whereas for biomolecular chains, such as DNA (Marko and Siggia, 1995), tropoelastin (Baldock et al. 2011) and tropocollagen
(Buehler and Wong, 2007), tend to be described by the WLC model, with a configuration of varying curvature.

Figure 11. Illustration of arterial wall with single element of network containing eight molecular chains (Zhang et al., 2005).

Figure 12 illustrates the two types of molecular chain models. $W_{\text{entropy}}$ and $W_{\text{repulsion}}$ give the anisotropic response that come from the fibrous network; $W_{\text{bulk}}$ is related to the isotropic response of the interstitial fluid or a ground substance. By using the two different types of molecular chains, the strain energy function $w(\rho)$ of a single chain can be expressed. For the FJC approach (Bischoff et al., 2002b),

$$w(\rho) = kT N \left( \frac{\rho}{N} \beta + \ln \frac{\beta}{\sinh \beta} \right)$$

(11)

For the WLC approach (Bischoff et al., 2002b),

$$w(\rho) = kT \left( \frac{\rho^2}{2\xi} + \frac{\xi}{4 (1 - \frac{\xi}{T})} - \frac{\rho}{4} \right)$$

(12)

where $\rho = r/l$ is the normalised deformed chain length. $N = \frac{a^2 + b^2 + c^2}{4}$ is related to the number of rigid links where $\sqrt{N}$ defines the finite chain extensibility describing the strain stiffening in macroscopic response. The parameter $\beta^{(i)}_{\rho} = L^{-1} \left( \frac{\rho^{(i)}}{N} \right)$, where $L^{-1}$ is the inverse Langevin function $k$ is the Boltzmann constant, and $T$ is the absolute temperature. In the WLC model, $\rho = r/A$ and $\xi = L/A$ where $L$ and $A$ are the contour and persistence lengths, respectively. The fully extended length corresponds to $\rho = \xi$. The SEF is then comprised of three contributions: the strain energy due to the configurational entropy of the unit cell ($W_{\text{entropy}}$), the strain energy due to interchain repulsive forces in the unit cell ($W_{\text{repulsion}}$) and the strain energy due to bulk
deformation ($W_{bulk}$). The $W_{entropy}$ and $W_{repulsion}$ give the anisotropic response that come from the fibrous network and $W_{bulk}$ is related to the isotropic response of the interstitial fluid or ground substance. The full equation of Bischoff et al. (2001a, b) SEF is given by the following equation:

$$W = w_0 + \frac{n}{4} \left[ \sum_{l=1}^{4} w(l) - \frac{1}{p} \left( \frac{dw(p)}{dp} \right)_{p=p} \ln \left( \lambda_a^2 \lambda_b^2 \lambda_c^2 \right) \right] + B [\cosh(J - 1) - 1]$$

where $w_0$ is constant, $B$ is a parameter that controls the bulk compressibility and $J$ is the volume ratio. The parameter $\beta_p = L^{-1}(\frac{P}{N})$, where $P = \frac{1}{2} \sqrt{a^2 + b^2 + c^2}$ is the normalised undeformed chain length. The parameter $n$ is related to the chain density per unit volume. Parameters $a$, $b$, and $c$ are the orientations of the chain in the unit cell along the principal axes, where $a$, $b$, and $c$ are aligned with the circumferential, longitudinal, and radial directions of the arterial wall (fig. 11), respectively, while $\lambda_a$, $\lambda_b$, and $\lambda_c$ are the stretches along those directions. It is noteworthy to mention that the $nkT$ term contributes to the initial stiffness. Here, the eight chain model was used to predict the uniaxial data and the biaxial one was captured by the orthotropic eight-chain (Bischoff et al., 2002a, b) model.

Figure 12. Illustration of molecular chain models (a) freely jointed chain model (b) worm-like chain model (Kuhl et al., 2005).

2.4. Characterisation Methods

To characterise the mechanical behaviour of the arterial wall, either uniaxial or biaxial testing can be performed. Due to its simplicity, uniaxial testing is often used to obtain the stress-strain response. In order to perform uniaxial tests, the arterial wall is typically cut open along its length and cut out in rectangular samples, either in the circumferential or axial direction. Guinea et al. (2010) conducted uniaxial testing on human aortic tissue. The aortic tissue was cut into dog bone specimen in the circumferential and longitudinal direction. The
The experimental setup is shown in fig. 13. The results revealed that the circumferential strength is always greater than the longitudinal strength. Also, the tensile strength and stretch at failure of healthy aortas exhibit a significant reduction with age, falling abruptly beyond the age of 30. On the other hand, Raghavan et al. (1996) assessed the mechanical behavior of AAA and healthy AA tissues in a uniaxial test, where the samples were cut into rectangular pieces in both directions. They found no significant difference between the circumferential and longitudinal strength. The results also found that the AAA tissue is stiffer and less extensible than healthy tissue, which is also reported by Xiong et al. (2008).

Uniaxial testing gives a poor representation of the complex loading under physiological conditions and does not sufficiently describe the anisotropic behavior of the tissue. Hence, biaxial testing may be used to provide a better understanding of arterial tissue behavior. However, biaxial testing can be more complicated than the uniaxial one because of the need to control boundary conditions.

Vande Geest et al. (2006) performed biaxial testing on 26 AAA tissue and 8 AA tissue samples. The testing was carried out using the following tension-controlled protocol, $T_{\theta \theta} : T_{LL} = 1 : 1, 0.75 : 1, 1 : 0.75, 0.5 : 1, 1 : 1, 1 : 0.5$ and $1 : 1$, where $T_{\theta \theta}$ and $T_{LL}$ stand for the circumferential and longitudinal directions, respectively. The maximum tension was 120 N/m, which is consistent with physiological pressure (113 mmHg). Each sample was preconditioned via 9 loading and unloading cycles for each tension rate. It was observed that there is a clear trend of increased stiffness and decreased extensibility in the circumferential direction for AAA, as shown in fig. 14, which suggests that AAA is stiffer than AA. The results also showed that the uniaxial response of AAA is much stiffer in the lower strain regions ($< 10\%$) and softer ($> 10\%$) in the higher strain region, than the biaxial response.

It was suggested by Fung (1993) that soft tissue should be preconditioned in order to orientate the molecular structure of tissues to its natural in vivo alignment. Preconditioning
will allow tissues to gradually adapt to loading and hence produce more consistent data in mechanical testing. This procedure can be done by imposing a cyclically varying strain until attaining a steady state. The preconditioning is associated with pseudoelastic behaviour of soft tissues and thus Fung (1993) also stated that soft tissues can be represented by separate elastic laws i.e. one elastic material in loading and another elastic material in unloading. Therefore, pseudoelasticity is not an intrinsic property of the material but is a convenient description of the stress-strain relationship in a specific cyclic loading. Moreover, other factors related to the physical and chemical environment, for example: temperature and pH can affect the mechanical behaviour of the tissue when tested under in vitro conditions. Furthermore, its mechanical properties may change due to biological degradation. Thus, arterial tissue should ideally be tested in appropriate physiological solutions, e.g. krebs-ringer's or phosphate buffer saline (PBS) solutions (Humphrey, 2002).

Figure 14. Biaxial responses in stress-strain curves for AA and AAA groups (Vande Geest et al., 2006).

As shown by Delgadillo (2008), the sample geometry can also affect the biaxial response of soft tissue. In Delgadillo's work, samples from pig aorta were cut into rectangular, square and cruciform pieces. The rectangular samples were used in the uniaxial tension and the square and cruciform sample were tested equibiaxially (fig. 15). For comparison, FE simulations were employed for each specimen. The results revealed that the cruciform sample performs better than that of the square one and showed a good agreement with the FE results.

Other factors such as thickness measurement methods can also influence the assessment on tensile stress. As soft tissue undergoes large deformation inaccurate measure of thickness can
lead to an inaccurate calculation of the stress. Lee and Langdon (1996) assessed five methods to measure the thickness of bovine pericardial tissue and found that the non-rotating thickness gauge is a good choice since it provides simplicity and inexpensive technique. A recent study conducted by O’Leary et al. (2013) has also found that the thickness gauge is a reliable method to measure the thickness in porcine aortic tissue (structured tissue) but fails to accurately measure unstructured tissue, e.g. intraluminal thrombus from AAA patient. They suggested that a micrometre should be used for unstructured tissue.

Figure 15. Experimental setup on (a) biaxial testing machine for (b) cruciform and (c) square samples (Delgadillo, 2008).

Another interesting technique that can be employed to test aortic tissue biaxially is to perform a bubble inflation test. This test is suitable for a membrane material, such as soft tissue, which cannot support out-of-plane shear. Some investigators have found this test to be superior to the planar biaxial method due to its simplicity and the ability of the inflated membrane when deformed into a spherical bubble to produce a truly equi-biaxial deformation at the pole of the bubble (Drexler et al., 2003, Haile et al., 2009). Also, unlike the planar biaxial technique, the bubble inflation test can also be utilised to investigate the failure properties of aortic tissue as shown by Mohan and Melvin (1983). However, there are also some limitations related to the bubble inflation test, including the difficulty of controlling the principal stretches independently, the limitations inherent to on-line video (Hsu et al., 1995) and also the fact that the test can only be used to determine the material properties for in-plane biaxial stretching (Wineman et al., 1979).
Chapter 3

Materials and Methods

3.1. Background

As mentioned earlier, either uniaxial or biaxial testing can be employed to characterise the mechanical behaviour of aortic tissue, where biaxial testing gives more representative loading of the physiological condition than the uniaxial one. In this study, both uniaxial and biaxial tests were carried out to give a better understanding related to the mechanical behaviour of aortic tissue. Typically, the recorded data from the machine is the force-displacement. The stress-stretch curves were then used to fit the parameters of the representative constitutive models.

In general, biaxial testing is more difficult than uniaxial testing, as two boundary conditions must be controlled. Particularly, the edges must be allowed to expand freely in lateral direction and the stress and strain in the central of target region should be uniform (Fung, 1993; Sacks, 2000). Moreover, different gripping methods have also been important in the application of biaxial testing. A computational study conducted by Sun et al. (2005) has suggested that suture attachments is the best suited gripping method for biaxial mechanical test of soft tissues.

In uniaxial test there are also experimental difficulties. One of the difficulties can arise from the specimen slippage from the clamp. Various methods have been proposed to prevent slippage during testing, such as using the ribbed clamps (Di Martino et al., 1999), applying cyanoacrylate glue between the specimen and the face of the clamp (Raghavan et al., 1996; Guinea et al., 2010), and covering the specimen with a plastic membrane (Xiong et al., 2008).

In this present work, biaxial and uniaxial tests were conducted. Rakes were used in both tests to apply the boundary conditions on the specimens and also to prevent the specimens from slippage. The advantage of using the biaxial machine to carry out the uniaxial test was to have a pre-conditioning on the specimen since the biaxial test machine allows for preloading and preconditioning on the specimen. However, the biaxial testing machine cannot apply a large strain or load the specimen to failure.
3.2. Materials

Ovine aortas were harvested from a local slaughter house and immediately placed in an iced cooler. The specimens were then transported to the lab. Upon arrival at the lab, the fatty residues and the connective tissues were removed using scissors and scalpel and were then rinsed with deionized water. Subsequently, the cleaned specimens were stored in a freezer at -20°C for 4 days. Prior to testing, the specimens were thawed overnight and equilibrated to a temperature 37° by immersion in fresh PBS afterward.

3.3. Biaxial and Uniaxial Testing Using the Mechanical Biotester

In biaxial testing, the tissue was cut into six (6) rectangular pieces by using a square die with dimensions 1.4 x 1.4 cm. The specimen thickness was measured by a digital vernier calliper. In this study, the BioTester 5000 (manufactured by Cellscale Biomaterials Testing, Waterloo, Ontario) testing machine was employed. Load cells of 5 N were utilised on moving beams in both axes.

In this study, the gripped area, or rake-to-rake length in the X and Y directions, was 9 mm x 9 mm. After mounting the specimen (fig. 16), a preload of 20 mN was applied along both axes to ensure the specimen lay flat. Equi-biaxial and nonequi-biaxial tests were carried out in deformation-controlled mode (load control has been used by other investigators, Vande Geest et al., 2006; Tong et al., 2011; Bellini et al., 2011). All specimens were tested with the following protocol: $\lambda_0 : \lambda_L = 1:1, 0.75:1, 1:0.75, 0.5:1, 1:0.5, 1:1$. The ratio $\lambda_0 : \lambda_L$ was kept constant for each specimen with maximum stretch $\lambda = 30\%$, which is the maximum achievable by the test machine. The test duration was 10s. Each specimen was preconditioned with 10 cycles of loading and unloading. Strain measurement using a 1280 x 960 pixel charge-coupled device (CCD) camera with images captured every 0.2 s was also conducted with 3 samples. This study was used to further investigate strain uniformity and compare with the computed strain data obtained by the rake-to-rake displacement. Speckles made by typical paint were randomly applied on the surface of the specimens (fig. 17). This was used to track point motions and to generate strain field maps using custom software (Labjoy 5.80). Following the biaxial tests, the gripped area of the square specimens was halved in the longitudinal and circumferential directions. The halved samples were uniaxially stretched up to 30% of nominal strain in the Biotester machine (see Fig. 18). A preloading of 20 mN and a preconditioning with ten cycles of loading and unloading were applied prior to the analysis. All tests were performed in saline at 37°C.
Figure 16. Experimental set up for biaxial testing

Figure 17. Image taken of speckles on the sample used in strain analysis

Figure 18. The halved specimen mounted in the Biotester machine to be tested uniaxially and the speckles were randomly distributed on the specimen to track the strain on the tissue.

To quantify the biaxial response of the aortic tissue, the Cauchy stress and stretch were calculated. By assuming no shear contribution and fully incompressible material response, the Cauchy stresses were determined as follows
where $t$ is the mean thickness of the tissue in the undeformed configuration; $f_\theta$ and $f_L$ represent the measured forces in the circumferential and longitudinal directions, respectively; $\lambda_\theta$ and $\lambda_L$ denote the stretches in each direction.

### 3.4. Material Parameters Identification

The orthotropic eight-chain model was implemented into MATLAB (Mathworks, Natick, MA) to determine the model parameters. The objective function of the residual between the experimental and the analytical values was minimised to obtain material parameters for each model. The `fsolve` function was employed and the residual function was defined as follows

\[
\text{Residual} = \sum_i \left( \sigma_{\text{exp}}(i) - \sigma_{\text{model}}(i) \right)^2
\]

where $\sigma_{\text{exp}}$ indicates the stress measured from the experiment and $\sigma_{\text{model}}$ the stress obtained from the model calculation. By following the procedure from Zhang et al. (2005), we set up the value of model parameters (see Fig. 11) as $a, b > c$ which is in agreement with the study from Holzapfel et al. (2000), indicating the medial and adventitial layers are modelled with fibre-reinforced material with no fibre contribution in the thickness direction. The orthotropic aspect ratios of the WLC model were kept similar to the FJC model. The coefficient determination $R^2$ from the results based on the eight-chain and the extended eight-chain models was calculated as follow (Matlab User's Guide, 2008)

\[
R^2 = 1 - \frac{\text{Residual}}{\text{Var} \sigma_{\text{exp}}}
\]

where Var $\sigma_{\text{exp}}$ stands for the variance of experimental stresses.

### 3.5. Statistical Analysis

Student’s $t$-tests were undertaken to compare the mechanical properties of the samples in circumferential and longitudinal directions and identify whether any differences are statistically significantly different. The values of initial slope, maximum slope, maximum stress and strain were examined. The initial slope was defined as the tangent to the linear region of the stress-stretch curve at the onset of the curve. The maximum slope was
determined as a tangent to the stress-stretch curve at the peak stress (see fig. 19). Those slopes are important to be investigated due to their relations to the elastic modulus of the tissue which could be different in magnitude for both directions. Moreover, a value of $p < 0.05$ (i.e. confidence interval $>95\%$) was used to consider conditions being statistically significant.

**Figure 19.** Determination of stiffness based on the initial and maximum slopes.
Chapter 4

Results and Discussions

4.1. Experimental Results

4.1.1. Biaxial and Uniaxial Testing

This study has evaluated mechanical properties of ovine aortic tissue and its response due to uniaxial and biaxial loading. Also, a validation of microstructural models based on statistical mechanics has been assessed. Fig. 20 demonstrates equi-biaxial response of ovine aortic tissue where the J-shape of stress-strain curve, a typical curve shape for soft tissues was shown. It was found that the tissue exhibited anisotropic and nonlinear behaviours. Generally, the stress-strain curve of soft tissue consists of three distinct regions; (1) an initial linear region, (2) an elbow region and (3) a final linear region. In the small stretch region ($\lambda < 1.1$), the curve is almost linear in both the longitudinal and circumferential direction. Since the tissue samples were subjected to a maximum stretch ratio of 1.3, the presence of a significant strain hardening response or a final linear region was not obvious in these tests. No damage was found in the samples. This was monitored carefully as the samples were retested uniaxially afterwards.

Fig. 21 shows the uniaxial responses of the ovine aorta samples where the curve in the longitudinal direction showed less nonlinearity than the circumferential one. Also, no significant strain hardening was found in the uniaxial response. It can be seen from these results that the circumferential samples demonstrated higher standard deviation than that of longitudinal samples.

Table 1 summarises the calculated initial and maximum tangent moduli and the maximum stress reached at a stretch ratio 1.3. It was found that there were statistically significant differences between the moduli from the uniaxial and equi-biaxial tests. In the uniaxial response, the maximum slopes were approximately twice that of the initial slopes in both directions whereas in the equi-biaxial response, the maximum slopes were approximately threefold and twice to that of initial slopes in circumferential and longitudinal directions, respectively. In term of maximum stress, the equi-biaxial showed higher stress values than that of the uniaxial stress in both directions.
It is known that elastin plays a significant role in load bearing mechanism at low stretch since there is no contribution from the collagen fibres due to the wavy and crimped configuration. In the physiological state, elastin is mostly responsible for carrying the load for the pressure range of 0-70 mmHg (~9.33 KPa). Most investigators (Holzapfel et al., 2000; Holzapfel, 2001, 2005b; Gundiah et al., 2007; Watton et al., 2009) have considered that elastin is isotropic and the anisotropic response only comes from the contribution of collagen fibres. However, our results from uniaxial and biaxial tests in the biaxial tensile tester showed that the initial tangent modulus was different in both directions (table 1), and it could be linked to the anisotropic behaviour of elastin.

Figure 20. The stress-stretch curves in circumferential and longitudinal directions and their standard deviations acquired from equi-biaxial testing on the ovine aortic tissue.

Figure 21. The stress-stretch curves in circumferential and longitudinal directions and their standard deviations acquired from uniaxial testing on the ovine aortic tissue.
Similar finding was obtained by studies of Zou and Zhang (2009) and Rezakhaniha et al. (2011) showing that elastin fibres are anisotropic with preferentially orientation in circumferential direction. Moreover, novel microscopy techniques using Serial Block Face-Scanning Electron Microscopy (SBFSEM) and confocal microscopy revealed that the elastin in the aorta has three unique forms i.e. lamellae, interlamellar elastin fibers (IEF) and radial elastin where IEF is preferentially oriented in the circumferential direction (O’Connell et al., 2008). Also, Rezakhaniha et al. (2011) suggested that an anisotropic model of elastin should be incorporated to accurately fit the experimental data.

**Table 1.** The magnitude (mean ± SD) of initial tangent modulus, maximum tangent modulus at stretch ratio of 1.3, and maximum stress of the ovine aortic tissue specimens in both directions from uniaxial and equi-biaxial tests.

<table>
<thead>
<tr>
<th></th>
<th>Circumferential (KPa)</th>
<th>Longitudinal (KPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial tangent modulus (uniaxial)</td>
<td>101.42 ± 17.13</td>
<td>68.73 ± 6.73</td>
</tr>
<tr>
<td>Initial tangent modulus (equi-biaxial)</td>
<td>110.60 ± 14.06</td>
<td>87.08 ± 6.34</td>
</tr>
<tr>
<td>Maximum tangent modulus (uniaxial)</td>
<td>224.67 ± 48.06</td>
<td>116.07 ± 25.58</td>
</tr>
<tr>
<td>Maximum tangent modulus (equi-biaxial)</td>
<td>305.49 ± 40.83</td>
<td>201.86 ±23.61</td>
</tr>
<tr>
<td>Maximum stress (uniaxial)</td>
<td>45.51 ± 7.95</td>
<td>24.51 ± 2.11</td>
</tr>
<tr>
<td>Maximum stress (equi-biaxial)</td>
<td>60.34 ± 7.91</td>
<td>41.81 ± 4.25</td>
</tr>
</tbody>
</table>

Our results have shown that the tangent moduli obtained from biaxial experimental curves were higher than from the uniaxial tests (Matsumoto et al., 2009). The initial tangent moduli obtained from uniaxial testing in our study were comparable to the value reported by Wells et al. (1998, 1999) showing the initial moduli values from in vivo and in vitro experiments of ovine aorta were about 100-350 kPa. As shown in table 1 the tangent modulus at stretch ratio 1.3 increased about three times in respect to the initial modulus. As discussed in the introduction, the explanation for this is that the collagen fibres start to be aligned toward the load so those fibres give a significant contribution to the instantaneous modulus of the tissue (due to the preferential orientation of the fibres).

Fig. 22 shows representative response of equi- and nonequi-biaxial response of ovine aortic tissue (only the results for $\lambda_0 : \lambda_L = 0.5:1$ and 1:0.5 are shown in here). The results indicate a decrease of applied stretch ($\lambda_0 : \lambda_L = 0.5:1$) in the circumferential direction and an increased anisotropic response whereas a decrease of applied stretch in the longitudinal decreased ($\lambda_0 : \lambda_L = 0.5:1$)
\( \lambda_L = 1:0.5 \) led to an increase of anisotropy. This result was in good agreement with the finding Zou and Zhang (2009) which carried out equi and non-equibiaxial testing in force-control. This could be explained by the preferential orientation of the elastin and collagen fibres in circumferential direction.

Figure 22. Representative stress-stretch curves of equi- and non-equibiaxial (\( \lambda_0 : \lambda_L = 0.5:1 \) and 1:0.5) responses of ovine aortic tissue.

4.1.2. Strain Measurement by Imaging Technique

The strain uniformity of the uniaxial and equi-biaxial tests in displacement-controlled was also examined. Fig. 23 demonstrates non-uniformity of the strain distribution in the equibiaxial test where the outer grids possess highest strain values and the distribution become more uniform for the grids at the centre. The figure also displays that the maximum engineering strain value in circumferential (\( E_x \)) and longitudinal (\( E_y \)) for that specimen were 0.257 and 0.283, respectively, which did not achieve the maximum applied strain (0.3). Fig. 24 shows non-uniformity of the strain distribution in the uniaxial test where the maximum engineering strain value was produced in the region near the rakes with maximum engineering strain value of 0.307. The maximum stretch ratio at the centre of the specimens did not reach a stretch ratio of 1.3 for both tests (fig. 25). It should be noted that the results in fig. 25 refer to the uniaxial stretches separately acquired for each direction (i.e. obtained in two separate tests). The magnitudes of the maximum stretch ratio measured by the image analysis were 1.25±0.01 and 1.26±0.01 for circumferential and longitudinal specimens in the
uniaxial tests and 1.20±0.02 and 1.23±0.02 for circumferential and longitudinal specimens in the biaxial tests, respectively. Therefore, in the equi-biaxial test, although equal displacements were imposed in each direction, the measured strain undergone by the specimens (i) was not equi-biaxial testing (ii) was non-uniform and (iii) was not consistent with the magnitude of the applied displacement. Similar trends were observed by Matsumoto et al. (2009). Their experimental results also showed that the strain value in the circumferential direction is lower than in the longitudinal direction. This could be due to the uneven length of each hook on the rakes in both directions which may affect the measured strain value. Eilaghi et al. (2009) also demonstrated that strain field uniformity in biaxial test can considerably decrease when subtle irregularities of attachment points positioning exist.

![Figure 23](image.png)

**Figure 23.** Strain mapping of image analysis on equi-biaxial test at maximum rake-to-rake displacement in (a) circumferential and (b) longitudinal directions where $E_x$ and $E_y$ terms stand for engineering strain in circumferential and longitudinal directions, respectively.

Also, an error related to marker tracking by the software was found where, in our experimentation, when speckles were not evenly distributed his could lead to an error of strain field mapping. However, despite these uncertainties, a number of investigators
(Gregory et al., 2011; Holmer et al., 2012) have calculated the strain-or stretch based on the applied displacement as conducted in this study. There is clearly a need to explore further and conduct a repeatable practice to employ this imaging technique to produce a reliable result. Also, although the measured strain can be determined by the imaging technique, one needs to consider the stress value and how it may be influenced by the non-uniformity of strain. Elliott et al. (2013) introduced a correction factor to predict the measured stress at the region of interest (i.e. the region with uniform strain field) which was based on FE analysis. However, their investigation was limited to planar clamped biaxial tension.

![Strain mapping of image analysis at maximum rake-to-rake displacement on uniaxial test.](image)

**Figure 24.** Strain mapping of image analysis at maximum rake-to-rake displacement on uniaxial test.

![Comparison of maximum strain measured by imaging technique in both directions for uniaxial and equi-biaxial tests done in the biaxial tensile tester.](image)

**Figure 25.** Comparison of maximum strain measured by imaging technique in both directions for uniaxial and equi-biaxial tests done in the biaxial tensile tester.

### 4.2. Determination and Validation of Model Parameters

Figs. 26 and 27 compare the prediction from the orthotropic FJC eight-chain model with the biaxial data from sample 6 which demonstrates a good agreement. The obtained model parameters of that sample were \( n = 3.30 \times 10^{24} \, (1/m^3) \); \( a = 2.44 \); \( b = 1.91 \); \( c = 0.61 \); \( N = 2.50 \). It was observed that the models with FJC and WLC approaches were able to fit the data \((R^2 = \)
0.93 ± 0.02 for FJC model and 0.92 ± 0.02 for WLC model, respectively) and the model parameters for the six samples are summarised in table 2 for FJC approach and in table 3 for WLC approach. Our results also did not show any significant difference between the two approaches in the orthotropic model which was in line with the study of Bischoff et al. (2002b). Further, the difference of the model parameters values was due to different theory that is used to govern those models.

Further, a comparison between uniaxial data and the prediction of the orthotropic eight-chain model with FJC approach is shown in fig. 28 in order to test their validity towards anisotropic behaviour of ovine aortic tissue. The mean value of the FJC model parameters \( n = 3.31 \times 10^{24} [1/m^3], a = 2.34, b = 1.93, c = 0.57, N = 2.38 \) was employed in the simulation. It can be seen the predicted stress value in the circumferential direction was somewhat lower than the experimental data whereas the experimental data in longitudinal direction was well predicted by the model. Furthermore, prediction of the uniaxial test data by using the model with the WLC approach was also carried out. The mean value of the model parameters with WLC approach \( n = 2.38 \times 10^{24} [1/m^3], a = 5.51, b = 4.56, c = 1.34, \zeta = 6.63 \) was used.

**Figure 26.** Representative stress-stretch data (symbols) of the ovine aortic tissue in the circumferential direction under five different biaxial deformation ratios \( \lambda_\theta : \lambda_L = 1:1 \) (rhombus), 0.75:1 (square), 1:0.75 (triangle), 0.5:1 (star), 1:0.5 (round) and the prediction from the orthotropic eight-chain model with FJC approach (solid lines).

The WLC approach produced a similar result as the prediction of the model with FJC approach (the results is not shown in here), and also the stress value predicted by the WLC approach was close to the value predicted by the FJC approach. This result emphasised the capability of the models to predict the mechanical response of the tissue. The non-linear
behaviour of the aortic tissue obtained from the biaxial tests was captured very well by the selected material models as shown in figs. 26, 27, tables 2 and 3. Zou and Zhang (2009) has confirmed the validity of the orthotropic eight-chain model by fitting the equi-biaxial data to acquire the model parameters, and those values were employed to predict the nonequi-biaxial response of elastin network of bovine aortic tissue. The results showed that the orthotropic model can predict the responses of elastin network under arbitrary biaxial loading conditions. Our results have also shown similar results but it was done in a different manner where the equi- and nonequi-biaxial were fitted towards the experimental data of biaxial testing to obtain the model parameters and the experimental data of uniaxial testing was employed to confirm the validity of the orthotropic model (fig. 28). Thus, these results confirm that the orthotropic eight-chain model can be employed for the study of anisotropic hyperelastic mechanical behaviour of ovine aortic tissue.

Figure 27. Representative stress-stretch data (symbols) of the ovine aortic tissue in longitudinal direction under five different biaxial deformation ratios $\lambda_D : \lambda_L = 1:1$ (rhombus), 0.75:1 (square), 1:0.75 (triangle), 0.5:1 (star), 1:0.5 (round) and the prediction from the orthotropic eight-chain model with FJC approach (solid lines).

4.3. Discussion

As mentioned previously, the model parameters of the eight-chain models are important, as they provide information related to the structure of the aortic tissue. This can be important in finding relevant mechanisms governing tissue disease development, by relating changes in model parameters to the increase in crosslink density with age, and/or the reduction in chain extensibility. Hence, it is clear that (micro)structurally-based models can give a better
representation of aortic tissues. Zhang et al (2005) showed that the chain density parameter \( n \) contributes significantly to the initial slope of the stress-strain curve, where the hypertensive arteries had higher values of this parameter compared to that of normotensive arteries. Moreover, the order of parameter \( n \) obtained in our results was similar to the value reported by Zhang et al. (2005) for the pulmonary artery and Zou and Zhang (2009) for the bovine aorta. Further, the chain extensibility parameter \( N \) plays a role in determining the onset of strain stiffening and the ratio of \( c/a \) has an effect on the prediction of tissue stiffness in the thickness direction relative to the stiffness in the in-plane direction as suggested from Zhang et al. (2005).

Table 2. Model parameters of the orthotropic eight-chain model with FJC (see eq. (5) and (7)).

<table>
<thead>
<tr>
<th>Sample</th>
<th>( n \times 10^{24} \text{ (1/m}^3) )</th>
<th>( a )</th>
<th>( b )</th>
<th>( c )</th>
<th>( N )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.81</td>
<td>2.27</td>
<td>1.97</td>
<td>0.54</td>
<td>2.33</td>
<td>0.91</td>
</tr>
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<td>Sample 2</td>
<td>3.22</td>
<td>2.27</td>
<td>2.02</td>
<td>0.61</td>
<td>2.40</td>
<td>0.94</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.61</td>
<td>2.36</td>
<td>1.87</td>
<td>0.52</td>
<td>2.34</td>
<td>0.91</td>
</tr>
<tr>
<td>Sample 4</td>
<td>3.81</td>
<td>2.37</td>
<td>1.95</td>
<td>0.54</td>
<td>2.43</td>
<td>0.94</td>
</tr>
<tr>
<td>Sample 5</td>
<td>3.12</td>
<td>2.31</td>
<td>1.88</td>
<td>0.58</td>
<td>2.30</td>
<td>0.91</td>
</tr>
<tr>
<td>Sample 6</td>
<td>3.30</td>
<td>2.44</td>
<td>1.91</td>
<td>0.61</td>
<td>2.50</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.31 ± 0.36</td>
<td>2.34 ± 0.07</td>
<td>1.93 ± 0.06</td>
<td>0.57 ± 0.04</td>
<td>2.38 ± 0.07</td>
<td>0.93 ± 0.02</td>
</tr>
</tbody>
</table>

Table 3. Model parameters of the orthotropic eight-chain model with WLC (see eq. (6) and (7)).

<table>
<thead>
<tr>
<th>Sample</th>
<th>( n \times 10^{24} \text{ (1/m}^3) )</th>
<th>( a )</th>
<th>( b )</th>
<th>( c )</th>
<th>( \zeta )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.90</td>
<td>5.25</td>
<td>4.56</td>
<td>1.24</td>
<td>6.24</td>
<td>0.90</td>
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<tr>
<td>Sample 2</td>
<td>2.35</td>
<td>5.37</td>
<td>4.77</td>
<td>1.45</td>
<td>6.72</td>
<td>0.93</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.54</td>
<td>5.53</td>
<td>4.39</td>
<td>1.22</td>
<td>6.42</td>
<td>0.89</td>
</tr>
<tr>
<td>Sample 4</td>
<td>2.80</td>
<td>5.63</td>
<td>4.63</td>
<td>1.29</td>
<td>6.85</td>
<td>0.93</td>
</tr>
<tr>
<td>Sample 5</td>
<td>2.20</td>
<td>5.40</td>
<td>4.39</td>
<td>1.36</td>
<td>6.28</td>
<td>0.90</td>
</tr>
<tr>
<td>Sample 6</td>
<td>2.53</td>
<td>5.88</td>
<td>4.61</td>
<td>1.48</td>
<td>7.25</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.38 ± 0.31</td>
<td>5.51 ± 0.22</td>
<td>4.56 ±0.15</td>
<td>1.34 ± 0.11</td>
<td>6.63 ± 0.39</td>
<td>0.92 ± 0.02</td>
</tr>
</tbody>
</table>

It is also important to notice some limitations of this study. The experiments were carried out with a small number of samples which may not produce a sufficiently robust data. Also, some considerations which may influence mechanical properties of the tissue such as age, location of the blood vessels, gender were not examined in our study. As mentioned before, the primary intention of this study was to characterise the passive mechanical properties of ovine aortic tissue and investigate the ability of the eight-chain based constitutive models for predicting the experimental data so some experimental issues were not considered here. Furthermore, examination of the active response of aortic tissue must account for the
behaviour of smooth muscle cells, as they contribute to the mechanical response of aortic tissue at physiological state by adding an active response with variable tone in circumferential direction.

**Figure 28.** Experimental data of uniaxial testing in both directions (symbols) and their predictions (lines) obtained by the orthotropic eight-chain model with FJC approach.
Chapter 5

Conclusions and Future Work

This work addressed the passive mechanical behaviour of healthy ovine aortic tissue. The tissue was subjected to biaxial and uniaxial displacement-controlled loading to retrieve its stress-stretch behaviour. Different biaxiality ratios were investigated to find the influence of tissue microstructure on its anisotropic response and the ability of the model to fit the experimental data. Two versions of the orthotropic eight-chain model (Bischoff et al., 2002a, b), i.e. the freely-jointed chain (FJC) and worm-like chain (WLC), were applied to capture the anisotropic response of the tissue. The main conclusions coming from this work are given below.

The ovine aortic tissue showed a nonlinear and anisotropic behaviour in biaxial and uniaxial testing. The circumferential direction was found to be stiffer than the longitudinal one, which resulted from the internal tissue structure formed by collagen and elastin. The response of the tissue was anisotropic even at low stretches, and the anisotropy increased with applied displacements. As the elastin is believed to govern the smaller-strain (stretch) response, it was concluded that it must be initially anisotropic i.e. it has some preferential orientation in the undeformed state. The increasing anisotropy in the response of the tissue was attributed to the straightening and reorientation of the collagen fibres.

The applied constitutive models captured well the biaxial response of the tissue at different biaxiality ratios. The predictions of the orthotropic models for stresses in two perpendicular directions were in a good agreement with experiments. The orthotropic versions of the eight-chain model were able to account for the tissue anisotropy with a single set of model parameters. Those parameters were found by fitting, but they were in a reasonable quantitative agreement with parameter values found in the literature for other tissues. The validity of the model has also been confirmed by employing the single set of model parameters obtained from the fitting of biaxial data in order to capture the uniaxial stress-stretch response of the tissue. This clearly suggests that the orthotropic eight-chain model is able to capture the anisotropic hyperelastic mechanical behaviour of ovine aortic tissue under different loading conditions with a single set of parameters and could be used for further study. For the uniaxial testing, it was found in general that the FJC version of the orthotropic model performed reasonably well. It was shown that the prediction in the circumferential
direction was somewhat lower than the experimental data, whereas the experimental data in the longitudinal direction was predicted very well by the model.

Also, for future work, it is suggested that the chain density parameter $n$ is determined through experiments such as swelling experiment or infrared spectroscopy in order to validate its determination from the curve fitting. Another aspect for further study is related to the viscoelasticity of aortic tissue, which may prove important in predicting more accurately its response as the tissue may undergo stress relaxation under constant strain and creep under constant stress conditions, respectively where our preliminary results have demonstrated these phenomena (results were not shown in this study).
References


