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The relationship between micro-structure and mechanical behaviour in passive skeletal muscle

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A thesis paper submitted to the University of Dublin Trinity College, in partial fulfilment of the requirements for the degree of

Doctor in Philosophy

Trinity College Dublin
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Declaration

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I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Michael Takaza

Dublin, Ireland, the 22nd of May 2014
I dedicate this thesis to the following:

1) Charity, Oisin and Fergal

2) All those special friends who we used to share those 5km bare footed runs to school on those cold winter morning in the early 70s and yet still spend the whole day without any food in the name of seeking education. Small and young as we were, it was those dreams we shared that kept us going. Several times, they talked me out of quitting school even when my body was at its limit, yet I found myself powerless to stop them quitting without even a secondary education.
He said "They are feeding on drowned yellow stone flies,"
I asked him, "How did you think that out?"
"All there is to thinking", he said, "is seeing something noticeable which makes you see something you weren't noticing which makes you see something that is not even visible"
I said to my brother, "Give me a cigarette and say what you mean."

*Norman Maclean, A River Runs Through It*
Summary

This thesis presents and discusses investigative work performed on characterising the behavioural response of skeletal muscle tissue that was subjected to large deformations. The passive mechanical properties of muscle tissue are important for many biomechanics applications, including impact biomechanics, tissue engineering and rehabilitation engineering. However, significant gaps remain in our understanding of the passive three-dimensional tensile and compressive response of skeletal muscle tissue. In particular, the tensile quasi-static soft tissue anisotropy remains unclear and the responses to loading at intermediate fibre directions, as well as the asymmetrical behavioural response of muscle tissue have not been investigated before.

Accordingly, tensile tests were performed along and perpendicular to the muscle fibre direction as well as at 30, 45 and 60 degrees to the muscle fibre direction using samples taken from freshly slaughtered pigs. Strain was measured using an optical non-contact method. The results show the transverse or cross fibre (TT') direction stress-strain response is broadly linear and is the stiffest (77kPa stress at a stretch of 1.1), but failure was observed to occur at very low stretches (approximately = 1.15). In contrast, the longitudinal or fibre direction (L) is nonlinear and much less stiff (10kPa stress at a stretch of 1.1) and failure occurs at higher stretches (approximately = 1.65). An almost sinusoidal variation in stress response was observed at intermediate angles. The following Poisson’s ratios were measured: \( v_{LT} = v_{LT'} = 0.47, \quad v_{TT'} = 0.28 \) and \( v_{TL} = 0.74 \). These observations have not been previously reported and they contribute significantly to our understanding of the three dimensional deformation response of skeletal muscle tissue.

Existing uniaxial compression data mostly relates to normalised strain rates of between 0.05%\( s^{-1} \) and 3200%\( s^{-1} \) and Split Hopkinson bar tests at normalised strain rates of above 54,000%\( s^{-1} \). Thus, data on fresh tissue in both the fibre and cross-fibre direction at normalized strain rates for the gap between 3200%\( s^{-1} \) and 54,000%\( s^{-1} \) is needed. Fibre and cross-fibre compression tests at strain rates varying from 11,600%\( s^{-1} \) to 37,800%\( s^{-1} \) were performed using a drop-tower testing rig. Results show a time dependent nonlinear stress-stretch relationship. The mean (standard deviation) engineering stress in the fibre direction at a stretch of 0.7 was 22.03kPa (1.5kPa) at a strain rate of 22,000%\( s^{-1} \) and 37.06kPa (3.0kPa) at 37,800%\( s^{-1} \). For the
cross-fibre direction, the respective engineering stresses were 5.95kPa (0.6kPa) at 11,600% s⁻¹, 25.88kPa (5.3kPa) at 22,000% s⁻¹ and 43.68kPa (1.4kPa) at 37,800% s⁻¹. However, significant local strain variations and an average 8% mass loss were observed.

The modelling results of compressive/tensile loading of freshly slaughtered porcine muscle is reported. The inverse finite element analysis shows that the elastic response in terms of both applied load and tissue deformation for each of the strain rates can be captured using a first order Ogden hyperelastic material law and which was extended with a three-term quasi-linear viscoelastic (QVL) expansion to model viscoelastic effects. An optimisation procedure was used to derive the optimal material parameters for which the error in the predicted boundary condition force at maximum compression was less than 3% for all three rates of testing (11,600% s⁻¹, 22,000% s⁻¹ and 37,800% s⁻¹). This model may be appropriate for whole body impact modelling at these rates.

Finally, the micro-structural deformation was investigated in order to understand and explain the muscle tissue's observed asymmetrical behaviour. Accordingly, freshly harvested skeletal muscle tissue was deformed by 30% and prepared for various microscopic analysis. For compressive or tensile stretch applied in the muscle fibre direction, the average measured muscle fibre cross-sectional area changes are in close correspondence with predictions based on global Poisson's ratio measurements and these deformation modes did not cause shape changes in the muscle cross-sections. However, muscle tissue reacted to applied cross-fibre deformation as follows: applied compression flattened muscle fibre cross-sections, aligning them perpendicular to the direction of the applied deformation while the applied tensile deformations stretched the cross-sections of muscle fibres aligning them parallel to the direction of applied deformation. No evidence of structural reorganisation of endomysium collagen fibres was observed. These observed responses appear to be significantly influenced by proximity to the perimysium network. The perimysium and its interaction with the surrounding muscle fibres is likely to be the predominant factor that is responsible for the tension/compression asymmetry observed in macroscopic tests of passive skeletal muscle.

In conclusion, the work described above has made significant additions and contributions to the knowledge data base in the skeletal muscle biomechanics field.
Acknowledgements

It is hard to believe it is now almost five years since I first spoke to Ass. Prof. Ciaran Simms about joining his group as a PhD student. I am in debt to a lot of people for their help with this work.

Firstly I would like to express my deep sincerest gratitude to my supervisor Asst. Professor Ciaran K Simms for his expert guidance, encouragement and continuous support. I would like to thank the zoology department (Histology section) especially Peter Stafford for his help with the histology techniques and allowing us to use his lab for our microscopic experiments.

I would like to acknowledge the workshop staff; these guys were always there and ready to attend to my needs whenever I needed them. I am also grateful to my colleague and good friend Gerard Cooney for useful interactions without which my research works would not have been so successful.

My special thanks to Dr Kevin M Moerman, a former work colleague, a friend as well as acknowledge the importance of the advisory role he played, it is through his constructive criticism and the high standard of his expectations from me that make this work as accomplished as it is. His advice and help with the modelling was invaluable

I would like to acknowledge all the financial support that I received from the Galway County Council.

Finally but not least, I would like to thank my wife Charity and my two sons (Oisin and Fergal), for their support and love during my PhD period.
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List of publication resulting from this study

First Author Journal Papers


Co-Authored Journal Papers


Conference Papers

1) M. Takaza, G. McManus, P. Stafford, C.K. Simms. “Microscopic analysis of muscle tissue that has been subjected to large deformation”, Bioengineering in Ireland, Limerick, 2014
2) M. Takaza, C.K. Simms. “Microscopic analysis of muscle tissue that has been subjected to large deformation”. World Congress of Biomechanics, July 2014, Boston. (invited to present in a soft tissue biomechanics session)


## Nomenclature

### Mathematical Notation

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>Scalar</td>
</tr>
<tr>
<td>( \mathbf{A} )</td>
<td>Vector</td>
</tr>
<tr>
<td>( \mathbf{e}_i )</td>
<td>Three mutual Orthonormal basis vectors</td>
</tr>
<tr>
<td>( \sum_{i=1}^{3} a_i \mathbf{e}_i )</td>
<td>Summation of the elements of ( \mathbf{a} ), top showing full notation, bottom equivalent for the Einstein's convection notation</td>
</tr>
<tr>
<td>( \mathbf{A} )</td>
<td>Tensor (matrix)</td>
</tr>
<tr>
<td>( \mathbf{A}^T )</td>
<td>The transpose of tensor or matrix ( \mathbf{A} )</td>
</tr>
<tr>
<td>( \mathbf{D}, \mathbf{A}^{-1} )</td>
<td>The inverse of tensor or matrix ( \mathbf{A} )</td>
</tr>
<tr>
<td>( \det(\mathbf{A}) )</td>
<td>The determinate of ( \mathbf{A} )</td>
</tr>
<tr>
<td>( \text{tr}(\mathbf{A}) )</td>
<td>The trace of tensor ( \mathbf{A} )</td>
</tr>
<tr>
<td>( I_i )</td>
<td>Invariants</td>
</tr>
<tr>
<td>( \Delta \mathbf{a} )</td>
<td>Change in ( \mathbf{a} )</td>
</tr>
<tr>
<td>( \nabla_x \mathbf{a} )</td>
<td>The gradient of ( \mathbf{a} ), defined as ( \frac{\partial d_i}{\partial x_i} )</td>
</tr>
<tr>
<td>( \Omega )</td>
<td>Continuum body</td>
</tr>
<tr>
<td>( \mathbf{X} )</td>
<td>Position vector in Lagrangian configuration</td>
</tr>
<tr>
<td>( \mathbf{X} )</td>
<td>Position vector in Eulerian configuration</td>
</tr>
<tr>
<td>( T )</td>
<td>Time</td>
</tr>
<tr>
<td>( V,V )</td>
<td>Volume in the Eulerian configuration</td>
</tr>
<tr>
<td>( \mathbf{F} )</td>
<td>Deformation gradient tensor</td>
</tr>
<tr>
<td>( \mathbf{U} )</td>
<td>Left stretch tensor</td>
</tr>
<tr>
<td>( \mathbf{V} )</td>
<td>Right stretch tensor</td>
</tr>
<tr>
<td>( \mathbf{C} )</td>
<td>Green or Lagrangian deformation tensor</td>
</tr>
<tr>
<td>( \mathbf{I} )</td>
<td>Unit or identity tensor</td>
</tr>
<tr>
<td>( \Lambda )</td>
<td>Stretch Ratio</td>
</tr>
<tr>
<td>( \Lambda )</td>
<td>Wavelength of light, Wavelength of Bragg signal and de Broglie wavelength,</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>( J )</td>
<td>Volume ratio or Jacobian of deformation gradient tensor (continuum mechanics)</td>
</tr>
<tr>
<td>( Me )</td>
<td>Mass of an electron</td>
</tr>
<tr>
<td>( Ve )</td>
<td>Velocity of an electron</td>
</tr>
<tr>
<td>( e,\varepsilon )</td>
<td>Strain</td>
</tr>
<tr>
<td>( G )</td>
<td>Green or Lagrangian strain tensor (continuum mechanics). Magnetic field gradient (physics of nuclear magnetic resonance)</td>
</tr>
<tr>
<td>( G )</td>
<td>relaxation function</td>
</tr>
<tr>
<td>( U )</td>
<td>Displacement tensor</td>
</tr>
<tr>
<td>( F,f )</td>
<td>Force (continuum mechanics).</td>
</tr>
<tr>
<td>( S,s )</td>
<td>Surface element in Eulerian, Lagrangian configuration</td>
</tr>
<tr>
<td>( N )</td>
<td>Surface normal vector</td>
</tr>
<tr>
<td>( T,t )</td>
<td>Traction vectors, in Eulerian, Lagrangian configuration, ( T ) is also known as first Piola Kirchoff vector</td>
</tr>
<tr>
<td>( \Delta )</td>
<td>Stress, Cauchy or true stress tensor (continuum mechanics), standard deviation of Gaussian function (statistics, image processing)</td>
</tr>
<tr>
<td>( P )</td>
<td>Piola-Kirchoff or Lagrangian stress tensor</td>
</tr>
<tr>
<td>( S )</td>
<td>Second Piola-Kirchoff stress tensor</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Kirchoff stress tensor</td>
</tr>
<tr>
<td>( E )</td>
<td>Infinitesimal or linear strain tensor</td>
</tr>
<tr>
<td>( T )</td>
<td>Temperature</td>
</tr>
<tr>
<td>( \Psi )</td>
<td>Strain energy, Helmholtz free-energy function</td>
</tr>
<tr>
<td>( \Psi_{iso} )</td>
<td>Isochoric strain energy</td>
</tr>
<tr>
<td>( \Psi_{vol} )</td>
<td>Volumetric strain energy</td>
</tr>
<tr>
<td>( E )</td>
<td>Young's modulus</td>
</tr>
<tr>
<td>( \mu )</td>
<td>A Lamé modulus: the shear modulus (continuum mechanics). Ogden parameter Mean (statistics)</td>
</tr>
<tr>
<td>( \kappa )</td>
<td>The bulk modulus</td>
</tr>
<tr>
<td>( H )</td>
<td>Hourglass parameter</td>
</tr>
<tr>
<td>( \nu )</td>
<td>Poisson's ratio</td>
</tr>
<tr>
<td>( N )</td>
<td>Order of Ogden strain energy formulation</td>
</tr>
<tr>
<td>( a_i )</td>
<td>Constant in Ogden strain energy formulation</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$\lambda_s$</td>
<td>Spring constant in rheological model</td>
</tr>
<tr>
<td>$\lambda_D$</td>
<td>Dashpot constant in rheological model</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Viscosity constant in rheological model</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>Voigt model based viscous material constant</td>
</tr>
<tr>
<td>$\sigma_t, \sigma_e, \sigma_v$</td>
<td>Total, elastic and viscous stress respectively</td>
</tr>
<tr>
<td>$G$</td>
<td>Relaxation modulus (continuum mechanics). Gradient of image (digital image processing)</td>
</tr>
<tr>
<td>$R$</td>
<td>Resolution</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wave length</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck’s constant</td>
</tr>
<tr>
<td>$\Pi_{int}$</td>
<td>Internal mechanical work</td>
</tr>
<tr>
<td>$n$</td>
<td>Refractive index</td>
</tr>
<tr>
<td>$P_{ext}$</td>
<td>External mechanical power</td>
</tr>
<tr>
<td>$K$</td>
<td>Kinetic energy</td>
</tr>
</tbody>
</table>

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEA</td>
<td>Finite Element Analysis</td>
</tr>
<tr>
<td>iFEA</td>
<td>Inverse Finite Element Analysis</td>
</tr>
<tr>
<td>KE</td>
<td>Kinetic Energy</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>F (L)</td>
<td>Fibre direction (Longitudinal)</td>
</tr>
<tr>
<td>XF (T)</td>
<td>Cross-fibre direction (Transverse)</td>
</tr>
<tr>
<td>$\nu_{LT}, \nu_{LT'}$</td>
<td>Poisson’s ratio (Longitudinal direction load, Transverse direction contraction)</td>
</tr>
<tr>
<td>$\nu_{TT'}$</td>
<td>Poisson’s ratio (Transverse direction load, Transverse direction contraction)</td>
</tr>
<tr>
<td>$\nu_{TL}$</td>
<td>Poisson’s ratio (Transverse direction load, Longitudinal direction contraction)</td>
</tr>
<tr>
<td>NA</td>
<td>numerical aperture</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Rationale of the study

This thesis concentrates on the investigation of behavioural response properties of skeletal muscle tissue that has been subjected to large deformations. The thesis’ main focus is on the passive mechanical properties and the micro-structural responses.

The primary function of skeletal muscle is to provide support and contractive forces that are responsible for the mobility and posture of the musculoskeletal system. Furthermore, muscle tissue makes up over 50% of human body weight (Tozeren, 2000, Wang et al., 1997, Chomentowski et al., 2011, Salem et al., 2006, Lieber, 2002). Any compromise or malfunction of muscle tissue on any part of the human body, is manifested with an associated reduction in the quality of life of the whole human body system (Kim et al., 2008). Therefore, it is imperative that muscle tissue should be thoroughly investigated and well understood. Most diseased states are when the muscles fail to perform to their required optimum level due to various medical reasons, one of them being the failure to provide the required specific mechanical properties.

A better understanding of the loading response of muscle will lead to improvements in injury minimisation and prevention medical science fields. For tissue engineers, this will mean developing adaptable engineered muscle tissue that possess correct mechanical properties, as this will lead to less implantation trauma and increased chances of success. Designers of surgical tools will have a better understanding of the targeted muscle’s mechanical properties and therefore will be designed to minimise injury.

The enhanced understanding of skeletal muscle soft tissue mechanical properties will benefit a lot of other science related fields (Taylor and Humphrey, 2009), including impact biomechanics (Muggenthaler et al., 2008, Ivancic et al., 2007, Snedeker et al., 2005, Li et al., 2007, Majumder et al., 2008, Forbes et al., 2005), surgical simulation (Lim and De, 2007, Audette et al., 2004, Famaey and Sloten, 2008, Sutherland et al., 2006, Anastakis et al., 2003), gait analysis (Mathur et al., 2010), rehabilitation engineering (Linder-Ganz et al., 2008, Linder-Ganz et al., 2007, Gefen et al., 2005, Ceelen et al., 2008, Portnoy et al., 2008, Portnoy et al., 2009), and soft tissue drug transport (Wu and Edelman, 2008, Swartz and Fleury, 2007, Wu et al., 2009, Pradhan et al., 2013, Zilberman and Elsner, 2008). There are also important
applications in tissue engineering, as the engineered skeletal muscle tissue needs mechanical properties that match those of the replaced native tissue (Hinds et al., 2011, Goldstein et al., 2001, Yan et al., 2007, Rizzi et al., 2012).

Data from the published literature show that the tensile mechanical property response of the passive skeletal muscle is anisotropic (Hernandez et al., 2011, Morrow et al., 2010) (some authors describe this as specifically being transversely isotropic (Blemker and Delp, 2005, Morrow et al., 2010)), nonlinear (Grieve and Armstrong, 1988, Morrow et al., 2010) and is dependent on the speed at which the tissue is deformed (Van Loocke et al., 2006, Song et al., 2007, Van Sligtenhorst et al., 2006, Grieve and Armstrong, 1988, Nie et al., 2011), (Martins et al., 1998, Myers et al., 1991, Yamada, 1970, Hernandez et al., 2011) Similarly, for compressive deformations, the following characteristics have been observed: anisotropic (Van Loocke et al., 2006, Van Loocke et al., 2007, Van Sligtenhorst et al., 2006, Van Loocke et al., 2008, Van Loocke et al., 2009) or specifically transversely isotropic (Song et al., 2007), nonlinear (Grieve and Armstrong, 1988, Song et al., 2007, Van Loocke et al., 2006) and viscoelastic (Grieve and Armstrong, 1988, Van Sligtenhorst et al., 2006, Song et al., 2007, Van Loocke et al., 2008, Van Loocke et al., 2009).

Taking accurate \textit{in vivo} measurements is not possible because when foreign bodies are introduced into the human body, this immediately activates the immune system response which leads to a different physiological environment (Nag and Banerjee, 2012, Franz et al., 2011, Wooley et al., 1996). In most cases, the foreign body activates the immune response process resulting in the encapsulation and isolation of the sensor which generally leads to reduced sensitivity of the sensors. There are also ethical issues to be considered before any living subject is subjected to such painful \textit{in vivo} experiments. In contrast, numerical modelling of muscle tissue can improve our understanding as well as predict all the parameters, thereby providing a better understanding of the underlying phenomena associated with the muscle mechanical response to external loading (Miller and Lu, 2013).

Finite element analysis (FEA) models of soft tissue can be used to predict deformations during momentary loading (Raul et al., 2008) or medical device model loading and simulations (McGarry et al., 2004, Prendergast et al., 2011, Wagner et al., 2011). The effectiveness of any numerical model is heavily dependent on accurate description of both the hard and soft tissue geometries, as well as a correct definition of their material properties under large deformations (Abraham et al., 2013, Morrow et al., 2010, Olesen et al., 2006, Sommer and Holzapfel, 2012). However, our
understanding of the deformation behaviour is not fully complete and the three-dimensional mechanical properties of the skeletal muscle tissue have not been adequately experimentally characterised, therefore this has been a major limitation for computational modelling. Since muscle tissue accounts for almost half of human body weight (Tozeren, 2000, Wang et al., 1997), the constitutive properties of muscle tissue are fundamental for any musculoskeletal models to possess the required accuracy.

In light of the points raised above, it is therefore important that any simulation and modelling of human body investigation takes into account a full understanding of the muscle tissue deformation responses and encompass correct mechanical properties of the muscle involved.

1.2 Objectives

The aim of this study was to characterise the 3D tensile and compressive behavioural response of skeletal muscle that has been subjected to large deformations. The detailed 3D quasi-static compressive data (including respective Poisson's ratios) was available from the experimental work performed by Van Loocke (Van Loocke et al., 2006). No similar data was available for the tensile deformation: therefore quasi-static tensile experiments were performed in order to generate the tensile data (including the Poisson's ratios). Microscopic deformation analysis at muscle fibre level was performed in order to understand and explain the observed macroscopic behavioural response. In addition: Impact experiments were performed at higher strain rates where viscoelasticity played a more significant role, a broad strain rate range was selected making sure the strain rate chosen covered human tissue loading rates that are similar to those experienced during automobiles crashes (for cars travelling within city limits). The chosen strain rate had no standardised published experimental data available; therefore it was important to generate more experimental data in this area. Finally, inverse analysis was needed in order to understand the behavioural response of skeletal muscle. A simple first order hyperelastic Ogden model was used to capture the elastic passive muscle tissue response and the model was later extended with a three term quasi-linear viscoelastic model in order to capture the strain rate dependant part.
These objectives can be divided into the following mini objectives:

- Tensile quasi-static uniaxial experiments were performed in order to characterise the response of skeletal muscle to external loading, as well as to characterise the influence of skeletal muscle fibre orientation.
- Comparison of the tensile quasi-static uniaxial experimental results with the published compression quasi-static data.
- Characterisation of the deformation response of micro-structural components of the muscle fibres.
- Perform compressive impact loading experiments at strain rates ranging from $11600\text{s}^{-1}$ to $378000\text{s}^{-1}$ in order to understand the impact behaviour at these rates.
- Perform inverse FEA using the first order hyperelastic Ogden model to capture the elastic response. The model was later expanded with a 3 term quasi linear viscoelastic model in order to capture the viscoelastic response.
1.3 Outline of the report

Theoretical framework
This chapter highlights the fundamentals and the basics of the theory of continuum mechanics.

Literature review
The anatomy and physiology of skeletal muscle tissue and its relevant published mechanical properties are discussed. Relevant experimental and computational modelling studies on the mechanical properties of skeletal muscle tissue are briefly discussed.

Study I: Uniaxial quasi-static Tensile Test
This study discusses the tensile uniaxial experimental work performed. The samples were loaded in the fibre direction and thereafter, the loading direction is rotated by $30^\circ$, $45^\circ$, $60^\circ$ and $90^\circ$ (when the muscle fibres are running at right angles to the loading direction). The Poisson's ratios were characterised for the fibre and cross-fibre directions.

Study II Impact Compressive Experiments
This study addresses the impact behaviour of muscle. Unconfined impact compressive experiments were performed in the fibre and cross-fibre direction at the following strain rates: $11600\text{s}^{-1}$, $22000\text{s}^{-1}$ and $37800\text{s}^{-1}$. The drop test rig, experimental set up, methods used and results are discussed in this chapter.

Study III Inverse Modelling of skeletal Muscle Tissue
This chapter discusses the inverse FEA investigation using a simple first order Ogden model to capture the elastic response. The first order Ogden hyperelastic material law is thereafter extended with a three-term quasi-linear viscoelastic (QVL) expansion in order to model viscoelastic effects.

Study IV Microscopic Experiments
This study discusses the investigations performed on the microscopic effects of tensile or compressive deforming a specimen by 30% compared. The samples were deformed and then fixed in their deformed state. The un-deformed samples (control) and the deformed samples were sliced and analysed under a compound light microscope and a polarised microscope. An SEM characterised results of the un-macerated and macerated muscle tissue are presented to support the polarised light results.
Discussion, conclusions and future work

The last chapter summarises the results from this thesis and discusses the implications, limitations of the work presented and recommendations for future work.

The flow chart presented below (Figure 1-1) help the reader to visualise and understand the whole thesis process.

![Flow Chart]

Figure 1-1: Presents the full thesis flow chart showing all thesis processes.
2 Theoretical Framework

This research work involves investigative tensile and compressive experimental work along with the modelling and micro-structural analysis of muscle components during different stages of passive loading. In order to provide an introduction and basic understanding of these areas, the following section will focus on introducing the relevant basics continuum mechanics. An understanding of this framework is of vital importance in designing appropriate testing rigs and in setting up the experiments to produce data that is usable by continuum mechanics based models.
2.1 Introduction (Continuum Mechanics)

This section will give a brief outline of the basics of continuum mechanics, four key books will be used as reference to this section, Holzapfel, Moore & Yaqub, Fung and Chandrasekharaih (Holzapfel, 2000, Moore and Yaqub, 1998, Chandrasekharaih and Debnath, 1994, Fung, 1993). The vector and tensor algebra will be briefly introduced, before progressing to discussing the section that concern motion, deformation, strain and stress. These topics are only briefly introduced here. Refer the reader to the above literature for a more detailed explanation.

2.1.1 The continuum hypothesis

Continuum mechanics is based on the assumption that the material body analysed is a continuum and is uniformly distributed throughout regions of space. The material is regarded as indefinitely divisible; it can be continually sub-divided into infinitesimal small elements which still exhibit the same properties as the bulk material. In practise this assumption is only valid up to a certain level (i.e. the mesoscopic scale where the dimensions of the body analysed are large in comparison to the characteristic lengths (e.g. a grain of sand, inter-atomic space) of the body (Spencer, 2004)).

2.1.2 Motion

Let us imagine a body $\Omega$ suspended in $\mathbb{E}^3$ with orthonormal basis $\mathcal{E} = \{e_1, e_2, e_3\}$, and this body is the composition of a set of particles or material point $P$ at time $t$ (Figure 2-1).

Figure 2-1: A continuum material body in the Lagrangian and Eulerian configuration

$\Omega$ is ingrained in a three dimensional Euclidean space at a given instant time $t$ and is in motion from its reference (Lagrangian) configuration $\mathcal{R}_0$ at time $t = 0$ to the new or current (Eulerian) configuration $\mathcal{R}_t$ at time $t = t$. $\mathbf{X}$ and $\mathbf{x}$ are the position vectors of any
point \( P \) belonging to \( \Omega \) defined with respect to the Lagrangian and Eulerian configuration, that have undertaken displacement defined by vector \( \mathbf{u} \). \( \chi \) of point \( P \) is then defined by an equation of the form:

\[
\mathbf{x} = \mathbf{x}(X, t) \quad \mathbf{x}_i = \mathbf{x}_i(x, t)
\]

2-1

Vector \( \mathbf{u} \) represents the displacement of the point \( P \) from its original to its final position. \( \mathbf{p} \) can be defined by:

\[
\mathbf{u}(X, t) = \mathbf{x}(X, t) - X \quad \mathbf{u}(x, t) = \mathbf{x} - \mathbf{X}(x, t)
\]

2-2

2.1.3 Deformation

Differentiation of equation 2-22 with respect to the Lagrangian coordinates leads to:

\[
\frac{\partial \mathbf{x}}{\partial X} = \mathbf{F} \frac{\partial \mathbf{X}}{\partial X} \quad \text{or} \quad \frac{\partial \mathbf{x}_i}{\partial X_j} = \sum_j \frac{\partial \mathbf{x}_i(X, t)}{\partial X_j} \frac{\partial X_j}{\partial X}
\]

2-3

where \( \mathbf{F} = \begin{bmatrix} \frac{\partial \mathbf{x}_i}{\partial X_j} \end{bmatrix} \) is the deformation gradient tensor, which transforms any elementary segment \( d\mathbf{X} \) of \( \Omega \) (defined with respect to the reference configuration) into a segment \( d\mathbf{x} \) (defined with respect to the current configuration). Equation 2.3 can also be defined as:

\[
\mathbf{F} = \begin{bmatrix} \frac{\partial \mathbf{x}_i}{\partial X_j} \end{bmatrix} = \nabla \mathbf{u} + \mathbf{l}
\]

2-4

The inverse of \( \mathbf{F} \) is known as \( \mathbf{D} \) and is defined as:

\[
\mathbf{D} = \mathbf{F}^{-1} = \frac{\partial \mathbf{X}}{\partial \mathbf{x}}
\]

2-5

and transforms any elementary segment \( \partial \mathbf{x} \) of \( \Omega \) in the Eulerian configuration into a segment \( \partial \mathbf{X} \) in the Lagrangian configuration. Now consider the deformation of a volume element (Figure 2-2).

Figure 2-2 A volume element in the Lagrangian (left) and Eulerian (right) configuration
A volume element in the Lagrangian configuration \( dV \) with its sides aligned with the orthogonal basis vectors is defined by:

\[
dV = \left( \partial X_1 \times \partial X_2 \right) \cdot \partial X_3
\]

and in the Eulerian configuration:

\[
dv = \left( \partial x_1 \times \partial x_2 \right) \cdot \partial x_3
\]

The Lagrangian and Eulerian configurations are related according to:

\[
dv = J dV
\]

The quantity \( J \) is referred to as the Jacobian of the deformation gradient tensor and is defined by:

\[
J = \frac{dv}{dV} = \det \left( \frac{\partial x}{\partial X} \right) = \det(F)
\]

In the Lagrangian configuration no deformation has occurred and therefore \( J \) equals 1. However \( J \) will also equal 1 during isochoric deformation (deformation without a change in volume). An incompressible material undergoes an isochoric deformation.

### 2.1.4 Stretch and rotation

In Figure 2-3, let \( \mathbf{a} \) and \( \mathbf{b} \) be unit vectors along \( \mathbf{dX} \) and \( \mathbf{dx} \) respectively and let \( dS \) be the length of an arc element in the Lagrangian configuration and \( ds \) the length of an arc segment in the Eulerian configuration.

![Figure 2-3: Lagrangian arc configuration on the left and Eulerian configuration on the right](image)

The following relationships can then be defined:

\[
\mathbf{dX} = dS \mathbf{a}
\]

\[
\mathbf{dx} = ds \mathbf{b}
\]

Equation 2-3 can then be written as \( ds \mathbf{b} = \mathbf{F} dS \mathbf{a} \) leading to:

\[
\lambda \mathbf{b} = \mathbf{F} \mathbf{a}, \text{ with } \lambda = \frac{ds}{dS}
\]
Where the $\lambda$ is known as the stretch ratio, or simply stretch. In the special case where the vector $a$ is aligned with $b$ the vector $a$ is an eigenvector of $F$ and $\lambda$ is an eigenvalue. However the vectors $a$ and $b$ are generally not aligned and thus the vector $a$ is not generally the eigenvector. This means that the deformation due to $F$ consists of two parts: a stretch and a change in orientation. These parts can be separately expressed following what is known as polar decomposition:

$$F = QU = VQ$$  \hspace{1cm} 2-12

Here $Q$ is an orthogonal tensor and $U$ and $V$ are (positive definite symmetric tensors) related to $F$ according to:

$$U^2 = F^TF$$
$$V^2 = FF^T$$  \hspace{1cm} 2-13

The tensor $Q$ is often referred to as the rotation tensor and $U$ and $V$ are known as the right and left stretch tensors.

Now equation 2-3 can then be rewritten:

$$dx = (QU)dX = Q(UdX)$$  \hspace{1cm} 2-14

The transformation from the Lagrangian to the Eulerian configuration due to $F$ can thus be seen as being composed of two operations: a tri-axial stretch due to the tensor $U$ and a rigid body transformation (rotation, translation) due to the tensor $Q$. Since $U$ is positive definite and symmetric it has one set of mutually orthogonal eigenvectors and three corresponding eigenvalues. The eigenvectors describe the direction of the eigenvalues, which are often referred to as the principal stretches $\lambda_1$, $\lambda_2$ and $\lambda_3$. A similar analysis can be made using the tensor $V$. However in this case the rigid body transformation due to $Q$ precedes the stretch transformations due to $V$. The tensor $V$ has the same eigenvalues as $U$. The tensors $Q$ (rotates, translates), $U$ (stretches) and $V$ (stretches) any elementary segment $dX$ of $\Omega$ in the Lagrangian configuration into a segment $dx$ in the Eulerian configuration. Recall equation 2-11, squaring both sides gives:

$$\lambda^2 = a \cdot (F^TF)a = a \cdot U^2a = a \cdot Ca$$  \hspace{1cm} 2-15

The tensor $U^2$ is better known as the tensor $C$ or the (right) Cauchy-Green tensor and can be used to calculate $\lambda$ when the vector $a$ is known. Similarly the inverse relationship can be obtained for the square of the tensor $V^2$:

$$\frac{1}{\lambda^2} = b \cdot (FF^T)^{-1}a = b \cdot B^{-1}b$$  \hspace{1cm} 2-16
The tensor $\mathbf{B}$ is often referred to as the Finger tensor and its inverse $\mathbf{B}^{-1}$ is known as the Cauchy deformation tensor or Eulerian deformation tensor. Because of their relation with the left and right polar decompositions of the deformation gradient tensor $\mathbf{F}$ the tensors $\mathbf{B}$ and $\mathbf{C}$ are also known as the left and right Cauchy-Green tensors respectively. Since the tensors $\mathbf{U}$ and $\mathbf{V}$ have the same eigenvalues (the principal stretches), the eigenvalues of the tensors $\mathbf{B}$ and $\mathbf{C}$ are also the same; the square of the principal stretches: $\lambda_i^2$. The invariants $I_i$ of $\mathbf{B}$ and $\mathbf{C}$ are defined by:

\begin{align*}
I_1 &= \text{tr}(\mathbf{C}) = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \\
I_2 &= \frac{1}{2} (\text{tr}(\mathbf{C})^2 - \text{tr}(\mathbf{C}^2)) = \text{tr}(\mathbf{C}^{-1}) \det(\mathbf{C}) = \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_1^2 \lambda_3^2 \\
I_3 &= \det(\mathbf{C}) = \det(\mathbf{F}^T \mathbf{F}) = \det(\mathbf{F})^2 = \lambda_1^2 \lambda_2^2 \lambda_3^2
\end{align*}

2.1.5 Stress

The continuum mechanics stresses in a body are due to two types of forces, external and internal forces. External forces act on part or the whole of the boundary surface. When an external force acts on the whole volume of the body (e.g. gravity) it is referred to as a body force. Internal forces act on (imaginary) surfaces within the body. Figure 2-4 shows a surface element $ds$ belonging to the continuum body $\Omega$ in the Lagrangian and a Eulerian configuration.

![Figure 2-4: Surface elements in Lagrangian configuration on the left and Eulerian configuration on the right](image)

The stress is defined as the force per unit area. The forces due to an infinitesimal force $(df)$ acting on a surface element $ds$ of $\Omega$ on Figure 2-4 can be defined using the following:
Where \( t \) represents the Cauchy traction vector (or force per unit area and is measured in the current configuration) exerted on \( ds \) with \( n \) at location \( x \) representing the outward normal vector. The traction vector \( T \) is the first Piola-Kirchhoff traction vector. The traction vector \( T \) is the force per unit area of the reference configuration. Then due to Newton’s law of action and reaction the following is obtained:

\[
t(x, t, n) = -t(x, t, -n) \quad T(X, t, N) = -T(X, t, -N)
\]

2-19

The Cauchy traction vector is the force per unit area of the current configuration. According to Cauchy’s stress theorem there exist two unique tensors \( \sigma \) and \( P \) such that:

\[
t(x, t, n) = \sigma(x, t)n \quad T(X, t, N) = P(X, t)N
\]

2-20

The Cauchy (or true) stress tensor \( \sigma \) and the first Piola-Kirchhoff or Lagrangian stress tensor \( P \) are related to each other as defined below:

\[
\sigma = J^{-1}P F^T \\
P = J\sigma F^{-1}
\]

2-21

In matrix notation \( \sigma \) is:

\[
\sigma = \begin{bmatrix} \sigma_{11} & \sigma_{21} & \sigma_{31} \\ \sigma_{12} & \sigma_{22} & \sigma_{32} \\ \sigma_{13} & \sigma_{23} & \sigma_{33} \end{bmatrix}
\]

2-22

The columns of \( \sigma \) are the components of the traction vectors acting on planes orthogonal to the basis \( E \). A graphical representation of the stress components acting on an infinitesimal cubic material element of \( \Omega \) aligned with \( E \) is show below in Figure 2-5. The Cauchy stress tensor is symmetric, therefore it has six independent components \((\sigma_{12} = \sigma_{21}, \sigma_{13} = \sigma_{31}, \sigma_{23} = \sigma_{32})\).

![Figure 2-5: Schematic representation of stress tensor components acting on a material element](image-url)
The Piola-Kirchhoff stress tensor is asymmetric and often symmetric stress formulations are preferred. However the Piola-Kirchhoff stress tensor can be decomposed as:

\[ P = FS \]  

Here the tensor \( S \) is symmetric and is known as the second Piola-Kirchhoff stress tensor and can be related to \( \sigma \) and \( P \) via:

\[ S = |F|^{-1} \sigma F^{-T} = F^{-1}P = S^T \]

\[ \sigma = J^{-1}FSF^T \]  

It is often more convenient to work with the so-called Kirchoff stress tensor \( \tau \) which differs from the Cauchy stress tensor by the Jacobian \( J \):

\[ \tau = J\sigma \]  

### 2.1.6 Strain energy

In a (thermodynamic) continuum the first law of thermodynamics states there must be a balance of both thermal and mechanical energy (Moran et al., 2011). In special cases where the thermal effects are ignored (isothermal conditions), the first law of thermodynamics is reduced to the following, in order to balance the mechanical energy:

\[ P_{ext}(t) = \frac{dK(t)}{dt} + \frac{d\Pi_{int}(t)}{dt} \]  

The external mechanical power \( P_{ext} \) equals the rate of change of the kinetic energy \( K \) of the mechanical system plus the rate of change of the internal mechanical work \( \Pi_{int} \). The internal mechanical work \( \Pi_{int} \) which is due to internal stresses can be stated as:

\[ \Pi_{int}(t) = \int \Psi dV = \int \Psi|^{-1}dv \]  

\( \Psi \) representing Helmholtz free-energy function (scalar function) which, under the isothermal conditions mentioned coincides with the internal strain energy and can therefore be considered solely as a function of a deformation or strain tensor. For an isotropic material the Helmholtz free-energy function is known as the strain energy density function and may be expressed purely in terms of deformation measures such that the tensors \( F, C \), the invariants \( I_i \) or the principal stretches \( \lambda_i \) are:

\[ \Psi(F) = \Psi(C) = \Psi(I_1, I_2, I_3) = \Psi(\lambda_1, \lambda_2, \lambda_3) \]  

Stress measures can be derived by taking derivatives of the strain-energy density functions with respect to deformation. If we take a homogeneous hyperelastic material,
the following relations can be established between the strain energy density function and selected stress tensors:

\[ P = \frac{\partial \Psi(F)}{\partial F} = 2F \frac{\partial \Psi(C)}{\partial C} \]
\[ S = F^{-1} \frac{\partial \Psi(F)}{\partial F} = 2 \frac{\partial \Psi(C)}{\partial C} = \frac{\partial \Psi(E)}{\partial E} \]
\[ \sigma = J^{-1}F \left( \frac{\partial \Psi(F)}{\partial F} \right)^T = 2J^{-1}F \frac{\partial \Psi(C)}{\partial C} F^T \]
\[ \tau = 2F \frac{\partial \Psi(C)}{\partial C} F^T = 2B \frac{\partial \Psi(B)}{\partial B} = \frac{\partial \Psi(\varepsilon)}{\partial \varepsilon} \]

2.1.7 Constitutive equations

2.1.7.1 Introduction

Elastic materials show a (non-)linear relationship between stress and strain, deform instantaneously due to stress and recover instantaneously once stress is removed. The aim of the constitutive equations is to relate parameters such as strain, strain-rate to the state of stress at any point in a continuum body at any time. Figure 2-6 shows a typical stress-strain curve for an industrial manufactured material. The deformation due to the stress applied can be separated into two regions elastic (recoverable) and plastic (un-recoverable) deformations.

Figure 2-6 A typical stress strain curve for a linear elastic material

The current thesis focusses on elastic soft tissue behaviour and permanent or plastic deformation is beyond the scope of this work. This section will discusses the following constitutive theories: linear elasticity, non-linear elasticity and viscoelasticity.
2.1.7.2 Linear elasticity

A linear elastic solid is a solid body that undergoes an infinitesimal recoverable deformation for which the governing material law is linear (Chandrasekhar and Debnath, 1994), see Figure 2-6. The theory of linear elasticity states that the stress at any time is directly proportional to the strain and is independent of strain history. The stress ($\sigma$) strain ($\varepsilon$) relationship in Figure 2-6 can be described by a special equation called Hooke's law ($\sigma = E\varepsilon$). $E$ is the Young's Modulus and defines the slope of the curve and effectively describes the stiffness of the material. The generalised form of Hooke's law for a homogeneous and isotropic linear elastic material relates the Cauchy stress $\sigma$ to the linear strain tensor $\varepsilon$ as:

$$\sigma = \lambda \text{tr}(\varepsilon) + 2\mu \varepsilon$$

$$\varepsilon = \frac{1}{2\mu} \left( \sigma - \frac{\lambda}{3\lambda + 2\mu} \text{tr}(\sigma) I \right)$$

Two new elastic moduli $\mu$ and $\lambda$ are introduced here and are known as the Lamé parameters. The relationship above can also be presented in a matrix form:

$$\begin{bmatrix} \sigma_{11} \\ \sigma_{22} \\ \sigma_{33} \\ \sigma_{21} \end{bmatrix} = \begin{bmatrix} 2\mu + \lambda & \lambda & 0 & 0 \\ \lambda & 2\mu + \lambda & \lambda & 0 \\ 0 & 0 & 2\mu + \lambda & 0 \\ 0 & 0 & 0 & \mu \end{bmatrix} \begin{bmatrix} \varepsilon_{11} \\ \varepsilon_{22} \\ \varepsilon_{33} \\ 2\varepsilon_{23} \end{bmatrix}$$

where $\varepsilon$ and $\nu$ represent the Young’s modulus and Poisson’s ratio respectively, and this can be connected to the Lamé parameters using the following:

$$E = \frac{\mu(3\lambda + 2\mu)}{\lambda + \mu}$$

$$\nu = \frac{\lambda}{2(\lambda + \mu)}$$

$$\lambda = \frac{2\mu\nu}{1 - 2\nu} = \frac{E\nu}{(1 + \nu)(1 - 2\nu)}$$

$$\mu = \frac{\lambda(1 - 2\nu)}{2\nu} = \frac{E}{2(1 + \nu)}$$

$$\kappa = \lambda + \frac{2}{3}\mu = \frac{\mu E}{3(3\mu - E)} = \frac{2\mu(v + 1)}{3(1 - 2v)}$$
where $\kappa$ represents the bulk modulus and the $\mu$ is the shear modulus.

Most of the synthetic engineered materials can be successfully modelled using linear elasticity especially at infinitesimal strains. However there exists another group of materials like polymers, rubbers and biological soft tissues behave in a non-linear manner and are capable of undergoing large deformations there by making the infinitesimal analysis and linearity assumptions invalid.

### 2.1.7.3 Non-linear elasticity

The limitation mentioned above can be overcome by working with the strain-energy density function $\Psi$ for the derivation of stress formulations. The constitutive laws derived based on a strain-energy density function are known as Green elastic or hyperelastic laws. Different forms of $\Psi$ have been proposed and some are based on physical laws while others on experimental observations. Only a few common constitutive laws will be presented here.

A common formulation is the polynomial hyperelastic (Rivlin and Saunders, 1951) given by the strain-energy density function:

$$\Psi(I_1, I_2) = \sum_{i,j=0}^{N} c_{ij}(I_1 - 3)^i(I_2 - 3)^j$$

where $I_i$ represents the strain invariants (see equation 2-17) and $c_{ij}$ represents the material constants (with $c_{00} = 0$). Numerous strain energy formulations can be derived using different permutations of these constants. For example when $N = 1$ and $c_{11} = 0$ the model reduces to the Mooney-Rivlin hyperelastic:

$$\Psi(I_1, I_2) = c_{01}(I_1 - 3) + c_{10}(I_2 - 3)$$

If $c_{10} = 0$ then the model reduces to the Neo-Hookean hyperelastic:

$$\Psi(I_1) = c_{01}(I_1 - 3)$$

The most commonly used model is the Ogden hyperelastic model (see also (Ogden, 1997, Ogden et al., 2004, Holzapfel, 2004, Ogden, 1972)). The Ogden hyperelastic model's strain energy function for incompressible materials is stated as below:

$$\Psi(\lambda_1, \lambda_2, \lambda_3) = \sum_{i=1}^{N} \frac{\mu_i}{\alpha_i} \left( \lambda_1^{\alpha_i} + \lambda_2^{\alpha_i} + \lambda_3^{\alpha_i} - 3 \right)$$

where $N$ is an integer which must always be greater than zero, in practise $N = 3$, which is normally adequate to succeed in giving a correct correlation (Holzapfel, 2004, Ogden et al., 2004, Ogden, 1997). If the following combination of constants is applied: $N = 2$,
\( \alpha_1 = 2, \alpha_2 = -2 \), the Ogden model is reduced to the Mooney-Rivlin model mentioned above in equation 2-34:

\[
\Psi(\lambda_1, \lambda_2, \lambda_3) = \frac{\mu_1}{2} (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) - \frac{\mu_2}{2} (\lambda_1^{-2} + \lambda_2^{-2} + \lambda_3^{-2} - 3) \quad 2-37
\]

When \( N = 1 \) and \( \alpha_1 = 2 \) are applied, then the Ogden model reduces to a Neo-Hookean as in equation 2-35:

\[
\Psi(\lambda_1, \lambda_2, \lambda_3) = \frac{\mu}{2} (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) \quad 2-38
\]

All the above mentioned models work successfully on incompressible materials \((J = 1)\) only. When dealing with compressible hyperelastic materials, the deformation gradient tensor \( F \) and Cauchy strain tensor \( C \) can be decomposed into a volume changing (volumetric) part, and an volume preserving (isochoric, deviatoric) part (Holzapfel, 2004):

\[
F = \frac{1}{3} \tilde{F} \quad 2-39
\]

\[
C = \frac{2}{3} \tilde{C}
\]

Here \( \frac{1}{3} \) and \( \frac{2}{3} \) called modified deformation gradient tensor \( \tilde{F} \) and the modified strain tensor \( \tilde{C} \) are associated with the isochoric deformation (thus \( \det(\tilde{F}) = \det(\tilde{C}) = 1 \)).

The strain-energy density functions defined a sum of the isochoric part \( \Psi_{\text{iso}}(\tilde{C}) \) and a volumetric part \( \Psi_{\text{vol}}(J) \) (which is a function of the Jacobian):

\[
\Psi(\tilde{C}, J) = \Psi_{\text{iso}}(\tilde{C}) + \Psi_{\text{vol}}(J)
\]

\[
\Psi(\lambda_1, \lambda_2, \lambda_3) = \Psi(\tilde{\lambda}_1, \tilde{\lambda}_2, \tilde{\lambda}_3, J) = \Psi_{\text{iso}}(\tilde{\lambda}_1, \tilde{\lambda}_2, \tilde{\lambda}_3) + \Psi_{\text{vol}}(J) \quad 2-40
\]

Several forms the volumetric part have been proposed including the following that is implanted by FEBio (FEBio, Musculoskeletal Research Laboratories, The University of Utah, USA):

\[
\Psi_{\text{vol}}(J) = \frac{1}{2} \kappa (\ln(J))^2 \quad 2-41
\]

where \( \kappa \) is the bulk modulus. Compressible hyperelastic formulations of the above mentioned constitutive laws may thus be obtained by replacing the role of \( \lambda_i \) with \( \tilde{\lambda}_i \) and by adding \( \Psi_{\text{vol}}(J) \).

### 2.1.7.4 Viscoelasticity

Viscous materials display a time dependent stress response. There are some materials that possess both the elastic and the viscous properties, resulting in these materials being called viscoelastic material (McDonald, 1996). In viscoelastic materials the stress
depends not only on the current deformation but also on the history of the deformation. When a deformation load is suddenly applied to a viscoelastic material, the material is suddenly deformed and if the strain is held constant afterwards, the corresponding stresses induced in the material will decay with time (stress relaxation observed by Fung (Fung, 1993)). If the stress is applied to the material and this stress is held constant over a period of time, viscoelastic materials are observed to undergo a time-dependent strain change called creep (Fung, 1993). Inducing a cyclic loads on viscoelastic materials will results in a different loading/unloading process (hysteresis (Fung, 1993)). This section will give an introductory discussion on common linear and non-linear viscoelasticity modelling approaches. The reader is referred to (Fung, 1993, Dill, 2007) for more detailed information.

2.1.7.5 Linear viscoelasticity

A spring and dashpot will be used in order to simulate viscoelastic material properties. The ideal spring (Figure 2-7a) can be used to represent the linear elastic solid.

![Figure 2-7 A spring (A) and dashpot model (B)](image)

The stress in an ideal spring is elastic and can be expressed (in 3D) by the formula (see equation 2-30):

$$\sigma_e = \lambda_S (\text{tr}(\varepsilon)) I + 2\mu \varepsilon$$  \hspace{1cm} 2-42

An ideal spring is able to allow instant deformation and the stress is dependent only on the strain applied and the spring (material) constants $\lambda_S$ and $\mu$. The dashpot exhibit strain rate dependent response (Figure 2-7b). The stress of an ideal dashpot is defined by:

$$\sigma_v = \lambda_D \left( \text{tr} \left( \frac{\partial \varepsilon}{\partial t} \right) \right) I + 2\eta \frac{\partial \varepsilon}{\partial t}$$  \hspace{1cm} 2-43
Instant deformation is not possible for a dashpot as the stress is dependent on the strain-rate $\frac{\partial \varepsilon}{\partial t}$ material parameters (constants $\lambda_D$ and $\eta$ (dashpot viscosity)).

The viscoelastic behaviour can be modelled by combining springs and dashpots. The Voigt model is shown below (Figure 2-8a), total stress is simply obtained by summing up all the elastic stress $\sigma_e$ due to the spring and the viscous stress $\sigma_v$ due to the dashpot. The Voigt model does not allow for instantaneous elasticity due to the parallel dashpot. This is achieved by adding another ideal spring in front of the dashpot (Kelvin model or standard linear solid model), see Figure 2-8b.

![Figure 2-8 A Voigt (a) and Kelvin or standard linear model (b)](image)

The major problem here is that the internal strain components and the strain histories are unknown. A more convenient way is to use the Boltzmann superposition principle which states that the total effect of applying several deformations is the sum of the effects of applying each one separately (see schematic representation in Figure 2-9). At time $\tau$ an infinitesimal strain increment $d\varepsilon$ will be accompanied by a corresponding infinitesimal stress increment $d\sigma$. The magnitude of this stress increment depends on history of the strain and stress applied (Spencer, 2004) and takes the form $d\sigma(t) = G(t - \tau)d\varepsilon(\tau)$.

![Figure 2-9 Schematic representation of the Boltzmann superposition principle for viscoelasticity](image)
Using Boltzmann's superposition principle leads to the following convolution integral for the total stress for the Kelvin model:

\[ \sigma(t) = \int_{-\infty}^{t} G(t-\tau) \frac{d\varepsilon}{d\tau} \, d\tau \]  

where \( G(t) \) is the relaxation modulus defined by:

\[ G(t) = \mu_\infty + \mu_1 e^{-\frac{t}{\tau_\varepsilon}} \]

\[ \tau_\varepsilon = \frac{\eta_1}{\mu_1} \]

The Kelvin model does not allow modelling of a continuous spectrum of relaxation. A more advanced general model of a linear viscoelastic material can be developed from an assembly of multiple springs and dampers (Dill, 2007) for instance by repeating the parallel spring and dashpot array \( n \) times (Figure 2-10).

![Figure 2-10: Generalised model](image)

When \( n = \infty \), a continuous spectrum of relaxation can be achieved. The stress takes the same form however this time the relaxation function becomes:

\[ G(t) = \mu_\infty + \sum_{i=1}^{n} \mu_i e^{-\frac{t}{\tau_{\varepsilon_i}}} \]

\[ \tau_{\varepsilon_i} = \frac{\eta_i}{\mu_i} \]

As is clear from these equations the relaxation function is composed of a series of negatively decaying exponentials. These series are known as Prony (or Dirichlet) series. The coefficient of the Prony series depends on the spring and dashpot constants which can be determined experimentally. The limitation of the linear viscoelasticity that it is an approximate theory only applicable to situations with infinitesimal strain and rotations (Spencer, 2004, McDonald, 1996).
2.1.7.6 Non-linear viscoelasticity

For finite deformation non-linear viscoelastic materials the non-linear stress-strain characteristics must be accounted for (Fung, 1993). Several non-linear viscoelastic constitutive laws have been derived (see review (Drapaca et al., 2007)) however, this research will focus on the quasi-linear theory of viscoelasticity by introduced by Fung in 1972 (Fung, 1993, Fung, 1972) since this is widely implemented in FEA software. For quasi-linear viscoelasticity (Puso and Weiss, 1998) the second Piola-Kirchhoff stress can be written in the following form:

\[ S(E, t) = \int_{-\infty}^{t} G(t - \tau) \frac{\partial S_e(E, \tau)}{\partial \tau} d\tau \]

with \( S_e \) the pure elastic stress derivable from \( \frac{\partial \Psi(E)}{\partial E} \) where \( \Psi \) may represent any suitable strain energy density function (e.g. a Mooney-Rivlin model (Puso and Weiss, 1998)) including anisotropic material laws. The discrete relaxation function is defined by:

\[ G(t) = \gamma_{\infty} + \sum_{i=1}^{n} \gamma_i e^{-\frac{t}{\tau_i}} \]

with the parameters \( \gamma_i \) and \( \tau_i \) dictating the viscoelastic behaviour. The parameters \( \gamma_i \) are constrained such that (ensuring that eventually \( S(E, t) = S_e \) following relaxation):

\[ \gamma_{\infty} + \sum_{i=1}^{n} \gamma_i = 1 \]

Alternatively the stress response may be written as:

\[ S(E, t) = \gamma_{\infty} S_e(E, t) + \sum_{i=1}^{n} \int_{-\infty}^{t} \gamma_i e^{-\frac{t-\tau}{\tau_i}} \frac{\partial S_e(E, \tau)}{\partial \tau} d\tau \]

As mentioned before, the elastic stress contribution \( S_e \) may be due to an anisotropic constitutive formulation. However to the best of the authors knowledge, no constitutive formulations have been proposed whereby the viscoelastic constants (e.g. \( \gamma_i \) and \( \tau_i \)) are orientation dependant.
3 Literature Review

3.1 Introduction

The structure of the human body is composed of compact solid bones and soft tissue. The bones, because of their mechanical properties, are responsible for giving the body structure and support in order to maintain the posture and shape (Abraham et al., 2013). The hard tissues undergo small deformations and displays linear elastic mechanical properties when subjected to externally applied loads (Valdez and Balachandran, 2013). By contrast, skeletal muscle has evolutionary and functionally modified and adapted to large deformations, and is normally accompanied by nonlinear (Calvo et al., 2010, Gras et al., 2012b, Gras et al., 2012c, Hernandez et al., 2011, Van Loocke et al., 2006, Van Loocke et al., 2008) and viscoelastic mechanical properties that have been well characterised by experimental tests (Van Loocke et al., 2008, Song et al., 2007, Van Sligtenhorst et al., 2006, Noonan et al., 1993, Myers et al., 1995, Gras et al., 2012a, Best et al., 1994, Chawla et al., 2009) or by relaxation behavioural tests (Gras et al., 2013, Anderson, 2001, Bosboom et al., 2001, Myers et al., 1995, Van Loocke et al., 2008, Van Loocke et al., 2009). Muscle tissue is important for the following physiological functions;

- Body movement
- Maintenance of posture
- Impact protection
- Respiration
- Production of body heat
- Constriction of organs and vessels
- Heart beat (Lieber, 2002)

Human locomotion is composed of complex movements which are the result of a combined coordination of the muscular skeletal muscles and the central nervous system. Human body movements are initiated by the central nervous system sending signals to the muscles via the nerves. On receiving the signals, the skeletal muscles initiate a movement by exerting a force on the body segments (Tortora and Derrickson, 2006, Lieber, 2002, Williams et al., 1995). The skeletal muscles are mainly involved in the first four functions listed above: therefore this study will use the phrase ‘skeletal muscle function’ in relation to the first four functions only.
This thesis is focussed on skeletal muscle mechanical properties, yet if mechanical experimental test results are to be meaningfully interpreted, then a good understanding of the skeletal muscle structure and physiological working processes is necessary. Skeletal muscle is made up of bundles of parallel long multinucleated fibres (Williams et al., 1995).

Human skeletal muscle is capable of producing active contractile forces well above 100 watts per kilogram of muscle (Knuth et al., 2006, Josephson, 1985, Gosselin et al., 1998), and extremely active animals like a cheetah for example, has muscles that can produce forces of up 400 watts per kilogram of muscle (West et al., 2013). Skeletal muscle was observed to be fatigue resistant: as demonstrated by Acker et al 1987, where skeletal muscle was shown to perfectly perform well at a cardiac level of work (Acker et al., 1987). Skeletal muscle was observed to be highly functionally adaptable, as demonstrated by the use of latissimus dorsi in cardiomyoplasty procedures (Hooper and Salmons, 1993, Hagege et al., 1990, Barron et al., 2001, Gharaibeh et al., 2012, Jondeau et al., 1995, Magovern et al., 1987, van Doorn et al., 1996).

The structure, function and mechanical properties of muscle have been studied for some time (Herzog, 2000), the study of muscle structure and geometrical arrangement can be traced as far back as 1680 (Borelli, 1680). The relationship between the magnitude of the force that the muscle can exert and its muscle shape, geometry and fibre orientation became a subject of intense research for many years (Gans and Bock, 1965, Benninghoff and Rollhäuser, 1952). The morphology and physiology of skeletal muscle will be discussed briefly in this introduction. A good understanding of the anatomy and physiology of the skeletal muscle is very important, before one can start discussing the mechanical properties of skeletal muscle. The anatomy and physiology of skeletal muscle will be followed by a brief review of the relevant passive skeletal muscle mechanical properties work that has already been published in the area, which also demonstrates the existence of knowledge gaps in the database.
3.2 Skeletal Muscle Background

3.2.1 Skeletal Muscle Physiology & Anatomy

There are three types of muscle in the human body: the smooth muscles, the cardiac muscles and the skeletal muscles (Williams et al., 1995). During the developmental phase, a number of satellite cells fused together, retained their nucleus and gave rise to individual multinucleated muscle cells (Gaudin, 1997, Williams et al., 1995). Muscle fibres are cells which are very long compared to any other cells in the body (Martini and Nath, 1997). The skeletal muscle's main function is to contract which ensures relative motion between bones. The muscle's second function is to provide support and protection by distributing applied loads, as well as cushioning the forces applied by the loads, before transferring these to the bones (Herzog, 2000, Williams et al., 1995). Muscle structure is defined by its fibre arrangements with respect to the direction of load.

![Figure 3-1: Structure of skeletal muscle. (Reproduced from http://www.gffi-fitness.org/muscle-tissues-structure/)](http://www.gffi-fitness.org/muscle-tissues-structure/)
Figure 3-1 shows a schematic representation and classification in architectural characteristics of skeletal muscles. The most common muscle architectures are the parallel fibred and the pennate muscles. Through aponeuroses (tendon-sheets) and tendons, the muscle fibres are attached to the bone structure at origin and insertion. The pennate muscle fibres are short and run at an angle to the direction of loading. The parallel fibred muscle's fibres run parallel to the loading direction. Some muscle fibres do not span the full length of the muscle; therefore the collagenous connective tissue plays a crucial role in transmitting the axial loads from these short muscle fibres to the tendons (Huijing, 1999, Purslow, 2010, Huijing, 2009, Sharafi and Blemker, 2011).

Skeletal muscles are externally activated by a signal from the nervous system. These signals set off a chemical chain reaction process that will initiate an actin filament and myosin filament connection. The connection between the actin filaments and the myosin filaments forms what is called a cross-bridge (Herzog, 2000). The fact that the muscle is attached to bones at both ends by the tendons means that a contracting muscle force generate a pulling force at both ends of the muscle (which is the force experienced by the attached tendons). The result of this muscle force is dependent on whether the force is large enough to cause the muscle length to shorten (concentric muscle contraction). This will be called isometric contraction if the force is too small to produce any change in length, or eccentric contraction if the muscle is still forced to elongate while the muscle is applying a contraction force (Herzog, 2000).

About 70-80% of muscle mass is made up of water (Heymsfield et al., 1983, Vignos and Lefkowitz, 1959), between 2 to 3% is fatty tissue and the collagenous connective tissue makes up to 10% of the muscle mass (Tortora and Derrickson, 2006, Williams et al., 1995, Vignos and Lefkowitz, 1959). These are average figures which differ from individual to individual and are muscle type dependent (Van Loocke et al., 2008, KJÆR, 2004). The health state of the muscle was shown to affect the above muscle content ratios, notably, significant differences occurred with collagen (KJÆR, 2004, Vignos and Lefkowitz, 1959). Collagen is composed of long-chain protein molecules, consisting of various amino acids and glycine, which are arranged in a triple helix. Collagen frequently adopts an oriented, fibrous form, so fibre orientation is a very important factor. The collagen fibrils are not isolated structures but exist in an extracellular matrix. These are superstructures that result from complex interactions between collagenous and non-collagenous components (Lethias et al., 1996).
Skeletal muscle exhibits a fibre-oriented structure; with each muscle composed of fascicles containing bundles of fibres which themselves are composed of parallel bundles of myofibrils (Figure 3-2). There is a dense network of connective tissues which surrounds groups of muscle fibres and this too has a hierarchical structure. Epimysium surrounds the whole muscle, perimysium surrounds bundles of muscle fibres and endomysium surrounds each individual muscle fibre.

### Table 3-1: Mechanical properties comparison between Skeletal Muscles fibres and collagen fibres (Martins et al., 1998).

<table>
<thead>
<tr>
<th></th>
<th>Young Modulus (GPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Strain to Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal Muscle</td>
<td>$6 \times 10^{-6} - 8 \times 10^{-4}$</td>
<td>0.1</td>
<td>0.161</td>
</tr>
<tr>
<td>Collagen</td>
<td>1</td>
<td>50-100</td>
<td></td>
</tr>
</tbody>
</table>

Collagen fibres were shown to be very stable and possessed very good mechanical properties compared to skeletal muscles (Table 3-1). The ratio of collagen fibre length
to collagen fibre diameter is 200 in tendons; therefore the tendons display very poor compressive properties (Quaglini, 2000).

3.2.2 Mechanical Properties of Skeletal Muscle

Advances in the other areas of science, engineering and in the medical field, require a fundamental and qualitative understanding of the biomechanical properties of skeletal muscles. The mechanical properties are very important to skeletal muscles as they help them to perform their biological function (Butler et al., 2000). There have been a number of skeletal muscle models that claim to have the capability to predict the nonlinear and transversely isotropic behaviour of soft tissue. The limitation of many of these models is a lack of experimental data to back up these predictive capabilities claims (Van Loocke et al., 2006). Most of the earlier mechanical testing carried out in the past has been centred around experimental tests on isolated muscle fibre, which led to the formulation of the sliding theory and the bridge theory of muscular contraction (Herzog, 2000, Huxley, 1957). The work carried out in the early seventies by authors like Huxley and Simmons met with great challenges, especially in isolating muscle fibres, testing and monitoring results at cross-bridge level (Herzog, 2000, Huxley and Simmons, 1970). The establishment by Herzog that the experimental test results will be the same, irrespective of whether the test is conducted at muscle level or fibre level, played a crucial role in eliminating the need for extracting the fibres from muscles, which in itself is a very difficult and time-consuming task (Herzog, 2000, Huxley and Simmons, 1970). This was however contradicted by a more recent work performed by Lewis and Purslow (Lewis and Purslow, 1990). Huxley and Simmons’ observation ignores the fibre to fibre relationship during tissue deformation, especially the ability of individual muscle fibres to mobilise by transferring force to adjacent muscle fibres when they are loaded close to the threshold (Street, 1983, Purslow, 2010, Huijing, 1999, Huijing, 2009). The first two study chapters will be investigating the mechanical response of skeletal muscle at macroscopic level and the last study chapter will concentrate at investigations of muscle fibre level micro-structural response.

Blemker and Delp hypothesised that muscle tissue can be considered as a transversely isotropic material with fibres (fibre direction) axis defining the plane of symmetry (Blemker and Delp, 2005). If this was to be adopted, then the transversely isotropic material will need to be characterised by testing in the fibre direction, cross-fibre direction as well as at intermediate fibre angles. Surprisingly, several researchers
have characterised muscle in the fibre direction (Anderson et al., 2002, Boriek et al., 2001, Gosselin et al., 1998, Hawkins and Bey, 1997, Van Loocke et al., 2006, Davis and Carlson, 1995, Davis et al., 2003, Gareis et al., 1992, Hete and Shung, 1995, Lin et al., 1999, Linder-Ganz and Gefen, 2004, Muhl, 1982), but only a few studies have characterised the cross-fibre directional response (Aimedieu et al., 2003, Van Loocke et al., 2006, Bosboom et al., 2001, Mathur et al., 2001, Morrow et al., 2010b) and no tensile tests have been reported at intermediate angles.

3.2.2.1 Tensile Strain behaviour of Muscles

Tensile response of skeletal muscle to external loading has been an area of research interest for a long time. The active response of skeletal muscle has been well researched, and the force displacement relationships are well documented (Davis et al., 2003). Only tensile data where tests were performed in more than one fibre load orientation will be discussed in detail. The tensile response of passive skeletal muscle also found to be nonlinear, viscoelastic (Martins et al., 1998, Meyers, 1994, Lissner et al., 1960, Yamada, 1970) and transversely isotropic (Blemker and Delp, 2005, Morrow et al., 2010b), several authors successively modelled skeletal muscle as transversely isotropic material (Heidlauf and Rohrle, 2013, Chaudhry et al., 2008, Holzapfel and Kuhl, 2012, Morrow et al., 2010a).

Morrow et al, 2010 investigated loading response of the rabbit’s extensor digitorum longus skeletal muscles (Morrow et al., 2010b), see Figure 3-3. The loading rate applied was 0.05% s⁻¹, and the fibre orientation tested were; fibre direction, the cross fibre direction and the longitudinal shear. Morrow et al, 2010 shows the fibre direction to be stiffer than the cross-fibre direction. Morrow’s data is presented in such a way that it is difficult to compare with other 3D tensile data. The uniqueness of Morrow’s data is that, to the best of the author’s knowledge is the only published data that reports that the muscle fibre direction response of freshly harvested skeletal tissue is stiffer than the cross-fibre response (see Figure 3-3).
Calvo et al, 2010 investigated the passive response of skeletal muscle to external loads using rat *tibialis anterior* skeletal muscles (Calvo et al., 2010), see Figure 3-4. The tissue was experimentally tested within 10 minutes after the animal has been sacrificed. The strain rates were 0.025% s⁻¹ and the stretches were up to 1.72. A fibre reinforced model was proposed by this paper using the energy density function. The model employed a large number of parameters. The model contains straightened fibres after a fibre stretch of approximately 1.79, which is well beyond the experimental achieved stretches of 1.72. Some parameters included in the model, do not have any contribution at all in the range tested in the experiments.

Freshly harvested porcine skeletal muscle samples were recently subjected to different tensile strain rates by Nie et al. 2011 (Nie et al., 2011). The results show a nonlinear anisotropic response which is dependent on the strain rate (Figure 3-5).
The viscoelastic effects are highlighted in the results and transversely isotropic behaviour is evident as the cross-fibre response is higher than the fibre direction response. In very recent work by Hernandez on stomach muscles there was unfortunately no attempt made to characterise the muscle fibre orientation and thereby making them incomparable with other published work by other authors (Hernandez et al., 2011).

Figure 3-5: Tensile skeletal muscle response observed by Nie et al, 2011 (adapted from Nie et al, 2011).

3.2.2.2 Conclusion

The tensile response of passive skeletal muscle is normally presented as nonlinear and viscoelastic (Nie et al., 2011, Calvo et al., 2010, Martins et al., 1998, Meyers, 1994, Grieve and Armstrong, 1988, Yamada, 1970, Lissner et al., 1960). Figure 3-6a and Figure 3-6b show the fibre direction tensile stress-stretch response observed by different authors, and some of the variability in the data may be a result of viscoelasticity. However, the data of Morrow et al shows the fibre direction to be stiffer than the cross-fibre direction, but the data of Nie et al indicates the opposite, see Figure 3-6(b), though Nie's work was at different strain rates. Morrow et al and Nie et al both report higher stresses than other researchers (Nie et al., 2011, Morrow et al., 2010b).
Figure 3-6: Literature findings for tensile stress - stretch response for skeletal muscle: (a) fibre direction responses, (b) comparison between fibre and cross - fibre direction responses.

3.2.3 Compressive Behaviour of skeletal muscle

Compressive behaviour of muscles has only started attracting research interest only recently. Grieve and Armstrong 1988 carried out unconfined compressive tests on porcine muscle tissue (Grieve and Armstrong, 1988). Experiments were carried out at different strain rates. The authors observed the following properties (see Figure 3-7), skeletal muscle tissue gave a viscoelastic nonlinear response.

Figure 3-7: Nonlinear viscoelastic unconfined compressive response of porcine muscle as observed by Grieve and Armstrong 1988 (Reproduced from Grieve and Armstrong 1988).
The samples were frozen on the day of the slaughter, no specific time between death and freezing is given, therefore Rigor Mortis cannot be ruled out. These samples were then defrosted for testing. Rigor Mortis investigation performed by Van Loocke show that it to be a significant factor after 2 hours (following death of the animal) (Van Loocke et al., 2006). The author does not mention if fibre direction was characterised.

Bosboom et al. 2001 managed to compressively load the *tibialis anterior* muscles of rats subcutaneously anaesthetised with a combination of ketamine (100 mg kg\(^{-1}\)) and xylazine (10 mg kg\(^{-1}\)) (Bosboom et al., 2001). Ramp and hold (held for 20 s) tests were performed at impact speeds of 25 m/s and the tests were performed to different stretch ratios. Stretch ratio dependant stress relaxation behaviour was established.

Dhaliwal used volunteers and cadavers to research low impact loading of the lower limb (Dhaliwal et al., 2002). In volunteer experiments, a pendulum test speed of up to 2.5 m/s was used to generate impact compressive loading data. Extrapolation was used to generate high impact data. High impact testing experiments were carried out on cadavers, but due to the amount of cadaver waxing, the results obtained remain an approximation (Dhaliwal et al., 2002). Muggenthaler used two sets of experimental volunteers for two different sets of experimental data for their models validation. A swinging pendulum and a drop test mass were used to deliver impact on the skeletal muscle tissue (Muggenthaler et al., 2008). They used the rebound height and dissipative energy to work out the muscle response to impact loading. While the displacement and acceleration graphs are presented, no effort is made to present the results in the normal stress/strain method.

Porcine *gluteus maximus* muscle tissue was cyclically compressively loaded at 5 to 30 Hz by Aimedieu (Aimedieu et al., 2003). However, their results were limited to the Kevin-Voigt model parameters. They observed an increase in stiffness parameter with increase in loading frequency.

Gefen et al. 2005 performed indentation experimental tests on exposed *gracilis* rat muscles (Gefen et al., 2005). They reported a long term modulus in the range of 0.345 to 0.730 kPa. Pelevski et al. also performed high speed indentation tests on *gluteus maximus* muscle samples (Palevski et al., 2006), and reported a long term shear modulus of around 0.7 kPa. Both these authors considered muscle as isotropic linear and elastic material.
Van Sligtenhorst et al. 2006 investigated the compressive behaviour of skeletal muscles at very high strains (Van Sligtenhorst et al., 2006). The author used bovine samples and observed a stiffness response that was highly dependent on the strain rate used due to viscoelastic nature of the muscle. The authors report observing an upward concave (nonlinear curves which are typical of all biological tissue response if load is applied in the fibre direction), but the curves presented appear to be broadly linear (see Figure 3-8). The tissue investigated was post Rigor Mortis.

![Figure 3-8: Bovine large strain compressive response (adapted from Van Sligtenhorst et al. 2006)](image)

Song et al. 2007 also investigated the behaviour of porcine skeletal muscle at very high strain rates. They conducted their tests at strain rates range of between 0.007s\(^{-1}\) to 3700s\(^{-1}\) and the compressive strain was up to 50% (Song et al., 2007).

![Figure 3-9: Porcine low strain rate to high strain rate response observed by Song et al. (Song et al. 2007).](image)
Song et al, 2007 observed a nonlinear, viscoelastic and strain dependent response in both the fibre and cross-fibre cases. Song et al, 2007 also observed the anisotropy behavioural response at the strain rates tested (Song et al., 2007), see Figure 3-9.

Van Loocke et al, 2006 investigated the quasi-static nonlinear elastic properties of skeletal muscle tissue. The author used freshly harvested porcine gluteus *maximus* skeletal muscle tissue, at strain rates of \(0.05\text{s}^{-1}\) and up to a 30% strain. The specimen loading direction with respect to the fibre direction was as follows; \(0^\circ, 30^\circ, 45^\circ, 60^\circ\) and \(90^\circ\) \((0^\circ\) being the fibre direction and \(90^\circ\) being the cross-fibre direction\), see Figure 3-10. Markers were placed on the specimen surface in order to track marker displacements; this enabled the author to calculate Poisson's ratios. All their tests were performed using freshly harvested porcine tissue, as aged tissue (post Rigor Mortis) is stiffer. Van Loocke observed the aged tissue to display different mechanical properties to fresh tissue in that, fresh tissue displays anisotropic behavioural accompanied by a very compliant toe for the fibre directional tests, while aged tissue presents a small toe to no toe at all and is isotropic response (Van Loocke, 2007), see Figure 3-11.

![Figure 3-10: The skeletal muscle response as observed van Loocke et al, 2006. F is the fibre direction, XF is the cross-fibre direction and 45F is 45 degrees to the fibre direction.](image-url)
An elastic nonlinear and tissue anisotropy behaviour response were observed, see Figure 3-10. The direction perpendicular to the muscle fibre direction was found to give the stiffest response when the skeletal muscle was loaded in compression at a quasi-static strain rate of 0.05%\(s^{-1}\). This was followed by the fibre direction, and the least stiff response was found to be when the skeletal muscle was loaded at 45 degrees to the muscle fibre direction. A Poisson’s ratio of 0.5 in both transverse directions (T and T’ see Figure 3-12) arising from the compressive load applied in the fibre or longitudinal (L) direction, indicating isotropic and nearly incompressible behaviour for this mode of loading. However, when a compressive load was applied in a transverse direction (T), the resulting expansion in the fibre (L) and the remaining transverse (T’) directions was characterised by Poisson’s ratios of 0.36 and 0.65 respectively, see Figure 3-12(Van Loocke et al., 2006), again indicating nearly incompressible but this time also anisotropic behaviour.

\[
\begin{align*}
v_{LT} &= v_{LT'} = 0.5 \\
v_{TT'} &= 0.65 \\
v_{TL} &= 0.36
\end{align*}
\]

Figure 3-12: Schematic illustration of muscle fibre directions: longitudinal (L) and transverse directions (T and T). On the right are the Poisson’s ratios reported by Van Loocke (Van Loocke et al., 2006).
In 2008, Van Loocke et al., conducted compressive investigation of freshly harvested porcine *gluteus maximus* muscles at much higher strain rates (0.5% s⁻¹, 1% s⁻¹, 5% s⁻¹ and 10% s⁻¹). The loading direction in relationship to the fibre orientation was as follows; 0°, 45°, 60° & 90°.

Stress–relaxation curves were also included in the author's published work. All these observation highlight the fact that skeletal muscle displays viscoelasticity in addition to being nonlinear and anisotropic when loaded at the reported strain rates (Figure 3-13).

### 3.2.4 Impact Response of Skeletal Muscle

The multi-axial compressive properties of fresh skeletal muscle at rates experienced during typical sports and automotive impacts are not well understood (Van Loocke et al., 2009). Therefore, although finite element human body models including muscle tissue are routinely used in impact biomechanics research, their utility remains limited mainly by uncertainties in the constitutive representation of the soft tissues.

Tests on fresh animal tissue at very low strain rates (approx. 0.05% s⁻¹) have shown that the compressive stress response in skeletal muscle depends on the strain
rate (Nie et al., 2011, Van Loocke, 2007, Song et al., 2007, Sacks, 2000, Van Sligtenhorst et al., 2006) and on the angle between the fibre orientation and the loading direction (Van Loocke et al., 2006, Nie et al., 2011, Morrow et al., 2010b). Van Sligtenhorst et al. investigated the compressive behaviour of bovine skeletal muscle samples at 10%s\(^{-1}\), 100000%s\(^{-1}\), 170000%s\(^{-1}\) and 230000%s\(^{-1}\) strain rates (Van Sligtenhorst et al., 2006) and observed a stiffness response that was highly dependent on the applied strain rate. Dhaliwal performed high impact testing experiments using cadavers (Dhaliwal et al., 2002) and low impact on volunteer tests on the lower limb were performed, but it was not possible to extract stress-strain data by this method.

Chawla performed compressive impact tests but they used surgical scraps of human tissue which had been frozen and then thawed and the state of Rigor Mortis is unfortunately unknown in those tests. Furthermore, they tested only in the cross-fibre direction and did not report quasi-static results so that the evolution of strain rate effects cannot be readily assessed (Chawla et al., 2009).

Comparison of the published data is difficult as experimental protocols vary and there is no standard manner of presenting the experimental data. To enable comparison, for each experimental protocol, the stress value from the lowest strain rate was taken as a reference and the ratios between this value and values obtained at higher rates were calculated. The resulting stress ratios were then combined to show the increase in stress from a reference strain rate of 0.05%s\(^{-1}\) (which now gives a ratio of 1), see Figure 3-14. Only data from Van Loocke and Song satisfied these requirements. While Chawla (Chawla et al., 2009) performed tests on human tissue at the relevant strain rates, they unfortunately used post Rigor Mortis samples and did not report quasi-static results so that the evolution of strain rate effects cannot be readily assessed. The normalised results from Song et al and Van Loocke are then plotted into a single graph which is presented in Figure 3-14 (a logarithmic scale was used to better visualise the results). Figure 3-14 shows that a gap exists for data between 3200%s\(^{-1}\) (highest rate for (Van Loocke et al., 2009)) and 54000%s\(^{-1}\) (lowest rate for (Song et al., 2007)).
An almost continuous evolution is observed in the results. A knowledge gap is observed between a strain rate of 3,200% s\(^{-1}\) to 54,000% s\(^{-1}\).

The need for experimental data for impact biomechanics applications can be assessed by the following order of magnitude comparison: in a 48 km/h unrestrained frontal vehicle impact, assuming the occupant’s body strikes the vehicle interior at 48 km/h, the tissue compression rate is 13.33 m/s. For a mid-body region muscle thickness of 5 cm, this represents a compression rate of around 25,000% s\(^{-1}\) (Van Loocke et al., 2009), but combined fibre/cross-fibre data at these rates is not generally available. Therefore, there is a need for more additional impact data, see also, see Figure 3-14.

### 3.2.5 Micro-structure analysis

The passive mechanical properties of skeletal muscle depend on the characteristics of its substructures, muscle fibres thickness, endomysium collagen diameter, perimysium collagen fibre diameters and the extracellular matrix thicknesses (Fang et al., 1999, KJÆR, 2004, MacIntosh et al., 2005). However, the individual contributions of these individual components remain unclear. Skeletal muscle is made up of parallel striated muscle cells, all surrounded and held in place by connective tissue (Williams et al., 1995). Skeletal muscle is the only muscle that animals have control over (Williams et
al., 1995, Tozeren, 2000, MacIntosh et al., 2005, Martini and Nath, 1997, Martins et al., 1998). Skeletal muscles are long parallel cells which are derivatives of small individual satellite cells (Tortora and Derrickson, 2006, Williams et al., 1995, MacIntosh et al., 2005, Martini and Nath, 1997).

Skeletal muscle fibres do not always span the whole length of the muscle, but terminate within the muscle (Tortora and Derrickson, 2006, Williams et al., 1995, Martini and Nath, 1997). Therefore this requires that the force within muscle fibres to be laterally transmitted to adjacent muscle fibres in order to initiate movement (Lieber, 2002, Ounjian et al., 1991, Street, 1983, Monti et al., 1999, Mass et al., 2003, Huijing, 2009). Skeletal muscle fibres do not always extend from one tendon plate to the other, which requires that a contractile force can be transmitted laterally to adjacent fibres to initiate movement (Ounjian et al., 1991, Huijing, 2009, Monti et al., 1999).

One of the most important breakthroughs was made by Street (1983) when he provided evidence that confirmed that two pathways of force transmission existed within muscles (Street, 1983). Their experiments demonstrated that forces within a muscle were transmitted along the muscle fibres as well as laterally through an extracellular matrix to endomysium and up to the perimysium as well (Monti et al., 1999, Street, 1983, Mass et al., 2003, Huijing, 2009). Street performed single muscle fibre tensile experiments on frog muscles. Street severed all skeletal muscle fibres, apart from the centre fibre. Street then applied a tensile load, which was found to be distributed to the surrounding muscle fibres (see schematic illustration in Figure 3-15).

![Figure 3-15: Illustration of Street's testing splint. All the myofibres in the 1 mm diameter splint were cut, except one which lay on the upper surface. An extending force was applied to the sarcomere of the intact myofibre, and the force was observed to have been laterally transmitted to the surrounding adjacent muscle fibres.](image-url)
Figure 3-15 schematic drawing illustrate why there is no need for muscles to span the whole muscle length, but this did not explain what role skeletal muscle structure played in these lateral force transmissions. Street's experiment showed that muscle fibres are able to transmit the load to neighbouring adjacent fibres, but did not show how the micro-structure components are affected by external load deformations. The changes of muscle fibre cross-sectional shape and cross-sectional muscle fibre orientation changes in response to different types of loading regimes have not been characterised by any published work.

Several authors have demonstrated that the extracellular matrix (ECM) tensile compliance is greater than that of myofibrils. However some authors suggest that the force transmission takes place through shearing mechanism (Huijing et al., 1998, Monti et al., 1999, Passerieux et al., 2007, Purslow, 2010, Purslow and Trotter, 1994, Sharafi and Blemker, 2010, Tidball and Chan, 1989).

Collagen fibres make up the bulk of the connective tissue, this provides the tissue with a good combination of flexibility as well as tensile stretch (Laurinavicius et al., 2011, Fang et al., 1999, Tortora and Derrickson, 2006, Williams et al., 1995). In 1991, Yang and Taber suggested that the extracellular matrix fluid movement has an effect on the viscoelastic behavioural response of passive myocardial tissue (Hamilton et al., 2012), this is similar to muscle tissue hypothesis suggested by Van Loocke later (Van Loocke et al., 2008) and used as bases for micro-structural model developed by Gindre (Gindre et al., 2013).

Skeletal muscle is made up of intramuscular connective tissue, which is functionally divided into 3 organisational levels: the epimysium encloses the whole muscle, the perimysium surrounds the fascicles, and the endomysium ensheathes individual muscle fibres (Freeman and Bracegirdle, 1982, Gaudin, 1997, Ross et al., 1989, Tortora and Derrickson, 2006, Williams et al., 1995).

### 3.2.5.1 Perimysium

The perimysium is a continuous network of connective collagen tissue that divides the muscle into fascicles commonly called muscle fibre bundles sometimes. The perimysium network was first observed by Rowe who described them as crimped fibres running through muscle fibres (Rowe, 1974). These muscle fibres bundles (fascicles not muscle fibres) span the full length of the muscle from tendon to tendon (Tortora and Derrickson, 2006, Williams et al., 1995), therefore transmit the force.
loads between tendons. At the ends, the muscle fibres form highly folded interdigitating joints (the myotendinous junction) with the tendon at this point (Purslow, 2002, Tortora and Derrickson, 2006, Williams et al., 1995, Huijing et al., 1998). The perimysial layers form a fenestrated network that extends across the entire cross-section of the whole muscle, and a shared structure lying between fascicles. At the surface of the muscle the perimysium merges and seamlessly joins with the epimysium (Nishimura et al., 1996, Purslow, 2010, Toldra, 2003, Tortora and Derrickson, 2006). In porcine semitendinosus muscle the degree of waviness has been observed to increase with animal age (Fang et al., 1999, Huijing, 2009, Monti et al., 1999). In a few muscles (e.g. bovine semitendinosus) there are substantial amounts of elastin fibres associated with the collagenous network (Purslow, 2010, Rowe, 1981). The perimysium was divided into secondary perimysium (very obviously thick perimysium collagen fibred network) and primary perimysium network (thinner perimysium normally sub-diving the fascicles already divided by the secondary perimysium (Fang et al., 1999, Ham, 1967). The perimysium was observed to undergo marked changes in fibre orientation as a function of sarcomere length (Purslow, 1989, Purslow, 2010, Rowe, 1974). The collagen fibres are arranged parallel to each other and lie at 55 degrees to the muscle fibre axis at the resting length of the muscle, see Figure 3-17. This angle is thought to change with a change in muscle length, varying from around 80 degrees at an extremely short sarcomere length of 1.1 mm to approximately 20 degrees at a long sarcomere length of 3.9 mm (Purslow, 2010, Podolsky, 1964). The perimysium is thought to be easily deformed in tension until the collagen fibres becomes almost aligned parallel to the stretching muscle fibres and the waviness in the perimysium collagen fibre network pulled out straight (Purslow, 2010). Passerieux suggested that the perimysium also transmit lateral forces (Passerieux et al., 2007).
Figure 3-16: Low magnification SEM photograph in the longissimus muscle. P; perimysium, E; endomysium (Nakamura et al., 2003). The PP; primary perimysium was added as an interpretation from (Fang et al., 1999)

Figure 3-17: SEM image of the perimysium collagen fibres, the green lines highlight the perimysium collagen fibre orientations and the blue lines highlight the muscle fibre orientation. The perimysium collagen fibres were observed to run in two parallel lines that were +55° and -55° to the muscle fibre axis. Reproduced from (Purslow, 2010)

3.2.5.2 Endomysium

At the bottom of the intramuscular connective hierarchical is the endomysium collagen fibre network. The endomysium collagen fibres are randomly oriented and organised into honey comb like structures (Nishimura, 2010, Nishimura et al., 1996, Passerieux
et al., 2007, Purslow and Trotter, 1994, Trotter and Purslow, 1992, Fang et al., 1999). These honey-comb structures house muscle cell fibres (see Figure 3-18). This is true even for the parallel muscle fibres that do not span the whole muscle length as some muscle fibres were observed to terminate mid muscle belly by tempering into the surrounding perimysium (Trotter, 1991, Monti et al., 1999, Bardeen, 1903, Gaunt and Gans, 1990, Huijing, 1999, Young et al., 2000). This immediately raises the question of how tension is passed from intrafascicular fibre terminations to the muscle tendon. As already pointed out, the tensional forces have been observed to be transmitted between adjacent myofibres through endomysium (Huijing, 2009, Huijing et al., 1998, Purslow, 2010, Sharafi and Blemker, 2011, Monti et al., 1999, Koo et al., 2013, Street, 1983). The endomysium network does not contain cells (Purslow, 2002, Trotter and Purslow, 1992, Tortora and Derrickson, 2006).

![Figure 3-18: SEM images after the bovine sternomanibularis muscle has been digested by NaOH. The cross-sectional view of the collagen structures of perimysium and endomysium (X100) (A), oblique view of approximately honey-comb-like endomysium structures (x3200) (B) and a closer look at randomly oriented endomysium collagen fibres (X122000 (C). (Reproduced from (Trotter and Purslow, 1994))](image)

3.2.5.3 Conclusion:

While published data available show the likelihood of the collagen fibres being responsible for the passive skeletal muscle response to external loading. Previous studies have been unable to accurately characterise the relationship between the muscle fibres micro-structural changes and change in strain and loading direction. Therefore the objective of the micro-structural thesis chapter was to characterise the micro-structural deformation response of skeletal muscle samples that have been subjected to large deformation.

3.2.6 Asymmetrical Behaviour

Skeletal muscle response from various published work by various authors was captured and plotted on the same graph (see Figure 3-19), While the tensile data is shown to be way stiffer than the compressive response, thereby highlighting the tension/compression asymmetrical response of skeletal muscle.
Figure 3-19: Published work data shows asymmetrical muscle fibre response to passive loading is shown. The tensile data from all authors show that the tensile response is several times stiffer than the compression response.

The compression data had to be re-drawn separately in order to show the amount of variation and magnitudes that existed within the data. Figure 3-20, presents typical stress-stretch curves extracted from literature. The Vannah et al. and Zheng et al. experimental data is compared to bovine in vitro data presented by Grieve and Armstrong. This is all then compared to theoretical model data based on in vivo and in vitro compression of rat muscles. The data presented by Vannah et al. was obtained from quasi-static strain rates. Zheng et al. data was obtained from indentation speeds ranging from 0.75 to 7.5 mms⁻¹. This corresponds to strain rates ranging from approximately 4 to 40%s⁻¹. A Viscoelastic material property was observed.
Figure 3-20: The compressive response which almost seems flat and almost similar due to the large tension scale in Figure 3-19 above, is shown to be more variable. Presented above is the porcine compression data from Van Loocke (referred to as VLM, this includes the aged tissue response), the bovine experimental results from *in vitro* from Grieve and Armstrong et al., the theoretical curves based on human *in vivo* indentation of human bulk muscular tissues from Vannah et al. and Zheng et al. and finally the rat *in vivo/in vitro* compression data from Bosboom et al.

### 3.3 Conclusions

The data from Morrow et al. shows the fibre direction to be stiffer than the cross-fibre direction, but the data of Nie et al. indicates the opposite, see Figure 3-6b. There appears to be no data on the Poisson’s ratios during tensile loading, though these data have been reported for compressive loading (Van Loocke et al., 2006). While the data from Morrow et al and Nie et al contradict each other in relation to anisotropy, both of their data sets are significantly stiffer in the fibre direction than previously observed by other authors, see Figure 3-6a. It is evident therefore that there remain significant gaps in our understanding of the three-dimensional tensile/compression response of passive muscle tissue to applied loading, particularly since the tensile response at intermediate fibre directions and the Poisson’s ratios have not been reported. An asymmetrical skeletal muscle response between compressive and tensile loading was observed (see Figure 3-19). This could not be explained by normal macroscopic observations and analysis. This was assumed to be the main contributing factor as to why all the finite element models are limited, even though these models are provided with both descriptions of the hard and soft tissue geometries. As a result of the
observed asymmetrical response, a major goal of this thesis chapter was to perform a micro-structural characterisation to explain this unique behavioural response. This work was performed to investigate the skeletal muscle component relationship during compressive/tensile loading at micro-structural level in order to provide more understanding of the asymmetrical response. However, the Nie et al. data is at a higher strain rate and shows an unusual concave (downwards) rather than the more normal convex (upward) stress-strain relationship, see Figure 3-6. Morrow et al. appear to have used aged tissue which has undergone Rigor Mortis. This would explain why their data is much stiffer than any other data performed at a similar strain rate. This may well explain the loss of the toe regions when the results of their experiments performed in the fibre direction are examined. The pre-load could as well have played a major factor as well.

In light of the all the knowledge gaps highlighted in this section, the thesis objectives are restated below:

- Quasi-static uniaxial tensile experiments were performed in order to characterise the quasi-static response of skeletal muscle to external tensile loading, as well as characterising the influence of the skeletal muscle fibre orientation.
- Comparison of the quasi-static uniaxial tensile experimental results to published quasi-static compressive data was done.
- Micro-structural characterisation of micro-structures was performed in order to understand the observed macroscopic behavioural response.
- Impact compressive loading experiments were performed for strain rates ranging from 11,600%\(s^{-1}\) to 37,800%\(s^{-1}\) in order to understand the muscle behaviour at viscoelastic levels.
- A simple hyperelastic Ogden model was used to capture the fibre direction quasi-static response. Inverse analysis was also performed on the impact data (the deformed configuration was taken as a starting geometry and then solved for the un-deformed geometry, thereby determining the amount of forces and stresses given in the deformed configuration.
4 Study 1: Uniaxial quasi-static Tensile Test

Figure 4-1, highlights the task and process routes involved in this chapter (highlighted in red) in order to achieve the objectives for this chapter. The overall thesis tasks and processes routes are included in Figure 4-1 in order to clarify where this chapter's work fits in with the rest of the thesis work.

![Thesis Flow Chart](image)

Figure 4-1: Presents the full thesis flow chart showing all thesis processes. Highlighted in red are the quasi-static uniaxial processes that are relevant to this chapter, but forms part of the whole thesis.
4.1 Introduction

The first work performed in this thesis was an experimental investigation into the passive tensile behavioural response of skeletal muscle when subjected to a large deformation. Skeletal muscles are composed of muscle fibres that are uniformly oriented in only one direction (anisotropic), therefore tensile tests were performed in various muscle fibre orientations with respect to the loading direction. The knowledge gaps that exist have been highlighted in section 3.2. The first objective of this chapter was to perform quasi-static uniaxial tensile experiments in order to characterise the quasi-static response of skeletal muscle to external tensile loading, as well as characterising the influence of the skeletal muscle fibre orientation. This involved characterisation of the corresponding Poisson's ratios as well. The second objective of this chapter was to perform a comparison of the quasi-static uniaxial tensile experimental results to published quasi-static compressive data and present the results.

4.2 Methods

4.2.1 Specimen Preparation

Fresh *Longissimus dorsi* skeletal muscle tissue was harvested from 3 month old female pigs. Five animals were used for the experimental work. All experiments were approved by the University of Dublin Ethics Committee for the protection of animals used for scientific purposes, according to the EU directive 2010/63/EU. Samples approximately 10 mm thick and 10 mm wide were prepared for quasi-static tensile testing. The sample length requirement according to the tensile testing standard ASTM E8/E8M were taken into account as much as was practically possible (ASTM International, 2011). It was not feasible to prepare samples with an exact dimensional specification due to the mobility of the freshly harvested skeletal muscle tissue (Van Loocke et al., 2006) and the limited availability of the tissue in the correct fibre orientation. The sample length variability was accounted for by adjusting the testing speed in order to maintain a constant strain rate of 0.05% s⁻¹. The width and thickness variability was accounted for by using image analysis to determine these two dimensions, see section 4.2.2 and section 4.2.3.
Six samples were prepared for each of the following muscle fibre orientations: the fibre direction (longitudinal), cross-fibre (perpendicular to muscle fibres, transversal) and 45 degrees to the muscle fibre orientation (see Figure 4-2 and Figure 4-4). Three samples were prepared for each of the following fibre orientations: 60 and 30 degrees. The first sample's fibre orientation was chosen at random, but the same orientation was not repeated until all other muscle fibre orientations had been tested. The test matrix is shown in Table 4-1 and Table 4-2. All the experimental tests were carried out within two hours after the death of the animal in order to minimise the Rigor Mortis effect on our results in accordance with previous Rigor Mortis investigation results (Van Loocke et al., 2006, Kobayashi et al., 1996). The experiments were performed on a Zwick Z005 tensile testing machine (Zwick GmbH & Co. Ulm, Germany), equipped with a 100 N load-cell. Grated plates were used on clamps, in order to prevent sample slippage from the clamps.

<table>
<thead>
<tr>
<th>Load direction</th>
<th>Sample size (N)</th>
<th>nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre (L)</td>
<td>6</td>
<td>L1, L2, ...L6</td>
</tr>
<tr>
<td>Cross Fibre (T)</td>
<td>6</td>
<td>T1, T2, ... T6</td>
</tr>
<tr>
<td>45 degrees (45L)</td>
<td>6</td>
<td>45L1, 45L2, ... 45L6</td>
</tr>
<tr>
<td>30 degrees (30L)</td>
<td>3</td>
<td>30L1, 30L2, 30L3</td>
</tr>
<tr>
<td>60 degrees (60L)</td>
<td>3</td>
<td>60L1, 60L2, 60L3</td>
</tr>
</tbody>
</table>

Table 4-1: Test matrix summary table showing testing direction, sample size and nomenclature employed

<table>
<thead>
<tr>
<th>Load direction</th>
<th>Length (mm) Avg</th>
<th>Std Dev</th>
<th>Width (mm) Avg</th>
<th>Std Dev</th>
<th>Thickness (mm) Avg</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre Direction</td>
<td>51.7 ±3.67</td>
<td>10.8</td>
<td>±0.89</td>
<td>10.3</td>
<td>±1.26</td>
<td></td>
</tr>
<tr>
<td>Cross-Fibre Direction</td>
<td>45.3 ±2.3</td>
<td>10.1</td>
<td>±1.69</td>
<td>10.5</td>
<td>±1.18</td>
<td></td>
</tr>
<tr>
<td>45 Degrees Direction</td>
<td>49.7 ±3.08</td>
<td>11.4</td>
<td>±0.79</td>
<td>9.9</td>
<td>±0.99</td>
<td></td>
</tr>
<tr>
<td>60 Degrees Direction</td>
<td>46.7 ±3.08</td>
<td>11.2</td>
<td>±0.33</td>
<td>10.1</td>
<td>±0.56</td>
<td></td>
</tr>
<tr>
<td>30 Degrees Direction</td>
<td>47.3 ±3.08</td>
<td>10.7</td>
<td>±0.79</td>
<td>10.2</td>
<td>±1.67</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-2: Test matrix summary table showing testing direction, sample size and nomenclature employed
4.2.2 Experimental Procedure

A sample of the required geometry was placed between grated plates, and then the clamps were clamped over the grated plates (Figure 4-3 represents an image of a grated plate surface). This improved the gripping capabilities of the clamps without over-tightening them, and helped to minimise the unnecessary introduction of stresses around the clamps. For strain evaluation, nine black dots were marked on the sample surface facing the camera (Figure 4-4 and Figure 4-5). The images were acquired using a CCD camera at a frequency of 1 frame per second. The camera CCD presented a resolution of 800 x 600 pixels. For dimensional analysis, each sample was photographed and the widths and thicknesses were recorded. During the test, phosphate buffer saline was continuously sprayed on the sample to avoid drying. Each sample was tested as soon as it was prepared to reduce unnecessary over-exposure to the environment after it had been cut into the required geometry. The testing temperatures ranged from 18-22 degrees. Samples were stretched at a strain rate of 0.05% s$^{-1}$. 

Figure 4-2: Schematic illustration of fibre orientation in different samples
A pre-load was determined by assuming that half the sample weight was supported by the top clamp and therefore half the sample mass was used as the pre-load. The pre-loads ranged from 0.1 N to 0.15 N. The muscle was then tested up to a stretch ratio of 1.3. For some samples, this stretch ratio was not reached due to premature failure. This pre-load method for this tissue was validated by taking measurements of the targeted tissue before harvesting, and then allowing the tissue to shrink after harvesting. It was then established that in order to bring the tissue to its original physiological working length, a tensile force equal to half the sample weight must be applied.

4.2.3 Data Analysis

A custom Matlab (The Mathworks, Natick Massachusetts, USA) script was used to calculate strains, as this eliminated the effects of clamp slippage as well as allowing the local strain variations to be analysed (see Figure 4-6). For each image, the central coordinates of each dot (determined using the mean of the segmented dot shapes) were recorded and compared to the coordinates of the image at the start of the experimental testing. The difference in coordinates was used to calculate the stretch ratios (Moerman et al., 2010).
Six local stretch ratios were calculated as follows:

$$\lambda_{iz} = \frac{idz}{ioz} \tag{4-1}$$

where $\lambda_{iz}$ represents the local stretch ratio of the respective region, $idz$ is the local length of the deformed sample and $ioz$ is the local initial length, see Figure 4-4. Six local stretch ratios were derived for each sample and then used to give the sample average stretch ratio. In all calculations, it was assumed that the samples tested were incompressible. The Cauchy stress was calculated as follows:

$$\sigma = \left(\frac{F}{A}\right)\lambda_z \tag{4-2}$$

where $\sigma$ is the Cauchy stress, $F$ is the current force, $\lambda_z$ is the stretch ratio in the vertical direction and $A$ is the original area.

Whilst the sample was tested in the Z-direction it elongated in that direction, it also suffers a contraction in the Y-direction (Figure 4-4 (y) versus Figure 4-4 (a)). This contraction was analysed using the following equation

$$\lambda_{iy} = \frac{idy}{ioy} \tag{4-3}$$

where $\lambda_{iy}$ is the local stretch of the respective region, $idy$ is the horizontal length after deformation of the sample, $ioy$ is the initial horizontal length and $\lambda_{iy}$, see Figure 4-4.
Finally the Poisson’s ratios were calculated using the vertical and horizontal stretches. The sample Poisson’s ratio was worked out as the average of the four regions shown in Figure 4-4. The Poisson’s ratio for each region was derived using the following formulae:

\[ \nu_{zy} = -\frac{\varepsilon_y}{\varepsilon_z} \]

where

\[ \varepsilon_y = \frac{1}{4} \sum_{i=1}^{4} \varepsilon_{iy} \quad \varepsilon_z = \frac{1}{4} \sum_{i=1}^{4} \varepsilon_{iz} \]

And

\[ \varepsilon_{iy} = \frac{1}{2} \left[ \ln \left( \frac{dy}{oy} \right) + \ln \left( \frac{dy}{oy} \right) \right] \quad \text{and} \quad \varepsilon_{iz} = \frac{1}{2} \left[ \ln \left( \frac{dz}{oz} \right) + \ln \left( \frac{dz}{oz} \right) \right] \]

where \( i = 1, 2, 3, 4 \)

\( j = i + 2 \)

### 4.3 Results

A typically tested sequence of photographs is shown in Figure 4-5. The first image is taken at the start of the tensile testing (\( \lambda = 1 \)); the second image is taken at the end of the test (\( \lambda = 1.35 \)). The coloured markings show the capability of the Matlab code in locating the centre of the markings on the tissue sample. The upper row is represented by circles, the middle row by plus symbols and triangles represent the bottom row. The blue colour shows the un-deformed configuration and the red indicates the final deformed configuration. The third image shows the marker dot displacements when the last image is superimposed on top of the first image.
Figure 4-5: Typical test sequence showing the un-deformed (initial, $\lambda=1$) image, the deformed (final, $\lambda=1.35$) image and a superposition of the two (final plus initial) for a fibre direction test.

Typical fibre directions within-sample stretch ratio variations at different time points are shown in Figure 4-6.

![Figure 4-6: Typical fibre direction within-sample stretch ratio variation at different time points (Sample 5 shown here). The individual region results (see Figure 4-4), the average and the stretch based on the Zwick machine displacement are shown.](image)

The results for the six regions shown in Figure 4-4 are displayed in Figure 4-7, along with the resulting average stretch and the stretch based on the Zwick machine displacement. This figure shows typical fibre direction within-sample stress variations as a function of applied stretch (derived from image analysis).
Figure 4-7: Typical within-sample stress variation (shown here is fibre direction sample L5).

Figure 4-8 shows the typical relationship between sample stress-stretch variations for the fibre direction tests. For each individual test the average stress was calculated based on the 6 regions shown in Figure 4-4c. The average across all the samples is also shown. An upwards curved graph with a clear noticeable toe region is presented.

Figure 4-8: Between-sample stress versus stretch variation for fibre direction tests.

Figure 4-9 shows almost similar results to those observed in Figure 4-8. The toe region is barely noticeable.
Figure 4-9: Between-sample stress versus stretch variation for the 30 degrees tests.

Figure 4-10 shows the stress-stretch response of samples loaded at 45 degrees to the muscle fibre direction. The toe region is almost gone, and it can be seen that the response stress magnitude is getting higher for the same stretch ratio as in the other test results presented above.

Figure 4-10: Between-sample stress versus stretch variation for the 45 degrees tests.

An increasing stiffness is observed for muscle fibres loaded at 60 degrees with respect to the muscle fibre orientation. The toe region is not present, seen in Figure 4-11.
Figure 4-11: Between-sample stress versus stretch variation for the 60 degrees tests.

Figure 4-12: Between-sample stress versus stretch variation for the cross-fibre direction tests.

Figure 4-12 shows the relationship between sample stress-stretch variations for the cross-fibre direction tests. The variation is higher than what was observed for the muscle fibre direction, the response is almost linear and the toe region has disappeared.

For clarity, the mean and the ± (plus/minus) standard deviation for each test direction are displayed in Figure 4-13. It can be seen that the muscle fibre response is compliant for the fibre direction. However, this response appears to become stiffer
according to the orientation of the muscle fibres with respect to the loading direction. The stiffest response is observed when they are loaded in an orthogonal direction to the muscle fibre alignment direction. The muscle fibres display anisotropic behavioural response and a nonlinear response (muscle fibre direction) that changes to linear response when the muscle fibre tissue is loaded in a transverse direction.

![Figure 4-13: Fibre direction responses (mean and plus/minus 1 standard deviation) shown for all tests performed: fibre, cross fibre and at 30, 45 and 60 degrees to the fibre direction.](image)

Finally, the measured Poisson's ratio values are presented as a function of stretch ratio in Figure 4-14. Since the linear regressions indicate that there is no influence of stretch ratio on measured Poisson's ratio. As such, the average values are presented in Table 4-3.

![Figure 4-14: The Poisson's Ratio averages and the solid lines are linear regression fits.](image)
**4.4 Discussion**

The observations presented for this experimental work contradict those observations presented by Morrow et al. (Morrow et al., 2010b). While the author acknowledges that the testing conditions and species may contribute to the observed dissimilarities, the differences are significant and cannot be accounted for by these reasons only. Using different animals may have given rise to the following problem: pigs (presented data) are bigger and live longer than rabbits (Morrow’s Data); therefore they take longer to reach maturity, which is the same when rats (Calvo’s data (Calvo et al., 2010)) and rabbits are considered. Morrow’s data is several times stiffer than the presented data or Calvo’s data. The fundamental fact is that the basic skeletal muscle structure is the same for all animals. However, due to the different working environments and functional mechanical property requirements which are also influenced by the animal’s gait, life-style, age and gender, the muscle remodels itself to accommodate. Different animals have different life cycles and therefore mature at different times. Morrow et al., 2010 performed their experiments on female New Zealand white rabbits that were 5 months old. These are almost adult rabbits (Frame et al., 1994, Macari and Machado, 1978). Calvo performed experiments on female Wistar rats that were 3 months old (mature rats (Rutledge et al., 1974)), whereas in this study experiments were performed on four month old female pigs. The strong evidence presented in the published data demonstrates that the pigs used for the experiments in this study were

<table>
<thead>
<tr>
<th>Fibre Direction</th>
<th>Effective Sample Size</th>
<th>Poisson’s Ratio</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>( v_{LT} = v_{LT'} )</td>
<td>24</td>
<td>0.47</td>
<td>±0.02</td>
</tr>
<tr>
<td>( v_{T'T} )</td>
<td>24</td>
<td>0.28</td>
<td>±0.04</td>
</tr>
<tr>
<td>( v_{TL} )</td>
<td>24</td>
<td>0.74</td>
<td>±0.016</td>
</tr>
</tbody>
</table>

Table 4-3: The three summarised Poisson’s ratios observed during tensile testing of muscle samples.
still in their developmental stages (Fang et al., 1999, Rehfeldt et al., 2008, Reiland, 1978). This would have resulted in a low stiffness response, yet the evidence presented in Figure 4-15, shows that the fibre directional data response almost matches the mature rat data that Calvo reported (Calvo et al., 2010). The cross-fibre response reported in this thesis is stiffer than the compliant fibre directional response, contrary to the response observed by Morrow (Morrow et al., 2010b). A slightly different mechanical response is therefore expected but not to the extent that was observed in Morrow's data.

![Graph showing comparison](image)

Figure 4-15: Comparison of current data with existing literature findings (Nie et al., 2011, Calvo et al., 2010, Morrow et al., 2010b).

More importantly, Morrow et al., 2010 froze their samples within an hour of the animal sacrifice, but no effort was made to discuss the thawing process they used. The thawing process is possibly the most important part of their experiments as it is likely to have allowed Rigor Mortis to set in and make the tissue much stiffer. The Rigor Mortis characterisation investigation performed on freshly harvested porcine tissue by Van Loocke, et al., 2006, showed that the testing window is two hours after death before Rigor Mortis starts influencing the passive response of porcine skeletal (Rutledge et al., 1974) muscles. The one hour window left after freezing makes it highly unlikely that Morrow was able to thaw and test the tissue in its fresh state. Therefore it is the view of this thesis that Morrow's experimental tests were probably performed on aged tissue (post Rigor Mortis). Furthermore, Morrow et al., 2010 performed their uniaxial passive testing on complete muscles which may have given rise to complicated geometric shapes, therefore resulting in incorrect stress calculations.
A thorough investigation was performed on pre-load. A pre-load will eliminate unavoidable initial compressive forces within samples and help straighten out muscle fibres in the sample without stretching them. However, it is the authors' view that the pre-load used by Morrow was too high, resulting in an almost loss of the toe region (fibre straightening and recruitment phase) and permanent damage of the cross-fibre samples resulting in the experimental tests on damaged samples. This is assumed to be the cause of the low cross-fibre linear response. Following extensive investigation, a pre-load based on half the specimen weight (mean pre-load was 0.11 N) was found to ensure this did not occur in these tests.

4.4.1 Testing method

Image displacement measurements were preferred over the machine grip displacement. In Figure 4-6, a difference between the displacement from image analysis and machine grip displacement is observed. The difference between the image analysis average stretch ratio and the Zwick machine stretch ratio is due to gripping clamp slippage. Further tightening the clamps may have addressed this problem, but would have also damaged the samples and created regions of high stress concentration. All stress-stretch results are therefore presented with stretches calculated from the more reliable image analysis techniques instead. While significantly more laborious, these methods are effective at showing local strain variations and are independent of clamp slippage whilst reflecting a more realistic determination of applied deformation.

Due to the micro-structural arrangement of collagen fibres in the connective tissue, the size of the sample chosen clearly plays an important role. This subject will be discussed in detail in the microscopic chapter. The reference to the 6 mm size limitation from Purslow is indeed based on cooked meat, but there is no evidence that this micro-structural effect would be influenced by the cooking process (Lewis and Purslow, 1989, Lewis and Purslow, 1990), therefore it was ensured that the samples used for this research did not go below the 6 mm thickness limit seen in Figure 4-16:
Figure 4-16: Schematic illustration of how an optimum thickness sized sample (b) cut from cuboidal muscle block (a) is wide enough to accommodate muscle fibre bundles (fascicles) that include several continuous perimysium fibres. Sample (b) thickness is very small and does not contain continuous perimysium fibres.

It can be hypothesised that when the tested muscle specimen Figure 4-16 (b) is in the vertical direction it has been sampled from a larger block Figure 4-16 (a), then the application of the stretching load will be resisted by the perimysium fibres. Due to incompressibility, the perimysium collagen fibres cannot simply reorient by collapsing the muscle fascicles, therefore the perimysium collagen fibres will bear the load until the weakest links between perimysium collagen fibres start failing. In a larger sample such as this, continuous perimysium collagen fibres can be traced from the top grip to the bottom grip. In contrast, if a thinner slice is prepared Figure 4-16 (c), then the perimysium collagen fibres may not be continuous from top to bottom. Applying a stretching load will then cause the perimysium sheets to be pulled away from the endomysium connections. Therefore the resistance that will be observed from this arrangement will be the result of the strength of the endomysium/perimysium collagen fibre connections, which is less than the resistance observed from the thicker perimysium collagen fibres themselves in Figure 4-16 (b). Thus a smaller sample should be weaker as it is less likely to contain continuous perimysium fibres. It has been observed that below about 6 mm, the sample thickness has a direct influence on the response (Lewis and Purslow, 1989), though this previous work performed its investigation on cooked meat, there is evidence to suggest that the micro-structure that gives rise to this behavioural response is not affected or changed by cooking, and this
was observed to be true by a more recent study performed by Blackburn (Blackburn, 2013).

Another factor that is important in tensile testing is the sample length, particularly if the effect of grip induced stress in the region of interest is to be reduced. For 6 out of the 24 samples tested for this study, the length fell short of the recommended ASTM E8 standards, but not by more than 10% (the length is required to be more than five times the width for rectangular specimens). Thinner samples were not feasible as this reduces the number of complete muscle fascicles in the sample. It has been observed that below about 6 mm, the sample thickness has a direct influence on the response (Lewis and Purslow, 1989, Lewis and Purslow, 1990). No significant difference in response was observed between the samples with a dimensional ratio slightly below the recommended ASTM E8 and those that met the standard (ASTM International, 2011).

4.4.2 Interpretation of results

The typical fibre direction within-sample stretch ratio variation (Figure 4-7) shows a range of the local variations in stretch within a sample, similar to findings observed by Palmer et al. for tensile testing of rat soleus muscle (Palmer et al., 2011). The corresponding within-sample stress variations (difference between maximum and minimum) as a function of applied stretch (Figure 4-7) are of lower magnitude and are relatively constant as stretch increases: 12% of mean stress at $\lambda=1.15$, 11% of mean stress at $\lambda=1.2$ and 11% of mean stress at $\lambda=1.3$ for the sample shown. These approximately constant stress variations are assumed to be due to the muscle fibre’s ability to transmit force to the surrounding muscle fibres. This seems to corroborate the previous findings that skeletal muscle fibres can laterally transmit 80% of their loads to the other adjacent muscle fibres (Monti et al., 1999, Huijing, 1999, Street, 1983, Huijing, 2009, Mass et al., 2003, Purslow, 2010). Figure 4-8, Figure 4-10 and Figure 4-12 show the between-sample stress-stretch variations for the fibre, cross-fibre and 45 degrees to the fibre direction tests, respectively. For the fibre direction (Figure 4-8) the typical nonlinear increase in stiffness with stretch is observed. The between sample variation is high as a percentage of the mean stress at low strains, but this decreases with stretch: 54% at $\lambda=1.1$, 31% at $\lambda=1.2$ and 26% at $\lambda=1.3$. For the cross fibre direction (Figure 4-12), a stiff and largely linear response was observed, with failure occurring at stretches beyond 1.15. The between-sample stress-stretch variation is
highest at low strains and again decreases relative to the mean stress at high strains: 59% at $\lambda=1.04$, 40% at $\lambda=1.08$ and 24% at $\lambda=1.12$. Most of the observed variation in the samples for the current study can be attributed to intra-animal variation, this is based on the fact that the samples were harvested from 5 different animals. Figure 4-10 shows the stress-stretch response of samples loaded at 45 degrees to the muscle fibre direction. Again, a mildly nonlinear relationship is observed. The between-sample variation as a percentage of mean is high at low strains, but this decreases at higher stretches: 83% at $\lambda=1.04$, 54% at $\lambda=1.08$ and 42% at $\lambda=1.12$. All stress-stretch results are shown in Figure 4-13, with only mean and ± (plus/minus) one standard deviation shown for each test direction for clarity. There is a clear influence of fibre direction on the stress-stretch relationships observed, with the cross fibre direction significantly stiffer than the fibre direction. To observe the change in stiffness with fibre angle, Figure 4-17 shows two views of a three-dimensional landscape plot, illustrating how stress increases with stretch and also with angle relative to the main muscle fibre direction. Furthermore, it can be seen that the relationship with angle is not linear, but rather follows a more sinusoidal shape. The fibre direction response was observed to be the most compliant, and this seems intuitive since it is the normal physiological working direction for muscle. The stiffest direction is the cross-fibre response, but this has a low failure stretch ($\lambda=1.15$). The fibre direction response reported in the current study was less stiff than the responses observed by both Nie et al. and Morrow et al. (Nie et al., 2011, Morrow et al., 2010b), but close to the findings of Calvo et al. (Calvo et al., 2010), see Figure 4-15 and Table 4-4.

**Figure 4-17:** The experimental Cauchy stress and stretch curves in 3D in relation to the muscle fibre angle. For visualisation a periodic cubic surface fit is shown (shaded towards stress magnitude), and this is shown with a rotation of the muscle fibre orientation with respect to the loading axis. In addition, surface plots (transparent grey) are shown for the anisotropy landscape (plus/minus 1 standard deviation).
Table 4-4: Showing varying tensile responses observed by different authors.

Calvo used a slightly lower strain rate of 0.025\% s\(^{-1}\), see Figure 4-15 and Table 4-4, and this may partially account for their lower stiffness results. It is well known that skeletal muscle is viscoelastic even at very low strain rates (Van Loocke et al., 2009). Nie et al., and Morrow et al. observed stiffer fibre directional responses than was observed in this chapter (Nie et al., 2011, Morrow et al., 2010b), and this may be partially attributed to different experimental protocols. Although Morrow et al., and Nie et al. (Nie et al., 2011, Morrow et al., 2010b), may have less reliable strain estimates since they did not use image analysis for strain estimation, this should mean they would underestimate stress. It is more likely that their higher stiffness values result from differences in the amount of perimysium in the skeletal muscle, which according to the literature can only vary between 0.4\% - 4.8\% of dry weight (Purslow, 2010, KJÆR, 2004, Purslow, 1999.). This may also explain why lower stretches to failure were observed in the present work, while Hernandez et al. observed stretch ratios up to 2.5 using samples harvested from rabbit external oblique muscle (Hernandez et al., 2011). The porcine *Longissimus dorsi* used in the tests for the current study may not be exposed to large stretches in the fibre direction and to only very small stretches in the cross-fibre during
physiological activities. The Nie et al. fibre response profile is unusual in that the typical toe region and increasing stiffness were not observed (Nie et al., 2011), and it is unclear why this is, though the use of different sample dimensional ratios can also contribute to the differences in Figure 4-15. The cross-fibre response observed in our work was in broad agreement with the Nie et al cross-fibre response up to a stretch ratio of 1.07, and their results also showed that the cross-fibre response is stiffer than the fibre response. This observation contradicts the findings of Morrow et al., and it is unclear why this is. This might be attributed to a different species used and a different experimental protocol. The typical toe region in soft tissue stretch due to the crimped collagenous fibres straightening out (Monti et al., 1999, Street, 1983, Trotter and Purslow, 1992) is absent from the Nie et al. data (Nie et al., 2011).

The Poisson's ratio results presented in Figure 4-14 and Table 4-5 show an important component of the deformation response in tension. To the author's knowledge this type of response has not been previously reported in literature. A ratio of 0.47±0.02 was observed when the sample was loaded in the fibre direction (longitudinal). Despite the fact that a small degree of fluid loss was observed, the passive response of skeletal muscle to tensile loading in the fibre (F or longitudinal direction) direction is therefore almost a volume preserving event, with equal contraction in the two transverse directions (T and T'). Due to the transversely isotropic and nearly incompressible nature of muscle tissue, this was expected, and it is in agreement with the assumed Poisson's ratios used by other authors (Beldie et al., 2010, Meier and Blickhan, 2000, Miller et al., 2000, Miller, 2011, Bilston, 2011, McAnearney et al., 2010, Behr et al., 2006, Weiss et al., 1996). Furthermore, it is also in agreement with the reported compressive Poisson's ratio of 0.5 ±0.04 for fibre direction loading (Van Loocke et al., 2006). The cross-fibre (T) tensile loading is more complex and gives rise to two Poisson's ratios. A Poisson's ratio of 0.28 was observed when the contracting side considered was the remaining orthogonal cross-fibre direction (T'). Whereas, a Poisson's ratio of 0.74 was observed when the contracting side considered was the fibre (F) direction. Thus, when a tensile load was applied in the transverse (T) direction, a volume preserving contraction response was observed in both the fibre direction (L) and in the other cross-fibre (T') direction. However, the magnitude of the fibre direction contraction was much greater than the cross fibre contraction, indicating that the cross-fibre (T) direction will require more internal force to contract than the less stiff fibre direction (L). The sum of the two Poisson's ratios
ratios was found to be close to 1 in both cases. This corroborates the assumption that the skeletal muscle tissue is nearly incompressible. The values for the Poisson’s ratios determined in the current study are different to the equivalent compressive Poisson’s ratios observed by Van Loocke (i.e., 0.64 and 0.36, respectively). Table 4-5 shows a complete Poisson’s ratio characterisation of quasi-static skeletal muscle tissue in tension and compression (Van Loocke et al., 2006). The Poisson’s ratio of 0.47 (rather than 0.5) may be due to the loss of biological fluids that was observed when the specimen was subjected to longitudinal tensile loading. For tensile loading in the cross-fibre (T) direction, the specimen was observed to be more compliant in the longitudinal contraction than in the other cross-fibre (T’) contraction. This is the opposite of what has been observed in compression (Van Loocke et al., 2006). While a nonlinear response was observed by van Loocke for the cross-fibre (T’) compression response, an almost linear response was observed for the cross-fibre (T’) tension response. The magnitude of stress response observed for the tensile test was way higher than the observed compressive response.

<table>
<thead>
<tr>
<th>Takaza et al. (Tension)</th>
<th>Van Loocke et al. (Compression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( v_{LT} = v_{LT'} )</td>
<td>0.47</td>
</tr>
<tr>
<td>( v_{T'T} )</td>
<td>0.28</td>
</tr>
<tr>
<td>( v_{TL} )</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 4-5: Tensile Poisson’s ratios observed in the present work, and compressive data observed by Van Loocke et al (Van Loocke et al., 2006).
4.5 Conclusion

The 3D tensile stress-stretch behaviour of samples from freshly slaughtered porcine *Longissimus dorsi* muscle has been reported in this chapter. Tests were performed along and orthogonal to the muscle fibre direction, as well as at 30, 45 and 60 degrees to the muscle fibre direction. The results show the cross-fibre direction was broadly linear and was the stiffest (77 kPa stress at a stretch of 1.1); however, failure occurred at low stretches (ca. $\lambda = 1.15$). In contrast, the fibre direction was nonlinear and much less stiff (10 kPa stress at a stretch of 1.1); however, failure occurred at higher stretches (ca. $\lambda = 1.65$). An approximately sinusoidal variation in stiffness was observed at intermediate angles. The following Poisson’s ratios were measured: $\nu_{LT} = \nu_{LT'} = 0.47$, $\nu_{T'T} = 0.28$ and $\nu_{TL} = 0.74$. These observations have not been previously reported and they contribute significantly to the overall understanding of the three dimensional deformation response of skeletal muscle tissue.
5 Study 2: Impact Compressive Experiments

Figure 5-1, highlights the task and processes involved in this chapter (highlighted in red) in order to achieve the objectives for this chapter. The overall thesis tasks and processes are included in Figure 5-1 in order to clarify where this chapter's work fits in with the rest of the thesis work.

Figure 5-1: Presents the full thesis flow chart showing all thesis processes. Highlighted in red are the impact experimental testing processes that are relevant to this chapter, but forms part of the whole thesis.
5.1 Introduction

During the course of the last century, a lot of research has been performed on vehicle safety and the research completed was primarily focused on the reduction of the automobile occupant fatalities in vehicle accidents. During this period, a lot of experiments were performed and significant progress was made towards the development of the following safety features; energy absorbing front and side structures, air bags, seat belts, collapsible steering columns and various crash avoidance devices (Du Bois et al., 2004).

Even with the implementation of the safety features mentioned, the annual published data show that around 1.24 million people are killed and 20 million more people are injured globally as a direct result of vehicle related accidents (Nantulya et al., 2002, WHO, 2013). This clearly shows that there is need for more work to be done in this research area. This chapter was developed with the view that most of the safety features mentioned above can be greatly improved if more understanding of how the tissue behaves under impact loading is made available. The strain rates considered in this chapter are relevant to those experienced by frontal occupants of automobile car crashes travelling within the standard city limit speeds.

The knowledge gap with missing data that is relevant to automobile crashes within the city limits has been established (see section 3.2.4 and Figure 3-14). The main objectives of this chapter was to design and build a drop test rig that can perform impact compressive loading experiments for strain rates ranging from 11600%\text{s}^{-1} to 378000%\text{s}^{-1}. This work will also increase the authors understanding of the skeletal muscle behaviour at viscoelastic levels.

The design of the impact drop test rig and impact drop test experiments for characterising skeletal muscle tissues is presented in this chapter. The impact drop test rig's design, force and deformation measurements are discussed first and then followed by the presentation and discussion of the results and the conclusions derived from this work.

5.2 Methods

The impact response of freshly harvested porcine muscle tissue to compressive impact loading was investigated using a custom designed drop test rig. In order for the drop test rig to functionally perform the required task, it had to satisfy the following conditions:
1) Dropping a known mass from a measured height and guide the mass to hit exactly the same point every time.
2) Reproducible impact velocity if the mass is dropped from the same height.
3) The impact velocity needs to be measured and controlled for reproducibility.
4) The force generated at impact, up to 50% compression of the sample needs to be measured.
5) A data acquisition method that suits the experiment needs to be developed.
6) An image data acquisition technique, that is capable of capturing and analysing the specimen deformation.

Taking the above six points on board, a suitable testing rig was designed, which allowed for the muscle’s response to be monitored and the resulting behavioural response analysed.

![Figure 5-2: Schematic drawing of the drop test rig and the top part of rig is highlighted on the right](image)

**5.2.1 Design & Method Justification**

In free space, the falling body's velocity increases with an increase in the falling distance. The instantaneous velocity can be mathematically described by the equation below:

\[
v = \sqrt{2gh}
\]

where \(v\) is the free fall velocity, \(g\) is the gravitational acceleration (9.81 m/s\(^2\)) and \(h\) is the vertical height of free fall. The falling mass was allowed to slide down the low
friction silver rods so as to be directed to impact the top of the specimen. The friction between the sliding mass and the silver steel rods was minimised as much as was practically possible by making the sliding mass slide on PTFE linear bearings (bushings), see Figure 5-3. The frictional loss was later calculated to be 23.3%.

A mass plate made from aluminium was designed to slide down the two 10 mm parallel silver steel rods. Aluminium was the material of choice for the falling mass plate due to its material properties, and silver steel was chosen for the parallel rods due to being able to achieve a high quality surface finish. A rough surface finish would have increased the amount of friction; therefore reducing the falling velocity and increasing the amount of wear and tear on the bearings. This would have resulted in an early failure of the PTFE bearings. The top and bottom plates were clamped and drilled together; this was to ensure there were no misalignments. A frame was built around the rig in order to give the rig the support. The frame and the top plate were joined together by a threaded bolt (see Figure 5-2). The silver steel rods needed to be kept absolutely parallel at all times. Tightening or loosening the tension bolt (see Figure 5-2), would either increase or decrease the tension in the silver steel rods, as such this would ensure the rods were always parallel.

5.2.2 Falling mass
The PTFE bearings were made in-house from purchased solid PTFE rods and drilling the holes for the silver steel rods to run through them. Bearing alignment plates were used to allow for fine-tuning (Figure 5-3). A dynamic accelerometer was mounted on top of the falling mass plate, which was used to measure the acceleration. The top force transducer was mounted facing downwards below the falling mass plate. From Figure 5-3, it is clear how the dropping mass will only impact the specimen through the top force transducer.
Figure 5-3: Schematic of the bottom part of the testing rig, the strain control plates are excluded for clarity.

The muscle sample schematically illustrated as a red cube in Figure 5-3 sat on top of the bottom dynamic force transducer, which was in turn mounted on the bottom base plate. The force transducers and the accelerometer transferred data into LabView via a signal-conditioning box.

Figure 5-4, shows a photo image of the drop test rig. The maximum compressive deformation of 50% was controlled by a strain control plate (Figure 5-5), which allowed the gap between the platens to be adjusted to the required maximum deformation while preventing complete destruction of the samples and the two transducer plates crashing into each other, which could damage them and, would require re-calibration. For the tests reported here, the gap was set at 50% of the sample height after which the falling mass plate struck the strain control plate and further specimen deformation halted. The design ensures the overall sample deformation rate is effectively constant between first contact of the falling mass plate with the tissue and contact of the falling mass plate with the strain control plate.
5.2.2.1 **Force Transducers Validation**

Two second-hand dynamic force transducers (Piezoelectric, model 208C04 ICP) were obtained from the electronics section. The force transducers were mounted below the falling mass and under the specimen as illustrated in Figure 5-5. The force transducer positioning below the falling mass is the lowest point of impact with the top surface of the specimen. The dynamic force transducer detects dynamics forces only. Newton's second law states that

\[ F = ma \]
Where the force in Newtons is $F$, $m$ is the mass in kgs and $a$ is the acceleration in ms$^{-2}$. Given the constant mass the change in variable $a$ will cause the dynamic force transducer to record a voltage reading. The piezoelectric dynamic sensor produces a voltage when a dynamic force is applied and the voltage output is proportional to the force applied. The sensitivity and accuracy of the dynamics force transducers was verified using a known input from an Instron tensile machine.

An Instron tensile testing machine (model 8501) was used to validate the force transducer output. The cyclic load alternating from minimum load (0 N) to maximum load (600 N for the first experiment and then 1000 N for the second experiment) was delivered by the Instron Machine Model 8501 tensile testing machine.

<table>
<thead>
<tr>
<th>Instron Input N</th>
<th>Force Transducer 1</th>
<th>Force Transducer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>1a</td>
<td>2a</td>
</tr>
<tr>
<td>Peak Voltage (V)</td>
<td>5.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Take out gain X10</td>
<td>0.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Convert to mV</td>
<td>560</td>
<td>580</td>
</tr>
<tr>
<td>Convert to Newton</td>
<td>498</td>
<td>516</td>
</tr>
<tr>
<td>Add pre-load</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5-1: Force transducer data output compared to input.

Table 5-1, shows that the two transducer outputs compared well with the Instron machine inputs, the differences are infinitesimally small. The force transducers were therefore considered validated for experimental use.

5.2.2.2 Accelerometer Validation.

The dynamic accelerometer (Piezoelectric model 353B03) was to be mounted on top of the dropping mass. The dynamic accelerometer measures the change in acceleration. The accelerometer was checked to see if it was capable of outputting a selected known input at a given frequency, as per the accelerometer user manual. A Bruel and Kjaer 4294 vibration calibrator was used, and the accelerometer was found to output the same signal as the Bruel and Kjaer 4294 vibration calibrator would input.
5.2.3 LabView Data Acquisition software
LabView was used for data acquisition duties. The data acquisition system was programmed to self-trigger once the de-acceleration accelerometer triggering signal rose above a threshold of 500 mV. The transducer signals were sampled at 25 kHz and the high speed video was sampled at 10 kHz.

A high impedance electrical signal generated by the force transducer was captured by LabView via a 4 channel signal conditioner (PCB Model 441A101). In the signal conditioner box, the signal is converted into low impedance and the selected gain applied before being transferred into LabView. The signal is processed and the voltage output is plotted against time and displayed on the screen. At the same time LabView digitally stores the data for further analysis. The data is stored in a format that is compatible with Matlab and Microsoft Excel.

5.2.4 Equipment Validation

![Graph showing force vs. time](image)

Figure 5-6: The graph shows the consistent similar profile and force variable data

The fully assembled drop test rig was checked to see if it was capable of consistently producing exactly the same results when given the same input, i.e. repeatable. If there was any scatter in the results, the results were analysed to see how widely spread the scatter was, along with a test to see what the rig percentage error at a 95% confidence interval was. The drop testing was repeated eleven times on vibration damping stiff rubber. The rubber is normally used to dampen vibrations on heavy machinery. Therefore it was assumed that the force involved in this experiment was not going to cause any changes to the mechanical properties of the rubber, considering that the damping rubber is manufactured to withstand large 3-D forces over millions of cycles.
at higher frequencies. The data output from the drop testing rig was analysed in Excel and the results from the drop testing were plotted on the same graph (see Figure 5-6). The maximum peak value for each experimental test run was recorded. The mean force of 381.33 N and a standard deviation of 23.52 were computed. The standard deviation was used to calculate the equipment's standard error. The standard error highlights spread of the mean of the completed tests, normal distribution was assumed at all times.

\[
\text{Standard Error (SE)} = \frac{\text{StDev}}{\sqrt{n}} \tag{5.3}
\]

Where \( n \) is equal to the size of sample (11) and \( \text{StDev} \) is the calculated standard deviation.

The standard error was calculated to be 7.092. Therefore the true population mean will be 381.33±7.09 N, which is considered to be very low as the forces involved are high. The standard percentage error of the equipment is calculated to be:

\[
\frac{7.092}{381.33} \times 100 = 1.86\%, \text{ standard percentage error at 95\% confidence interval.}
\]

The percentage standard error of 1.86\% on the mean value was considered acceptable to proceed with this experiment.

### 5.2.5 High Speed Video Camera

A high speed camera (Mikrotron HiSpec) shown in Figure 5-7 was used to capture specimen deformation. LabView was used to control and monitor the accelerometer signal and a rising edge signal was used to trigger data acquisition (see section 5.2.3), including the camera. The video was first saved onto a buffer file by default settings. The saving of images to the buffer enables the camera to save at the rate the video was acquired, and was considerably faster than the speed with which the computer could save the images. The high speed video camera default settings saved 3 seconds of data before the trigger was activated and 3 seconds of data after the trigger was activated. The video was then reviewed and only those parts in the targeted range of interest were saved.
5.2.6 Specimen Preparation

Uniform geometric samples need to be prepared so that deformation can be monitored. A consistent geometrical shape is needed to minimise data scatter. Freshly harvested Longissimus dorsi porcine samples were prepared from 3 month old pigs to produce approximately cuboid samples. Samples were compressed either in the cross-fibre (compression applied to face A or C) or the fibre direction (compression applied to face B), see Figure 5-8a. The height of the sample for all tests was approximately 10 mm and nominally cubic samples were used for the cross-fibre direction tests. For the fibre direction tests, the width and depth were set to approximately 20 mm to reduce the buckling effects (Van Loocke et al., 2006, Van Sligtenhorst et al., 2006, McElhaney, 1966). Producing cubic samples is very difficult given the very low stiffness of fresh skeletal muscle tissue. To avoid the effects of Rigor Mortis, testing was performed within 2 hours after the death of the animal (Van Loocke et al., 2006).

5.2.7 Data Analysis

Each specimen was marked with 9 dots painted on a side face of the samples prior to testing (using a black fine line waterproof Mitsubishi, Uni-ball eye pen) as illustrated in Figure 5-8B. The displacement of the marker dots was then tracked using Matlab-based image analysis methods. The strain for the overall deformation of the specimen was obtained using the platen displacement from the video data. For local deformations within the samples, the region of interest shown in Figure 5-8B was divided into 6 sub-regions as labelled and the local stretch ratios ($\lambda$) were calculated by dividing the current height ($L_1$) by the original height ($L_0$):
Engineering stress ($\sigma$) is presented in favour of Cauchy stress, as the deformation was found to be not uniformly distributed from top to bottom. The engineering stress computed by dividing the force by the original cross-sectional area:

$$\sigma = \frac{F}{A_0}$$

where $A_0$ is the original area, and $F$ is the current force.

Figure 5-8: (A) Specimens illustrating the cross-fibre, fibre and 45 degrees to the fibre direction. The surfaces are marked A (cross-fibre direction), B (fibres direction) and C (cross-fibre direction). (B) shows schematic regions of interest used during image strain analysis.

### 5.2.8 Testing Matrix

A total of thirty one compressive impact tests were performed at different strain rates in the fibre and cross-fibre direction as shown in Table 5-2.

<table>
<thead>
<tr>
<th>Testing Direction</th>
<th>Specimen Qty</th>
<th>Impact Velocity ms$^{-1}$</th>
<th>Avg Specimen height</th>
<th>Strain Rate %s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-Fibre</td>
<td>4</td>
<td>1.16</td>
<td>10.03± 0.21</td>
<td>11600</td>
</tr>
<tr>
<td>Cross-Fibre</td>
<td>12</td>
<td>2.2</td>
<td>9.78 ± 0.44</td>
<td>22000</td>
</tr>
<tr>
<td>Cross-Fibre</td>
<td>5</td>
<td>3.78</td>
<td>10.29± 0.36</td>
<td>37800</td>
</tr>
<tr>
<td>Fibre</td>
<td>5</td>
<td>2.2</td>
<td>20.23± 0.38</td>
<td>22000</td>
</tr>
<tr>
<td>Fibre</td>
<td>5</td>
<td>3.78</td>
<td>20.03± 0.44</td>
<td>37800</td>
</tr>
</tbody>
</table>

Table 5-2: Shows the testing matrix of our experiment
5.3 Results

Analysis was carried out to verify that the applied strain rate is constant over the very small distances relevant to the sample compression. Figure 5-9 shows that the platen displacement is effectively constant after tissue compression commences at approx. 1.1 ms, thereby ensuring an effectively constant strain rate during the tests.

![Figure 5-9: Upper compression platen displacement time history](image)

Figure 5-10 shows a typical time history sequence of the upper and lower force transducers and the applied load approximated using the accelerometer signal (unfiltered) by combining it with the mass of the falling plate. The upper force transducer curve presented is inertia-compensated to account for the mass of the upper platen. It can be seen that up to the time of peak sample compression around 1.6 ms, the three force estimates are very similar, which seems to show that the force is equilibrated throughout the sample during compression.

A typical cross-fibre direction specimen deformation during compression is presented in Figure 5-12 (i.e. compression applied to cut face C in Figure 5-8A). The first row (R1) shows a camera view on a face perpendicular to the muscle fibres (face B in Figure 5-8A). The middle row (R2) shows a camera view on a face parallel to the muscle fibres (face A in Figure 5-8A). Since only one high speed camera was available, the first row and second row in Figure 5-12 represent images from tests on different samples. The bottom row shows the capability of the Matlab code in tracking the marker centres initially (R3a) and at the end (R3b). The initial (red plus symbol) and
final (blue circle symbol) marker locations are overlaid on the un-deformed image in R3c.

Figure 5-10: Typical accelerometer and force transducer results (the corrected upper transducer reading accounts for the platen inertia)

Figure 5-11: Percentage difference between lower and upper transducers, dynamic equilibrium was reached after roughly 0.6ms (at 23% of the strain)
Figure 5-11 shows that the muscle tissue material reached dynamic equilibrium 0.06ms (23% of the required stretch) after impact. The dynamic equilibrium is calculated by taking the percentage difference between the top transducer reading and the bottom transducer reading, see Figure 5-11. No further radial inertia investigations like the work performed by Warren and Forrestal was performed as the top force transducer reading was in close agreement with the bottom transducer reading (Warren and Forrestal, 2010).

Typical within-specimen strain variation for cross-fibre direction compression (compression applied to cut face C in Figure 5-8A) is shown in Figure 5-13 and Figure 5-14, with the strains found from tracking the dots located on a cut face perpendicular (surface B in Figure 5-8A) and parallel (surface A in Figure 5-8A) to the fibres respectively. Since each sample was tested only once, Figure 5-13 and Figure 5-14 represent tests on different samples. Assuming a uniform strain based on the platen displacement and a uniform stress distribution throughout the cross-section of the sample at each time point, nominal stress-stretch ratio curves were derived for comparison with other published work, see Figure 5-15 and Figure 5-16 for the cross-fibre and fibre directions respectively.
Figure 5-12: Cross-fibre experimental results: The first row (R1) shows the cross-fibre face (B in Figure 2 Left), the middle row (R2) shows the along fibre face (A in Figure 1 Left), the bottom row shows the capability of the marker tracking algorithm in finding the marker centres in the initial (R3a) and final deformed state (R3b). The initial and final marker locations are overlaid on the un-deformed image in R3c. The overall applied stretch level is indicated by $\lambda$. 
Figure 5-13: Typical strain variation within the regions of interest for cross-fibre direction testing. Rows 1 to 3 show the strain variation of the left, middle and right column (see Fig. 4B) respectively as observed from a cut face parallel to the muscle fibre direction (Marked as A in Fig. 4A).

Figure 5-14: Typical strain variation within the regions of interest for cross-fibre direction testing. Rows 1 to 3 show the strain variation of the left, middle and right column (see Fig. 4B) respectively as observed from a cut face perpendicular to the muscle fibre direction (Marked as B in Fig. 4A).
Figure 5-15: Nominal Stress-stretch ratio curves of the porcine skeletal muscle tissue for loading in the cross-fibre direction (ie when the loading is perpendicular to the fibre direction).

Figure 5-16: Engineering Stress-stretch ratio curves of the porcine skeletal muscle tissue in the fibre direction (ie when the loading is parallel to the muscle fibre direction).
Figure 5-17: Engineering Stress-stretch ratio curves of the porcine skeletal muscle tissue when loaded at a strain rate $37800\,\text{% s}^{-1}$.

Figure 5-18: Summary of all engineering stress-stretch ratio curves of all porcine skeletal muscle tissue.
5.4 Discussion

The goal of this work was to perform dynamic uniaxial compressive tests on uniformly shaped samples of freshly harvested skeletal muscle tissue in both the fibre and cross-fibre direction at rates relevant to automotive injuries. However, as can be seen from Figure 5-12 (R1a), preparation of uniform samples is difficult due to the extremely soft nature of the tissue which deformed significantly even under its own weight. Accordingly, Figure 5-12 shows that some shearing as well as axial compression occurred during the sample deformation. This is the result of a number of factors including the difficulty in preparing cubic specimens, the interaction of the micro-structural components of solid matrix and fluid, and the impact nature of the applied deformation. These results are evident from the strain time histories in the different regions presented in Figure 5-13 and Figure 5-14. Nonetheless, these kinds of tests appear to be the closest this work could get to pure uniaxial compression for these tissues.

The marker tracking software was found to successfully locate the displacement of the nine dots on the images, see Figure 5-12 (R3), and this provided a reliable local strain measure. The resulting strain time history data presented in Figure 5-13 and Figure 5-14 are typical individual specimen results for cross-fibre direction impacts.

Figure 5-13 shows the typical evolution of strain in the different regions of the sample as a function of time for the cross-fibre direction tests when viewed orthogonal to the muscle fibres (face A in Figure 5-8A). It can be seen that the strain in the upper regions is initially greater than in the lower regions, but this equalizes towards the end of the test. Furthermore, the strain is greater at the left and right edges than in the middle of the sample. This may be because fluid expulsion is easier at the free edges and the muscle fibres collapse earlier at these two edges. This could trap the fluid located at the specimen centre, unless it can pass through the connective tissue matrix, and this would be consistent with lower rate results obtained previously by Van Loocke (Van Loocke et al., 2008), where the fluid expulsion appeared to be along rather than across the muscle fibres. The fluid mass loss was 8% on average during the testing.

Figure 5-14 shows the equivalent results for a cross-fibre direction test when viewed parallel to the muscle fibres (B in Figure 5-8A). It is again evident that the strain is initially greater at the top of the sample than at the bottom, but now the strain at the left and right edges is similar to the strain in the middle of the sample. The result highlight the dependency of the strain on the chosen region (see Figure 5-8B) and the
results are significantly different to the stretch calculated from the platen displacements.

The speed of an elastic stress wave in a fluid is around 1500 ms\(^{-1}\) (Jolliffe et al., 1966, Arfken et al., 1989). For the sample geometries used, this elastic stress wave would pass through the sample in microseconds and cannot be observed in the high speed video images (sample rate 10 kHz), but it may be the reason why the upper and lower force time histories shown in Figure 5-10 match well. A separate densification wave, where the tissue is seen to collapse was observable at a much lower speed of approximately 10 ms\(^{-1}\) and is evident in the strain time history in Figure 5-13 and Figure 5-14 and is shown schematically in Figure 5-19.

![Figure 5-19: Schematic illustration of the strain process during impact loading](image)

Given the observation that the tissue compression moves through the sample in a wave-like manner (the initial compression in the upper portions of the sample is greater than in the lower portions of the sample as shown in Figure 5-13 and Figure 5-14), it is surprising that the load measured at the top and bottom of the sample appears to be substantially the same (when correcting for the inertia of the upper platen) up to the time of contacting the strain control plate, see Figure 5-10. One explanation for the equalizing of the force between the top and bottom of the sample could be the high fluid content in the tissue. Nonetheless, the result is surprising since it indicates that the tissue is behaving similar to a fully confined fluid in this respect.
These equilibrated force measurements indicate that the inertia force associated with accelerating portions of the tissue during the test are negligible.

The ultimate goal was to measure stress-stretch relationships for the tissue at strain rates relevant to automotive impacts. Clearly, given the complex deformation behaviour observed, this can only be done in an average sense and more detailed inverse finite element modelling would be necessary to attempt to extract truly local material properties. Nonetheless, the engineering stress evaluations presented in Figure 5-15 and Figure 5-16 do provide useful information on the dynamic deformation behaviour of fresh porcine skeletal muscle tissue in both the fibre and cross-fibre direction at automotive impact rates. The cross-fibre stress-stretch data presented in Figure 5-15 are averages of four tests at 11,600% s⁻¹, twelve tests at 22,000% s⁻¹ and five tests at 37,800% s⁻¹ and the standard deviations are also shown. The fibre direction data seen in Figure 5-14 are averages of five repeated tests at 22,000% s⁻¹ and five tests at 37,800% s⁻¹, and again the standard deviations are shown. The results show nonlinear stress-strain relationships as well as clear stiffening with increasing strain rate, which is qualitatively similar to results from lower rate testing (Van Loocke et al., 2006, Van Loocke et al., 2008, Chawla et al., 2009, Song et al., 2007, Van Sligtenhorst et al., 2006). The stress at 30% compression was approximately 9 times higher than the strain rate of 37800% s⁻¹ compared to 11600% s⁻¹, which is qualitatively similar to observations at other strain rates (Van Loocke et al., 2006, Van Loocke et al., 2008, Zheng et al., 1999).

In most of the published lower strain rate literature, the cross-fibre direction was observed to be stiffer than the fibre response at a given strain rate (see example in Figure 3-10) (Song et al., 2007, Van Loocke et al., 2006, Van Loocke et al., 2008, Zheng et al., 1999) but the differences observed here between the fibre and cross-fibre direction responses were observed to be strain dependent (compare Figure 5-15 to Figure 5-16).

Direct comparison with the frozen and then thawed human tests by Chawla et al (Chawla et al., 2009) is difficult as slightly different strain rates were used, but a broad comparison shows that the stress at a stretch of 0.7 for the cross-fibre direction tests at both 11,600% s⁻¹ and at 22,000% s⁻¹ was about two to three times more compliant for the current tests than for the results of Chawla et al. The same differences were observed by Van Loocke when the comparison of aged tissue to fresh tissue was performed, see Figure 3-11). This may be due to the effects of Rigor Mortis on their
results, as discussed earlier, but may also reflect other differences. As Chawla et al did not test in the fibre direction it is not possible to compare those results, and they did not comment on fluid expulsion in their testing.

In order to assess the evolution of stress with strain rate, Van Loocke (Van Loocke, 2007) used a stress normalisation method to compare different experimental data obtained from different protocols and this is applied again here. For each experimental protocol, the stress value from the lowest strain rate was taken as a reference and the ratios between this value and the values obtained at higher rates were calculated. The resulting stress ratios were then combined to show the increase in stress from a reference strain rate of 0.05% s\(^{-1}\) (which now gives a ratio of 1), see Figure 5-18. A continuous evolution by comparison with the ramp and cyclic tests reported in the literature (Van Loocke, 2007, Van Loocke et al., 2006, Van Loocke et al., 2008, Van Loocke et al., 2009) is evident, but the results presented here show a stiffer response than the split Hopkinson bar tests results observed by Song et al (Song et al., 2007).

![Figure 5-20: Relative increase in stress with compression rate at $\dot{\lambda} = 0.7$ for tests performed by Van Loocke et al and Song et al (Song et al., 2007, Van Loocke, 2007) and the experimental results from the current tests.](image)
Overall, this chapter provided new 3D data on the impact response of fresh passive porcine skeletal muscle tissue at rates relevant to automotive impacts. Both the fibre and the cross-fibre direction have been tested on fresh porcine tissue prior to the onset of Rigor Mortis. Furthermore, this study utilised the same tissue preparation and testing protocol as in earlier quasi-static testing work, and this has allowed calculation of the evolution of stress increase with compression rate in a way that was not previously possible. Finally, an attempt to characterise the strain variation within the samples and the fluid behaviour within the tissue during the impact process has been performed. This is considered to be novel because to the best of the author’s knowledge, this has not been reported before.
5.5 Conclusion

Freshly slaughtered cuboidal shaped porcine muscle samples were compressively impact loaded using a custom-designed drop tower testing rig. At a stretch ratio of 0.7, the following engineering stress responses magnitudes were observed: 5.95 kPa ±0.6 kPa, 25.88 kPa ±5.3 kPa and 43.68 kPa ±1.4 kPa at strain rates of 11,600% s⁻¹, 22,000% s⁻¹ and 37,800% s⁻¹ respectively for a load applied in the cross-fibre direction. For loading applied in the fibre direction, the following engineering stress response magnitudes were observed: 22.03 kPa ±1.5 kPa and 37.06 kPa ±3.0 kPa at strain rates of 22,000% s⁻¹ and 37,800% s⁻¹ respectively. A compressive stress wave was observed to propagate downwards and muscle fluids were ejected at the front of the wave resulting in an average sample mass loss of 8%. For the strain rates tested, which are relevant to automotive impact cases, skeletal muscle displays a nonlinear stress stretch relationship as well as a clear rate dependency.
6 Study 3: Inverse FEA

Figure 6-1, highlights the task and processes involved in this chapter (highlighted in red) in order to achieve the objectives for this chapter. The overall thesis tasks and processes are included in Figure 6-1 in order to clarify where this chapter's work fits in with the rest of the thesis work.

Figure 6-1: Presents the full thesis flow chart showing all thesis processes. Highlighted in red are the modelling processes that are relevant to this chapter, but forms part of the whole thesis.
6.1 Introduction

A review on the micro-structural composition of skeletal muscle tissue is given in sections 3.2.1. The complexity of the micro-structured muscle tissue that is functionally divided into three organisational levels (Gaudin, 1997, Freeman and Bracegirdle, 1982, Ross et al., 1989), may relate the tissue's passive anisotropic behaviour response (see Figure 3-18).

Sections 3.2.1 of the literature review discussed the work from several authors who have performed experimental tests on skeletal muscle in the fibre and cross-fibre direction. This has been represented in models as either isotropic or using single (longitudinal) fibre family reinforced transverse isotropy models. However, compressive experimental work performed by Van Loocke showed that there is an interesting passive behavioural response at intermediate angles (0°, 30°, 45°, 60° and 90°), (Van Loocke et al., 2006) and in chapter 4, of this thesis it has been shown that muscle tissue exhibits a complex anisotropic behaviour at intermediate angles (see also (Takaza et al., 2013).

In this study a simple first order isotropic hyperelastic Ogden model was used to capture the fibre direction elastic response. The inverse finite element analysis (iFEA) has been applied to identify soft tissue material parameters with great success in the past (Ahn and Kim, 2010, Samur et al., 2007), including recently published work (Böl et al., 2013, Miller and Lu, 2013, Abyaneh et al., 2013, Wittek et al., 2013, Heiland et al., 2013, Badir et al., 2013). Inverse analysis was performed on the experimental data (the deformed configuration was taken as a starting geometry and then solved for the un-deformed geometry, thereby determining the amount of forces and stresses present in the deformed configuration). Major emphasis of this work was placed on the impact strain rates as they include the viscoelastic properties.

Therefore the first objective of this chapter was to perform an inverse finite element analysis using a simple first order hyperelastic Ogden model to capture the fibre direction elastic response. The deformed configuration was taken as a starting geometry and then solved for the un-deformed geometrical parameters, thereby determining the amount of forces and stresses given in the deformed configuration. The second objective was to compare the tensile and the compressive responses based on the Ogden elastic parameters that are required to capture the experimental response. The third objective was to perform an inverse finite element analysis on the impact data by using a QLV model with three branches.
6.2 Methods

Inverse FEA was performed using the freely available FEBio software (open source version 1.6, Musculoskeletal Research Laboratories, The University of Utah, USA (Maas et al., 2012)). The simple routine codes were developed in MATLAB (8 R2013b The Mathworks Inc., Natick, MA) to enable data processing and visualisation. Inverse analysis was performed using MATLAB based control and parameter optimisation. Explicit formulation was used since the simplicity formulation was observed to be very slow and used larger time steps that introduced unnecessary errors.

6.2.1 Experimental Procedures

A detailed description of the experimental procedure, test rig validation and the raw experimental data can be found in chapters 4 and 5, (see also (Takaza and Simms, 2012, Takaza et al., 2013)).

6.2.2 Finite Element Modelling

Simulation of the cross-fibre experimental tests were performed using finite element analysis (FEA) with the freely available FEBio software (version FEBio 1.5, MRL University of Utah), and inverse analysis was performed using MATLAB based control and parameter optimisation.

6.2.3 Finite Element Model Description

Figure 6-2: (A), FEBio compressive muscle model and compression plate (blue) model. (B), Shows the FEBio tensile muscle model.
For simplicity, an idealised geometry model was used even though non-ideal geometries occurred in the experiment; see Table 4-1 and Table 4-2 (and further in Figure 5-12). The compressive muscle samples were represented by a 10x10x10 mm cube which was uniformly meshed using 10x10x10 solid 4 node tri-linear hexahedral elements (total of 1000 elements). The tensile muscle samples were represented by a 50x10x10 mm which was uniformly meshed using 50x10x10 solid 4 node tri-linear hexahedral elements (total of 5000 elements). This was assumed to be a good average representation as some tissue dimensions were above as well as below the ideal geometrical shape. This was found to have no influence on the optimised parameter as the input data was based on the averages experimental response and the shape standard deviation was observed to be low (see Table 4-1 and Table 4-2). The compression plates in both cases were meshed using a 45x45, 0.01 mm thick quadrilateral shell elements (total 2025 elements) and assigned aluminium alloy (5251_H26) material properties (Young's Modulus = 70 GPa, Poisson's ratio = 0.35 and density = 2780 kg/m$^3$). Contact between the compression plate and the sample top surface was modelled using a frictionless sliding interface (Ateşhian et al., 2010, Al-Mayah et al., 2009) to replicate the experimental condition. The top surface elements of the tensile model were prescribed an upward displacement of 15 mm (30% stretch) or downwards displacement of 3 mm (30% compression). A mesh convergence test was performed to determine the required mesh density (Chawla et al., 2009, Miller et al., 2000). The hour-glassing parameter in FEBio was investigated in dynamic preliminary work and it was established that a value of 15 effectively suppressed the hour-glassing mode without significantly affecting the model response. In the compressive dynamic simulations, the muscle samples were subjected to dynamic unconfined compression by prescribing the compression plate displacement to match the experimental conditions for all three rates of testing (11,600%$s^{-1}$, 22,000%$s^{-1}$ and 37,800%$s^{-1}$). An initial spacing of 0.2 mm between contact surfaces was used to allow the contact to develop.
6.2.4 Constitutive modelling

6.2.4.1 Bulk Modulus

The ability of the current employed model to resist uniform compression (bulk modulus) was investigated separately before the optimisation process. Figure 6-3, shows the effect of the Bulk Modulus parameter $\kappa$ on the boundary condition force at 30\% compression: for a Bulk Modulus above 20 MPa the effect on the predicted boundary condition force is constant. Accordingly, the Bulk Modulus was set to 20 MPa.

![Figure 6-3: (left) Influence of varying Bulk Modulus on boundary condition force versus muscle stretch and (Right) Influence of varying Bulk Modulus on peak boundary condition force at $\lambda = 0.7$.](image)

To verify that incompressibility was achieved when the Bulk Modulus is 20 MPa, the relative volume was assessed. Figure 6-4, shows that when $\kappa = 20$ MPa, the relative volume is effectively 1 at the peak compression of $\lambda = 0.7$.

![Figure 6-4: Volume preservation of the model at peak compression for Bulk Modulus K = 20MPa.](image)
The bulk modulus of 20 MPa was observed to enforce the required nearly incompressible behavioural response. However bulk the modulus used here is 100 times less than that of incompressible water (2000 MPa). Nevertheless, convergence was investigated and it was observed that increasing the bulk modulus above 20 MPa did not improve the results, but started making it difficult for FEBio to complete iterative steps.

6.2.4.2 Hourglass suppression mode

The value prescribed to the hour-glassing parameter was observed to have an effect on the dynamic simulation results. The hour-glassing parameter in FEBio was investigated after the final parameters were established through the optimisation process. Hourglass is considered to be under control if it is observed to be 10% of the total internal energy (Jacquotte and Oden, 1984, Murray et al., 2005), but due to FEBio's inability to output the internal energy, the method described below was adopted. The hour-glassing parameter was gradually increased from 0.001 to 35 and the resulting boundary condition force recorded and plotted against time, and these results are presented in Figure 6-5 below.

Figure 6-5: (left) The effect on the boundary condition force time history of adjusting the hour-glassing parameter (H) and (Right) Influence of varying the hour-glassing parameter (H) on peak boundary condition force at compression of $\lambda = 0.7$. 
Figure 6-5 and Figure 6-6 shows that the effect of adjusting the hour-glassing parameter $H$ on the boundary condition force is small when $H$ is set at approximately 15, and this was therefore the value chosen for $H$. It is considered that this value provides effective hourglass mode suppression without significantly affecting the stiffness response of the tissue. The resulting deformation behaviour for $H = 15$ is shown with the result when $H = 1$, see Figure 6-6.

![Hour-glass parameter set at 1](image1) ![Hour-glass parameter set at 15](image2)

Figure 6-6: The hour-glass effect is shown above. Setting the hour-glass at 15 also allowed a faster less dense mess to be used.

### 6.2.4.3 Elastic behaviour

The constitutive modelling framework presented employs the following deformation metrics: $F$ is the deformation gradient tensor (see equation 2-3), $J = \det(F)$, the Jacobian or volume ratio (see equation 2-9); $C = F^TF$ (see equation 2-13 and 2-15), the right Cauchy-Green tensor and the principal stretches $\lambda_i$ (with $i = 1,2,3$). In addition, for uncoupled representations the following modified or deviatoric deformation metrics are employed (denoted $\tilde{\bullet}$): $\tilde{F} = J^{-\frac{1}{3}}F$, $\tilde{C} = J^{-\frac{2}{3}}C = F^T\tilde{F}$, and $\tilde{\lambda}_i = J^{-\frac{1}{3}}\lambda_i$ (see equation 2-39).

The elastic behaviour of muscle tissue was modelled using the following uncoupled hyperelastic strain energy density formulation:

$$\Psi(\tilde{C}, J) = \Psi(\tilde{C}) + \Psi_{\text{vol}}(J)$$

6-1

Here $\Psi$ defines the deviatoric elastic response of muscle tissue and is defined as an isotropic non-linear hyperelastic first order Ogden constitutive law (Ogden, 1972), similar to Bosboom et al. (Bosboom et al., 2001) (reader is referred back to nonlinear elasticity section 2.1.7.3):

$$\Psi(\tilde{\lambda}_1, \tilde{\lambda}_2, \tilde{\lambda}_3) = \frac{\mu}{\alpha} (\tilde{\lambda}_1^\alpha \tilde{\lambda}_2^\alpha + \tilde{\lambda}_3^\alpha - 3)$$

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where the behaviour is dictated by the material parameters $\mu$ and $\alpha$. The volumetric contribution is given by:

$$\Psi_{\text{vol}}(J) = \frac{k}{2} (\ln J)^2.$$ 

where the material parameter $k$ is the Bulk Modulus. To satisfy near incompressibility (Maas et al., 2012), a bulk modulus of 20 MPa was used, see section 6.2.4.1 above. The tissue was prescribed a material density of 1060 kg/m$^3$ (Patel et al., 2003).

The second Piola-Kirchoff stress tensor $\mathbf{S}$ for the elastic behavior can be written (Maas and Weiss, 2013):

$$\mathbf{S} = 2 \frac{\partial \Psi}{\partial \mathbf{C}} = 2 \frac{\partial \Psi}{\partial \mathbf{C}} + p\mathbf{C}^{-1} = J \frac{2}{3} \text{Dev}(\bar{\mathbf{S}}) + p\mathbf{C}^{-1},$$

where use was made of the reference frame deviatoric operator $\text{Dev}(\mathbf{S}) = (\mathbf{S}) - \frac{1}{3} ((\mathbf{S}) : \mathbf{C}) \mathbf{C}^{-1}$, $\bar{\mathbf{S}} = 2 \frac{\partial \Psi}{\partial \mathbf{C}}$ is the deviatoric second Piola-Kirchoff stress and $p$ is a pressure term given by:

$$p = \frac{\partial U}{\partial j} = \frac{k}{j} \ln J.$$ 

6.2.4.3.1 Compressive Contact Definition and Boundary Conditions

The compression plate was prescribed a downward displacement to achieve an overall sample compression of 30% and the time taken was set to match the different experimental conditions. Vertical displacements of the nodes of the bottom surface of the muscle sample were constrained and the central node of the bottom surface was pinned in order to anchor the model. Predicted boundary condition force measurements were from the reaction forces between the top surface of the muscle and the compression plate.

6.2.4.3.2 Inverse optimisation based constitutive parameter identification

The Compressive Ogden elastic parameters ($\mu$ and $\alpha$) were obtained in an optimisation run by simulating the quasi-static 0.05%$s^{-1}$ cross-fibre direction experimental data from Van Loocke (Van Loocke et al., 2006). Similarly, the tensile Ogden parameters were obtained quasi-static 0.05%$s^{-1}$ cross-fibre direction experimental data from chapter 4.

The optimisation process was controlled through a custom MATLAB code (Moerman et al., 2013) which:

- Generated FEBio input files containing the model description and appropriate material parameters.

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• Started an FEBio simulation and imported results once analysis was terminated (e.g. simulated force curves)

• Computed the sum of squared differences between simulated and experimental force/stretch ratio data which here served as the optimisation penalty parameter. The inverse optimisation routine was based on the Nelder-Mead simplex search algorithm (implemented using the MATLAB \texttt{fminsearch} function, see also (Lagarias et al., 1998)). The objective function ($O$) for optimisation was defined as:

$$O(\mu, \alpha) = \sum_i \sum_j \left( F_{\text{exp}}(\lambda_i, \dot{\varepsilon}_j) - F_{\text{sim}}(\lambda_i, \mu, \alpha, \dot{\varepsilon}_j) \right)^2,$$

Which describes the objective function as the sum of squared differences between the experimental ($F_{\text{exp}}$) and simulated force ($F_{\text{sim}}$) data across all stretch levels $\lambda_i$ and all three strain rates $\dot{\varepsilon}_j$ (see Figure 6-7).

Convergence was defined as when the parameter variation (sum of squared differences) was less than 0.01\% or 1000 iterations reached. Parameter convergence was also investigated for different initial parameter values and results were insensitive to the initial guess. The model predictions were assessed in relation to the experimental results in two ways:

i. Comparison of the boundary condition force in the contact between the upper plate and the tissue sample and

ii. Comparison of the displacements of the markers in the experiment and their equivalent location in the model.

The following summary statistics will be computed with respect to the experimental data:

i. R squared

ii. Root mean square (RMS) of the differences

iii. Sum of squared differences (SSQD)

iv. The maximum difference (Max.Diff.).
6.2.4.4 Viscoelastic behaviour

The viscoelastic behaviour of muscle tissue is modelled using discretised quasi-linear viscoelasticity (QLV) (Puso and Weiss, 1998, Maas and Weiss, 2013) combined with the uncoupled hyperelastic formulation (Hassan et al., 2012, MARC, 1991). Since the viscoelastic behaviour is defined only for the deviatoric stress, the Second-Piola Kirchoff stress can be written:

$$S(t) = \int_{-\infty}^{t} \frac{2}{3} \int_{-\infty}^{t} G(t-s) \frac{\partial \text{dev}(\tilde{S}^e)}{\partial s} ds + pI C^{-1},$$

where $\tilde{S}^e$ is the pure elastic deviatoric Second Piola-Kirchoff stress (equation 6.7). The function $G$ defines a discretised relaxation function given by:

$$G(t) = 1 + \sum_{i=1}^{n} \gamma_i e^{-\frac{t}{\tau_i}},$$

where $\gamma_i$ and $\tau_i$ define proportional and time decaying viscoelastic behaviour respectively and it is clear that the long-term deviatoric response reduces to the pure elastic response described earlier. In the modelling, a three-term relaxation function was employed. Referring to (Puso and Weiss, 1998) and (FEBio-Manuals-Version-1.5, 2012) will provide a more detailed description.
The Ogden elastic parameters ($\mu$ and $\alpha$) obtained in an initial optimisation run by simulating the quasi-static 0.05\%s$^{-1}$ cross-fibre direction experimental data from Van Loocke (Van Loocke et al., 2006) were used for dynamic Inverse FEA. The three viscoelastic time constants ($\tau_1, \tau_2, \tau_3$) were fixed at 0.015 ms, 0.0015 ms and 0.00015 ms respectively to span the time interval of the experimental tests, similar to the approach of Van Loocke et al. (Van Loocke et al., 2008). The remaining three viscoelastic parameters ($\gamma_1, \gamma_2, \gamma_3$) were then obtained through inverse analysis of the experimental cross-fibre tests at the three different compression rates $\dot{\varepsilon}_i$ ($\dot{\varepsilon}_1 = 11,600\%s^{-1}, \dot{\varepsilon}_2 = 22,000\%s^{-1}$ and $\dot{\varepsilon}_3 = 37,800\%s^{-1}$), where the elastic parameters ($\mu$ and $\alpha$) were fixed. The optimisation process was controlled through a custom MATLAB code (Moerman et al., 2013) as described in section 6.2.4.3.
6.3 Results

6.3.1 Elastic Inverse FEA

Optimisation was performed in order to establish the first order hyperelastic Ogden parameters. The Ogden material law is an isotropic model; therefore it does not take into account the influence of the fibre direction loading. The optimised elastic parameters are presented in Table 6-1.

<table>
<thead>
<tr>
<th>Test Type (0.05% s⁻¹)</th>
<th>μ (kPa)</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tension</td>
<td>341.6264</td>
<td>3.2339</td>
</tr>
<tr>
<td>Compression</td>
<td>1.685</td>
<td>15.434</td>
</tr>
</tbody>
</table>

Table 6-1: Shows the elastic isotropic Ogden Material parameters (Compression experimental data from Van Loocke)

Statistical analysis of the differences between the presented experimental data and the presented model prediction are presented in Table 6-2.

<table>
<thead>
<tr>
<th>Measure of Statistical difference</th>
<th>Compression</th>
<th>Tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSQD (kPa) Avg</td>
<td>0.0002632</td>
<td>0.9863</td>
</tr>
<tr>
<td>Max_Deviation (kPa) Avg</td>
<td>0.1367</td>
<td>1.896</td>
</tr>
<tr>
<td>R Squared Avg</td>
<td>0.9975</td>
<td>0.9176</td>
</tr>
<tr>
<td>RMS (kPa) Avg</td>
<td>0.00124</td>
<td>0.1295</td>
</tr>
</tbody>
</table>

Table 6-2: Summary statistics for the first order hyperelastic Ogden material law. The statistics are averages for all the loading directions.

The R squared (also known as the coefficient of determination) value show how close the model prediction is to the experimental data. The maximum deviation of our experimental data from the experimental data was observed to be 1.896 kPa. The root mean square of the differences gives the sample standard deviation of the differences between the Ogden model's predicted values and the experimental values as 0.00124 kPa for compression and 0.1295 kPa for tension. The RMS is another measure of how accurate the model predictions are.

Table 6-1 shows the elastic material parameter for the isotropic Ogden model for the compressive and the tension experiments experimental data. The isotropic Ogden model provided a good prediction of the van Loocke experimental data seen in Figure 6-8. If the symmetrical behavioural response was assumed between tension and
compression, then the material parameters obtained from the compressive quasi-static optimisation were used to predict the tensile behavioural response, see Figure 6-9. The predicted tensile response was then plotted in a graph, which is presented in Figure 6-9. This data was then compared to the real literature based experimental data, presented in Figure 6-10. The predicted tensile response was found to be around 100 times less than the compressive experimental data. This highlights the asymmetrical behavioural response of skeletal muscle. So far no known model possesses the required predictive capabilities to capture the compression and tension response using the same material properties.

Figure 6-8: Van Loocke compressive data fitted with an isotropic Ogden Model fit.

Figure 6-9: Symmetrical behavioural response prediction if the compressive optimised parameter (Van Loocke) were used to predict tensile behavioural response
Figure 6-10: Predictions of an Ogden model prescribed the compressive parameter from the Van Loocke (VLM) experimental data.

Figure 6-11 (A) shows the anisotropic experimental data and isotropic Ogden model response (blue) in the same graph. The first order isotropic Ogden model provides a very good fit even though individual optimisation had to be repeated for each loading direction in order to get a perfect fit on all muscle fibre orientation data. Figure 6-11 (B) shows the tensile response landscape, and the predicted isotropic first order Ogden model response (Shown in blue for the fibre direction), which used a tensile result optimised parameter. The tensile response was observed to change from nonlinear to linear response as the muscle fibre testing angle is changed from 0 to 90 degrees. Therefore an isotropic model was observed to be poor in predicting the anisotropic response.

Figure 6-11: (A) Shows the quasi-static compressive response landscape obtained by applying the elastic compressive material parameters presented in Table 6-1. The blue coloured plots are the isotropic Ogden predicted response. (B) Shows the quasi-static tensile response landscape obtained by applying the elastic tensile material parameters presented in Table 6-1. The isotropic Ogden predicted is plotted in blue.
Table 6-3 presents the first order Ogden model parameters that have been obtained through the inverse analysis process and also the bulk modulus (held constant during optimisation).

<table>
<thead>
<tr>
<th>Strain rate % $s^{-1}$</th>
<th>$\mu$ (kPa)</th>
<th>$\alpha$ (-)</th>
<th>$\kappa$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11600</td>
<td>4.00e-03</td>
<td>19.92</td>
<td>20</td>
</tr>
<tr>
<td>22000</td>
<td>1.698e-02</td>
<td>19.43</td>
<td>20</td>
</tr>
<tr>
<td>37800</td>
<td>3.849e-02</td>
<td>18.85</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6-3: Cross-fibre optimised Ogden parameters

<table>
<thead>
<tr>
<th>Measure of Statistical difference</th>
<th>Strain rate % $s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11600</td>
</tr>
<tr>
<td>SSQD (N)</td>
<td>0.00223</td>
</tr>
<tr>
<td>D. Max. Rel (N)</td>
<td>0.1952</td>
</tr>
<tr>
<td>R Squared</td>
<td>0.09960</td>
</tr>
<tr>
<td>RMS (N)</td>
<td>0.001325</td>
</tr>
</tbody>
</table>

Table 6-4: Summary statistics for the first order hyperelastic Ogden material law.

Comparisons of the model boundary condition force using the optimised parameters in Table 6-3 for the cross-fibre direction tests at 11,600% $s^{-1}$, 22,000% $s^{-1}$ and 37,800% $s^{-1}$ are presented in Figure 6-12, Figure 6-13 and Figure 6-14.

Figure 6-12: Comparison of the predicted model boundary condition force with experimental observation for cross-fibre direction tests at 11,600% $s^{-1}$ using optimised model parameters.
The model predictions are good, but optimisation has to be performed every time the impact velocity is changed (new parameters required for different impact speeds). This is tedious, time consuming and limits the model usage to only the impact speeds applied in the experiments.
6.3.2 Viscoelastic Inverse FEA

In order to solve the above limitation, the viscoelastic behaviour of muscle tissue is modelled using discretised quasi-linear viscoelasticity (QLV), (Maas and Weiss, 2013, Puso and Weiss, 1998). Table 6-5 shows the first order Ogden model parameters obtained through the inverse analysis processes and also the bulk modulus (held constant during optimisation) as well as the elastic parameters ($\mu$ and $\alpha$) obtained in the initial optimisation in simulating the quasi-static 0.05% s-1 cross-fibre direction experimental data from Van Loocke (Van Loocke et al., 2006).

<table>
<thead>
<tr>
<th>$\gamma_1$</th>
<th>$\gamma_2$</th>
<th>$\gamma_3$</th>
<th>$\tau_1$ (ms)</th>
<th>$\tau_2$ (ms)</th>
<th>$\tau_3$ (ms)</th>
<th>K (MPa)</th>
<th>$\mu$ (kPa)</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0715</td>
<td>2.49</td>
<td>0.277</td>
<td>0.015</td>
<td>0.0015</td>
<td>0.00015</td>
<td>20</td>
<td>1.685</td>
<td>15.434</td>
</tr>
</tbody>
</table>

Table 6-5: Optimised viscoelastic Ogden parameters (The elastic parameters are obtained from section 6.3.1).

<table>
<thead>
<tr>
<th>Measure of Statistical difference</th>
<th>Fibre angle with respect to load direction (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSQD (N)</td>
<td>2.632</td>
</tr>
<tr>
<td>D. Max. Rel (N)</td>
<td>0.1</td>
</tr>
<tr>
<td>R Squared</td>
<td>0.9649</td>
</tr>
<tr>
<td>RMS (N)</td>
<td>0.1295</td>
</tr>
</tbody>
</table>

Table 6-6: Summary statistics for the QLV

Figure 6-15 presents the experimental boundary condition force (mean and standard deviation) at the three testing rates together with the predicted FEA result for the optimised parameters from Table 6-5. Figure 6-16 and Figure 6-17 present the experimental and optimised model shapes at the peak compression of $\lambda = 0.7$ for the three different testing rates. The third row presented in Figure 6-17 provides a comparison of the measured and predicted marker locations at peak compression. Figure 6-18 and Figure 6-19 respectively show the measured versus predicted marker displacement time histories of the top, middle and bottom rows of markers on the sample for vertical and horizontal motion of the sample during compression.
Figure 6-15: Comparison of the predicted model boundary condition force with experimental observation for cross-fibre direction tests at 11,600% s^-1, 22,000% s^-1 and 37,800% s^-1 using optimized model parameters. The red plots are the experimental fibre direction data, the blue is the cross-fibre experimental data and the black are the model predictions of the cross-fibre direction tests.

Figure 6-16: overlay of experimental shape change and test marker locations (o) and model predictions (+).
Figure 6-17: First row: images of the specimen at $\lambda = 0.7$ at testing rates of 11,600% s$^{-1}$, 22,000% s$^{-1}$ and 37,800% s$^{-1}$ respectively; Second row: corresponding model image at 30% compression, the blue corresponds to the highest vertical displacement of 3.2mm while the red colour shows areas with the least or no displacement at all; Third row: overlay of experimental test marker locations (o) and model predictions (+).

Figure 6-18: Comparison of average experimental and model vertical displacement of the 9 marker dots for cross-fibre direction tests at the 3 different a strain rates.
Figure 6-19: Comparison of average experimental and model horizontal displacement of the 9 markings for cross-fibre direction tests at the 3 different strain rates.
6.4 Discussion

Inverse optimisation was used to identify the elastic material constants for a first order isotropic Ogden law, and the first order isotropic Ogden law was then expanded with a three term quasi-linear viscoelastic (QVL) approach to capture the dynamic rate dependent effects. The long term elastic parameters ($\mu$ and $\alpha$) in Table 6-1 were determined from the quasi-static cross-fibre data from Van Loocke et al. (2006), for which the tissue was harvested from the same species and tested under similar experimental conditions. The first optimisation minimised the difference between the model boundary condition stress and the experimental observations of the muscle fibre testing, see Table 6-1. Since the isotropic Ogden model provided only an average fit to the average tissue response, the prediction capabilities of the model for the compressive response as the loading angle is changed were observed to be still good, yet the results were somewhat very poor for the tensile response. Figure 6-11(B) Shows the quasi-static tensile response landscape obtained by applying the elastic tensile material parameters presented in Table 6-1. The isotropic Ogden predicted response is plotted in blue. This was due to the fibre directional parameter having been optimised on a nonlinear response; but the observed response changed from nonlinear to linear as the loading angle was rotated to 90 degrees.

The second optimisation minimised the difference between the model boundary condition force and the experimental observations for the three individual impact testing rates (11,600% s\(^{-1}\), 22,000% s\(^{-1}\) and 37,800% s\(^{-1}\)), see Table 6-3. This resulted in three separate individual optimised parameters for each strain rate. The optimised parameters presented in Table 6-3 were used, the resulting predicted model force response was observed to match the experimental data well as seen in Figure 6-12, Figure 6-13 and Figure 6-14. The QLV modelling worked well for our work, partly because the range of strain rates is limited. And that perhaps at higher strain rates the approach may not yield good results.

The main optimisation minimised the difference between the model boundary condition force and the experimental observations at all three testing rates (11,600% s\(^{-1}\), 22,000% s\(^{-1}\) and 37,800% s\(^{-1}\)) seen in Table 6-5. When these optimised parameters were used (Table 6-5), the resulting predicted marker displacements of the marker locations matched the experimental results very well for all three testing rates; see Figure 6-16 - Figure 6-19 for vertical and horizontal deformations respectively, even though an isotropic model was employed.
In this chapter, the parameter identification using the optimisation technique was performed only for the cross-fibre direction as it was observed that at high strain rates the muscle tissue behaved in a more or less isotropic manner (see section 5.4 and Figure 5-18). Using a first order uncoupled viscoelastic Ogden material model extended with three term QLV to capture a good response at each of the different strain rates when inverse analysis is performed. Further to this, a good comparison to both the fibre and cross-fibre direction experimental results are achieved, since the tissue anisotropy is relatively small at these dynamic loading rates, see Figure 6-15.

The experimental data presented in this chapter are also of use for future evaluation of constitutive models. In particular, the analysis presented in Figure 6-18 and Figure 6-19 showing the horizontal and vertical displacement time histories of the different regions in the sample provide a rich dataset for model evaluation.

6.5 Conclusions

Provided in this chapter is an inverse finite element analysis of the experiments, whereby muscle was represented by a first order Ogden hyperelastic model extended with a three term QVL expansion to account for rate dependent effects. This indicated a good response at each of the different strain rates tested for both the boundary condition force and the tissue deformation observed.
7 Study 4: Micro-structural Deformation analysis

Figure 7-1, highlights the task and routes involved in this chapter (highlighted in red) in order to achieve the objectives for this chapter. The overall thesis tasks and routes are included in Figure 7-1 in order to clarify how this chapter's work fits in with the rest of the thesis work.

Figure 7-1: Presents the full thesis flow chart showing all thesis processes. Highlighted in red are the micro-structural processes that are relevant to this chapter, but forms part of the whole thesis.
7.1 Introduction

The asymmetrical tension/compression passive responses of skeletal muscle samples when they are subjected to external large deformations are not fully understood. The tensional mechanical response magnitude is in the order of approximately 100 times higher than the compressive mechanical response (see Figure 7-2). In compression, the 45 degree response is the lowest, the whole muscle fibre testing angles present a nice upside down “U” shape. In tension, the muscle fibre direction is the most compliant; the whole muscle fibre testing angles describing a sinusoidal (see Figure 4-17) shape response.

![Figure 7-2: The anisotropic nonlinear elastic behaviour of skeletal muscle tissue in compression (A) and tension (B). Thick solid curves are the mean experimental data, thin curves represent the mean response plus and minus. Coloured (toward Cauchy stress kPa) surfaces are periodic surface fits to the mean response and grey transparent surfaces are for the standard deviation offsets. Circled in red are the stretch comparison figures and circled in yellow is the fibre orientation response comparison.](image)

Given the observed tension-compression asymmetrical behavioural response of the skeletal muscle tissue at the macroscopic stress-strain level, the objective of this chapter was to ascertain at the micron scale whether the structures responsible for the asymmetrical can be observed.

The goal was to assess whether the light microscope shows evidence of limits to the continuum behaviour of skeletal muscle tissue at the micro-scale. If such limits could be observed, this could provide clues in relation to the structures responsible for the overall tension-compression asymmetrical behaviour. This asymmetrical behavioural response of tissue has not been investigated before, and is the objective of this microscopic study.
7.2 Methods

Freshly harvested porcine muscle tissue was deformed and fixed in a fixative chemical. The deformed and un-deformed fixed samples were then prepared for light microscopic analysis including staining with different stains. Running parallel to this, fixed samples were prepared for the high resolution SEM analysis to verify the light microscope results.

The use of animals in this study was approved by the University of Dublin Ethics Committee for the protection of animals used for scientific purposes, according to EU directive 2010/63/EU. The tissue was fixed in a solution of 10% formaldehyde solution, embedded in paraffin wax, sectioned at a thickness of 8 μm and stained with the appropriate dye. For the analysis the samples were stained with pirco Sirius red (Puchtler et al., 1973, Rich and Whittaker, 2005). The thin sections were analysed using an Olympus IX81-long focal length fluorescent microscope. For polarised analysis, a Nikon polarising (model Eclipse E400 POL) microscope that was fitted with circular filters to provide polarised light was used. For the SEM analysis a Carl Zeiss Supra SEM microscope was used. The microscope image analysis was performed using the naked eye as well as quantitatively using Image J tools (Image J is a freely available image processing and analysis program), (ImageJ, 2012)

7.2.1 Specimen Preparation

In order to investigate the tensile micro-structural response of deformed muscle tissue, a custom designed deformation rig was developed (see Figure 7-3). Samples were stretched by 30% using this newly developed uniaxial tensile deformation device. After the specimens were deformed to the required stretch, the whole rig was immersed into the formaldehyde solution in order to allow chemical fixation to take place on the mechanical fixed samples. The aim of the experiment was to analyse the muscle fibre micro-structural response when tissue is deformed by 30%. The specimen compressive deformation was achieved by using uniquely designed clamps, but available commercially.
Freshly harvested *longissimus dorsi* skeletal muscle tissue was harvested from 3 month old female pigs. Tensional stretches or compressive deformation of 30% were applied with respect to the muscle fibre orientation as shown in Figure 7-4. A schematic illustration of how the samples were deformed is shown in Figure 7-4 below:

![Figure 7-4: Schematic drawing of the rig, showing how stretch deformation were applied and the stretch kept constant while the formaldehyde was chemically fixing the samples. The deformed samples mechanically fixed in this position by the rig, the un-deformed samples and the SEM samples were immersed in the formaldehyde in order to facilitate chemical fixation.](image)

![Figure 7-4: Four deformation directions with respect to the muscle fibre direction are shown above. The illustrations on the left are for the tension and on the right are for compression.](image)
The formaldehyde solution chemically fixed the tissue micro-structure and kept it in that deformed state to allow for the microscopic analysis of the tissue micro-structures. A 10% formaldehyde solution (with 2% glycerol so that the tissue retained its softness) was used. A ratio of 3 times more formaldehyde solution volume to specimen was used in order to make sure that the formaldehyde molecules were not used up in the solution during the fixation period. The mechanically fixed samples were left immersed in the formaldehyde for 60 hours because of their extra thickness. The immersion of freshly harvested tissue into formaldehyde arrests the tissue living processes, thereby arresting any post mortem autolysis, putrefaction and Rigor Mortis processes. Formaldehyde fixation preserved the biological tissue as close to their natural state as was practically possible. The fixative also acted on biomolecules especially proteolytic enzymes, which if not fixed would have started the digestion processes thereby damaging the tissue. The formaldehyde fixative also gave extra protection from extrinsic damage.

Formaldehyde is poisonous to most microorganisms; therefore it helped stop their multiplication and colonisation, which could have easily led to the digestion or damage of the tissue. Formaldehyde fixative altered the tissue at micro-level, which resulted in an increased mechanical strength and stability. The new acquired strength and the rigidity (as a direct result of cross linking) helped the tissue maintain its shape and structure. The length of the samples were measured before and after the chemical fixation process so that verification could be made if there was a change in length during the fixation phase or following the removal of the clamps. No length change was observed; this therefore led to the conclusion that the chemical fixation of the specimen was successfully performed.

The samples were then dehydrated to allow paraffin wax to be embedded into the tissue. The paraffin wax gave the tissue support during the sectioning phase. The samples were hydrated using the following graded alcohol; 50%, 70%, 80%, 95%, 100% and then immersed in xylene. The tissue was then further cut into smaller pieces to allow them to fit inside the white plastic holder that was going to hold the tissue and molten liquid paraffin during the molten wax cooling phase. The paraffin wax blocks with the tissue embedded inside were afterwards mounted on the microtome machine, ready for slicing, see Figure 7-5.
A Leica RM2255 automated microtome machine was used to slice 8 μm thick sample slices (see Figure 7-6 (a)). After the microtome slicing, the thin samples were mounted on the microscope glass slides. These were left for twenty four hours to air dry before any staining could be attempted. Xylene was used to remove paraffin from dried microscope slides prior to staining. The tissue was stained using picro Sirius red, Mallory's trichrome triple stain and Haematoxylin & Eosin (H & E) similar to the work performed by Rich and Whittaker (Rich and Whittaker, 2005). The stained microscope slides (see, Figure 7-6 (b)) were then analysed under an optical microscope and a polarised light microscope.

Figure 7-5: Shows the samples that have been embedded in paraffin wax and ready for microtome slicing.

Figure 7-6: (a): Thin slice cutting microtome machine. (b) Different sample microscope slides are shown on the left.
The 8 \( \mu m \) microscopic sections were stained with Haematoxylin & Eosin in order to enable the detection of slides with technical artefacts and histo-pathological changes. Microscopic sections that contained un-necessary extreme above features were excluded from the study and were not analysed quantitatively thus, they were excluded from any further studies.

7.2.2 Cell Maceration and Scanning electron Microscopy

The main objective of using the high resolution SEM was to give more confidence to the results observed using the optical microscope. The cell maceration technique that was first reported by Ohtani (Ohtani et al., 1988) was used. The SEM requires the specimen to be analysed in a vacuum chamber. This is because air contains a lot of ions that may interfere and influence the negatively charged electron beam. If a moist specimen is placed in a vacuum chamber, it will result in the moisture (ions, molecules and atoms) being found in the vacuum space, which will cause interference with the electron beam therefore resulting in increased focusing difficulties and low quality images. The specimen going into the SEM chamber must therefore be fully dehydrated. Each specimen was dehydrated by first placing it in a series of increasing percentage of ethanol solution before using a critical point drying machine to dry the specimen to the required SEM standard.

The removal of the water from the specimen could have resulted in the alteration of the specimen micro-structure. Therefore the specimens were chemically fixed first before being dehydrated. Glutaraldehyde or formaldehydes were the preferred fixation chemical. The above fixatives do not fix the lipids and membranes; therefore Osmium tetra-oxide was also used to make sure that the lipids and membranes were chemically fixed.

A scanning electron microscope (AML-Carl Zeiss Ultra SEM), was used for the SEM analysis. Different accelerating voltages were used in order to limit specimen charging. Samples were analysed un-coated where possible, as this allowed more detailed analysis and accurate measurement to be taken. The main problem to this was that this was only possible when an accelerating voltage of 3kV and below were used. This low accelerating voltage gave less depth and some focusing difficulties. Where this was not possible the samples were coated with gold, this allowed more depth and higher accelerating voltage to be used, resulting in improved focusing capabilities, but with less nanoscale details visible.
7.2.3 Cross-Sectional Areas of Muscle Fibres.

Thin 8 μm slices of the each sample were extracted using a microtome slicing machine, see section 7.2.1 (see also illustration in Figure 7-7). The CSA of muscle fibres found on the cross sectional areas of individual slices were measured using Image J tools. The average muscle fibre CSA from the control samples was compared to the average muscle fibre area from the tension/compression deformed samples. A comparison analysis was also performed on the tension/compression data.

![Figure 7-7: Schematic of a 8 um slice that is being extracted to be used for image analysis, as well as showing the area referred to as the cross-sectional area](image)

7.2.4 Ellipse Fit.

All the cross-sectional muscle fibres areas were fitted with the best fitting ellipse. From the ellipse parameter the following could be analysed: major axis angle, major axis length and minor axis length. The angles of the major axis were measured (see Figure 7-8). The major and minor axes were recorded and the ratio between the two analysed. An orientation comparison between major axis angles from deformed specimen, major axis angles from control specimen and other major axis angles from other deformed specimens were made. This was performed in order to observe if the muscle fibres have a preferred cross-fibre cross-sectional fibre major axis orientation when in their physiological resting position and whether this preferred cross-fibre, cross-sectional major axis angle orientation changes when a passive external load is applied. In the figures, this is referred to as major axis angle (or muscle orientation angle). Any
changes at muscle fibre level that could be captured by using the best fit ellipse method were characterised.

Figure 7-8: Schematic illustration of a best fit ellipse that is being illustrated being fitted on one of the muscle fibre cross-sectional area. The major axis is the longest diameter of the best fitted ellipse and the shorter diameter is called the minor axis. The angle that the major axis makes with the horizontal edge of the image is called the major axis angle of the muscle fibre.

7.2.5 Feret's Diameter.

The Feret's maximum diameter is the distance between two parallel tangents that are drawn on the longest distances apart on the points along the boundary of the muscle fibre cross-sectional perimeter (see Figure 7-9). This is repeated for the orthogonal distances as shown in Figure 7-9. The ratio of the Feret's maximum diameter and the Feret's minimum diameter were analysed to observe if there are any quantifiable differences between the un-deformed specimen and the deformed specimen. This is similar to a method used by Ferreira et al., to analyse mass transfer of a bubble (Ferreira et al., 2012). The angle of the Feret's maximum diameter was also analysed similar to the method discussed in section 7.2.4, the two different muscle fibre cross-section angle distributions (the ellipse major axis angle and the Feret's maximum diameter angle) were compared were fond to complement each other, therefore, only results from the best fitting ellipse were presented here.
Figure 7-9: Schematic illustration of a Feret’s diameter method. The Feret’s diameter (FD\_Max) is the longest arrow, and the shorter blue arrow is the minimum Feret’s diameter. The angle that the FD\_Max makes with the horizontal edge of the image is called the FD\_ angle of the muscle fibre.

### 7.2.6 Image J verification

The dimensions of the scale bar with known dimensions (see Figure 7-10) were used to validate the accuracy of the Image J analysis tool kit.

Figure 7-10: Showing the scale bar with known dimensions
7.3 Results

From the results presented in Figure 7-11, (A) shows that the Glutaraldehyde fixations is too aggressive and causes the muscle fibres to contract, resulting in large artefacts between the fibres. This was judged not to be a suitable method to compare the muscle fibre structures of the un-deformed and deformed samples. This method was judged to be unsuitable since the sample preparations protocol altered the sample microstructure. Figure 7-11 (B), the trichrome failed to reveal very thin collagen fibres (especially endomysium collagen fibres), which could have easily led to difficulties in
characterising the shape of the muscle fibres and under estimation of the collagen network characteristics (as observed by these authors (Kiernan, 2002, Whittaker et al., 1994), combined with its tendency to fade with time (also observed by (Sweat et al., 1964)) made it an unsuitable choice for the work. The specimen contained a lot of artefacts. Figure 7-11 (C) & (F) shows slice sections that were fixed with the mild formaldehyde and then stained with H & E stain to check for sample preparation induced artefacts. In this case, the slices from the same sample were accepted as properly prepared and could then be stained with suitable stains for microscopic analysis, (D) is a formaldehyde fixed sample and stained with the more suitable picro-sirius red. All the collagen fibres are clearly visible (including the smaller thinner endomysium collagen fibres). Figure 7-11 (E) is the same as in (D); the difference being that the polarised light is used to illuminate the specimen.

7.3.1 Light Microscope Results

7.3.1.1 Cross-sectional area

The cross-sectional areas of the muscle fibres were analysed to quantify and understand if deforming skeletal muscle tissue is accompanied by an equal muscle fibre cross-sectional area change (Figure 7-12).

![Figure 7-12: Cross-fibre cross-sectional area analyses are shown above. The term XF CSA tells the reader that all analysis was on cross-fibre (XF) cross-sectional areas (CSA). The third and fourth words describe loading orientation with respect to muscle fibre orientation and the loading direction. The top left is the summary of all the cross-sectional area deformation and the plot on the top right is the undeformed cross-sectional area analysis. The second row presents the Fibre tensional loading (left) and the cross-fibre tensional loading (right). The bottom row presents the compressive area analysis (Fibre compression on the left and cross-fibre compression on the right). The black solid thick line on all plots is the control samples' average muscle. The red lines indicate the individual deformation response.](image-url)
The average muscle fibre area for the control samples was found to be $1094 \pm 455$ $\mu m^2$. The average muscle fibre area for a specimen subjected to a tensile deformation of 30% was observed to reduce be $877 \pm 407$ $\mu m^2$, which is 20% less than the control average area. The 30% cross-fibre stretch was found to have an average muscle fibre area of $1674\pm838$ $\mu m^2$, which was an increase of approximately 50%. The 30% compressive deformation in the fibre direction gave an average muscle fibre area of 1638, which was about a 50% increase. The 30% compressive deformation in the cross fibre showed a reduction of muscle fibre areas by almost 44% (Average muscle fibre area of 1577$\mu m^2$). In Table 7-1, the compressive published Poisson’s ratio data was obtained from published van Loocke data and tensile data from chapter 4 (Van Loocke et al., 2006). The applied epsilon ($\varepsilon$) was found by taking the log of the stretch. The predicted epsilon associated with Poisson’s ratio effects was computed by multiplying the applied $\varepsilon$ with the macroscopic observed Poisson’s ratio. The predicted stretch associated with Poisson’s ratio can then be calculated taking the exponential of the predicted $\varepsilon$. The final predicted ratio was then calculated by squaring the predicted stretch, since the area of a square is the base squared (or height squared). These results were also compared to the published Poisson’s ratios (see Table 4-5), and found to closely match the theoretical calculated deformation changes using the Poisson’s ratios. From Table 7-1, it is clear that the microscopic observed deformations are in good agreement with the analytical computed deformations using Poisson’s ratios obtained from the macroscopic analysis.

<table>
<thead>
<tr>
<th></th>
<th>Avg muscle fibre CSA</th>
<th>Def Ratio (case/control)</th>
<th>Macro. Poisson' s Ratio [Lit.]</th>
<th>Applied $\lambda$</th>
<th>Applied $\varepsilon$ (log $\lambda$)</th>
<th>Pred. $\varepsilon$ associated with Poisson's ratio effect</th>
<th>Pred. $\lambda$ associated with Poisson's ratio effect</th>
<th>Pred. Def. Ratio</th>
<th>% Diff Pred. Vs Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1094 ±455</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre Tension</td>
<td>877 ±407</td>
<td>0.80</td>
<td>-0.47</td>
<td>1.30</td>
<td>0.26</td>
<td>-0.12</td>
<td>0.88</td>
<td>0.78</td>
<td>2.5%</td>
</tr>
<tr>
<td>Fibre Compr.</td>
<td>1638 ±838</td>
<td>1.50</td>
<td>-0.50</td>
<td>0.70</td>
<td>-0.36</td>
<td>0.18</td>
<td>1.20</td>
<td>1.43</td>
<td>5%</td>
</tr>
</tbody>
</table>

Table 7-1: Deformation of Cross-Fibre cross-sectional areas was compared to the Poisson’s ratio computed theoretical deformation (Van Loocke literature for compression data and chapter 4 for tensile data 4) (Obtained Using Poisson’s computations). The actual deformation (blue) is compared to the theoretical deformation (red), and the difference between prediction and actuals is given in black.
7.3.1.2 Ellipse Fitting.

Ellipses were fitted on the cross-sectional areas of muscle fibres using an elliptical tool in Image J (see section 7.2). The major axis and minor axis of the best fitted ellipses (see Figure 7-8) were outputted from the Image J software. The values of these axes are presented in plots below (see Figure 7-13). This allowed the plots to be presented in the same graph for visual scale comparison. The plots were also plotted in their individual graphs, with the averages for the control plotted in all graphs (solid black lines). The red lines showed the individual case average. The yellow colour was used to show the minor axis in all cases.

![Ellipse Fitting](image)

Figure 7-13: The major axis and the minor axis of the best fit ellipse are shown. Top left plot shows the summary of all major and minor axis together. Top right is the major and minor axis for the control samples. The second row is for the tensional deformation (Second row left is the fibre and right is the cross-fibre deformation). The third row is for the compressive deformations (the left is the fibre compression and the image on the left is the cross-fibre compression). All the minor axis are plotted in yellow in all the plots above. The black solid thick line on all plots is the control samples' average and the two broken black thinner lines are the standard deviations. The red lines indicate the particular deformation response.

The ratios of the major and minor axis were computed. These ratios are presented in a graphical form shown below in (Figure 7-14) and table format (Table 7-2). The ratios also show how the major axis and minor axis change with each particular deformation.
Figure 7-14: Major/minor axis ratios are presented above. Top left plot shows the summary of all major and minor axis ratios together. Top right is the ratio for the control samples. The second row is the tensional ratio (Second row left is the fibre and right is the cross-fibre ratios). The third row is for the compressive deformations (the left is the fibre compression and the image on the left is the cross-fibre compression). The black solid thick line on all plots is the control samples' average and the two broken black thinner lines are the standard deviations. The red lines indicate the particular deformation response.

An average ratio of around 1.65 was observed for both the control and the 30% fibre directional stretch (see Figure 7-14 above and Table 7-2 below). No statistical significant difference was observed between the fibre tensional loading and the control results.

<table>
<thead>
<tr>
<th>Major/Minor Axis Ratios</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.65</td>
</tr>
<tr>
<td>30% Tension in F</td>
<td>1.66</td>
</tr>
<tr>
<td>30% Tension in XF</td>
<td>2.58</td>
</tr>
<tr>
<td>30% Compressive F</td>
<td>1.76</td>
</tr>
<tr>
<td>30% Compressive F-</td>
<td>1.68</td>
</tr>
<tr>
<td>Revised</td>
<td></td>
</tr>
<tr>
<td>30% Compressive XF</td>
<td>2.241</td>
</tr>
</tbody>
</table>

Table 7-2: Major/minor axis ratios. The revised, is where outliers were removed.

130
The ratio of 1.65 showed that the muscle fibres were elongated in one direction (oblong). This relationship ratio was maintained when the muscle fibres were deformed (tension or compression) in the fibre direction. This clearly implies that the fibre directional deformations do not change the shape relationship of the muscle fibres, but only scales them up (compression) or scales them down (tension). The cross fibre deformations were observed to increase the ratios significantly (Table 7-2). The cross-fibre deformation therefore deforms the muscle fibres, elongating them in one direction and aligning them in response to the deforming load. A statistical analysis was performed at 95% confidence level and it was observed that there is no significant difference between the control and the muscle fibre direction deformed specimen (see Table 7-3).

<table>
<thead>
<tr>
<th></th>
<th>Significant Difference</th>
<th>Outliers Removed</th>
<th>Significant Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre Tension</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cross-fibre Tension</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fibre Compression</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cross-fibre Compression</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 7-3: Statistical results showing if there was a significant different between the control and the deformed samples. The fibre direction compressive statistical significance changed from significant to insignificant difference when the sheared muscle fibres were removed. The last two columns show whether there is a change if the outliers are removed, and only the compression in the fibre direction changes from significant difference to insignificant difference.

### 7.3.1.3 Major Axis Angle

The angles of the major axis of the best fitted ellipses were analysed to see if there was an orientation change of the muscle fibres when they were subjected to passive external loading (see Figure 7-8). The orientation angles of the major axes of the control samples were found to have a random distribution (see results presented in Figure 7-15 and Figure 7-16). The major axis angles of the fibre direction deformed samples were found to be uniformly distributed and no showing overall preferred orientation, but further analysis show that the control samples have an average orientation angle of 44.36 ± 16.48 degrees. The tensile deformation slightly reduces this to 40.93 ± 14.87 degrees. The compressive deformation changes the average orientation angles to an average of 55.86 ± 22.04 degrees. This shows that even though
Figure 7-16 show the control, fibre tensile and compressive fibre deformations show a uniform, almost evenly distributed muscle fibre cross-sectional orientations, Figure 7-17 show that their averages are different, with the compressive deformation causing a wider standard deviation. The major axis angles for the cross-fibre deformed muscle fibres were found to have a preferred orientation in relationship to the loading direction. Basic orientations biased were observed following different deformation regimes. In order to quantify these different major axis orientations, the data was placed into 10 equally spaced bins. The frequency of the data in each bin was computed and presented in histogram plots presented in Figure 7-15 and Figure 7-16.

The major axis angles for the control and fibre direction deformed samples were observed to have no preferred orientation angles. The major axis angles for the cross-fibre deformed specimens were observed to orient themselves in response to the loading regime that the muscle specimen has been exposed to.

![Figure 7-15](image1.png)

Figure 7-15: Shows the major axis angle of the muscle fibres analysed on the cross-fibre sectional area. Top left plot shows the summary of all major and minor deformations together. Top right is the major and minor axis for the control samples. The second row is for the tensional deformation (Second row left is the fibre and right is the cross-fibre deformation). The third row is for the compressive deformations (the left is the fibre compression and the image on the left is the cross-fibre compression).
Figure 7-16: Top left is the major axis angle orientation for the control samples. The second row is for the tensile deformation (Second row left is the fibre and right is the cross-fibre deformation). The third row is for the compressive deformations (third row left is the fibre compression and third row right is the cross-fibre deformation).

Figure 7-17: Shows the muscle fibre cross-sectional area orientation effects of compression and tension deformation. The detailed analysis here shows that the control samples have an average muscle fibre CSA orientation angle of 44.36 ± 16.48 degrees. The tensile deformation slightly reduces this to 40.93±14.87 degrees. The compressive deformations changes the average orientation angles to an average of 55.86±22.04 degrees.
7.3.1.4 Image and Quantitative analysis

Figure 7-18: (A) Shows the muscle fibres following a 30% tensile stretch. The muscle fibres were fitted with best fit ellipses and then the angle of the major axis analysed (see section 7.2.4). This resulted in muscle fibres having no preferred orientation (A2). Analysis of small local areas (B1) (notice the localised preferred orientation for this area show in B2). C1 shows localised areas that have maintained the no preferred orientation behaviour. D1 shows the loading direction.

Figure 7-18 shows the results of a fibre direction tensile load. The top bar chart shows that there are some local variations (preferred orientations in regions B1 and orientation analysis presented in B2), yet the global orientation for the whole image (A1) shows no preferred orientation (A2).

The cross-fibre tension loaded specimen was equally examined. The major axes of the fitted ellipse were found to show that the muscle fibres were oriented with the major axis of the fitted ellipse aligning itself in the direction of the stretch (see Figure 7-19).

For a muscle fibre directional compression, some small areas were observed to have some localised preferred orientations. In Figure 7-20 (A1), the small little black arrows show the alignment direction of the surrounding muscle fibres. The overall effects of these localised alignments were captured in Figure 7-20 (A2). A Cartesian coordinate system was adopted (shown in A4), the muscle fibre orientation for (A2) is shown as if it prefers being oriented in x and y directions. However, the global bar chart (A3) shows no preferred orientation, when the whole sectioned slice images of the same sample are taken into consideration.
Figure 7-19: Shows the muscle preferred orientation when the specimen that was tensional loaded in the cross-fibre direction. The first bin corresponds to alignment in the loading direction and the 10th bin corresponds to the direction orthogonal to the loading direction.

Figure 7-20: Shows a polarised light microscope image of a specimen that was compressively loaded by 30% in the fibre direction. (A2), shows the alignment as a results of analysing the major axis angle of the muscle fibres in image A1. However when all images that cover the whole slice are considered, no preferred orientation is observed (A3).
7.3.2 Polarised Light Microscopy

By rotating the analyser in relationship to the polariser, a good alignment was found where the stained organised collagen fibre network were allowing the maximum light through (the same collagen fibre network appeared brighter than other parts) see Figure 7-21. It was observed that the thicker perimysium collagen fibre network allowed for these settings changes with some corresponding colour changes, while no corresponding colour changes was observed for the endomysium achieved. The endomysium collagen network, even though composed of collagen fibres as well, did not allow for such conditions to be achieved. The assumption was made that the perimysium collagen network was organised while the endomysium network was randomly oriented. This agreed with the finding of the work published by other authors (Nishimura, 2010, Nishimura et al., 1996, Purslow, 2002, Purslow, 2010, Purslow and Trotter, 1994, Trotter and Purslow, 1992). In Figure 7-21, the perimysium collagen fibre organisational network were observed to be wavy (Rich and Whittaker, 2005) and banded, for the control and the compressed specimens, but straightened out and un-banded for the tensile specimens.

Figure 7-21: Polarised light techniques used to establish which collagen networks are organised. The perimysium collagen networks appear to be organised while the endomysium collagen network dull colour imply that they are randomly organised. The waviness and loss of waviness is highlighted between the compressed and the tensile deformed specimen as shown on the top and bottom images respectively.

Following the observations made above, the magnifications on the perimysium collagen network for the compressive specimen were increased to the microscope maximum (400X). The magnification was close to the limit of the optical light...
resolution, and yet the individual collagen fibres could not be identified, however, it was observed that the dark bands that exists are caused by a direction change of the collagen fibres network, therefore at these points the organisation was assumed to be lost, see Figure 7-22.

Figure 7-22: The perimysium collagen network from a cross-fibre compressed specimen is examined. (A): shows the image of taken from the specimen compressed by 30% in the cross-fibre direction and magnified 100x. (B) Shows part of the perimysium network that has been magnified 400x, the perimysium collagen fibres appear as a rope made up a group of individual collagen fibres. (C) Further magnification results in the image being made bigger but with no improvement in resolution.
7.3.3 SEM Results

Figure 7-23: Specimen undergoing cell macerations (after 7 days in NaOH)

The maceration of the muscle tissue took 14 days on average; a muscle sample undergoing maceration using sodium hydroxide is shown in Figure 7-23. When the maceration is complete the whole tissue appears whitish and almost transparent. The maceration had to be monitored twice a day towards the end, as given enough time; sodium hydroxide can digest any biomaterial. There was a need therefore to safeguard against over exposing the collagen fibre networks to unnecessary sodium hydroxide digestion.

Figure 7-24: Poor results due to HMDS forming thick layers

HMDS formed a thick layer of the resin, therefore making it difficult to perform any meaningful analysis (Figure 7-24). The use of HMDS was discontinued as it was judged to be unsuitable for experimental use required for this work.

The SEM user manual recommendation for biological samples is that they should be coated with gold to improve electron conductivity. For the analysis performed for this work, samples were coated or un-coated for the reason discussed below. Due to the small nanometre diameter of collagen fibres and gaps between collagen fibres, the gold coating was found to greatly influence the measured collagen fibre dimension. The advantage of the coating was that there was a free flow of
electrons to the ground, and a high energy beam could be used, which improved the field depth and the focusing abilities thereby resulting in a good image. The un-coated specimen had to be worked on using a very low energy beam of electrons; this made it difficult to focus but gave an image with more detail. Electron build up was a problem as well (manifested in the form of bright glowing spots). Therefore, in order to view more detail, the specimens were analysed uncoated using very low energy beams to minimise charging (a term used when electrons build up and glowing of these parts is observed making it difficult to focus).

![Figure 7-25: Comparison of micro-structures that as viewed using electron beam and optical microscope. Top Left: shows the muscle fibres from an SEM image at 150x. Top Right: shows the muscle fibres when viewed using the light microscope at 200x. Below Left: the SEM magnification is then increased to 400x which compared to the polarised light microscope image at the same magnification (Bottom Right).](image)

Figure 7-25, shows the images acquired using an electron beam and ordinary light. The same features can be observed on both images, which more or less show these micro-structures at the same magnification scale. The detail of the electron beam images is better even though the SEM images are in black and white. This was also repeated on cross-sectional areas in Figure 7-26.
Figure 7-26: Comparison of cross-sectional areas of muscle fibre images obtained using SEM and optical microscope. The formaldehyde was used to fix both samples and the optical image was stained with H & E stain.

Figure 7-26 shows the cross-sectional areas of the muscle fibres from the SEM (the left shows the optical microscope image, and the right images on the right is from the optical microscope). This shows that the same structures can be observed using an optical microscope or when using low magnification SEM mode (all must be at the same magnification). This therefore gave the author more confidence when performing the SEM that was being used for high magnification and resolution analysis (see Figure 7-27 and Figure 7-28.

In Figure 7-27, images of un-macerated skeletal muscle samples are presented, from low magnification (150X) to very high magnification (2500X). In the top right image of Figure 7-27, is an image taken from a tensile deformed specimen (500X) and the muscle fibres appear to be crimped and tightly packed. This is different to the observation made from the bottom left image, which is taken from a specimen that has been compressed by 30% in the fibre direction. The muscle fibres appear loose and not tightly packed. The bottom right image is included to illustrate the problem encountered when a gold coated sample was used. The details of the specimen are lost, and as the energy of the beam is increased in order to get more resolution, the specimen ends up charging (glowing brightly) more than the case for un-coated samples. Collagen muscle fibres were still observable inside the gaps of the gold coated specimens.
A macerated sample that had been chemically fixed with formaldehyde solutions was analysed using an SEM microscope (see Figure 7-28). The first image on the left: a 5kV electron energy beam was used for better depth and better focusing, on a macerated specimen (without being gold coated), and a honey comb structure of a collagen network can be clearly seen. The endomysium collagen network is clearly visible (marked by green arrows) as well as the perimysium network (marked by blue arrows), gaps can be seen where the muscle fibres have been removed (shown by red arrows). The magnification was further increased to 4.13kx in order to examine the collagen fibres in detail. The endomysium collagen fibres in Figure 7-28 (centre) show the endomysium collagen fibres to be randomly oriented. This was in agreement with the polarised light observations that were presented in see section 7.3.2, Figure 7-21. The polarised light analysis showed that the endomysium collagen fibres remain
randomly oriented even after passive external deformations loading of 30% (see section 7.3.2). The last image on the right of Figure 7-28, imaged at the point indicated by the first blue arrow on the image on the right hand side. The perimysium collagen fibre shows some organised alignment and waviness as shown by the polarised light image in Figure 7-22.

![Image of muscle fibres and arrows indicating perimysium network](image)

**Figure 7-28:** 1st Image shows sample with the muscle fibres removed using NaOH. 3rd Image. The blue arrows are pointing to perimysium network, the green arrows pointing to endomysium network and the red arrows are pointing to where the muscle fibres have been removed. The centre SEM image is showing randomly oriented endomysium collagen fibre network when magnified at 4.13kx. The last image shows the somewhat organised perimysium collagen fibres.

The endomysium randomly oriented collagen fibres diameter was measured and found to have an approximate average diameter of 65 nm. The perimysium collagen fibres were observed to have a varying diameter, ranging from approximately 80 nm to 125 nm, depending on where the perimysium is located (see section 7.3.4).
Figure 7-29: Shows the results of a coated specimen that is magnified to 5.36kx, using an electron energy beam of 15kV. The gold coating was found to fill in the small gaps between the collagen fibres as well as increase the dimension of the micro-structural components. An unknown body was observed that joined the perimysium wall to the muscle fibre walls.

Figure 7-26, Figure 7-27, and Figure 7-27 show failures occurring within or between the muscle fibre interface and the perimysium network. Some specimens were coated and analysed, this allowed more penetration and allowed for further examination inside the gaps. Intermittent connection bodies (see Figure 7-29) similar to those reported by Passeriux (Passerieux et al., 2007) were observed between the perimysium and fascicle membranes, however their function is unknown.
7.3.4 Microscopic Cross-Fibre Sections Image Visual analysis

Image of the muscle fibre layout of un-deformed specimen (Control Specimen), the perimysium is divided into secondary perimysium (SP) and primary perimysium (PP). (see section 3.2.5.1)

30% Fibre direction tensile deformed specimen. 30% cross-fibre tensile deformed specimen.

30% Fibre direction compressive deformed specimen. 30% Cross-fibre compressive deformed specimen.

Figure 7-30: Image of the control specimen and the images following different deformation regimes. These images are presented here for the benefit of the reader. This gives the reader a glance at the differences that will be discussed in detail in this section.

Some parts of the perimysium network were seen to respond to deformation more than others. Only the secondary perimysium networks (SP in Figure 7-30) were observed to have responded to the deformation (shown with the blue and black arrow
in Figure 7-31). The deformation of the secondary perimysium network was observed to be dependent on the loading path.

**Figure 7-31:** Contracted parts of skeletal muscle specimen that has been subjected to a 30% tensile deformation. The blue arrow is pointing to what is thought to be waviness of the perimysium network that has been pushed together, therefore appear thicker. The black arrow is pointing to a primary perimysium that is showing the same effects.

Figure 7-31 shows parts of the image where contracted secondary perimysium networks were thought to be undergoing contraction deformation. The perimysium waviness was seen to bunch together (see where blue arrow is indicating in Figure 7-31). The black arrow shows the same effect as well on the primary perimysium. Comparing Figure 7-21 and Figure 7-31, it is clear that there are parts of the specimen where the perimysium collagen network was stretched and parts that have increased collagen fibre network waviness.

In Figure 7-32, the muscle fibres appear to have aligned themselves parallel to the secondary perimysium marked SP1. The other muscle fibres close to SP2 are observed to have aligned themselves parallel to this secondary perimysium, which is almost orthogonal to the alignment of SP1. From these observations we can only conclude that these two secondary perimysiums have a direct influence on how the surrounding muscle fibres are responding to the external applied cross-fibre tensile deformation load.
Figure 7-32: Cross-fibre moderate response to a 30% stretch shown. Note the alignment of the muscle fibres in parallel to SP1 and SP2.

Figure 7-33: Sheared muscle fibres as a result of two or more perimysium networks at different angles pulling away from this area.

In Figure 7-33, the local area is thought to be subjected to two or more transmitted loads by perimysium (labelled A, B, and C) pulling away from this area at different angles. The magnitude of these forces is thought to determine how much of a shearing force will be created. The loading here appears to be complex and the number of perimysium forces pulling from an observed local area are assumed to manifest themselves in a sheared appearance local area (see Figure 7-33).

In Figure 7-34, the main vertical secondary perimysium is thought to be vertically displaced by a vertical pulling force. The secondary perimysium which are...
always wavy when in their resting length, are observed to be thinned out and straightened out as well (labelled P fully stretched). The waviness and the straightness of the main pulling secondary perimysium network were found to differ along the perimysium network observed length. The secondary perimysium network is still wavy towards the bottom of the image (labelled P still un-stretched in Figure 7-34).

Figure 7-34: Cross-fibre stretch in the vertical direction. An upward displacement of the main perimysium caused the other attached smaller perimysium to be dragged along, deforming them and the enclosed muscle fibres to deform in response to these stretch loads. The red arrows show the direction of the pulling force. The black arrow is pointing to an area further down where the perimysium is still un-stretched and the muscle fibres below that are still un-deformed.

In their resting length, the secondary perimysium network appear to be wavy and thick, therefore a stretching deformation will straighten out the waviness and make them thinner as they stretch out (see comparison in Figure 7-21). In Figure 7-34, the primary perimysium (marked PP partially stretched) appears to be aligning themselves to accommodate the stretching deformations being experienced. The alignment towards the top of Figure 7-34 is more pronounced than towards the top than towards bottom of the image. This is different to compressive cases in Figure 7-35.
where the perimysium collagen fibre network is observed to increase in thickness due to the waviness being pushed together by compression.

Figure 7-35: Compressive response of skeletal muscle fascicles and the enclosed muscle fibres is shown using polarised light, it is shown to cause the perimysium collagen network to increase its thickness by the waviness pushed together

This is schematically illustrated in, Figure 7-36, showing that 30% compressively deformed cross-fibre image, and causes the fascicles and the enclosed muscle fibres to align themselves in an orthogonal direction to the direction in which the deforming load is applied. The compressive loads were applied in the horizontal direction, and the fascicles and the enclosed muscle fibres elongated in the vertical direction, as shown on the schematic illustration below.
Schematic illustration of an un-deformed muscle fibre

A horizontal deformation will flatten the muscle fibre, yet forcing it to expand vertically.

Figure 7-36: Schematic illustration of how the compressive cross-fibre deformation causes the muscle fibres to re-align themselves.

According to macroscopic observations made by Van Loocke (Van Loocke et al., 2006), see Table 4-5, the 30% fibre directional compression (illustrated in Figure 7-37) skeletal muscles was observed to cause the muscle fibres to expand in equal proportions in all the directions orthogonal to the muscle fibre alignment. The secondary perimysium shown in Figure 7-37 is assumed to have undergone a stretch due to the increase in the cross-sectional area of the whole specimen, as well as being displaced to the right by the pressure building inside the enclosed fascicle. The muscle fibres in contact with the secondary perimysium are sheared upwards as well as stretched to the right. The complicated loading regime in the primary perimysium networks results in the muscle fibres around PP1 being almost vertically stretched, therefore aligning themselves in this vertical direction. The primary perimysium PP2 has the muscle fibres being stretched and aligned in an almost horizontal direction. These observations show that the muscle tissue local deformations are complex and are accompanied by complex orientation as well, which all cancel and balance out to what was observed at a macroscopic level in chapter 4.
Figure 7-37: Shows the 30% fibre direction compression. In the case above the compressive displacement is into the page.
7.4 Discussion

Microscopic image analysis plays a very important role in pathology (Janin et al., 2012, Hamilton et al., 2012, Laurinavicius et al., 2011, Calvi et al., 2012), especially in terms of accurate diagnosis which arises from precise measurements and counting of different features in their relationship to their deviation from the established standard. Quantitative data obtained from morphological characterisation of tissue and cellular analysis is now widely used for diagnostic and prognostic purposes, especially for cancer patients (Kong et al., 2013, Mukherjee et al., 2009). Individual human eyes are affected by different factors including the following: luminosity, variations in contrast and brightness. This therefore makes the visual acquired results open to subjective interpretation. Great caution was taken when interpreting the microscope images for this work as in the past some bacteria artefacts (mesosome) were misinterpreted as part of a cell’s organelle (Hudson, 2003), yet this was later shown to be artefacts when superior and better resolution electron microscopes came into use.

The small gaps between endomysium collagen fibres and their corresponding diameters were observed to affect the effectiveness of HMDS. The HMDS usage was found to effect the efficient working of the SEM machine. The vacuum chamber took six times the normal time it normally takes to pump to the required vacuum. The HMDS image was observed to be very difficult to focus on which was thought to be due to some random HMDS molecules still subliming into the vacuum space (see section 7.2.2). The author did not observe any of the advantages of using HMDS as mentioned by other authors (Braet et al., 1997, Nation, 1993). HMDS was observed to instead form a thick layer of the resin, making it difficult to make any meaningful analysis of the micro-structural details (Figure 7-24). The author discontinued the use of HMDS for the microscopic work, and reverted back to the widely preferred and used critical point drying machine (Bray, 2000, Bray et al., 1993).

The SEM images showed that the endomysium collagen fibres that enclose the muscle fibres are randomly orientated (see Figure 7-28), which is in agreement with other authors (Purslow and Trotter, 1994, Purslow, 2001, Trotter and Purslow, 1992, Trotter and Purslow, 1994, Nishimura, 2010, Nishimura et al., 1996, Fang et al., 1999) when the muscle is at rest. No evidence of alignment at the reported deformations was observed as was reported by Purslow (Purslow, 2002), this could be due to our data resulting from analysis performed on the cross-sectional plane, while Purslow analysis were performed on the longitudinal plane. However, our data is in agreement with the...
same author's work in 1998 when he was investigating collagen orientation during viscoelastic experiments (Purslow et al., 1998). The SEM analysis performed for this work was also supported by observations made using the polarised light microscope Figure 7-38. Huijing and Purslow pointed out that such honey comb structure images allow the reader to comprehend the extra cellular matrix of collagen fibres more easily in an extensive 3D set of organised connected tunnels in which muscle fibres operate (Huijing, 1999, Purslow, 2002).

Figure 7-38: Shows the SEM images alongside the polarised light microscope image (compressed by 30% in the fibre direction). The bright coloured parts of the collagen network shows some organised orientations, while the dull colouring on the endomysium network show lack of organised orientation. The SEM structure compares well with the polarised light acquired image on the left.

The diameters of endomysium collagen fibres (approximately 50 nm) and the perimysium collagen fibres (approximately 80-125 nm) explains why the 200 nm resolution limit of the optical microscope prevented the author from identifying individual collagen fibres when using light microscopes. Equally the perimysium collagen fibres fell below the resolution limit of the optical microscope. These observed collagen measured diameters are in close agreement with Nakamura’s observation, who reported measured diameters of 50-100 nm for type I collagen fibres and 50 nm for type III collagen fibres (Nakamura et al., 2003). The collagen types were not characterised in our experiments. However in an adult animal, 70 to 80% of muscular collagen fibres are type I collagen fibres (Nishimura et al., 1997, Listrat et al., 1999). Therefore an assumption was made that most of the collagen fibres we measured were collagen type I, and the low diameters we measured were accounted for being due to specimens having been harvested from young pigs. The perimysium collagen fibres were observed to have a varying diameter, ranging from approximately 80 nm to 125 nm, depending on where the perimysium is located (see section 7.3.4).
In the past it has also been established that the colour of collagen fibres stained with Picro-sirius red and viewed with polarised light depends upon the fibre thickness: the colour changes from green to yellow, orange and red were associated with an increase in collagen fibre thickness (Junqueira et al., 1982, Rich and Whittaker, 2005). This was the correct relevant polarising filter and circular analyser setting for this type of analysis. However the settings for this work were for the collagen organisation only as can be shown by different colours on the same collagen network when direction changes. The change in direction is thought to affect the organisation only and not the fibre thickness (Noorlander et al., 2002, Frohlich, 1986, Boyde and Riggs, 1990, Bromage et al., 2003), thereby resulting in dark bands and no colour changes as mentioned above.

The perimysium structures and patterns observed using polarised light microscopy and scanning electron microscope were different to what was observed and described by Purslow (Purslow, 2008, Purslow, 2002, Purslow, 1989, Purslow and Trotter, 1994) see also their illustration in Figure 7-39 below. Purslow claims that the orientation of the perimysium collagen fibres is approximately 55° to the muscle fibre axis at the physiological muscle resting length (Purslow, 2008, Purslow, 2002, Purslow, 1989, Purslow and Trotter, 1994). However we did not make the same observation as that reported by Purslow mainly due to the thesis data resulting from analysis performed on the cross-sectional plane, while Purslow analysis were performed on the longitudinal plane. Our observations support the observations made by other authors, (see Figure 7-39), (Nishimura et al., 1996, Fang et al., 1999). Our observations support the observations made by other authors (see Figure 7-40), (Nishimura et al., 1996, Fang et al., 1999).
Figure 7-39: SEM image of the perimysium collagen fibres, the green lines highlight the perimysium collagen fibre orientations and the blue lines highlight the muscle fibre orientation. The perimysium collagen fibres were observed to run in two parallel lines that were +55° and -55° to the muscle fibre axis. Reproduced from (Purslow, 2010)

Figure 7-40: Our perimysium observed perimysium pattern and structure agrees with published SEM images from Fang (Fang et al., 1999) image on the right hand side as well as the polarised light images. The SEM image was acquired from 30% cross-fibre compressed specimen of a 3 months old female pig, while the Fang's SEM image was obtained from a 55 months old pig.

The Picro-Sirius red stained images for the control showed that all the endomysium collagen networks are randomly oriented. This was shown to be true using the SEM analysis (see Figure 7-28). All the deformed Picro-Sirius red stained images showed that the endomysium collagen network did not develop any kind of orientation following both 30% compression and tensile deformation. Therefore no further expensive SEM analysis was performed. Based on these experiments, it was concluded that there was no evidence to support that the micro-structures at muscle fibre by themself have a direct contribution to any passive skeletal muscle response.
The tensile stretch in the fibre direction was observed to decrease the muscle fibre cross-sectional areas, while the compressive stretch in the fibre direction was observed to increase the muscle fibre cross-sectional areas. Tensile stretch and compressive stretch in the cross fibre direction were observed to increase the cross-sectional area of the muscle. Macroscopic results and microscopic results at the micron length scale were in agreement (theoretical results obtained through Poisson's ratios), but if we examine the microscopic standard deviations we observe that there is a lot of responses data away from the mean. This standard deviation is almost half of the average cross-sectional area of muscle fibres (this is true for the control and fibre directional deformation responses) see Figure 7-12 and Table 7-1. Comparing major axis to minor axis: fibre directional stretches showed no changes on average in the deformed state compared to the control. Changes were observed for the cross-fibre direction. Cross-sectional muscle fibre orientations were not affected by fibre directional deformation, but large shape changes and orientation changes were observed for cross-fibre deformations.

The muscle fibre cross-sectional area analysis presented showed that the only factor in the observed differences was whether the load was applied in the fibre direction or in the cross-fibre direction (see section 7.3.1.1). The same observation was made for the analysis performed using the best fitted ellipse (refer to section 7.3.1.2 to section 7.3.1.4). The results show that the passive response of skeletal muscle tissue is more dependent on the loading direction in relation to the muscle fibre orientation than on whether the test is performed on compression or in tension. The visual observational analysis in section 7.3.4 shows that at fascicle level, perimysium deformation contributes significantly to the macroscopic observed asymmetrical differences (see Figure 7-21).
7.5 Conclusion

This chapter shows that there is no evidence at muscle and endomysium collagen fibre network level to support that the muscle fibre structures are responsible for the asymmetrical behavioural response of skeletal muscle. However, visual examination of the images at muscle’s fascicle level including perimysium collagen fibre network showed that some differences between compression and tension exist. This requires further investigation. It was therefore concluded that the perimysium and the interactions with the surrounding muscle fibres and endomysium networks are likely to be the predominant factors responsible for the tension/compression asymmetry observed in the microscopic test of passive skeletal muscle.
8 Discussion, Conclusion and Future work

Figure 8-1, shows the task and processes that will be discussed in this chapter (highlighted in red) in order to achieve the objectives for this chapter. All other processes are included for clarity.

This thesis focuses on improving our understanding of how skeletal muscle behaves when subjected to large external strains. This was investigated at quasi-static rates (tension and compression), at compressive impact levels and the quasi-static muscle fibre micro-structural level deformation. The tension/compression asymmetrical behaviour is highlighted and an isotropic first order Ogden hyperelastic material law is used to capture the elastic muscle tissue response and support the highlighted asymmetrical behavioural response of skeletal muscles, see Figure 6-9. The asymmetrical behaviour that could not be explained using the normal macroscopic experimental protocols, was investigated using the microscopic techniques to verify
that muscle fibres and the surrounding extracellular matrix at muscle fibres level have a direct influence on this macroscopic observed asymmetrical phenomena. Figure 8-1, shows the tasks and processes performed for this thesis and how each of these are interlinked and related to each other.

In study one (chapter 4), uniaxial quasi-static tensile tests (at 0.05%\text{s}^{-1}) were performed on freshly harvested porcine \textit{longissimus dorsi} skeletal muscle tissue from five, 3 month old female pigs. Custom Matlab codes were developed to analyse the local displacement of selected specimen areas using marker analysis. The experimental results were presented in the following graphical forms:

- Cauchy stress vs Stretch ratio.
- Poisson's ratios; $\nu_{LT}$ and $\nu_{LT'}$, characterising contraction in one of the transverse directions ($T$ or $T'$) when tensile load was applied in longitudinal ($L$) directions.
- $\nu_{TT'}$, characterised contraction in the transverse directions ($T$) when tensile load was applied in the other transverse direction ($T'$)
- $\nu_{TL}$ and $\nu_{T'L}$, characterised contraction in the longitudinal direction when tensile load was applied in one of the transverse directions ($T$ or $T'$).

The cross-fibre direction response was the stiffest (77kPa at $\lambda = 1.1$), broadly linear and failed at low stretches (ca. $\lambda = 1.15$). In contrast the fibre direction is much less stiff (10kPa at $\lambda = 1.1$) and nonlinear, but failure occurs at higher stretches (ca. $\lambda = 1.65$). As the fibre angle is rotated from perpendicular, to align with the load axis, the stiffness gradually decreased in an approximately sinusoidal fashion, see Figure 8-2.
Figure 8-2: (Top) Fibre direction responses shown for all tests performed and the Poisson’s ratio. (Bottom) Sinusoidal periodic surface fit

The passive response of skeletal muscle to tensile loading in the longitudinal (L) direction (Poisson’s ratio = $\nu_{LT} = \nu_{LT'} = 0.47$) is therefore almost a volume preserving event, with an equal contraction in both transverse directions ($T$ & $T'$). The response to tensile loading in the transverse ($T$) direction results in Longitudinal ($\nu_{TL} = \nu_{T'L} = 0.74$) and transverse ($\nu_{TT'} = 0.28$) contractions. The sum of both sets of Poisson’s ratios was close to 1 in both cases, which corroborates the assumption that skeletal muscle tissue is nearly incompressible. Van Loocke et al observed the following compressive Poisson’s ratios; $\nu_{LT} = \nu_{LT'} = 0.5$, $\nu_{TT'} = 0.56$ and $\nu_{TL} = \nu_{T'L} = 0.36$ (Van Loocke et al., 2006). This shows that the skeletal muscle displays asymmetrical anisotropic tension/compression mechanical properties.

In study two (chapter 5), existing published data mostly related to uniaxial compression tests (ramp or cyclic about a mean compression level) at normalised strain rates of between 0.05%$s^{-1}$ and 3200%$s^{-1}$ (Van Loocke, 2007, Van Loocke et al., 2008, Van Loocke et al., 2009) and split Hopkinson Bar tests carried out in experiments at normalised strain rates of above 54000%$s^{-1}$ (Van Loocke, 2007). Thus stress-strain data at low strain rates and very high strain rates are available in the published literature, but there appears to be none at normalised strain rates of between 3200%$s^{-1}$ and 54000%$s^{-1}$ that are relevant to automotive and sports injury biomechanics impacts see Figure 8-3. A drop tower testing rig was designed and built in-house and used to deliver a falling mass impact at velocities of 1.16 m/s, 2.2 m/s and 3.78 m/s,
corresponding to strain rates of 11600%\,s^{-1}, 22000%\,s^{-1} and 37800%\,s^{-1} respectively for samples of 10 mm nominal height.

The results show nonlinear stress strain behaviour as previously reported at lower strain rates. The tissue also exhibits the classic strain rate dependency, with the stress at 30% compression approximately 9 times higher for a strain rate of 37800%\,s^{-1} compared to 11600%\,s^{-1}. The cross fibre direction is stiffer than the fibre direction, but the difference reduces as the strain rates increase. The compression of the specimen was observed to be non-uniform throughout the depth of the specimen, with a compressive wave observed to propagate down the specimen. Biological fluid was observed to be forcefully expelled starting from the top and moving down with a compressive wave; all these observations are still being investigated, but the orientation of the muscle sample significantly affects the fluid expulsion.

The nonlinear and strain rate dependent response was expected and is in broad agreement with published work at higher and lower strain rates. A comparison of the stress ratio at 22000%\,s^{-1} and 37800%\,s^{-1} relative to 11600%\,s^{-1} shows an almost continuous evolution by comparison with the lower rate ramp and cyclic tests reported in the literature, but indicates significantly stiffer behaviour than very high rate results reported from split Hopkinson bar testing (Song et al., 2007), see Figure 8-3. The non-uniform compressive strain response throughout the depth of the specimens has not been reported before, and is a result of the movement of the compressive strain wave from top to bottom of the specimen. The fluids were found to be forcefully expelled from the fibre end of the tissue when the compression was in the cross fibre direction. A larger proportion of the fluid was found to escape from the end of the fibre than across the fibres when compression was in the fibre direction, therefore more force was needed to force the fluids across the fibre extra cellular matrix than forcing its way between the compressing platens and the tissue. The observed fluid movement is in agreement with the hypothesis put forward by Van Loocke (Van Loocke, 2007).
Skeletal muscle tissue gives a nonlinear and strain dependent response to impact loading at rates relevant to automotive impacts. The strain is non-uniform and moves through the specimen in a wave like propagation. The connective tissue matrix surrounding the muscle fibres opposes fluid movement across the fibres.

Study three (chapter 6) investigated the capability of an isotropic first order Ogden hyperelastic material law model to capture the elastic skeletal muscle response. An optimisation procedure was used to derive optimal material parameters for which the error in the predicted boundary condition force at maximum compression was less than 3%. The first order Ogden hyperelastic material law was thereafter extended with a three-term quasi-linear viscoelastic (QVL) expansion in order to model viscoelastic effects for the data obtained from chapter 5.

The impact compression was observed to start from the top region and progress downwards in a wavelike manner. The inverse FEA derived model fitting force
response was found to compare well with the observed experimental response (see Figure 8-4).

The inverse analysis shows for the first time that the mechanical response, in terms of both applied load and tissue deformation for each of the strain rates can be captured using a first order Ogden hyperelastic material law extended with a 3 term QLV. Using inverse FEA a good match for each of the different strain rates can be obtained. The responses for cross fibre and fibre loading are observed to be similar and therefore it appears that the effects of anisotropy are relatively small at these load rates.

When the predicted deformations are compared to the observed experimental deformation, a poorer comparison is observed towards the top of the sample, which may be explained by the fact that for the hyperelastic material nearly incompressible behaviour was enforced and damage development is not modelled, while in the experiments, fluid expulsion and some permanent deformation was observed. Thus, damage modelling remains a topic for future development of the model.

A further limitation of the finite element modelling is that the initial geometry of the muscle was cuboid, while the experimental samples were only approximately cuboid due to difficulties in samples preparation, and this may have some influence on the predicted parameters (Böl et al., 2012).

A few authors have pointed out that Fung's quasi-linear viscoelasticity (QLV) is imprecise for higher strain rates. The paper by Wu in the International Journal of Artificial Organs (Lu et al., Wu, 2006), itself refers to much older papers by Roger Haut (Haut and Little, 1972) and Savier Woo (Woo et al., 1981) for the statement that the QLV produces problems at higher strain rates. However, there could be many reasons for the reported lower success with this approach (number of viscoelastic branches used, different tissue types etc), and there is nothing theoretical about the formulation of the QLV that prevents it from being used at higher rates, and in the intervening decades many researchers have successfully done just that. A few of these recent cases are discussed below:

Untaroiu et al (Untaroiu et al., 2005) applied the QLV model to medial collateral ligaments and obtained good results at strain rates up to 12500% s⁻¹ (the thesis computed this strain rate by applying their impact speed of 2.5m/s to the given length of 20mm also given in their paper). Ledoux and Blevins investigated the material properties of cadaveric plantar soft tissue (Ledoux and Blevins, 2007). They then
successfully applied the QLV to predict the viscoelastic response for the following strain rates $0.6\% s^{-1}, 1.2\% s^{-1}, 0.8\% s^{-1}, 105.7\% s^{-1}$ and $1080\% s^{-1}$. Doehring et al (2004) also applied the QVL on the aortic valves in order to simulate experiments performed at strain rates up to $800\% s^{-1}$ (Doehring et al., 2004). Sparrey and Keaveny (2011) applied the QLV to porcine spinal cord white matter for strain rates ranging from $0.5\% s^{-1}$ to $500\% s^{-1}$ (Sparrey and Keaveny, 2011). They used the first order Ogden model to capture the loading response of spinal cord white matter, and then a viscoelastic material model combining the first order Ogden model with a 3 term Prony series. Using this method they managed to capture the effect of strain rate and relaxation response.

A practical problem with the QLV method is well described by Carew, who points out that to apply QVL at high strain rates, the main problem is the difficulty in obtaining accurate experimental data that doesn’t include errors like overshoot, vibration and inaccurate measurements. Carew et al used the QVL model for strain rates up to $400\% s^{-1}$ (Carew et al., 1999), and this was also supported by Funk et al (Funk et al., 2000). These problems have reduced with the rapid advancement and continuous improvement of modern experimental methods, and probably explain the more recent successes with the QLV approach at higher rates.

A review paper of classical nonlinear viscoelastic models and an effort to provide a unifying framework using the continuum mechanics formalism by Drapaca in 2007 (Drapaca et al., 2007), acknowledges that “in many practical applications, the QLV model of Fung has proved to be the most successful nonlinear model”. No limitation was raised any reviewer was mentioned.

For the anisotropic behavioural response, Moerman (Moerman, 2012) used the Gaussian modulated spherical fibre distributed Ogden hyperelastic model (GMSFD) for both tension and compression. The GMSFD model was able to accurately predict the compressive response well (see Figure 8-5 and Figure 8-6) (Moerman, 2012).
Moerman (2012), was able to use this anisotropic GMSFD model to accurately predict the experimental tensile response of skeletal muscle tissue discussed in chapter four of this thesis. Two views are shown for clarity (Moerman, 2012), Figure 8-6.

While Moerman (2012), was able to successfully use the GMSFD model for both the tensile and compressive responses. The model does not address the asymmetrical behavioural response of skeletal muscle; therefore different parameters are needed for tensional and compressive behaviour in order to successfully predict the tissue response (Moerman, 2012).
Study four (chapter 6) discusses the investigation of the microscopic effects of tensile and compressive deformation of the specimen by 30% compared to an undeformed specimen. The samples were deformed and fixed in their deformed state. The un-deformed samples (control) and the deformed samples were sliced and analysed under a compound light microscope and a polarised microscope. The deformed state results were characterised against the un-deformed states. An SEM analysis was performed on un-macerated and macerated results.

Freshly harvested skeletal muscle samples were divided into four groups.

1. Group 1: muscle fibre specimen were stretched/compressed by 30%, and then chemically fixed with formaldehyde, sliced, mounted on microscopic slides and then stained with picro-sirius red.
2. Group 2: un-deformed control samples were also prepared for the light microscopic analysis as in group 1. Two more groups of samples were prepared for SEM analysis.

3. Tissue maceration was performed on group 3, thereby exposing the collagen fibre network.

4. Group 4: samples were left un-digested and SEM analysis performed on complete intact muscle tissue.

Analysis of the muscle fibre cross-sectional areas was performed using Image J (Image J 1.48C). The average areas were compared between the control and deformed states. Ellipses were fitted on all muscle fibre cross-sectional outlines and the ratio of the major and minor axis compared. The muscle fibre orientations obtained by using the angle of the ellipse major axis was characterised. SEM analysis of muscle was also performed.

The tensile stretch in the fibre direction was observed to decrease the muscle fibre cross-sectional areas, while the compressive stretch in the fibre direction was observed to increase the muscle fibre cross-sectional areas. Tensile stretch and compressive stretch in the cross fibre direction increases the cross-sectional area of the muscle. Macroscopic results and microscopic results at the micron length scale were in agreement (theoretical results obtained through Poisson’s ratios).

The muscle fibre cross-sectional areas were fitted with the best fitting ellipse. Using the best fitted ellipse components, the relationship between major axis and the minor axis characterisation showed that the fibre directional stretches does not induce any changes, when comparison was made with the control. However changes were observed for the cross-fibre direction deformations. The relationship between the average major axis angle for the deformed specimen and the control was observed and the results are re-presented in Figure 8-7 for clarity.
Figure 8-7: Typical results showing muscle fibre cross-sectional orientation obtained through analysing the fitted ellipse’s major axis angles

The analysis using polarised light highlighted that the endomysium is made up of randomly oriented collagen fibres, and the randomly orientated collagen fibres were not affected by the 30% deformation. There was no difference observed between the compression deformed specimens and the tensile deformed specimen. The images from a high resolution SEM confirm that the endomysium collagen fibres are randomly oriented; this is in agreement with other published work. However, evidence of differences between the compressions deformed perimysium collagen networks and tension deformed perimysium collagen networks were observed (Figure 8-8).

Figure 8-8: Polarised light images showing the difference tension/compression behavioural response of perimysium network
Tensile deformations were observed to cause the secondary perimysium to straighten out. Compressive deformations increase the muscle fibre thickness and the waviness of the perimysium network is pushed together.

Therefore, this study indicates that the source of the tension/compression asymmetry of skeletal muscle is not at the individual muscle fibres/endomysium level, but at fascicle/perimysium level. Since the polarised light perimysium images were showing different shapes between tensile stretch specimens compared to compressive deformation specimens, it is proposed that the hierarchal organisational at the fascicle and its interaction with other micro-structures level were responsible for the asymmetrical behavioural response observed in macroscopic tests on muscle tissue. The use of an animal in its developmental stage also brought some extra complications in that extra-cellular matrix components and cellular cells were still undergoing functional development as highlighted by the study of Fang and Nishimura (Fang et al., 1999, Nishimura et al., 1996), see Figure 8-9.

Figure 8-9: SEM images of porcine muscle tissue still undergoing functional development (Fang et al., 1999). On the left is the image taken from pig at birth and on the right is an image of a mature pig (55 months).

Figure 8-9 compares two images of porcine skeletal muscle harvested from two separate pigs, the first pig is in its early stages of the developmental phase and the second image is of an adult pig. In order to eliminate the unknown elements associated with dealing with the differences of tissue harvested from pigs in their developmental stages, the author recommends that all future work should use adult pigs. It is a known fact that no two animals develop at the same pace, therefore usage of tissue from 3 month old pigs could have contributed to some of the variances observed in this thesis.
(see sections 4.3). The three-dimensional distribution and structure of the extracellular matrix components within the muscle were also shown to vary with the age of the animal (Purslow, 2004, Nishimura et al., 1996, Nishimura, 2010).

8.1 Concluding Remarks

The work in this thesis has provided new skeletal muscle quasi-static tensile response data. This data includes the muscle fibre response at intermediate angles as well as the quasi-static Poisson’s ratio for freshly harvested skeletal muscle. The novel landscape shape response that gives the reader some form of 3D visualisation of how the response changes with the change in the loading angle is presented. This is all new data that has been contributed to the overall knowledge base as a result of this thesis.

There existed a gap in knowledge for impact data, in particular of relevance to automobile car crashes within the city limits. The data presented in this thesis addresses this gap. This data was obtained using freshly harvested skeletal muscle before the Rigor Mortis phase. This data appears to fit well with the existing data (forming a nice continuous evolution, see Figure 8-3). This is also new data that is being added to the existing data.

A simple easy to use first order Ogden material law has been shown to be able to predict the experimental data very well. This model does not extend to the viscoelastic behavioural response. Therefore a three branch QLV model was applied in order to capture the viscoelastic part. While the models used are not novel, the method that has been used in applying the usually unstable QLV at high strain rate is new.

This work has shown that no evidence exists to show that the deformation response of muscle fibres and the surrounding extracellular matrix is responsible for the asymmetrical response observed at macro-structural level. The high standard deviations show that there is a lot variability in the individual muscle fibre response. Those muscle fibres close to secondary perimysium were observed to be affected more by the perimysium collagen fibre network response (this includes straightening out the waviness, hence stretching part of the muscle fibres there are in contact with). This therefore led to the observed wide range of the standard deviations. The work also shows that at low deformation strains used in the thesis work, the endomysium collagen network remains randomly oriented. The observed behaviour response that was different when tension or compression deforming loads were applied was the
perimysium straightening out or the waviness bunching together. This was only visually analysed, and more quantitative analysis need be performed in order to fully elucidate the cause of this. The concluding remarks go as far as summarising the work carried out for this thesis and noting that this work has not been reported before and will be crucial for the future use of the passive micro-structural model approach.

8.2 Future Recommendations and Limitations
The most important limitation is that the skeletal muscle work reported in this thesis was all performed using freshly harvested porcine tissue due to the un-ethical and impracticalities associated with acquiring freshly harvested human skeletal muscle samples. The author would like to acknowledge that there exist variations in the mechanical properties in the skeletal muscle tissue obtained from different species. However, these differences are only quantitative rather than qualitative as all skeletal muscles are built from the same components. The differences in muscle fibre sizes and quantity of connective tissue have been put forward as the reason behind these differences (KJÆR, 2004).

The future work may be centred on trying to establish the relationship between skeletal muscle mechanical properties and structural parameters such as muscle fibre size or extra cellular matrix collagen muscle fibre thickness. Use of mature animals (animals past their developmental phase) is recommended, as this eliminates the age related variations.

A limitation of the inverse finite element modelling is that the initial geometry of the muscle was cuboid, while the experimental samples were only approximately cuboid due to difficulties in the preparation of the samples, and this may have some influence on the predicted parameters (Böl et al., 2012). It is impossible to experimentally test tissue without inducing damage as shown by tensile low strain cross-fibre failure, therefore it is suggested that future experiments and models incorporate damage modelling. No failure was investigated in this thesis, combining the continuum damage mechanics with existing hyperelastic Ogden model will improve the models’ ability to predict damage and failure, especially for cross-fibre tensile stretches (Rich and Whittaker, 2005, Untaroiu and Lu, 2013, Volokh, 2010).

For the anisotropic behavioural response, Moerman (2012), used the Gaussian modulated spherical fibre distributed Ogden hyperelastic model (GMSFD) for both
tension and compression. The GMSFD model was able to accurately predict the compressive response well (see Figure 8-5 and Figure 8-6), (Moerman, 2012). The model was not able to capture the asymmetrical behavioural response of tissue, thus leading to the necessity of different parameters for tension and compression. The main limitations of the modelling employed was that the first order Ogden material law can only capture the isotropic elastic behavioural response of the tissue, the QLV can only capture the viscoelastic behaviour, therefore the anisotropy and the asymmetrical properties need to be incorporated in the future models.

Future work will also focus on exploring the passive micro-structural model suggested by Gindre (Gindre et al., 2013). However, further microscopic work is still needed in order to fully understand the micro-structural deformation before a further attempt at developing a 3D model capable of capturing the asymmetrical behaviour is developed.

Future work should also investigate these perimysium shape differences by identifying these local areas and performing a more detailed analysis using the new advanced Helium Ion Microscopy Analysis. While this new technology is functionally similar to SEM, it provides better focusing capabilities using a smaller probe size and has a smaller interaction volume at the surface, thereby creating less charging and far superior resolution of less than 0.3nm using energy acceleration of between 25-30kV. Helium atoms are huge compared to electrons, so the de Broglie wave length is short enough that the focused probe is not affected strongly by diffraction effects which greatly affect the traditional SEMs. Helium atoms create far superior signals and as such the better signals creates a higher contrast that enables the user to have a superior view of the edges of the material of interest (Rice et al., 2013, Scipioni, 2008, Terpstra et al., 2013, Postek and Vladar, 2008, Inai et al., 2007).
9 References


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