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THE MECHANICS OF VASCULARISED TISSUE

ADAM MICHAEL REEVE

Supervised by Professor Poul Nielsen,
Professor Martyn Nash
and Associate Professor Andrew Taberner

A thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy in Bioengineering,
the University of Auckland, 2014.
Biological tissues consist of a mixture of fluid and solid components, and the mechanical behaviour of a tissue can be influenced by the fluid within that tissue. This thesis investigated how fluid pressure affects tissue mechanics, and how this influence can be incorporated in continuum-level models of whole organs.

Firstly, a physical phantom model of vascularised tissue was constructed using silicone gel. Mechanical experiments were performed on this phantom to determine how it responded to changes in fluid pressure. Replicating the nonlinear, strain-stiffening behaviour of some tissues was attempted by incorporating a strain-stiffening wool-yarn into the gel.

Following this, a representative volume element model of vascularised tissue was developed that explicitly modelled vessels within tissue. This model predicted that anisotropy in the constitutive behaviour of a tissue’s solid components causes anisotropic swelling and stiffening, and that anisotropic vascular structure also contributes to anisotropic swelling.

It was demonstrated that poroelasticity can be used to model increases in stiffness with fluid pressure, provided that the poroelastic material’s constitutive relation is strain-stiffening, and the strain-stiffening terms are volume dependent. Approaches for incorporating anisotropic vascular structure in poroelastic models were then investigated and compared.

A poroelastic model with anisotropic constitutive behaviour was used to model the effect of perfusion pressure on the passive mechanics of the left ventricle of the heart. This model could reproduce the swelling deformation of myocardium, but further development of constitutive relations is required to accurately reproduce anisotropy in stiffness changes.

Finally, the effect of perfusion pressure on the mechanics of the rat tibialis anterior muscle was investigated. No significant change in muscle stiffness was observed between perfusion pressures of 5 kPa and 20 kPa, but a small swelling deformation was measured.
ACKNOWLEDGEMENTS

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**Certification by Co-Authors**

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
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INTRODUCTION

1.1 MOTIVATION

Mathematical models of the mechanics of biological tissues are useful in numerous applications. Patient specific models of the heart may be used in clinical decision making and diagnosis (Aguado-Sierra et al., 2011), and mechanical models of the breast allow tracking of tumours across multiple imaging modalities to assist in surgical planning (Rajagopal et al., 2010). Aside from direct clinical applications, improving understanding of how tissues act mechanically can provide valuable insight into how the human body functions.

Modelling the mechanics of tissues presents a number of difficulties when compared to modelling most engineering materials. Tissues often undergo large deformations during normal function, requiring non-linear governing equations to describe their motion. Their stress–strain relationship is usually nonlinear, with stiffness increasing with strain due to the straightening of fibrous structures, such as collagen and elastin. Biological tissues also consist of closely coupled solid and fluid materials, and the interaction between these components can play an important role in determining their mechanical behaviour.

Most tissues are vascularised and contain a complex network of blood vessels. The fluid within these vessels is pressurised by the pumping of the heart, and this perfusion pressure may vary over a wide range, adding complexity to a tissue’s mechanical behaviour. Interaction between the fluid and surrounding tissue acts in both directions; stress within tissue can alter the shape of blood vessels, changing their resistance and affecting fluid flow. Fluid pressure within vessels can also exert force on the surrounding tissue to alter its mechanical behaviour.

It is known that increasing vascular perfusion pressure can cause an increase in the stiffness of a tissue. This phenomenon is named the “garden hose” effect, as an
analogy to the stiffening and straightening of a garden hose when it becomes filled with water at high pressure. McCulloch et al. (1992) hypothesised that in order to exhibit stiffening with increased vascular pressure, the stress–strain relationship of a tissue must be strain-stiffening. In such cases, an increase in perfusion pressure that causes a tissue to swell will lead to the tissue operating in a steeper region of its stress–strain curve.

This dependence of mechanical behaviour on perfusion pressure has important consequences for tissue mechanics models. All mathematical models of tissue mechanics rely on constitutive relations that determine stress as a function of strain. These constitutive relations may be based on knowledge of the tissue’s microstructure, but nearly always contain parameters that must be estimated from experimental results. Due to the difficulty of performing mechanical tests on tissues in-vivo, experiments are usually performed on excised, unperfused tissue. As perfusion pressure can influence the mechanics of tissue, it is important to determine the error introduced by using constitutive parameters obtained from experiments on unperfused tissue. If this error is large, it may be accounted for by using a mathematical model to determine the change in mechanical behaviour with perfusion. Alternatively, experiments can be performed on perfused tissue when determining constitutive behaviour.

In some cases, normal physiological variations in perfusion pressure may affect mechanical behaviour. For example, arterial pressure in the heart varies throughout the cardiac cycle, which may have an effect on the passive mechanics of the myocardium. In such cases, the difference between perfused and unperfused tissue cannot be simply accounted for by modifying static constitutive behaviour, but dynamic changes in perfusion must be modelled and coupled to the solid tissue mechanics.

As well as vascular fluid, fluid in other tissue compartments may also influence tissue mechanics. For example, Desai et al. (2008) showed that acute oedema can affect cardiac mechanics. Oedema is also known to influence brain mechanics (Mchedlishvili et al., 1989). Approaches for modelling the effect of vascular fluid on tissue mechanics should also be applicable to modelling the influence of other fluids.

1.1.1 Cardiac Mechanics

May-Newman et al. (1994) presented a set of experiments performed on isolated, perfused dog hearts, in which strain within the myocardium was measured while
varying perfusion pressure. Strain was measured across the left ventricular free wall by inserting sets of radiopaque markers and imaging the heart using a biplane x-ray system. The reconstructed positions of these markers showed that with a cardiac perfusion pressure of 15 kPa, the strain in directions transverse to the muscle fibres when loaded by ventricular pressure was much less than with no perfusion pressure. The strain in the muscle fibre direction did not show a significant change. Swelling of the myocardium could also be measured from the positions of the radiopaque beads; perfusing at 15 kPa with zero ventricular pressure resulted in a swelling of 7% at the epicardium and over 15% at the endocardium. The perfusion-induced strain was found to be much greater in the directions transverse to fibres than in the fibre direction.

May-Newman et al. (1994) carried out these experiments in order to evaluate an explanation for Gregg’s phenomenon (Gregg, 1963), in which an increase in perfusion pressure leads to increased contraction force. It was hypothesised that an increase in perfusion pressure would increase the length of myocytes, leading to an increase in contraction force through the Frank-Starling mechanism. However, May-Newman et al. showed that a perfusion pressure increase does not cause a significant length increase in the muscle fibre direction, and thus concluded that this explanation is not likely to be the mechanism behind Gregg’s phenomenon. It is now believed that Gregg’s phenomenon acts through the opening of stretch-activated ion channels, which increases intracellular calcium and calcium sensitivity (Schouten et al., 1992; Westerhof et al., 2006).

Although the results obtained by May-Newman et al. did not support the idea that the garden hose effect could alter cardiac contractility, they demonstrated that changes in cardiac perfusion could significantly alter the passive mechanics of myocardium. Inclusion of this effect in mechanical models of the heart would improve their accuracy if properly validated, improving the clinical predictive ability of such models.

1.1.2 Other Tissues

May-Newman et al. (1994) have published the most comprehensive investigation to date into the effect of perfusion pressure on the mechanics of a biological tissue. However, fluid interaction has also been shown to be important in the mechanics of a range of other tissues.

The most obvious tissue type where fluid content and pressure is important for function is erectile tissue. This is present in the male and female sex organs, but
can also be found in other non-sex organs. The turbinate of the nose, for example, are formed from erectile tissue and control heat and mass transfer during breathing (Ng et al., 1999).

Gefen and Margulies (2004) showed that physiological levels of perfusion pressure in the brain do not lead to a difference in stiffness when compared with unperfused tissue in-situ. However, a decrease in the viscoelastic stress relaxation time was observed for unperfused brain tissue. Although perfusion pressure does not have a significant influence on brain mechanical properties, the pulsatile nature of this pressure may play an important functional role; pulsatile swelling caused by perfusion leads to pulsatile motion of cerebrospinal fluid within the ventricles (Stadbauer et al., 2010). Modelling the coupled mechanics of swelling brain tissue and cerebrospinal fluid flow may improve understanding of cerebrospinal fluid’s role as a transport medium in the brain. Shaken baby syndrome research may also benefit from improved understanding of the mechanisms and effects of tissue swelling, as brain swelling due to leakage from intracranial veins has been hypothesised to be a factor in the pathogenesis of shaken baby syndrome (Geddes et al., 2003).

In the lung, there is mechanical interaction between air, blood, and tissue. Experiments have shown that increasing vascular pressure increases vascular volume and decreases airway volume (Wagner and Mitzner, 1996). Wildhaber et al. (1998), and Peták et al. (2002) found that increasing either vascular pressure or vascular flow rate decreased lung compliance and increased resistance to flow in airways. These studies illustrate that vascular pressure is important to consider when modelling the mechanics and function of the lung.

In the breast, not only may blood pressure influence mechanics, but fluid pressure changes during lactation play a significant mechanical role. Milk production in the mammary glands can lead to painful levels of engorgement and risk of infection (Hill and Humenick, 1994). Research into the mechanics of this process may lead to treatments for reducing pain or help identifying mothers at risk of developing engorgement. Accounting for the change in mechanical behaviour of the breast during lactation will become important in mechanical models used for tumour tracking, as breast cancer is becoming increasingly common during pregnancy and during breast-feeding (Woo et al., 2003).
1.2 THESIS OVERVIEW

1.2.1 Objectives

There is a wide range of tissues where fluid pressure and flow may play an important mechanical role. However, there has been little investigation into how to evaluate the significance of fluid effects on mechanics, and how to incorporate these effects into mathematical models of tissue mechanics.

The aim of this thesis is to develop and validate mathematical models of biological tissue that incorporate the effects of fluid pressure on a tissue’s mechanical behaviour.

1.2.2 Thesis Outline

Chapter 2 reviews experimental studies into the effect of perfusion on tissue mechanics, as well as approaches for modelling the interaction between fluid mechanics and the mechanics of tissues and whole organs. Areas requiring further study are identified, which this thesis aims to address.

Chapter 3 presents a novel approach for constructing anisotropic, vascularised phantoms using silicone gel. These phantoms incorporated strain-stiffening elements analogous to the collagen fibre network in many biological tissues. The aim of constructing these phantoms was to produce a mechanical model of biological tissue that stiffens with increasing fluid pressure. This would serve to improve understanding of the interaction between vascular fluid and tissue mechanics, and provide experimental data useful for testing mathematical models. This approach was not successful in producing pressure-driven stiffening behaviour, but did provide insight into fluid-tissue interactions.

Chapter 4 presents a representative volume element model of vascularised tissue where the solid and fluid tissue constituents are modelled discretely. This model is an improvement on previously published representative volume element models of vascularised tissue, as it allows varying the anisotropy of the vascular geometry. The effects of porosity, anisotropy of the vasculature, and anisotropy of the solid stress-strain constitutive behaviour are investigated. It is shown that the anisotropy in the constitutive behaviour of myocardium is the most significant factor causing its anisotropic stiffening, while anisotropic swelling behaviour is also dependent on the vascular structure.
Chapter 5 describes a static model of poroelasticity and investigates constitutive relations for poroelastic models, examining the form of constitutive relation required for a material to exhibit stiffening with increased fluid pressure. This is a novel contribution to the field of poroelasticity, as forms of constitutive relations are identified that are suitable for modelling materials that exhibit pressure-driven stiffening.

Chapter 6 presents novel constitutive relations for poroelasticity that account for anisotropy in vascular structure, which can result in anisotropic swelling deformation. The behaviour of these poroelastic models is compared to the RVE model presented in Chapter 4.

Chapter 7 applies an anisotropic poroelastic constitutive relation to modelling the left ventricle of the heart. Changes in the left ventricle’s mechanical behaviour with perfusion pressure are compared to experimental data obtained by May-Newman et al. (1994), and it is shown that a poroelastic model does a reasonable job of reproducing the swelling deformation of the ventricle and changes in passive myocardium mechanics with perfusion.

Chapter 8 presents experimental results from mechanical testing of the rat tibialis anterior muscle. Transverse indentation and axial extension experiments were performed with the muscle perfused at two different pressures. No change in the mechanical response was observed with increased perfusion, in contrast with the behaviour of myocardium. This is the first time that the influence of perfusion pressure on skeletal muscle mechanics has been investigated.

Chapter 9 summarises the results from this thesis, considering the main outcomes and limitations. Possible directions for further research into the mechanical interaction between the solid and fluid components of tissue are discussed.
MECHANICAL INTERACTION BETWEEN TISSUE AND VASCULATURE

Experiments on biological tissue have shown that mechanical behaviour is affected by blood perfusion (May-Newman et al., 1994; Wildhaber et al., 1998). The interaction occurring at the microstructural level to produce these changes in behaviour is complex, and acts through multiple mechanisms. These mechanisms can be purely mechanical or can involve chemical signalling.

In this chapter the mechanisms of interaction between the vasculature and surrounding tissue are reviewed, along with approaches for modelling this interaction. Areas requiring further study are identified for investigation in this thesis.

2.1 THE CIRCULATORY SYSTEM

Blood vessels in the human body may be grouped into two systems, named the pulmonary and systemic circulation. The pulmonary circulation moves blood from the right ventricle of the heart to the lungs, where it is oxygenated, then back to the heart and into the left ventricle. The left ventricle pumps oxygenated blood through the systemic circulation, distributing it throughout the body and returning deoxygenated blood to the right ventricle. In both the systemic and pulmonary circulations, blood vessels are organised into a hierarchical, branching network. Arteries are large vessels that transmit blood away from the heart. Within an organ, arterioles branch off arteries and carry blood to small capillaries, where oxygen and other chemicals are exchanged between the blood and surrounding tissue. Blood in capillaries then flows through to venules, which transmit the blood to veins. Veins then return blood to the heart.

Blood vessels are made up of separate layers. The innermost layer is the endothelium, which is attached to a basement membrane. This is surrounded by layers of smooth muscle, which are encased in a connective tissue layer named the adventitia.
This general structure is modified at different levels of the vascular hierarchy to adapt vessels to their specific role (Tortora and Derrickson, 2006). Arteries and arterioles have a much thicker smooth muscle layer than veins and venules, as they contain blood at a higher pressure. Veins and venules are therefore much more compliant than arteries and arterioles. Veins may also contain valves to prevent reverse flow. Capillaries are designed to permit efficient exchange of nutrients; they have no smooth muscle layer and their endothelium layer is only a single cell thick.

Most blood vessels, except for capillaries, are mechanically active structures; their smooth muscle layer can alter its contractile state in response to a number of different factors. Smooth muscle exhibits a myogenic response, meaning it contracts in response to stretch. Therefore, when vascular pressure increases, the resulting stretch of smooth muscle cells leads to an increase in vascular tone to maintain a constant vessel diameter (Grände et al., 1979). Blood flow is sensed by cilia on the surface of the endothelial cells that line blood vessels. Nitric oxide is released by the endothelium when flow rate increases, which leads to relaxation of the vascular smooth muscle in order to maintain a constant flow rate (Koller and Kaley, 1991). Vascular tone may also be altered in response to energy requirements, which are signalled through the release of chemical messengers from the surrounding tissue. The largest pressure drop in the vasculature occurs at the level of arterioles, and it is arterioles that contribute most to pressure and flow regulation. Tune et al. (2004) provide an in-depth review of mechanisms involved in blood flow regulation, focussing on myocardium.

2.1.1 Blood Vessel Mechanics

The mechanical behaviour of blood vessels has been the subject of much experimental research and modelling, as it plays a significant role in the function of the circulatory system. The passive contribution of vasculature to the large-scale mechanical properties of tissues has not been well explored, although Allaart et al. (1995) showed vasculature could have a significant effect on the mechanics of papillary muscle. This study is described further in Section 2.2.2.

Weizsäcker et al. (1983) measured passive mechanical properties of isolated rat arteries at a range of internal pressures and extension ratios, finding that increasing the interior pressure of a vessel increases its zero-stress length and increases its longitudinal stiffness. This indicates that the vasculature itself may contribute to the increased stiffness of tissues observed with increased perfusion pressure.
Chuong and Fung (1983) described the mechanical behaviour of rabbit arterial wall using an exponential, anisotropic constitutive relation. Non-uniform stresses across the vessel wall were found, with stress attenuating rapidly away from the interior surface. Holzapfel and Weizsäcker (1998) developed an improved constitutive relation for arteries, where the strain energy density contained separate contributions from isotropic and fibrous, anisotropic components, corresponding to elastin and collagen, respectively. This relation was able to accurately reproduce the behaviour of the aorta and arteries. The collagen structure within arteries was incorporated into an anisotropic constitutive relation by Alastrué et al. (2009). A microsphere-based approach was used, with collagen fibre orientations described by a continuous orientation distribution.

2.1.2 Vascular Network Geometry

An important functional feature of the vasculature is its branching, hierarchical structure, which must be accounted for in computational models to accurately determine pressure-flow relations and tissue perfusion. Both measuring the vascular network geometry of an organ, and representing this geometry in computational models, present considerable challenges, due to the large range of vessel sizes and large number of vessels present in any given organ.

Kassab (2000) reviewed an approach for imaging and reconstructing the geometry of the vascular network of the heart. Silicone elastomer casts were used to determine the structure of vessels with a diameter greater than 50 µm, and histology was used to measure the morphometry of smaller arterioles, venules, and capillaries. The branching of the vascular tree was described by a modified Strahler centripetal ordering scheme, with capillaries numbered zero, arteries given positive numbers, and veins given negative numbers.

Smith and Kassab (2001) discuss the use of such reconstructed vascular geometries in computational models of the heart, where models of blood vessel mechanics are used to couple blood flow to the ventricular mechanics. Wijngaard et al. (2013) reviewed approaches for imaging and reconstructing the coronary vasculature, focusing on new developments in imaging and computational modelling that enable high resolution reconstructions of vasculature geometry. These new approaches include cryomicrotome imaging of small vessels (Goyal et al., 2013) and micro-computed tomography using radiopaque contrast agents (Beighley et al., 1997).

The first full reconstruction of the vascular network of the heart, containing approximately $27 \times 10^6$ vessel segments, was presented by Kaimovitz et al. (2010). Recon-
struction of the capillary and venous networks was completed and combined with previous arterial data (Kassab et al., 1993). Due to the large number of capillaries present in tissue, capillaries were not represented individually, but grouped into functional units of up to 30 capillaries.

The destructive imaging techniques used to reconstruct high-resolution vascular network geometries cannot be applied to clinical use in humans. In this situation, magnetic resonance imaging (MRI) can be used to determine capillary orientation distributions (Karampinos et al., 2010; Vignaud et al., 2006).

An aspect of vascular geometry that is important when considering its effect on tissue mechanics is the orientation of vessels within a tissue. This is less important than vessel segment lengths and branching angles when considering blood flow, so is not usually reported in studies of vascular morphometry. Bassingthwaighte et al. (1974) used silicone casting with light microscopy to estimate capillary densities and lengths in dog myocardium. Capillaries were observed to be aligned along the muscle fibre directions, but their orientations were not quantified. Mathieu et al. (1983) quantified the anisotropy of capillaries in skeletal muscle and showed that their orientation could be represented by a Fisher axial distribution (Weibel, 1980), with capillaries preferentially aligned with the muscle fibre direction. Poole and Mathieu-Costello (1990) validated the use of a Fisher axial distribution for describing the orientation of capillaries in the rat heart, and May-Newman et al. (1995) quantified the anisotropy of capillaries in the dog heart using the same approach. Some terminal arterioles in myocardium have been shown to also be preferentially aligned with the muscle fibre direction. However, these vessels are tortuous and there is no evidence for other vessel types having a nonuniform orientation distribution (Kassab et al., 1993).

### 2.2 Interaction Mechanisms

Blood vessels are tethered to surrounding tissues by bundles of collagen fibres within the extracellular matrix (Caulfield and Janicki, 1997). Blood vessels can also contact tissue cells directly, and hydrostatic stress can be transmitted between the vasculature and tissue through the extracellular fluid.

The relative importance of these different mechanisms to the interaction between blood vessels and their surrounding tissue is not well understood. Abovsky et al. (1996) used a mathematical model to determine the effect of collagen struts on the deformation of capillaries within myocardium. Simulations suggested that these
collagen struts have a significant influence on the deformation of capillaries and prevent their collapse at low internal pressures. Lamberts et al. (2004) experimentally investigated the importance of collagen on the impediment of blood flow by cardiac muscle contraction. Reducing extracellular collagen by treating myocardium with a collagenase did not significantly affect the impediment of blood flow with contraction. This result suggests that although the extracellular collagen network may be important for maintaining vascular structure within a tissue, it is not required for transmission of forces between tissue and the vasculature.

2.2.1 Tissue Mechanics Affects Vascular Fluid

Deformation of tissue can affect vascular resistance by deforming blood vessels. Yamamoto et al. (1999) found that compression of myocardium transverse to vessels increased their resistance, but compression in the vessel axis direction did not affect resistance. This behaviour can be explained by transverse compression causing a decrease in vessel cross-sectional area, which increases resistance. An isotropic hydrostatic pressure in tissue can also affect vascular resistance (Hartsock et al., 1998). The pressure difference between the interior and exterior of a vessel is named transmural pressure, and a vessel’s diameter is related to transmural pressure through its compliance. When hydrostatic pressure in the tissue surrounding a vessel increases, transmural pressure decreases, decreasing vessel diameters. Arteries generally have low compliance, meaning their diameter changes very little for a given change in transmural pressure, whereas veins have much higher compliance, but generally contain blood at a lower pressure.

The compliance of a vessel in-vivo depends not only on the vessel itself, but also on the properties of the surrounding tissue, as inflating a vessel requires displacing the tissue around it (Hamza et al., 2003; Vis et al., 1995). In veins and capillaries, the pressure-diameter relation of a vessel is determined mostly by the surrounding tissue, whereas in arteries and arterioles, the arterial wall itself contributes most to vessel compliance. This contribution of the tissue to vessel compliance is important to consider when investigating the influence of perfusion pressure on tissue mechanics, as the extent to which perfusion pressure deforms the surrounding tissue depends on both the vessel mechanical properties and the mechanical properties of the surrounding tissue.

The influence of tissue mechanics on vascular flow has been studied most extensively in the heart. Coronary artery disease is the leading cause of death worldwide (Finegold et al., 2013), and is commonly associated with the accumulation of plaque
in coronary arteries, which reduces blood flow. Therefore, understanding the mechanics of blood flow in the heart has considerable importance and has been the focus of much research. It has been found that the mechanics of the beating heart can have a significant effect on blood flow. Westerhof et al. (2006) provide a comprehensive review of the mechanisms of interaction between vasculature and myocardium. Myocardium is especially interesting when considering vascular interactions, as the myocardium vasculature experiences stress transmitted from the ventricles, as well as stress due to local contraction of muscle fibres.

Experimental studies of coronary flow have shown that arterial flow is lower during systole, when the heart contracts and blood flows out of the ventricles, than in diastole, when the heart relaxes and the ventricles are filled (Hoffman and Spaan, 1990). Venous flow is increased during systole, resulting in an overall decrease in the total blood content of the myocardium (Kajiya et al., 1985). Arterial flow impediment is greater in the endocardium than epicardium (Downey et al., 1974), and at low perfusion pressures, reversal of arterial flow has been observed (Hoffman and Spaan, 1990; Kajiya et al., 2008; Spaan et al., 1981).

A number of models have been proposed to explain these experimental results. The vascular waterfall model (Downey and Kirk, 1975) and intramyocardial pump model (Spaan et al., 1981) both explain flow impediment by using an intramyocardial pressure that varies linearly from ventricular pressure at the endocardium to atmospheric pressure at the epicardium. The change in intramyocardial pressure during systole decreases transmural pressure across vessel walls, reducing vessel diameters and therefore increasing vascular resistance. The vascular waterfall model predicts the collapse of blood vessels at large intramyocardial pressures, such that flow becomes proportional to the difference between arterial pressure and intramyocardial pressure, rather than arterial pressure and venous pressure, thereby reducing flow. Collapse of vessels has not been observed in-vivo, providing no evidence to support the vascular waterfall model. The intramyocardial pump model introduces capacitance of the vasculature and assumes that contraction of the heart acts to pump intramyocardial vascular fluid the same way ventricular fluid is pumped. This model is able to reproduce the experimentally observed negative arterial flow during systole with low perfusion pressure. Bovendeerd et al., 2006 developed improvements to the waterfall and intramyocardial pump models that incorporated radial intramyocardial wall stress and fluid exchange. The ventricular wall was modelled as a number of nested thin shells, and this model was able to describe a greater range of cardiac mechanical behaviour than previous models.

Although these models could reproduce many experimental results, measurement
of blood flow during an isobaric contraction, in which left ventricle pressure is maintained at zero, showed the same reduction in flow as an isovolumic contraction (Krams et al., 1989). As both the intramyocardial pump model and vascular waterfall model predict no flow impediment in this situation, a new model was needed to explain this result. The time-varying elastance model was introduced, which defines the elastance of a vessel as a function of the local myocardium contraction state. The muscle shortening and thickening model (Vis et al., 1997), and vascular deformation model (Westerhof et al., 2006) were also developed to explain this behaviour.

The muscle shortening and thickening model explains the impediment of blood flow by the thickening of myocytes. Myocytes maintain their volume when deforming because they are filled with incompressible cytoplasmic fluid. Therefore, contraction of a myocyte requires it to thicken. This thickening can reduce vascular space, increasing vascular resistance. It has also been shown that when muscles contract, vessel tortuosity increases (Mathieu-Costello, 1987), and increased tortuosity increases vessel resistance (Pries et al., 1997). This behaviour is accounted for by the vascular deformation model (Westerhof et al., 2006). These models of myocardium–vasculature interaction based on local contraction do not explain the increased flow impediment observed at the endocardium unless a difference in muscle contraction between the endocardium and the epicardium is introduced.

Algranati et al. (2010) showed that no single mechanism from the above models could reproduce all of the major features of coronary flow observed experimentally. However, a combination of both muscle contraction induced intramyocardial pressure and ventricle induced intramyocardial pressure could reproduce most of the experimental results.

2.2.2 Vascular Fluid Affects Tissue Mechanics

The above models of myocardium–vasculature interaction consider the effect of ventricular mechanics on blood flow. However, blood pressure can also influence tissue mechanics. When fluid pressure within a vessel increases, it causes an increase in vessel diameter dependent on the compliance of the vessel and surrounding tissue. Increasing vessel diameter causes a deformation of the surrounding tissue, which can affect its mechanical behaviour. May-Newman et al. (1994) showed increased transverse stiffness in dog myocardium with increased perfusion pressure, as described in Chapter 1. This transverse stiffening is believed to be a purely mechanical effect caused by the strain-stiffening behaviour of myocardium. Perfusion-induced
deformation of tissue can also have an effect on mechanics through chemical pathways. For example, the Gregg effect is an increase in cardiac muscle contractility in response to an increase in perfusion pressure. This is believed to be caused by the opening of stretch-gated calcium channels, which results in a subsequent increase in calcium sensitivity (Lamberts et al., 2002). Axial stretch of myocytes can also increase their contractility through the Frank-Starling effect and the Anrep effect. The Frank-Starling effect describes the increase in ventricular stroke volume with increased filling volume, such that ventricular output volume matches the input volume. A proposed mechanism for this effect is that the axial stretching of myocytes increases calcium sensitivity, thereby increasing the number of cross-bridges between actin and myosin (Hanft et al., 2008). The Anrep effect has a similar result but acts more slowly and is related to an increase in the amplitude of calcium transients (Cingolani et al., 2013).

As well as affecting the passive mechanics of tissue, perfusion pressure can also affect the active contraction of muscle through a purely mechanical method. Muscle cells thicken as they contract, such that their volume remains constant. Pressure within vessels can inhibit contraction by opposing the transverse thickening of muscles (Willemsen et al., 2001).

Allaart et al. (1995) investigated the effect of perfusion pressure on the axial stress–strain relation of isolated rat papillary muscles. Axial stiffness was found to increase with perfusion pressure at all levels of strain. A mathematical model was developed to explain this increase in axial stiffness. The stiffness of vessels was modelled as a function of perfusion pressure according to the arterial pressure–stiffness data of Weizsäcker et al. (1983), and the stiffness of the extracellular matrix and muscle fibres was assumed to remain constant. The model was able to reproduce the experimental results from papillary muscle, showing that the vasculature contributes significantly to the mechanical stiffness, and suggesting that the change in the stress–strain response of the vasculature alone could explain the increased stiffness of perfused papillary muscle. One limitation of this model is that the experimental pressure–stiffness data was based on an excised vessel, whereas in-vivo, surrounding tissue opposes vessel inflation, limiting how much it can stiffen. This model also fails to explain why stiffening occurs transverse to the muscle fibre direction in myocardium, which was observed by May-Newman et al. (1994).

As well as increasing blood vessel diameters, increased blood pressure may also straighten blood vessels. Redaelli and Pietrabissa (1997) developed a cardiac mechanical model where fluid flow was described by the waterfall and intramyocardial pump models, which were modified to incorporate vessel straightening. Increased
perfusion pressure led to a straightening of the cardiac blood vessels, which caused the ventricle to enlarge, altering its mechanics.

The contribution of different levels of the vascular hierarchy to tissue mechanical behaviour will vary according to their internal pressure, volume fraction, and vessel compliance. Arteries and arterioles have high internal pressure which may result in more tissue deformation than other vessel types. However, they also have thick, strong vessel walls that may shield the surrounding tissue from their internal pressure. Capillaries contain the largest proportion of blood volume and have thin vessel walls, but contain blood at low pressure. The mechanical effects of different vessel types are difficult to measure independently, but it may be possible to predict them using computational simulations.

Fluid pressure within vessels exerts both normal stress on the vessel wall proportional to its hydrostatic pressure, and shear stress due to its velocity. It is well known that blood velocity can affect tissue mechanics indirectly through signalling molecules released by the endothelium. The direct mechanical effect of fluid shearing on the surrounding tissue has not been thoroughly investigated, but its effect is likely to be small compared to hydrostatic pressure (Westerhof et al., 2006).

The effect of perfusion on tissue mechanics can be negated by autoregulatory mechanisms that act to maintain constant vessel dimensions, such as the myogenic contraction of smooth muscle within vessels walls. In the study of May-Newman et al. (1994), discussed in Chapter 1, adenosine and nifedipine were both included in the perfusate. Adenosine increases levels of cyclic adenosine monophosphate, resulting in vasodilation, and nifedipine blocks L-type calcium channels, preventing contraction of vascular smooth muscle (Rang et al., 2012). May-Newman et al. did not investigate the response of myocardium in the absence of these molecules. However, other studies in myocardium have failed to show a mechanical effect of perfusion pressure without the inhibition of autoregulatory mechanisms (Westerhof et al., 2006). Depending on the application, this may mean that the influence of perfusion on mechanics can be ignored. Alternatively, this result can be used to argue that autoregulatory mechanisms need to be accounted for in mechanical models of blood vessels.

2.3 Modelling Vessel–Tissue Interaction

This section reviews approaches for developing computational models of the interaction between perfusion and tissue mechanics. These range from models that con-
sider solid–fluid interaction at a microstructural level to approaches for modelling the solid and fluid mechanics of a whole organ.

2.3.1 Vascular Network Flow Models

Vascular network models consider flow through a network of one-dimensional vessel segments embedded in tissue. Smith (2004) modelled blood flow in the three-dimensional geometry of the heart, accounting for the effects of tissue stress and deformation. Six branching generations of the coronary arterial vasculature were modelled using a one-dimensional form of the Navier-Stokes equations. The ventricular mechanics model developed by Nash (1998) was solved at time steps throughout the cardiac cycle, independent of blood flow. The mechanical model solution was then used to determine the deformation of the vasculature and intramyocardial pressure. Intramyocardial pressure was calculated as the average stress normal to the vessel walls, and was found to vary approximately linearly from ventricular pressure at the endocardium to atmospheric pressure at the epicardium, in agreement with the intramyocardial pump model. Stress due to local tissue contraction was found to significantly decrease fluid flow during the ejection phase of the cardiac cycle.

Kassab et al. (2013) recently reviewed the use of such one-dimensional network flow models coupled to ventricular mechanics and discussed how the effects of both ventricular pressure and local contraction on blood flow can be included. Although these models can accurately reproduce the effect of tissue mechanics on vascular flow, their ability to model the effect of perfusion on tissue mechanics has not been investigated.

A limitation of network flow models is that the number of vessel segments they can model is limited by computational memory and processing time, such that it is not feasible to model all generations of the vasculature. To circumvent this issue, explicit network models can be coupled to continuum models describing flow through the microvasculature (Michler et al., 2013).

2.3.2 Explicit Vessel Models

A brute-force approach to modelling the interaction between a solid tissue and the fluid in blood vessels at small scales is to model the vessel geometry explicitly. Fluid flow through vessels may be modelled using the Navier-Stokes equations, or simplifications of these, and the effect of fluid pressure on the solid component may
be included through pressure boundary conditions on the vessel walls. When the fluid mechanics are not important, the fluid component can be modelled implicitly using pressure boundary conditions on the solid component.

Bilston (2002) modelled a small block of tissue containing a single blood vessel using a two-dimensional, plane strain, finite element model. A Mooney–Rivlin constitutive relationship was used with stiffness parameters decaying exponentially with time to model viscoelasticity. The results showed increased material stiffness with an increase in vessel pressure. The response to pressure was non-linear, with increased sensitivity at higher pressures. Increasing the initial vessel diameter increased tissue stiffening, and adding viscoelastic terms also enhanced the effect of pressure on stiffness.

A similar approach was taken by Jor (2005) to develop a three-dimensional vessel model. A quarter of a blood vessel and the surrounding tissue were modelled using both a neo-Hookean and an exponential constitutive relation. A neo-Hookean relation represents materials with a relatively linear stress–strain relationship, whereas an exponential relation represents materials that exhibit significant strain-stiffening. This model showed that the nonlinearity of the exponential constitutive relation was required for stiffening of the tissue with increased fluid pressure; the neo-Hookean model did not exhibit significant stiffening. The stiffening effect was also found to be more pronounced as the initial fluid volume proportion increased, in agreement with Bilston (2002).

These two models provide valuable insight into how blood pressure alters the mechanics of tissues. However, both models only represent tissue with parallel vessels, and cannot model variation in the anisotropy of blood vessel arrangement. Anisotropy of the solid constitutive relation was not considered either. To understand the experimental results of May-Newman et al. (1994), which showed that myocardium stiffens predominantly in directions transverse to the muscle fibres, it is important to investigate the influence of these two types of anisotropy.

2.3.3 May-Newman and McCulloch’s Model

May-Newman and McCulloch (1998) developed a continuum-level model of perfused myocardium to explain their previous experimental results (May-Newman et al., 1994). These results showed that myocardium swells in directions transverse to the muscle fibres. It was hypothesised that this transverse swelling is due to the alignment of capillaries in the muscle fibre direction; fluid pressure acts normal
to the vessel walls, leading to swelling transverse to the vessels and therefore transverse to the muscle fibres.

The computational model developed by May-Newman and McCulloch (1998) was based on finite deformation elasticity with an additional dependent variable representing vessel diameter stretch ratio. Deformation of the myocardium was decomposed into deformation due to vascular fluid pressure and deformation due to external boundary conditions (Figure 2.1). The deformation due to fluid pressure was assigned by assuming that vessels are aligned in a single direction, such that the perfusion deformation gradient in a coordinate system aligned with the vessels is given by

\[ \mathbf{F}_V = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \lambda_V & 0 \\ 0 & 0 & \lambda_V \end{bmatrix} \] (2.1)

where \( \lambda_V \) is the vessel diameter stretch ratio. The vessel diameter was related to the difference between vascular pressure and intramyocardial pressure through a compliance parameter, where intramyocardial pressure was calculated as the average of stresses perpendicular to the vessel direction. An anisotropic constitutive relation was used to represent the behaviour of myocardium, based on the relation developed by Guccione et al. (1991), and the standard incompressibility constraint of finite deformation elasticity was modified to account for changes in fluid volume.

Figure 2.1. The total deformation gradient tensor \( \mathbf{F} \) is composed of deformation due to the fluid pressure, \( \mathbf{F}_V \), and subsequent deformation of the solid component due to external forces, \( \mathbf{F}_S \). Redrawn from May-Newman and McCulloch (1998).
This model was implemented in a static finite element model of the dog left ventricle and compared to the previous experimental results. The predominantly transverse swelling and stiffening of myocardium were reproduced, as well as the approximately linear increase in swelling volume ratio from the epicardium to the endocardium. Although this model is simple, it could be extended in a number of ways to more accurately represent myocardium. For example, the deformation due to perfusion could be decoupled into deformation due to pressure in different types of vessels by incorporating accurate morphometry data. Rather than assuming a constant vascular pressure, a more accurate model of blood flow and pressure could also be used to represent the mechanics of the heart in-vivo.

One limitation of this model is that it does not account for the mechanics of surrounding tissue on the deformation due to perfusion, but assumes the vessel compliance is constant. As Vis et al. (1995) showed, the surrounding tissue contributes significantly to this pressure-diameter relation, especially in capillaries. As stress–strain relations of tissue are anisotropic and nonlinear, the influence of surrounding tissue on perfusion deformation may be important to model. The transverse swelling and stiffening of myocardium could be explained by incorporating the mechanical behaviour of surrounding tissue. As myocardium is anisotropic, the less stiff transverse directions may allow more stretch than the fibre direction, leading to the observed stiffening in transverse directions.

2.3.4 Poroelastic Models

Poroelasticity is a continuum approach to modelling the mechanics of materials consisting of a solid component and fluid component, where the fluid can move through pores in the material. The concept of a poroelastic material originates with Karl von Terzaghi’s study of soil mechanics (Terzaghi, 1943), and a general linear theory of poroelasticity was developed by Maurice Biot (Biot, 1941). Poroelasticity was originally developed to describe consolidation of soils, which consist of a skeleton of solid particles saturated with water. However, the same principles can be extended to biological tissues, where the fluid component may be blood within vessels, intracellular fluid, or extracellular matrix fluid. Poroelastic models have been applied to a wide range of biological tissues including cardiac muscle (Chapelle et al., 2010), skeletal muscle (Vankan et al., 1998), cartilage (Lai et al., 1981) and brain tissue (Smith and García, 2009). Poroelasticity has also been used to model the mechanics of individual cells (Moeendarbary et al., 2013).

In Biot’s original poroelastic theory, the solid component mechanics were described
using linear elasticity, and the fluid mechanics were described by Darcy’s equation. Darcy’s equation (Darcy, 1856) linearly relates the average flow in a porous material to the pressure gradient using a permeability tensor. The original theory of Biot has been reformulated and extended numerous times. Bowen (1980) developed a nonlinear porous media theory for cartilage where the solid component is incompressible and large deformations are permitted. Poroviscoelastic models have been developed where the solid component exhibits viscoelastic behaviour. Such models have been used to model liver tissue (Raghunathan et al., 2010) and myocardium (Huyghe et al., 1991).

A useful concept developed by Terzaghi (1943) is the idea of effective stress. Effective stress is the stress that acts to deform the solid skeleton of a porous material, and can be calculated as

$$\sigma_{\text{eff}} = \sigma - pI$$

(2.2)

where $\sigma_{\text{eff}}$ is the Cauchy effective stress, $\sigma$ is the total Cauchy stress within the solid and fluid mixture, $p$ is the fluid hydrostatic pressure, and $I$ is the identity tensor. It should be noted that neither the effective stress nor the total mixture stress are necessarily pure deviatoric stresses; both may contain a hydrostatic stress component separate to the fluid pressure. Buhan et al. (1998) formulated the governing equations for a poroelastic material under large deformations using a boundary integration method at the microstructural level and found that an effective stress formulation is valid when the solid component is incompressible.

When modelling vascularised tissue, the intracellular and extracellular fluid are generally contained in the solid component, such that the fluid component only represents blood within the vasculature. The porous structures of soil and vascularised tissue are illustrated in Figure 2.2. In contrast with water in soil, blood in vascularised tissue is contained in a network of vessels, which may have a preferred orientation. Biological tissues generally experience larger deformations than soil and have more nonlinear and anisotropic constitutive behaviour.

To accurately model blood flow in tissues requires accounting for the hierarchical structure of the vasculature. Huyghe and Campen (1995a,b) extended poroelasticity to account for this hierarchical structure by adding a fourth dimension to the equations governing fluid flow, representing position within the vascular hierarchy. A conductance tensor was introduced, analogous to the permeability tensor in the standard Darcy equation, that controls both flow within a hierarchical level and flow between levels of the hierarchy. A formal averaging procedure was used to formulate the governing equations of this extended Darcy equation.
The same equations were derived by Vankan et al. (1996) using the theory of mixtures. In this approach each component of the mixture is given a volume fraction, but the discrete nature of the components is not considered. Vankan, Huyghe, Drost, et al. (1997) applied the finite element method to the extended Darcy equation so that it could be used to describe organs with complex geometries and boundary conditions. Using this finite element method, Vankan et al. (1998) developed a model of contracting calf muscle to determine the effect of muscle contraction on blood flow. This model was validated by comparing with a lumped-parameter model of blood flow. It was not compared with any experimental results due to the difficulty of imaging blood flow through a tissue. However, this may be possible with techniques such as diffusion tensor imaging. Comparison with an actual calf muscle experiment would be important for validation. The effect of fluid pressure on the mechanical behaviour of the muscle was not considered with this model.

An extension introduced by Vankan et al. (1998) to the general poroelastic method is that the hydrostatic pressure in the solid component and the fluid pressure are not the same, but are assumed to be related by a vessel compliance term and the difference between these two pressures contributes to elastic energy stored in the vessel wall.

Chapelle et al. (2010) developed a general poroelastic model for finite deformation, based on the work of Coussy (2004), and applied this to blood perfusion in the heart.
The constitutive relation included terms to prevent the porosity becoming negative, which is not possible in reality but may occur with very large deformations in poroelastic models. A Mooney–Rivlin based constitutive relation was used to describe the mechanics of the solid component. This relation does not reproduce the anisotropic and strain-stiffening behaviour of myocardium, but could be substituted for a more suitable relation. The main contribution of this work was the development of a new poroelastic formulation using a compressible solid component and allowing large deformations, based on thermodynamic considerations.

Cookson et al. (2012) introduced a novel multi-compartment method for modelling the hierarchical organisation of vasculature in poroelastic models, based on the double-porosity concept of Coussy (2004). Rather than representing flow through the vascular hierarchy using a fourth dimension, vessels are grouped into discrete sets representing a level of the vascular hierarchy. Flows between levels of the vascular hierarchy are then represented by source and sink fields, which are calculated using an intercompartment coupling tensor. This approach appears to be an improvement over the method of Huyghe and Campen (1995b) as spatial flows at different vascular hierarchy levels are described separately. Cookson et al. (2012) showed that this multi-compartment Darcy framework can also be applied to modelling geometrically separate perfusion regions, as found in the heart.

Michler et al. (2013) considered the use of the multi-compartment method in pure Darcy flow problems. A reduced formulation, in terms of pressure variables only, was compared to the full formulation in terms of velocity and pressure, finding that the pressure only formulation provided significant reductions in memory usage and computation time without increasing error. Michler et al. (2013) also demonstrated how an explicit network model of large arteries can can be used to provide source terms for a Darcy flow model.

2.3.4.1 Determining Continuum Flow Parameters

Flow through a poroelastic material is described at a continuum level and controlled by the material’s permeability tensor, which is defined by the solid skeleton structure and fluid viscosity. The permeability tensor for a vascularised tissue can be calculated from a geometric network of vessel segments. If a repeating vessel arrangement is assumed then mathematical homogenisation techniques can be used to determine permeability (Chapman et al., 2008). However, determining permeability from measured vessel morphometry data requires alternative approaches.
Huyghe et al. (1989) presented a formal averaging procedure for calculating permeability from a vascular network. This method was validated by comparison to network flow solutions computed from a rigid network of vessels. Vankan, Huyghe, Janssen, Huson, et al. (1997) conducted a similar analysis using the finite element method and a computer generated vascular network, and also investigated approaches for quantifying the hierarchical level of vessel segments for use in a four-dimensional extended Darcy formulation. Huyghe and Campen (1995b) extended the averaging approach of Huyghe et al. (1989) to poroelastic models where fluid flows through deforming tissue.

Recently, Hyde et al. (2013) evaluated various methods for calculating permeability fields from vascular network models. The method of Huyghe and Campen (1995b) was compared to an isotropic permeability scaled by porosity and a new projected principal component analysis method. A three compartment Darcy formulation, based on the multi-compartment method of Michler et al. (2013), was used to model a computer generated vascular network. Confocal imaging data of rat myocardium was also used to generate a single compartment model. Averaged solutions from the network flow models were compared to the Darcy solutions. None of the investigated methods provided an accurate estimate of the permeability tensor unless a scaling factor was estimated in a post-processing step. After application of the scaling factor, the method developed by Huyghe and Campen (1995b) was found to be the most accurate of the three methods investigated, and could reproduce the pressure and flow behaviour of the network flow model with a reasonable level of error. This study also presented a method for determining parameters describing flow between fluid compartments, and investigated the effect of varying the radius over which averaging was performed.

2.3.4.2 Relations Between Permeability and Strain

As studies of flow within myocardium have shown, deformation of tissue can affect flow through that tissue (Yamamoto et al., 1999). This effect can be incorporated in poroelastic models by defining the permeability tensor to be dependent on deformation. A number of approaches have been used to specify the dependence of permeability on strain. Vankan et al. (1998) simply weighted permeability by the square of fluid volume content divided by initial fluid volume, based on the assumptions that vessels remain circular and volume changes are proportional to the change in squared vessel diameter. This approach neglects the different effect on permeability of strains in different directions, assuming that permeability remains isotropic. The
effect of deformation on flow through cartilage was modelled with poroelasticity by Holmes and Mow (1990), with permeability defined by an exponential function of the volume ratio. This was able to accurately reproduce experimental data from compression of bovine cartilage, but also assumed permeability remained isotropic.

Kubik and Sawczuk (1983) developed a theory of anisotropic consolidation, based on linear poroelasticity. The permeability tensor was formulated as a function of the initial permeability and the strain tensor, and an initially isotropic porous skeleton was shown to become anisotropic under deformation. Markert (2007) developed a poroelastic formulation for materials undergoing large deformations, applying this model to polyurethane foam. The influence of deformation on strain was determined using an idealised model of orthogonal tubes, and modelled by factoring the permeability tensor into permeability in the reference state and a tensor function of the deformation. The foam used for experimental validation of this model had an isotropic pore structure so further validation is required for application to anisotropic materials.

2.3.4.3 Influence of Fluid on Tissue Mechanics

The effect of fluid on a material’s mechanical behaviour with a poroelastic model has not been well investigated. Cookson et al. (2012) used a poroelastic model with an isotropic exponential strain-energy function for the solid component to simulate the passive inflation of the left ventricle in perfused and unperfused states. Ventricular stiffness was observed to increase in the perfused state. However, the strain caused by a perfusion pressure increase was primarily in the ventricular longitudinal direction, rather than the radial direction as observed experimentally by May-Newman et al. (1994).

Bogen (1987) considered the mechanics of poroelastic tissues modelled with a power-law-based constitutive relation. It was found that stiffness increased as the material swelled if the power-law exponent was large. However, for exponents less than three, the model showed a decrease in stiffness.

2.4 Conclusion

There is complex, two-way mechanical interaction between tissue and fluids. While the effect of tissue mechanics on vascular flow has been extensively studied, particularly in myocardium, the influence of the vasculature on tissue mechanics has
been less well explored. Poroelastic models have the potential to incorporate this behaviour but more experimental data is required to determine appropriate constitutive relations and validate these models.

The most detailed experimental study investigating the effect of blood pressure on tissue mechanics is that of May-Newman et al. (1994), where three dimensional strains were calculated in dog myocardium, and swelling and stiffening were found to occur transverse to the muscle fibre direction. The complex geometry of the heart makes interpretation of this data difficult, and experimental studies on simpler structures such as skeletal muscle may provide further insight into the mechanics of vascularised tissue. Chapter 8 therefore presents results from experiments studying the influence of perfusion pressure on the mechanics of the rat tibialis anterior muscle. Finite element models using explicit microstructural geometry can also be used to improve understanding of how tissue properties and vascular structure both contribute to the effect of vascular fluid on tissue mechanics, and these are investigated in Chapter 4.

May-Newman and McCulloch (1998) developed a homogenisation-based model of myocardium that could reproduce its transverse stiffening behaviour, by assuming that blood pressure within vessels causes transverse swelling that is controlled by a constant compliance term. However, this model only considers the fluid pressure to be a static field, and does not incorporate the influence of surrounding tissue mechanics on the deformation due to perfusion.

In contrast, poroelasticity can model the dynamic two-way interaction between fluid and solid mechanics, and has proven to be useful for modelling the influence of solid mechanics on vascular flow within the whole heart. Importantly, different levels of the vascular hierarchy can be modelled using a multiple-compartment method. Poroelastic models can also be coupled to explicit geometry models of larger vessels to provide more detailed flow information. However, the ability of poroelasticity to model pressure-driven stiffening has not been thoroughly investigated. The effect of constitutive behaviour on the response of a poroelastic material to perfusion pressure is therefore investigated in Chapter 5. Current poroelastic models also fail to reproduce the anisotropic swelling and stiffening of myocardium. Novel poroelastic models for anisotropic poroelasticity are presented in Chapter 6 to address this. The ability of an anisotropic poroelastic model to reproduce the mechanics of the perfused dog heart is then investigated in Chapter 7.
A VASCULARISED GEL PHANTOM

3.1 INTRODUCTION

Determining the response of perfused tissue to changes in blood pressure requires experimental investigation. However, using tissue experiments to derive insight into the relationship between microstructure and constitutive behaviour is complicated. The complex heterogeneous and anisotropic microstructure of tissues, as well as their complex geometry, complicates the interpretation of experimental results.

An alternative approach to better understanding the mechanics of biological tissue is to perform experiments on physical models, or phantoms. Phantoms are constructed such that they reproduce specific properties of tissue that are of interest. Compared to actual biological tissues, the structure of a phantom can be much simpler, to aid in interpreting results and simplify the development of mathematical models. Performing experiments on a phantom is more straightforward than on live tissue, and results are generally more repeatable.

Tissue phantoms are commonly used for evaluating medical imaging devices, such as magnetic resonance imaging scanners and computed tomography scanners. They have also been applied to soft tissue mechanics problems. Babarenda Gamage et al. (2011) used silicone gel phantoms to investigate how the different mechanical properties of components within a heterogeneous tissue can be identified. Chung et al. (2008) used a silicone gel phantom to validate the use of a finite element model of breast deformation for tumour tracking.

The aim of this chapter was to develop a phantom representing vascularised tissue, which would reproduce the swelling and stiffening exhibited by myocardium. By reproducing pressure-driven stiffening behaviour, this phantom would help to explain the mechanisms of pressure-driven stiffening in tissues. The microstructural features that lead to this behaviour could then be linked to features of constitutive
relations used to describe vascularised tissue in computational models. This phantom should also provide experimental data for validation of computational models of coupled solid and fluid mechanics.

In addition, creating a phantom that exhibits increasing stiffness with perfusion pressure may have useful engineering applications, enabling the development of pressure-driven actuators with dynamic stiffness.

It is believed that strain-stiffening behaviour is required for a material to exhibit pressure-driven stiffening (McCulloch et al., 1992). An increase in stiffness causes stretching of strain-stiffening elements, moving a material into a steeper region of its stress–strain curve. Therefore, a phantom that aims to produce this behaviour should incorporate a strain-stiffening material. Additionally, myocardium has been shown to swell and stiffen in directions transverse to the muscle fibre direction, which is believed to result from the alignment of blood vessels parallel to the muscle fibre direction (May-Newman et al., 1994). A phantom model in which the effect of anisotropic vascular structure could be investigated would therefore be useful for studying the anisotropic swelling and stiffening behaviour of myocardium.

3.2 Methods

3.2.1 Phantom Construction

Appendix A describes alternative approaches for constructing porous phantoms that were investigated before arriving at the method described in this chapter.

Porous phantoms were constructed using Sylgard® 527 silicone gel from Dow Corning, USA. This is a two-part gel where the ratio of parts can be varied to control the stiffness of the resulting gel. A mixture of 1 part A to 1.5 part B by mass was used for all phantom models, where part B is the linking agent.

The silicone gel was moulded into a 45 mm × 27 mm × 60 mm rectangular cuboid. The mould was constructed from 10 mm thick acrylic (polymethyl methacrylate). The acrylic end-plates at the top and bottom of the mould had a layer of thick paper glued to one side with epoxy. This paper faced inward towards the silicone gel, providing a surface for the gel to adhere to. This configuration meant that one end-plate could be used to attach the gel to a base for controlling its internal fluid pressure, while the other end could be sealed.

Aligned vessels were incorporated in the gel by including an array of silicone tubes in the mould. Each tube had an inner diameter of 1.5 mm and an outer diameter of
2 mm. These tubes were threaded through the two acrylic end-plates. Six rows of nine tubes were used, to provide a vascular volume fraction, or porosity, of 0.079. This is of a similar order of magnitude to myocardium, which has a vascular volume fraction of approximately 0.125 (Kassab et al., 1993).

The tensile stiffness of the silicone used in the tubes was 394 kPa, and the tensile stiffness of the Sylgard® 527 silicone at a ratio of 1 to 1.5 was 14.7 kPa. An alternative approach for phantom construction was investigated using a stiffer silicone gel that matched the stiffness of the silicone tubes. However, these gels were found to be too stiff to deform significantly without cracking when fluid pressure was applied. Phantoms were also created by moulding gel around removable rods rather than including tubes, as described in Appendix A, but these could not contain high fluid pressures without leaking. The difference in material properties between the silicone tubing and surrounding silicone gel makes modelling the mechanics of the phantoms more complicated but better mimics the tissue microstructure, where blood vessel walls can have different constitutive properties to the surrounding tissue.

Two different types of phantom were constructed. In one phantom, wool yarn was included to act as a strain-stiffening element. The yarn represents fibrous structures in tissue such as collagen and elastin. This embedded-wool phantom was compared to a silicone-only phantom without any embedded fibres. In the embedded-wool phantom, lengths of yarn were aligned in parallel with the silicone tubes and positioned evenly between them. This design mimics the arrangement of blood vessels and fibres in skeletal muscle and myocardium, where blood vessels are predominantly aligned parallel to the muscle fibre direction (Poole and Mathieu-Costello, 1990). An array of seven by ten lengths of yarn was used. The yarn was threaded loosely through the end-plates of the mould so that it was slack in the reference state of the gel phantom. The nonlinear stress-strain response of a single strand of this wool yarn under extension is demonstrated in Figure 3.1. Significant hysteresis can be observed in the response.

3.2.2 Pressure Control

After the silicone gel was cured, it was removed from the mould and mounted in a specially constructed aluminium base, which is shown in Figure 3.2. Detailed drawings of this base are provided in Appendix B. Fluid was pumped into the base so that it filled the silicone tubes within the gel. The top acrylic plate of the phantom was then dried and sealed with 5-minute Araldite® epoxy resin. Figure 3.3 shows
Figure 3.1. Load against extension ratio for a single length of wool yarn, which was used as a strain-stiffening element in silicone gel phantoms. The load-extension response is highly nonlinear and also exhibits significant hysteresis.

Figure 3.2. Aluminium base used for perfusing the gel phantom. The acrylic base plate of the gel is clamped into the rectangular indentation and sits on a rubber O-ring, which is visible in black.
3.2 Methods

3.2.1 Sealed gel end-plate
- Silicone gel
- Clamping plate
- Open end-plate
- Rubber O-ring
- Fluid inlet
- Fluid outlet

Figure 3.3. Porous silicone gel phantom mounted in the aluminium base for perfusion experiments. The top end-plate has been sealed with epoxy while the bottom end-plate remains open to the fluid source and is clamped to the base.

A diagram of the silicone gel phantom mounted in the base, and Figure 3.4 shows a photograph of the silicone gel phantom with embedded wool fibres.

During experiments, the fluid pressure was controlled by using a compressed air source with a precision pressure regulator (IR1000-01, SMC) to pressurise a fluid reservoir. Pressure was measured at the inlet to the aluminium base using a pressure transducer (24PCFFA6G, Honeywell).

3.2.3 Mechanical Tests

Mechanical tests were performed on the perfused phantom models using the testing device presented in Section 8.2.2. Although this device was developed specifically for testing small skeletal muscles, it is also useful as a general mechanical testing instrument. Briefly, the testing device consists of a voice coil motor driving a shaft that is mounted by two flexure bearings. A potentiometer is used to measure the shaft displacement and a load cell at the end of the shaft measures force. The device is operated using open-loop voltage control, rather than controlling on force or displacement.

Axial extension, axial compression, and transverse indentation experiments were performed on both the silicone-only phantom and the silicone phantom with embedded wool yarn. For axial extension experiments, the top acrylic plate of the phantom was clamped and pulled away from the base. For axial compression
experiments, a probe attached to the load cell of the testing device was brought down onto the top acrylic plate surface, then pushed downward. In the transverse indentation experiments, a 10 mm × 10 mm square indenter was used to indent the surface of the gel in the centre of the 60 mm × 45 mm face.

For each experiment, the gel perfusion pressure was varied from 0 kPa to 50 kPa in 10 kPa increments. Twenty load cycles were repeated at each pressure to ensure that the response had equilibrated and to provide additional data for analysis. Data from the first ten load cycles was discarded and only the last ten cycles at each loading set were analysed. It should be noted that a fluid pressure of 50 kPa is much greater than the blood pressure experienced by most tissues, which is typically less than 16 kPa. This higher pressure was required to produce significant deformation of the gel phantoms. Pressures greater than 50 kPa were tested and found to cause leaks through fluid splitting the silicone gel.

After tests were performed in ascending pressures, they were repeated, descending in pressure to 0 kPa, in 10 kPa decrements. This cycle of increasing and decreasing fluid pressures was repeated four times.

For each load cycle, the tangent stiffness at 0.5 N and the zero-stress intercept were measured. Tangent stiffness at 0.5 N was measured by fitting a line to the load-displacement curve between 0.3 N and 0.7 N using a least-squares approach.

For transverse indentation and axial compression experiments, the zero-stress intercept was calculated by fitting a line to the load-displacement curve between 0.05 N
and 0.2 N, and extrapolating this line to 0 N. For axial extension experiments, the zero-stress intercept was calculated by fitting a line to the load-displacement curve between −0.2 N and 0.2 N. Changes in the zero-stress intercept provide an indirect measurement of the relative surface position of the gel, which changes as the gel swells with increasing fluid pressure.

3.3 RESULTS

3.3.1 Axial Extension

Figure 3.5 plots the measured zero-stress position for all sets of axial extension load cycles, relative to the surface position for the first set of load cycles at zero fluid pressure. These results show that for both the silicone-only and embedded-wool...
phantoms, the zero-stress position at a given pressure depends not only on the fluid pressure but also on the previous fluid pressures; the mechanical response of the phantoms exhibits hysteresis with respect to the internal fluid pressure. The application of fluid pressure causes some change to the phantom structure that is not reversed within the time between load sets. This structure change may be a shift between the silicone gel and the silicone tubing or wool fibres, indicating that the deformation of the silicone tubes and wool fibres may not be perfectly coupled to the deformation of the surrounding silicone gel.

Figure 3.6 plots tensile load against displacement for axial extension experiments, and compares the response of the silicone phantom with and without embedded wool. Compressive loads were also measured in the initial portion of each load cycle. The shift to the right with increasing fluid pressure demonstrates swelling of the gel in the axial direction. The tensile stiffness of the silicone gel phantom with embedded wool is much greater than the silicone-only phantom. The wool phantom has a tangent stiffness at 0.5 N of 2.85 MPa, compared to 0.0934 MPa for the silicone-only phantom. The high stiffness of the embedded-wool phantom means that the displacement range measured is small, resulting in a noisy displacement signal. There is also a small amount of nonlinearity in the load-displacement curve due to error in the displacement transducer signal, which had a linearity rating of ±0.07 mm.

Both phantoms show linear load-displacement behaviour. The linear behaviour of the silicone phantom was expected. However, it was expected that the phantom with embedded wool would show strain-stiffening behaviour, due to the strain-stiffening response of the wool yarn. Although the wool yarn was slack in the mould, the combined silicone and wool phantom is very stiff about the zero load point. This may be due to the wool fibres being tightly coupled to the surrounding silicone gel, such that even small deformations about the zero-stress state require stretching of wool fibres.

Figure 3.7 plots the measured zero-stress surface positions and the change in tangent stiffness at 0.5 N. The zero-stress positions correspond to the zero load intercepts in Figure 3.6. These results were taken from the final series of loading sets where fluid pressure was increasing. The silicone-only phantom shows a very nonlinear response of surface displacement to fluid pressure, with surface displacement increasing more rapidly at higher pressure. The maximum displacement of 0.345 mm at 50 kPa fluid pressure corresponds to an axial Cauchy strain of $5.7 \times 10^{-3}$. The embedded-wool phantom showed much lower surface displacements, with a maximum axial Cauchy strain of only $0.55 \times 10^{-3}$. 
Figure 3.6. Load against displacement for axial extension at a range of fluid pressures of (a) the silicone-only phantom and (b) the phantom with embedded wool yarn. Load-displacement curves are the final loading in each load set, in the final set of load cycles with increasing pressure. Negative loads indicate compression of the gel. The shift to the right of the intercept at zero load indicates swelling of the phantom as fluid pressure increases. Note that both the load and displacement scales are different between the two plots.

Figure 3.7. (a) Calculated surface displacement and (b) relative tangent stiffness at 0.5 N against fluid pressure for the silicone-only phantom and the phantom with embedded wool, under axial extension. Tangent stiffnesses have been normalised with respect to the tangent stiffness at zero pressure to facilitate comparison of the relative level of stiffness changes between the two phantoms. Plotted values are the mean from ten load cycles, and error bars indicate the standard deviation.
The silicone-only phantom shows a small increase in tangent stiffness at 0.5 N of 16% between 0 kPa and 50 kPa fluid pressure. The tangent stiffness at 0.5 N for the embedded-wool phantom appears to decrease slightly for pressures up to 30 kPa, then increase for pressures above 40 kPa. However, there is a large standard deviation in the tangent stiffness calculated for the embedded-wool phantom, due to the noisiness of the displacement signal and the small change in displacement, so the actual change in stiffness with fluid pressure is unclear. A significant increase in stiffness with fluid pressure was expected for the embedded-wool phantom, due to the strain-stiffening response of the wool fibres, but this behaviour was not observed.

3.3.2 Axial Compression

Figure 3.8 plots load against displacement at pressures from 0 kPa to 50 kPa for both the silicone-only and embedded-wool phantoms. The shift to the left with increasing fluid pressure demonstrates swelling of the gel in the axial direction. Because fibres buckle under compression and do not contribute compressive stiffness to most tissues, it could be expected that the embedded-wool phantom would be much more compliant in compression, compared to extension in the axial direction. However, the axial compression response is very similar to axial extension for both phantoms. The tangent stiffness at 0.5 N compressive load for the embedded-wool phantom was 1.93 MPa at 0 kPa fluid pressure, compared to 2.85 MPa for axial extension at a tensile load of 0.5 N. This compressive stiffness is much greater than that of the silicone-only phantom, which was 0.0586 MPa at 0 kPa fluid pressure, indicating that the presence of wool yarn is causing a significant increase in the axial compressive stiffness as well as the tensile stiffness of the phantom.

Small negative spikes in the load-displacement loops can be seen on the returning portion of the loop for the silicone-only phantom, which were due to sticking of the indenter probe to the epoxy on the top plate of the phantom. This did not affect data analysis, which was only focussed on the increasing load portion of the load-displacement loop.

Figure 3.9 plots the measured zero-stress surface positions and the relative tangent stiffness at 0.5 N, for the axial compression experiments. The results are very similar to those calculated from the axial extension experiments. The silicone-only phantom shows nonlinear axial swelling with fluid pressure; the surface displacement increases more rapidly at higher pressures. There is also a small amount of stiffening as pressure increases; the tangent stiffness at 0.5 N and a fluid pressure of 50 kPa is
Figure 3.8. Load against displacement for axial compression at a range of fluid pressures of (a) the silicone-only phantom and (b) the phantom with embedded wool yarn. Load-displacement curves are the final loading in each load set, in the final set of load cycles with increasing pressure. The shift to the left of the intercept at zero load indicates swelling of the phantom as fluid pressure increases. Note that the displacement scales are different between the two plots.

Figure 3.9. (a) Calculated surface displacement and (b) relative tangent stiffness at 0.5 N against fluid pressure for the silicone-only phantom and the phantom with embedded wool, under axial compression. Tangent stiffnesses have been normalised with respect to the tangent stiffness at zero pressure to facilitate comparison of the relative level of stiffness changes between the two phantoms. Plotted values are the mean from ten load cycles, and error bars indicate the standard deviation.
18% greater than the stiffness at zero fluid pressure. The embedded-wool phantom shows a small, linear increase in the top surface position with increasing fluid pressure. The tangent stiffness at 0.5 N decreases relative to the zero fluid pressure stiffness for fluid pressures from 10 kPa to 40 kPa, but the stiffness at 50 kPa fluid pressure is slightly greater than at 0 kPa. Again, the standard deviations of the calculated stiffnesses for the embedded-wool phantom are large due to the low signal-to-noise ratio in the displacement data.

3.3.3 Transverse Indentation

Figure 3.10 plots load against displacement for transverse indentation experiments of the silicone-only and embedded-wool phantoms. For these plots, swelling in the transverse direction causes a shift to the left of the load-displacement curves. The embedded-wool phantom is stiffer than the silicone-only phantom, but the stiffness difference is not as great as for axial compression or extension. The tangent stiffness at 0.5 N with 0 kPa fluid pressure was 44.0 kPa for the embedded-wool phantom, compared to 26.6 kPa for the silicone-only phantom. The load-displacement curves are again very linear and hysteresis is evident for both phantoms.

Figure 3.11 plots the zero-stress surface displacement and relative tangent stiffness at 0.5 N for transverse indentation of both phantoms. The surface displacement with increasing fluid pressure is very similar for the two phantoms; displacements are only slightly greater for the more compliant silicone-only phantom. The increases in surface displacement at 50 kPa fluid pressure represent transverse Cauchy strains of $28 \times 10^{-3}$ for the silicone-only phantom and $27 \times 10^{-3}$ for the embedded-wool phantom.

Both phantoms shown an increase in tangent stiffness at 0.5 N as fluid pressure increases. The change in stiffness is similar up to a pressure of 20 kPa. For fluid pressures from 30 kPa to 50 kPa, stiffness continues to increase for the embedded-wool phantom, but the increase in stiffness is less for the silicone-only phantom.

3.4 Discussion

Physical phantoms representing vascularised tissue were created using silicone gel. A phantom built from silicone gel with embedded silicone tubes was compared to a phantom that also contained embedded wool yarn. The aim of including wool yarn was to reproduce pressure-driven stiffening behaviour, which has been demon-
Figure 3.10. Load against displacement for transverse indentation at a range of fluid pressures of (a) the silicone-only phantom and (b) the phantom with embedded wool yarn. Load-displacement curves are the final loading in each load set, in the final set of load cycles with increasing pressure.

Figure 3.11. (a) Calculated surface displacement and (b) relative tangent stiffness at 0.5 N against fluid pressure for the silicone-only phantom and the phantom with embedded wool, under transverse indentation. Tangent stiffnesses have been normalised with respect to the tangent stiffness at zero pressure to facilitate comparison of the relative level of stiffness changes between the two phantoms. Plotted values are the mean from ten load cycles, and error bars indicate the standard deviation.
strated in some vascularised tissues. When fluid pressure within the phantom increased, it was expected that this would cause swelling that would stretch the wool yarn, pushing it further up its stress–strain curve into a stiffer region, increasing the tangent stiffness of the phantom. Because the wool yarn was oriented in the axial direction of the phantom, it was expected that this pressure-driven stiffening would be most evident in axial extension experiments. For axial extension stiffness to increase with fluid pressure, a fluid pressure increase would have to cause axial swelling of the phantom, which would lengthen the fibres. The fibres would have to contribute little tensile stiffness in the zero-pressure state of the phantom, and their stiffness contribution would increase as they lengthened.

This behaviour was not produced in the embedded-wool phantom. Instead, the axial stiffness at zero fluid pressure was large, preventing significant axial stretch with fluid pressure increases. The compressive axial stiffness was also similar to the tensile stiffness, which was not expected, as the wool fibres should only contribute tensile stiffness, but buckle under compression. This response is likely to be due to the use of wool yarn, rather than individual fibres, combined with the yarn being penetrated by the surrounding silicone gel and tightly bonded to it. Because yarn consists of wound fibres, the fibres are not perfectly aligned along a single axis. This means that although the yarn was initially slack before the silicone gel was moulded around it, any deformation about the reference state of the phantom still requires individual wool fibres to stretch.

Although the embedded-wool phantom did not exhibit any pressure driven stiffening in the axial direction, the silicone-only phantom showed some stiffening for both axial compression and axial extension. This result was not expected, as silicone has a linear stress-strain response, and Jor (2005) found no change in stiffness when using a neo-Hookean constitutive relation to describe a representative volume element model of tissue surrounding a single vessel. The phantom actually shows strain-softening behaviour under axial extension; the tangent stiffness of the silicone-only phantom at a tensile load of 0.5 N was 1.89 kN m$^{-1}$, compared to 1.73 kN m$^{-1}$ at a load of 3 N. Therefore, the increased stiffness of the silicone-only phantom as fluid pressure increases is likely to be due to the externally applied load acting against the fluid pressure directly, rather than the internal pressure affecting the mechanics of the surrounding silicone gel. Axial extension of the gel requires transverse compression, due to the gel’s incompressibility, which acts to radially compress the silicone tubes in the phantom against their internal fluid pressure.

Indentation is a more complex mode of deformation than axial extension or compression. Due to the presence of rigid end-plates in the phantom, homogeneous
deformations cannot be applied, and transverse compression cannot be applied to the entire surface of the phantom. Therefore, indentation experiments were performed to evaluate the effect of fluid pressure on the transverse mechanics of the phantoms. Indentation involves compression of the gel directly under the indenter probe, resulting in lengthening in directions orthogonal to the indentation direction. High shear strains are also present around the edges of the probe. The length scales involved in the indentation deformation were of a similar magnitude to the scale of the wool and tube structure within the phantoms, so it may not be appropriate to treat the phantom as a continuum when interpreting these experiments. The embedded-wool phantom was constructed with wool yarn closest to the surface of the gel, and silicone tubes lying underneath the layer of wool. This arrangement means the wool yarn has a greater effect on the response of the phantom to indentation, compared to an arrangement with yarn further below the gel surface.

In contrast to the axial experiments, both the silicone-only and embedded-wool phantoms showed similar levels of swelling in the transverse direction. The axial orientation of the fibres meant that they did not significantly hinder transverse swelling. When performing indentation experiments on the embedded-wool phantom, it was anticipated that the compression underneath the indenter probe would result in lengthening of the wool fibres. Increasing fluid pressure would stretch these fibres, increasing their stiffness and increasing the phantom’s resistance to indentation. The indentation results showed increases in stiffness with fluid pressure for both the embedded-wool and silicone-only phantoms, and for this deformation mode, the embedded-wool phantom showed greater relative stiffening than the silicone-only phantom.

Because of the anisotropic structure of the embedded-wool phantom, it is likely that compression of the gel under the indenter would result in greater lengthening in the direction orthogonal to both the indentation direction and the fibre direction, compared to lengthening in the fibre direction. Greater levels of pressure-driven stiffening may be achievable by orienting fibres in two orthogonal directions, both orthogonal to the indentation direction, such that the phantom matrix is transversely isotropic with respect to the indentation direction.
3.4.1 Phantom Limitations

The major shortcoming of these phantom models is that the embedded-wool phantom did not produce strain-stiffening behaviour. In a tissue such as skeletal muscle, fibres are bound to each other, and to other tissue structures including vessels, by collagen struts. These components exist within a fluid-filled extracellular space that allows relatively free movement. In comparison, the embedded-wool phantom contains fibres that are tightly bound to the surrounding silicone gel. This resulted in a material that exhibited high stiffness in the reference state, for both tension and compression experiments.

The transverse strains caused by increasing pressure to 50 kPa were approximately 0.03 for both phantoms. This is lower than the transverse strains measured by May-Newman et al. (1994) in myocardium at a perfusion pressure of 15 kPa, which varied from 0.05 to 0.1 in the ventricular radial direction. The low bulk compliance of the phantoms is likely to be mainly due to the high stiffness of the silicone tubing and embedded wool, but is also contributed to by the constraints introduced by the rigid end-plates. Increasing the vessel volume fraction of the phantoms could be used to increase the bulk compliance.

An alternative approach for constructing anisotropic phantoms was developed by Qin et al. (2013), for investigating the use of magnetic resonance elastography to measure tissue anisotropy. Elastic fibres with a diameter of 36 µm were embedded in polyvinyl alcohol hydrogels. Individually, these fibres have linear mechanical behaviour. However, an array of many linear fibres can lead to strain-stiffening behaviour. If the fibres are slack in the reference state, they will straighten and begin to contribute stiffness to the material as it deforms. The gradual recruitment of many linear fibres leads to a strain-stiffening response. Applying this method to the production of porous phantoms could result in a material with more nonlinear, strain-stiffening behaviour, better reproducing the behaviour of strain-stiffening tissues. This should lead to greater increases in stiffness with perfusion pressure, so future work on developing vascularised phantoms should investigate this approach.

The single fibre orientation in the embedded-wool phantom was also a limitation of this approach to phantom construction. Most strain-stiffening tissues exhibit strain-stiffening in all directions, but may have a preferred, stiffest direction. May-Newman et al. (1994) showed that myocardium stiffens in directions transverse to the fibre direction when perfused, and suggested that stiffening of transversely oriented fibres leads to this behaviour. The possibility of developing an isotropically strain-stiffening vascularised phantom was investigated using polyvinyl alcohol
hydrogel, as discussed in Appendix A. However, these phantoms were not found to exhibit enough strain-stiffening behaviour to produce pressure-driven stiffening. A possible alternative approach would be to develop a phantom that contains three orthogonal sets of fibres.

A further limitation observed with this method for producing vascularised phantoms was that the deformation exhibited hysteresis with respect to fluid pressure. This was observed for both the silicone-only phantom and the embedded-wool phantom. An advantage of most phantom models is that they provide more repeatable, reliable results than the more complex systems they represent. For the purpose of understanding the static mechanical interaction between fluid pressure and surrounding materials, this hysteresis adds unwanted additional complexity to the phantom’s mechanical behaviour.

Rigid end-plates were used during construction of the gels to align the silicone tubing and wool yarn, and to perfuse the gels through the base plate during mechanical tests. However, these end-plates constrained the gel deformation and meant that homogeneous modes of deformation could not be applied. Improved designs for gel phantoms that removed the requirement for these end-plates would enable a wider range of mechanical testing. Performing full transverse compression experiments on vascularised phantoms may provide better information about the effect of fluid pressure on the transverse mechanics of materials with aligned vessels. The ability to perform both transverse compression and extension would also be advantageous, and this may require using an adhesive to attach a probe to the gel surface.

3.5 Conclusion

An attempt at developing a vascularised gel phantom for investigating the effect of internal fluid pressure on a material’s mechanical behaviour was presented. Previously, mechanical experiments have been performed on isotropic porous materials such as foam (Markert, 2008), but as far as the author is aware, no attempts have been made to produce strain-stiffening porous phantoms, or phantoms with anisotropic pore structure.

A number of limitations were found with the investigated approach for phantom construction. The strain-stiffening behaviour exhibited by fibrous tissues was not replicated, meaning that the level of stiffening with increased fluid pressure was small. Only transverse indentation experiments exhibited greater stiffening with the embedded-wool phantom compared to the phantom without any strain-stiffening
elements. This result demonstrates that the initial low-stiffness region in the stress-strain response of tissues is critical for producing a pressure-driven stiffening response.

The phantoms also exhibited hysteresis with respect to the internal fluid pressure, and the rigid end-plates required for controlling the fluid pressure within the phantoms limited the mechanical experiments that could be performed.

Further investigation into approaches for developing strain-stiffening, vascularised phantoms will be important for better understanding the interaction between internal fluid pressure and the mechanics of a material, as well as providing reliable data for validation of mathematical models representing fluid-filled tissues.

Due to the limitations of these vascularised gel phantoms, alternative approaches for studying the mechanics of fluid interaction in tissues were investigated. Chapter 4 presents a representative volume element model of tissue microstructure, and Chapter 8 presents a study of the influence of blood perfusion pressure on the mechanics of the rat tibialis anterior muscle.
4.1 INTRODUCTION

In order to better understand how perfusion pressure influences tissue mechanics, it is helpful to construct a model of tissue at the microstructural level where the solid and fluid components may be modelled discretely using the finite element method. This approach does not scale to modelling whole organs, but does provide insight into how to incorporate perfusion pressure dependent effects in continuum level models.

Bilston (2002) developed a two-dimensional finite element model of brain tissue surrounding a single circular vessel and investigated how this model responded to changes in vessel pressure. The Mooney–Rivlin constitutive relation (Mooney, 1940; Rivlin, 1947) was used to represent the solid tissue component, and the effect of a viscoelastic solid component was also investigated. Jor (2005) developed a similar model in three-dimensions and compared the behaviour of a neo-Hookean constitutive relation to an exponential isotropic relation. It was found that the strain-stiffening exponential relation exhibited an increase in stiffness with perfusion pressure, whereas the neo-Hookean relation did not.

These two models provide some insight into the behaviour of tissue when perfused. However, there is much scope for further investigation. Both models considered only tissue with perfectly aligned vessels and an isotropic constitutive relation for the solid component. Although capillaries are predominantly aligned with the muscle fibre direction in cardiac and skeletal muscle, larger vessels are not, and these contribute significantly to the fluid volume fraction of tissue (Kassab et al., 1993). Other tissue types such as brain and lung also show more isotropic vascular organisation (Weibel, 1984). Many biological tissues, including myocardium and skeletal muscle, also demonstrate anisotropic stress-strain behaviour. Therefore,
this study investigated how anisotropy in both blood vessel arrangement and the stress-strain constitutive relation of the solid component affect the mechanics of perfused tissue.

Myocardium was modelled in order to compare simulation results with the experimental results of May-Newman et al. (1994). May-Newman et al. compared the strain due to left ventricle inflation between perfused and unperfused myocardium, showing that myocardium stiffens when perfused, predominantly in directions transverse to the muscle fibre direction. By incorporating realistic parameters for myocardium in this microstructural model, the mechanisms behind the results of May-Newman et al. may be better understood. The level of vascular anisotropy was based on experimental measurements, and the Holzapfel–Ogden constitutive relation (2009) was used to describe the solid component mechanics with model parameters estimated from measurements performed on myocardium (Dokos et al., 2002). As both the solid component constitutive anisotropy and the anisotropy of the vessel structure may contribute to the anisotropic stiffening behaviour observed experimentally, both factors were simulated independently to determine how significantly each influences the behaviour of perfused myocardium. Variations in constitutive behaviour and vascular anisotropy were also considered to investigate how other tissue types may respond when perfused.

4.2 REPRESENTATIVE VOLUME ELEMENT GEOMETRY

To model vessels embedded in tissue, a single representative volume element (RVE) was considered. This represents a periodically repeating unit cell within a block of tissue. Stresses and strains at the whole organ level vary at a much larger length scale than the length of the RVE, such that the deformed geometry of the RVE can be assumed to repeat periodically within the tissue (Charalambakis, 2010). RVE models are the foundation for homogenisation techniques in which the continuous deformation of large scale structures is determined by solving the microstructural RVE problem at multiple locations within the whole problem domain (Rohan et al., 2006).

4.2.1 Model Geometry

The geometry of the RVE consists of a rectangular cuboid containing three orthogonal vessels of equal radius. The predominant vessel direction was set to align with the $x$ coordinate, and is referred to as the axial direction. The two transverse vessels
were aligned with the $y$ and $z$ coordinates. Varying the overall dimensions of the RVE varies the anisotropy of the vascular network represented by the repeated RVE model. For example, increasing the $x$ dimension of the RVE decreases the density of vessels oriented in the $y$ and $z$ directions, as the spacing between vessels oriented in these directions is increased. In the limiting case of vessels that are perfectly aligned in one direction, the RVE consists of a single vessel within a block of tissue. Figure 4.1 shows the RVE geometries for the totally anisotropic case with a single vessel, the isotropic case with equal dimensions, and an intermediate model with dimensions set to approximate the anisotropy of the myocardial vasculature, as described in Section 4.2.2.

Figure 4.1. Finite element meshes for one eighth of the representative volume element models for (a) the case of totally anisotropic geometry, (b) the case of isotropic geometry, and (c) an intermediate, anisotropic case representing myocardium. This one eighth geometry was used when modelling symmetric deformations. All models have a porosity of 0.125.
Lengths in the RVE were non-dimensionalised by normalising with respect to the transverse RVE dimension. The vessel wall was not modelled separately but lumped into the solid component, as Bilston (2002) and Vis et al. (1995) showed that vessel properties have a negligible effect on swelling in comparison to the properties of the surrounding tissue. If the effect of vasoconstriction were to be included then modelling the vessel wall as a separate component would be required.

OpenCMISS-Iron (Bradley et al., 2011) was used to solve the governing equations of finite deformation elasticity with the finite element method. The fluid within the vessel space was not modelled explicitly, but its influence on the solid was modelled using pressure boundary conditions on the vessel surfaces. Quadratic Lagrange interpolation was used to represent the reference and deformed geometric fields, while linear Lagrange interpolation was used for the hydrostatic pressure field that acts as a Lagrange multiplier to enforce incompressibility of the solid component.

4.2.2 Vascular Anisotropy

Poole and Mathieu-Costello (1990) showed that the orientation distribution of capillaries within myocardium may be modelled using a Fisher axial distribution, with the probability density function:

\[ f(\psi) = \frac{1}{n(K_c)} e^{K_c \cos(2\psi)} \] (4.1)

where \( \psi \) is the angle from the preferred capillary direction, \( n(K_c) \) is a normalising function, and \( K_c \) is a concentration parameter that defines how tightly concentrated the vessel orientation is around the preferred direction, and is analogous to the variance of a normal distribution. The length of vessels per unit volume, \( J_v \), can be calculated from the number of vessels transecting a surface according to the equation:

\[ J_v = c(K_c, \theta)Q_A \] (4.2)

where \( c(K_c, \theta) \) is a proportionality function dependent on \( K_c \) and the angle between the surface normal and preferred vessel direction (\( \theta \)), and \( Q_A \) is the density of vessels per unit area in the surface. For sections normal to the preferred vessel direction, \( \theta = 0 \). When the vessel orientation distribution is completely isotropic, \( K_c = 0 \) and \( c(K_c, 0) = 2 \). For a fully anisotropic structure with parallel vessels, \( K_c = \infty \) and \( c(K_c, 0) = 1 \) (Weibel, 1980).

May-Newman et al. (1995) used light microscopy analysis of glutaraldehyde-fixed canine hearts to estimate the volume fraction and anisotropy of capillaries. They
found capillary volume fractions in the range of 4% to 6% and determined a value of 1.13 for $c(K_c, 0)$. Based on a capillary volume fraction of 5%, and assuming that the capillaries make up 40% of the total intramyocardial vascular volume (Kassab et al., 1993; Spaan, 1985), the overall volume fraction of the RVE was set to 0.125.

The RVE model with three orthogonal vessels has a discontinuous vessel orientation distribution, with three orthogonal sets of vessels, so cannot be described by a Fisher axial distribution except in the totally anisotropic case. The RVE was assumed to be transversely isotropic, with vessels aligned preferentially in the axial direction ($x$), and equal vessel densities in the two transverse directions ($y$ and $z$). Anisotropy was controlled by the ratio of the transverse dimensions to the axial dimension, $r_d$, which varies from zero to one. Ratios greater than one represent structures with a preferred plane of orientations and were not considered. By normalising the RVE model dimensions with respect to the transverse dimensions, the non-dimensionalised surface density of vessels and vessel length density are given by:

$$Q_A = 1, \quad J_v = \frac{2 + \frac{1}{r_d}}{1 \times 1 \times r_d} = 2r_d + 1 \quad (4.3)$$

As both $c(K_c, 0)$ and $r_d$ linearly relate the vessel length density to the surface density, the $r_d$ value for the RVE geometry can be set to represent an experimentally calculated $K_c$ parameter according to:

$$r_d = c(K_c, 0) - 1 \quad (4.4)$$

Therefore, if only the myocardium capillaries were represented, an $r_d$ value of 0.13 would be used based on the results of May-Newman et al. (1995). However, the influence of larger vessels must also be accounted for. These were assumed to be arranged isotropically. Although Kassab et al. (1993) found that approximately 50% of terminating arterioles run parallel to capillary beds, these arterioles are very tortuous, giving them a more isotropic orientation distribution. There is no reported evidence for other vessels having a preferred orientation. Based on capillary volumes accounting for approximately 40% of vascular volume (Spaan, 1985), the dimension ratio for the RVE representing the combined vasculature was set according to a simple linear weighting:

$$r_d = 0.4r_{dc} + 0.6r_{di} = 0.652 \quad (4.5)$$

where $r_{dc} = 0.13$ is the dimension ratio for a capillary only model and $r_{di} = 1$ is the dimension ratio for an RVE model representing the isotropic vessels. The vessels
in the RVE represent the lumped contribution of all levels of the vascular hierarchy. Due to this, the varying pressure and vessel properties across different levels of the vasculature cannot be accounted for with this model. The RVE model representing myocardium, with \( r_d = 0.652 \), is illustrated in Figure 4.1 (c).

4.2.3 Mesh Convergence Analysis

Before performing simulations, a mesh convergence analysis was conducted to determine the most computationally efficient finite element mesh. Pressure boundary conditions of 15 kPa were applied to the vessel wall surfaces of the myocardial RVE model. As the RVE repeats within the whole tissue, and the external swelling deformation does not involve any rotation, the external surfaces of the RVE were constrained to remain in plane when deformed. One eighth of the full RVE was modelled to take advantage of the problem symmetry, and the internal surface planes at \( x = 0 \), \( y = 0 \) and \( z = 0 \) were constrained to remain in their respective planes. The constitutive relation developed by Holzapfel and Ogden (2009) was used with the parameters estimated for myocardium by Göktepe et al. (2011). The application of this constitutive relation to the RVE model is described in Section 4.3. The absolute errors in the displacement of the external RVE surfaces with respect to the most refined mesh are plotted in Figure 4.2. From this result, the mesh with 1179 degrees of freedom was selected for all further simulations.

4.3 Solid Component Constitutive Relation

LeGrice et al. (1995) used scanning electron microscopy to show that myocardium has a complex orthotropic structure, with bundles of muscle fibres arranged in sheets separated by cleavage planes (Figure 4.3). Capillaries lie within the muscle layers and on their surface. At any point in the myocardium, a local material coordinate system can be established with base vectors oriented in the fibre (F), sheet (S) and normal (N) directions. The fibre direction is aligned with the muscle fibre orientation, the sheet direction is orthogonal to the fibre direction and in the plane of the fibre layers, and the normal direction is orthogonal to the other two directions. Dokos et al. (2002) performed shearing experiments on excised blocks of pig myocardium from the left-ventricular wall, and showed that the orthotropic structure of myocardium leads to orthotropic constitutive behaviour. Cubes measuring 3 mm × 3 mm × 3 mm that were aligned with the fibre, sheet and normal coordinates were extracted from six animals, and six modes of deformation
were tested for each animal. These six shearing modes are defined by the direction perpendicular to the sheared face and the direction of shearing. For example, the FS mode denotes shearing in the sheet direction of the face perpendicular to the fibre direction. Shear displacement values were defined as a percentage of the sample dimension in the direction perpendicular to the sheared face, and shear displacements of up to 50% were applied.

The results showed that the fibre direction is much stiffer than both the sheet and normal directions, and the sheet direction is stiffer than the normal direction. No statistically significant differences in stiffness were found within the pairs of FS and FN, SF and SN, and NF and NS shear modes, when considering the data from all six animals. Individual samples did, however, show relatively large differences between the FS and FN, and SF and SN modes. Most animals did not show significantly different behaviour between the NF and NS deformation modes. This difference was inconsistent between animals, and for some samples, the positive and negative shearing directions exhibited different behaviour. This highlights that more comprehensive experimental data is still required in order to better understand the relation between myocardium’s microstructure and its mechanical behaviour.
Figure 4.3. Microstructure of myocardium showing the fibre (F), sheet (S) and normal (N) directions. Sheets of myocytes are separated by cleavage planes, with perimysial fibres forming connections between sheets.

Based on the results of Dokos et al. (2002), Holzapfel and Ogden (2009) developed a structurally based, orthotropic constitutive relation for myocardium. This constitutive relation can reproduce the different responses of the fibre, sheet and normal directions, as well as differences between the FS and FN, and SF and SN modes of deformation. The form of the strain energy density function is:

$$\Psi = \frac{a}{2b} \exp(b(I_1 - 3))$$

$$+ H(I_{4f} - 1) \frac{a_{f}}{2b_{f}} \left[ \exp \left( b_{f}(I_{4f} - 1)^2 \right) - 1 \right]$$

$$+ H(I_{4s} - 1) \frac{a_{s}}{2b_{s}} \left[ \exp \left( b_{s}(I_{4s} - 1)^2 \right) - 1 \right]$$

$$+ \frac{a_{fs}}{2b_{fs}} \left[ \exp \left( b_{fs}I^2_{8fs} \right) - 1 \right]$$

(4.6)

where $a, b, a_{f}, b_{f}, a_{s}, b_{s}, a_{fs}$ and $b_{fs}$ are material parameters that must be estimated experimentally. $H$ denotes the Heaviside step function, which is zero for negative inputs and one for inputs greater than or equal to zero. $I_1$ is the first invariant of $\mathbf{C}$, $I_{4f} = C_{ff}, I_{4s} = C_{ss}$, and $I_{8fs} = C_{fs}$, where $\mathbf{C}$ is referred to the microstructural fibre, sheet and normal coordinates. The first term based on $I_1$ represents strain energy due to deformation of the isotropic extracellular matrix. The terms in $I_{4f}$ and $I_{4s}$ represent strain energy due to stretching of fibres in the muscle fibre and sheet directions, respectively. The Heaviside step function is used so that fibres only contribute strain energy when they are under tension. Fibres buckle when compressed so have no compressive stiffness. The final term based on $I_{8fs}$ represents
strain energy due to pure shear deformation in the FS and SF modes, to reproduce responses where these deformation modes show a stiffer response than the FN and SN modes, respectively.

Holzapfel and Ogden (2009) estimated parameters for their constitutive relation using the data published for one individual animal, labelled SP20, from Dokos et al. (2002). Unfortunately, Holzapfel and Ogden assumed that the FS shearing mode is always stiffer than the FN mode. As SP20 demonstrated a stiffer response in the FN direction, it was incorrectly assumed that Figure 6 from Dokos et al. (2002) was mislabelled, and constitutive parameters were estimated based on this assumption. Göktepe et al. (2011) and Wang et al. (2013) have also used Dokos et al.’s shearing data to estimate parameters for the Holzapfel–Ogden constitutive relation (2009), and unfortunately both studies used the same incorrect interpretation of the data.

4.3.1 Methods

In order to accurately represent the orthotropic constitutive behaviour of myocardium in the current RVE model, the constitutive relation of Holzapfel and Ogden (2009) was selected, and the shearing data from Dokos et al. (2002) was used to estimate material parameters. This constitutive relation was developed to describe the mechanics of myocardium at the whole-heart scale, with the vasculature lumped into a continuum model including all other tissue components. In the RVE model the constitutive relation represents only the solid component and excludes the space occupied by blood in the vasculature. Therefore, it was not appropriate to use previously published constitutive parameters, and parameters were estimated specifically for the RVE model.

Material parameters for the RVE model were estimated using the experimental results from a single individual, SP17. This individual was selected because it showed stiffer responses in the FS and SF shearing modes than the FN and SN shearing modes, respectively, such that the Holzapfel–Ogden constitutive relation could be used without modifying the $I_{sts}$ term. Some features of the results from SP17 are not well explained by this constitutive relation; these samples exhibited a large difference in stress between the SF and SN deformations, with very little difference between the SN, NF and NS deformations. This could be due to variation in the sheet orientations across the sample, as changes in orientation of up to 30° were observed in some samples.

The fibre coordinate system was aligned with the global coordinate system of the RVE. The fibre direction was aligned with the preferred vessel orientation, which
corresponded to the \( x \) coordinate in Figure 4.1, and the sheet and normal directions were aligned with the \( y \) and \( z \) coordinates, respectively.

The repeated RVE model should reproduce the behaviour of myocardium at the macroscale. Assuming the experimental shearing procedure produces a homogeneous deformation, then the deformed boundary of the RVE must match the shearing deformation of the whole tissue sample. This assumption of homogeneous deformation introduces a small amount of error, as shear deformation generally includes a non-homogeneous bulging due to the Poynting effect (Poynting, 1909; Schmid et al., 2008).

The experimental results of Dokos et al. (2002) presented shear stress as a function of shear deformation percentage. For the RVE model to be representative of myocardium microstructure, the same shear deformation applied to the RVE should reproduce the same average stress over the sheared face as measured for the whole tissue sample. As the sheared face contains voids due to the presence of vessels, this average shear stress is the total shear force acting on the sheared face, divided by the total area of the face, which includes both the solid and vessel surface areas. As the experiments were carried out on excised tissue, the fluid pressure within the vessels was assumed to remain at zero throughout the deformation. For each shearing deformation mode, the surface opposite the sheared face was fixed in the reference position, and the sheared face was fixed at the appropriate displacement in the direction of shear to match the experimental data. For example, for a 50\% shear deformation in the NS mode, the surface perpendicular to the N direction was displaced in the S direction by 50\% of the RVE dimension in the N direction. The simulation setup is illustrated for the NS deformation mode in Figure 4.4.

It should be noted that while external faces were constrained to remain in plane for uniaxial and pressurisation simulations, these constraints would not allow the rotation necessary for shear deformation. Ensuring that the RVE model represents a repeating unit cell when applying shear deformation would require general linear constraints, which are not yet available in OpenCMISS-Iron. Therefore, there is some small error in the RVE shear results used for parameter estimation, due to the lack of these constraints.

The eight constitutive parameters of the Holzapfel–Ogden constitutive relation (2009) were estimated by minimising the root-mean-squared error between the experimental shear stress values and the simulated shear stresses. The L-BFGS-B optimisation routine (Zhu et al., 1997) was used through SciPy (Jones et al., 2001). The experimental stress values were decimated to give 21 to 24 total stress points.
Figure 4.4. Shearing deformation in the NS mode applied to the whole tissue sample (3 mm × 3 mm × 3 mm), and the shearing of a representative volume element within the tissue sample, which incorporates the microstructural vascular geometry.

for each deformation mode, to reduce the computational time required to run the parameter estimation procedure.

As well as the Holzapfel–Ogden constitutive relation, the RVE swelling behaviour with two isotropic constitutive relations was also considered. Parameters for these were also estimated from the experimental shearing data using the same method applied to the Holzapfel–Ogden relation. The first isotropic relation was the linear neo-Hookean relation, with strain energy density function:

$$\Psi = c_1 (I_1 - 3)$$

(4.7)

The second was an isotropic exponential constitutive relation, with strain energy density function:

$$\Psi = \frac{c_1}{2c_2} (e^{c_2 (I_1 - 3)} - 1)$$

(4.8)

These two constitutive relations were investigated to compare the behaviour of linear and strain-stiffening materials, to confirm that the RVE model shows pressure-driven stiffening with a strain-stiffening material but not with a linear constitutive relation, as observed by Jor (2005). Simulations were also performed with the isotropic exponential constitutive relation to investigate the effect of geometric anisotropy without the influence of constitutive anisotropy.
4.3.2 Results

Table 4.1 shows the estimated constitutive parameters for the myocardial RVE model, compared to parameters fitted to the same experimental data assuming a homogeneous shearing deformation and the parameters obtained by Göktepe et al. (2011), which were based on the experimental results from a different animal (SP20). Figure 4.5 compares simulated shear stress results from the RVE model with estimated parameters to the decimated experimental data. The behaviour of myocardium in all six shear deformation modes is well represented by the model.

First comparing the parameters for the homogeneous model (based on SP17 data) and the parameters estimated by Göktepe et al. (2011) (based on SP20 data), it can be seen that the parameters are mostly of the same order of magnitude. The fibre direction for SP17 is less stiff, and in the sheet direction, \( a_s \) is estimated to be much lower but \( b_s \) is greater, indicating lower initial stiffness with some stiffening at high strain. This is a consequence of the SN deformation mode exhibiting a similar response to the NF and NS deformation modes. Comparing the sets of parameters fitted to the homogeneous model and the RVE model (both using SP17 data), it can be seen that the estimated parameters for the RVE are in general larger, which was expected, as there is less solid material so the tissue stiffness must be greater to match the behaviour of the homogeneous case.

It is also interesting to note that the simulation results in Figure 4.5 show a small increase in stiffness in the NF deformation mode compared to the NS mode. In the Holzapfel–Ogden relation (2009) the NF and NS deformation modes exhibit the same behaviour, so this difference is due to the geometry of the RVE providing greater stiffness in the fibre shearing direction.

For the isotropic exponential relation (Equation 4.8), the estimated parameters were \( c_1 = 4.32 \text{ kPa} \) and \( c_2 = 5.84 \). For the neo-Hookean relation (Equation 4.7), the \( c_1 \) parameter was estimated to be 5.62 kPa.

Table 4.1. Parameters fitted to the Holzapfel–Ogden constitutive relation (2009) for the microstructural RVE model compared to those fitted by Göktepe et al. (2011), and parameters fitted following the same procedure and assuming a homogeneous deformation.

<table>
<thead>
<tr>
<th></th>
<th>( a ) (kPa)</th>
<th>( b ) (kPa)</th>
<th>( a_f ) (kPa)</th>
<th>( b_f ) (kPa)</th>
<th>( a_s ) (kPa)</th>
<th>( b_s ) (kPa)</th>
<th>( a_{fs} ) (kPa)</th>
<th>( b_{fs} ) (kPa)</th>
</tr>
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<tbody>
<tr>
<td>Myocardial RVE model</td>
<td>1.26</td>
<td>9.42</td>
<td>7.44</td>
<td>17.1</td>
<td>6.31 \times 10^{-9}</td>
<td>110</td>
<td>0.335</td>
<td>14.3</td>
</tr>
<tr>
<td>Homogeneous model</td>
<td>0.709</td>
<td>8.63</td>
<td>6.39</td>
<td>17.9</td>
<td>0.0414</td>
<td>51.6</td>
<td>0.202</td>
<td>13.0</td>
</tr>
<tr>
<td>Göktepe et al. (2011)</td>
<td>0.496</td>
<td>7.21</td>
<td>15.2</td>
<td>20.4</td>
<td>3.28</td>
<td>11.2</td>
<td>0.662</td>
<td>9.47</td>
</tr>
</tbody>
</table>
To investigate the change in mechanical behaviour of myocardium with perfusion pressure, uniaxial extension simulations with extension ratios up to 1.12 were performed in the three fibre coordinate directions of the RVE, with vascular pressures varied from 0 kPa to 10 kPa. The maximum extension ratio of 1.12 was chosen to match the maximum extensions in the fibre, sheet and normal directions in the shear experiments used for parameter estimation, as the estimated parameters may not be appropriate for modelling stretches outside of this range. The initial tangent stiffnesses of the RVE for uniaxial extensions about the swollen geometry were also measured at a range of pressures by calculating the change in Cauchy stress on each face of the RVE with an increment in the extension ratio of $1 \times 10^{-3}$.

As the RVE structure and applied boundary conditions are both symmetric, the external surfaces of the RVE must remain in plane when deformed to enforce the condition of a periodically repeating unit cell. This was achieved by mapping the degrees of freedom for the normal direction displacement of nodes in the external
RVE surfaces to a single degree of freedom in the solved system of equations. For example, the $z$ component of the deformed geometric field was constrained to be equal for all nodes in the external surface oriented in the $z$ direction. Another consequence of the problem symmetry was that one eighth of the full RVE could be modelled to reduce computation time, as shown in Figure 4.1.

When applying extension ratios to the perfused and swollen RVE, pressure boundary conditions were first applied to the internal vessel surfaces such that the RVE swelled, and the swollen geometry was recorded. Uniaxial extension was then applied to the swollen state by prescribing displacement conditions on the extended face, according to the extension ratio referred to the swollen dimension. The Cauchy stress on each face was calculated as the sum of nodal forces on the face divided by the total deformed surface area of the RVE face, including the space occupied by the vessel.

### 4.4.1 Myocardium RVE Behaviour

The pressurised geometry of the myocardial RVE at 10 kPa vessel perfusion pressure is shown in Figure 4.6. Figure 4.7 plots Cauchy stress against extension ratio in the three orthogonal directions of the myocardial RVE model at a range of perfusion pressures up to 10 kPa. Stiffening with perfusion pressure is evident in the sheet and normal directions, but the fibre direction stiffness initially decreases with perfusion pressure. The stress–strain behaviour for all three directions changed most significantly from 0 kPa to 2 kPa perfusion pressure, with a comparatively smaller change from 2 kPa to 10 kPa. In the fibre direction, stiffness decreases when pressure increases from 0 kPa to 2 kPa, but then shows a small increase in stiffness as pressure increases further. Strain stiffening behaviour can be observed in all three directions. The fibre direction is stiffer than the sheet direction, which is stiffer than the normal direction.

Figure 4.8 plots the swollen volume and stretch of the RVE as a function of perfusion pressure, as well as the change in initial tangent stiffness for uniaxial extensions applied to the swollen geometry. Swelling stretch in each direction was calculated as the swollen RVE dimension divided by the undeformed RVE dimension, and the volume ratio was calculated as the ratio between the perfused and unperfused states of the overall volume of the RVE including the fluid space. As pressure increases, the RVE volume initially increases rapidly, then less so as pressure increases further. This response explains the large shift in the stress–strain behaviour between 0 kPa and 2 kPa shown in Figure 4.7. Because the constitutive behaviour of myocardium
is nonlinear and strain–stiffening, the model swells significantly at low pressure but then begins to stiffen. This initial large amount of swelling causes a large shift in the stress–strain response of the tissue.

Figure 4.8 (b) shows that the increase in volume with perfusion pressure is due to swelling in the normal and sheet directions, while the fibre direction contracts as pressure increases. This contraction in the fibre direction is due to the different response of the microstructural fibre direction to tension and compression. The fibre direction has high tensile stiffness, strongly opposing any swelling deformation in that direction, but has low contractile stiffness, allowing contraction normal to the vessel wall, and resulting in an overall contraction of the RVE in the fibre direction.

The lower tensile stiffnesses in the sheet and normal directions result in swelling in these directions. Extension in the sheet and normal directions also contributes to contraction in the fibre direction through the incompressibility of the matrix material. Above pressures of 2 kPa, the dimensions in the sheet and fibre directions remain relatively constant, and only the normal direction continues to swell significantly. The volume ratio of 1.11 at 10 kPa pressure is comparable to the volume ratios of 1.07 to 1.15 observed by May-Newman et al. (1994) at a coronary artery pressure of 15 kPa.

Figure 4.8 (c) shows an initial discontinuity in the fibre direction tangent stiffness as pressure increases above zero. This is a result of the discontinuity in the gradient of
Figure 4.7. Cauchy stress against extension ratio for the myocardial RVE. Uniaxial extensions in the fibre, sheet and normal directions are plotted at perfusion pressures varying from 0 kPa to 10 kPa. Stiffening with increasing perfusion pressure is exhibited in the sheet and normal directions. Stiffness in the fibre direction is lower at 2 kPa than at 0 kPa, but increases slightly as pressure increases further. Note that the stress axes have different ranges.
Figure 4.8. (a) Volume ratio, (b) swelling stretch and (c) initial tangent stiffness against perfusion pressure for the myocardial RVE. Swelling stretch and tangent stiffness are plotted for each of the fibre, sheet and normal directions. Swelling is greatest in the normal direction, followed by the sheet direction. In contrast, the fibre direction contracts as pressure increases. Initial tangent stiffness shows a discontinuity in the fibre direction; it is much greater than the other two directions in the reference state but decreases as the tissue initially swells and contracts in the fibre direction. All three directions then increase in stiffness with fluid pressure and exhibit similar tangent stiffnesses.
the strain energy function with respect to the microstructural fibre direction stretch (Equation 4.6). In the reference state, a small extension in the fibre direction of the overall RVE results in fibre direction stretches greater than one within the RVE solid component. As pressure increases, the fibre direction contracts, slackening the fibres, such that they do not contribute any extra stiffness to the solid. The fibre direction stiffness is then only provided by the isotropic exponential term of the strain energy function. After this initial slackening of the fibre direction, the three directions all stiffen as pressure increases and show very similar stiffnesses, as the stiffness in each direction is predominantly due to the isotropic exponential term. Although the fibre direction continues to contract with increasing pressure, its stiffness still increases due to interaction with the other directions through the isotropic exponential term in $I_1$.

Figure 4.8 (c) shows similar changes in initial tangent stiffness for each direction after the initial discontinuity in the fibre direction. However, Figure 4.7 shows much greater stiffnesses in the fibre and sheet directions than the normal direction for uniaxial extension, due to the large amount of strain-stiffening behaviour in these directions. The swelling deformation in the sheet direction is not enough to cause a significant stress contribution through the sheet terms in Equation 4.6, but the sheet direction stiffness does increase significantly with further extension. The swelling also causes a shift in the stress-strain curve for the sheet direction that results in a larger amount of stiffening in the sheet direction than the normal direction, due to its greater level of strain-stiffening.

4.4.2 Vessel Structure Variations

To investigate the influence of vascular structure independent of constitutive anisotropy, variations in the RVE geometry were considered with the isotropic exponential constitutive relation from Equation 4.8.

Figure 4.9 shows the swollen volume and initial uniaxial tangent stiffness of the RVE with an isotropic geometry at a range of porosities. The swollen volume ratio increases rapidly at low pressures but less so as pressure increases further. The tangent stiffness in the unperfused state decreases as porosity increases, but as pressure increases, stiffness increases more rapidly for higher porosities, such that the stiffness of the high porosity models becomes greater than lower porosity models.

Results for models with constant porosity and varying dimension ratio are shown in Figure 4.10. The dimension ratio ($r_d$) is the ratio between the transverse and axial
Figure 4.9. RVE volume ratio and initial uniaxial tangent stiffness against perfusion pressure at a range of porosities ($\phi$). The isotropic exponential constitutive relation was used with the isotropic vascular structure model, so all directions show the same response. The swollen volume ratio increases as porosity increases. Increasing porosity decreases the initial tangent stiffness but results in increased stiffening as vessel pressure is increased.

dimensions of the RVE. The RVE in this case is transversely isotropic, so stretches and stiffnesses in both the axial and transverse directions are shown. As the level of structural anisotropy increases, the swelling stretch in the transverse direction increases, and stretch in the axial direction decreases. For highly anisotropic structures with $r_d <= 0.4$, the axial direction compresses when swelling, which is similar to the inflation of a thick walled cylinder.

From the plot of stiffness against pressure (Figure 4.10, bottom plot), it can be seen that tangent stiffness increases with pressure in both the axial and transverse directions for all dimension ratios, but the increase in stiffness is greater in the transverse direction, except for the isotropic case where $r_d = 1$. It is interesting
Figure 4.10. Swollen dimensions of the RVE model and axial and transverse stiffnesses against perfusion pressure with a range of RVE dimension ratios \( (r_d) \), when using the isotropic exponential constitutive relation. As the model has transverse symmetry, only the axial and transverse directions are shown. The porosity was 0.125 for all models. For this constitutive relation, vascular anisotropy significantly affects swelling deformation but has a limited effect on stiffness.

To note that even for \( r_d \leq 0.4 \), where the axial dimension remains relatively constant or compresses, axial stiffness increases, and the stiffness increase is not significantly less than that in the transverse direction. This is due to the coupling between strain directions in the strain energy function for this particular constitutive relation (Equation 4.8). The \( e^{c_2/(I_1-3)} \) term determines the stiffness in all directions, and increases with stretch in any one direction. Therefore, the swelling stretches in the transverse directions also stiffen the axial direction.

From the stiffness intercept in Figure 4.10, it can be observed that altering the dimension ratio \( (r_d) \) of the RVE affects its initial stiffness, making its stress–strain response anisotropic at zero vessel pressure. The stiffness in the axial direction is
slightly greater than the transverse direction stiffness. For the totally anisotropic geometry \( r_d = 0 \), the axial direction is 15% stiffer than the transverse direction. This stiffness difference will contribute to the greater swelling in the transverse directions, but is negated as the RVE swells and the transverse direction stiffens more than the axial direction.

The effect of anisotropic vascular structure on stiffness is demonstrated further in Figure 4.11, which plots stress against extension ratio for uniaxial extension of the RVE model with an isotropic exponential constitutive relation and an anisotropic vascular structure. The myocardial RVE geometry was used, which has a dimension ratio of 0.652. The response to extension in the transverse direction is very similar to the axial direction at zero pressure, and as pressure is increased, stiffness in the transverse direction increases only slightly more than in the axial direction.

![Figure 4.11](image_url)

Figure 4.11. Cauchy stress against extension ratio for uniaxial extension of the RVE model with an isotropic exponential constitutive relation and anisotropic vascular structure, with \( r_d = 0.652 \). Stresses are plotted for perfusion pressures from 0 kPa to 10 kPa. The transverse direction stiffens slightly more than the axial direction as pressure increases.
4.4.3 The Influence of Constitutive Behaviour

Figure 4.12 compares the behaviour of the isotropic exponential constitutive relation (Equation 4.8) with the neo-Hookean relation (Equation 4.7), using the RVE model with isotropic geometry. As pressure increases in the neo-Hookean model, the volume ratio increases. This swelling gradient is shallow at first, then steeper at higher pressures. The initial tangent stiffness shows a decrease with swelling, reflecting the increased compliance seen in the pressure-volume relationship. This result matches that previously reported by Jor (2005), where an exponential constitutive relation showed pressure-driven stiffening but a neo-Hookean constitutive relation did not.

The influence of the anisotropic constitutive behaviour of myocardium was simulated with an isotropic RVE geometry so as to determine its effect independent of geometric anisotropy. The swelling stretch and uniaxial tangent stiffness in each of the three myocardial directions is shown as a function of perfusion pressure in Figure 4.13 (solid lines), and is compared to results from the RVE model with anisotropic geometry based on myocardial vasculature (dashed lines). Both models used the anisotropic Holzapfel–Ogden constitutive relation (2009) with the parameters estimated using myocardium data in Section 4.3. The model with isotropic geometry swells less in the sheet and normal directions than the anisotropic geometry, and contracts less in the fibre direction. However, the swelling deformation

![Graph showing volume ratio and initial uniaxial tangent stiffness against perfusion pressure](image)

Figure 4.12. Volume ratio and initial uniaxial tangent stiffness against perfusion pressure for the isotropic exponential and neo-Hookean constitutive relations, with the isotropic RVE geometry. The exponential relation exhibits increased stiffness with perfusion pressure, whereas the neo-Hookean relation shows a decrease in stiffness.
Figure 4.13. RVE swelling stretches and initial tangent stiffness against perfusion pressure with an anisotropic constitutive relation and isotropic vessel structure, compared to the myocardial RVE geometry with the same constitutive relation and porosity. The isotropic geometry with an anisotropic constitutive relation shows different swelling deformation but similar stiffening behaviour.

is still highly anisotropic. This indicates that the constitutive anisotropy plays the most significant role in determining the swelling deformation of the RVE, but the anisotropic vascular structure still has a strong influence.

The change in initial tangent stiffness is very similar between the two models, especially in the myocardial sheet and normal directions. The model with isotropic geometry stiffens more in the fibre direction than the anisotropic model, due to the decreased contraction in this direction. This result shows that the anisotropic geometry has little effect on the stiffening of the model, which is mostly dictated by the anisotropy in the constitutive behaviour.

4.5 DISCUSSION

The presented representative volume element model of myocardium provides insight into how the mechanics of this tissue change when perfused. The highly nonlinear and anisotropic nature of myocardium leads to a complex response.

May-Newman et al. (1994) showed experimentally that an increase in coronary perfusion pressure leads to myocardial stiffening, predominantly in directions transverse to the muscle fibre direction. They did not differentiate between the
response in the myocardial sheet and normal directions, but did show that the strain
due to perfusion is greatest in the radial direction of the heart, which is mostly
aligned with the muscle sheet direction at the ventricular mid-wall. The results
obtained from this RVE model reproduced the combination of greater swelling
stretch and stiffening in the transverse directions. The normal dimension swelled
significantly more than the sheet direction, and the fibre direction contracted as the
RVE swelled. Each direction exhibited similar changes in initial tangent stiffness.
However, greater stiffening in the sheet and normal directions was observed in
uniaxial extension simulations, which applied larger extension ratios of up to 1.12.
Although the RVE model predicted greater swelling stretch in the normal direction
for an isolated block of tissue, the experimentally observed radial stretch may be
due to the geometry and boundary conditions present in the whole heart. This is
investigated further in Chapter 7, where a poroelastic model of passive ventricular
inflation is developed.

Results from the RVE model with an isotropic constitutive relation and varied
vascular anisotropy showed that the preferential alignment of blood vessels leads
to swelling predominantly in the transverse directions, but stiffening occurs in both
the transverse and axial directions (Figure 4.10). This response is dependent on
the choice of constitutive relation, and it is unknown whether myocardium exhibits
this behaviour. When geometric anisotropy was removed from the myocardial RVE,
the swelling deformation changed, but changes in tangent stiffness with perfusion
pressure were very similar (Figure 4.13).

An important implication of this study is that only constitutive anisotropy needs
to be taken into account to model the change in constitutive behaviour with perfu-
sion pressure, but vascular anisotropy must be modelled to accurately determine
deformation due to perfusion.

In the homogenisation based model of May-Newman and McCulloch (1998), per-
fusion stretch is applied equally in all directions transverse to the muscle fibres,
based on a vessel compliance, but this RVE model showed that when incorporating
anisotropy in the constitutive relation, swelling induced stretch is greater in the
myocardial normal direction than the sheet direction due to its high compliance.
Subsequent modelling approaches should therefore account for the effect of consti-
tutive anisotropy on swelling behaviour.

The difference in response between the sheet and normal directions may be reduced
if the vessel wall was introduced as a discrete component in the model with less
compliance than the surrounding tissue. However, Bilston (2002) showed that the
stiffness of the vessel wall has relatively little influence on the swelling behaviour of the tissue when compared to the surrounding tissue. The RVE model also showed swelling volume ratios comparable to those observed experimentally by May-Newman et al. (1994), indicating that the bulk compliance of the RVE matches that of myocardium to a reasonable approximation, without including a discrete vessel wall.

It is interesting to compare this model with that of Bilston (2002), which used the Mooney–Rivlin constitutive relation to model perfusion in the brain. The $c_2$ Mooney–Rivlin parameter used in that model was a similar magnitude to the $c_1$ parameter, such that the constitutive behaviour showed some strain-stiffening, but not as much as exhibited by myocardium. This model showed increased stiffness with perfusion pressure, but significant stiffness increases were only seen with perfusion pressures above 12 kPa. In contrast, the current model showed the most change in behaviour in the low perfusion range, with relatively less change in behaviour within physiological perfusion levels. The overall relative stiffening predicted was also much greater, due to the more nonlinear constitutive behaviour. This indicates that for nonlinear, strain-stiffening tissues such as myocardium, it is important that the effect of tissue perfusion is accounted for when using excised, unperfused tissue to determine the tissue’s constitutive stress–strain behaviour. However, changes in myocardium mechanical behaviour in-vivo with normal fluctuations in perfusion pressure may be small.

This RVE model has several limitations that should be noted. Only uniaxial deformation was considered. Modelling shear deformation would require general linear constraints to maintain a repeating unit cell structure, which are not yet available in OpenCMISS-Iron. The experimental shearing results from Dokos et al. (2002) indicated that stiffness due to pure shear in myocardium is relatively insignificant compared to the effects of fibre stretching, due to the similarity between the FS and FN modes, SF and SN modes, and NF and NS modes. Therefore, it is likely that shear simulations would exhibit a similar pattern of stiffness changes as those shown with uniaxial simulations.

The RVE geometry considered was also simple, with only three orthogonal vessels used to represent the extremely complex vascular network of myocardium. All tissue components outside of the vessel lumen were modelled as a continuum, using the Holzapfel–Ogden (2009) constitutive relation. However, microstructural features of the tissue, including muscle fibres, have a similar size scale to capillaries. Therefore, treating them as a continuum at the small length scale of the RVE is an approximation. Using this simplified model allowed porosity and vessel
anisotropy to be varied easily, while avoiding errors introduced by differences in mesh structure.

The simplified RVE structure also assumes that the influence of each level of the vascular hierarchy on the tissue is proportional to its volume fraction. As larger vessels have thicker vessel walls, they may exert less influence on the tissue, making the influence of the anisotropic capillaries more substantial in comparison.

The fluid–structure interaction in this model only accounted for the hydrostatic pressure of the fluid acting normal to the vessel wall. Shear stresses due to fluid flow may also affect the mechanics of perfused tissue and could be simulated by explicitly modelling the fluid mechanics of blood in the vessels. Modelling fluid shear stresses would also enable incorporating the vasodilation and vasoconstriction of vessels in response to changes in blood flow (Koller and Kaley, 1991).

Although the RVE model used simple geometry and boundary conditions, these simplifications reduced the complexity of the model to more clearly illustrate how perfusion pressure influences the mechanical behaviour of tissue.

4.6 Conclusion

A representative volume element model of vascularised myocardium was developed to provide an understanding of the mechanical response of myocardium to changes in blood perfusion pressure. Due to the strain-stiffening behaviour of the myocardial constitutive relation, significant stiffness increases were observed even for low pressures of 2 kPa. Stiffness increases occurred predominantly in the myocardial sheet and normal directions, and swelling stretch occurred mostly in the myocardial normal direction.

The anisotropic change in stiffness with perfusion pressure is primarily due to the anisotropy of the myocardial solid constitutive relation. However, the anisotropic structure of the myocardial vascular network does have an effect on how it deforms when perfused. These results motivated the development of a poroelastic model that incorporates the effect of anisotropic vascular structure on the constitutive behaviour of vascularised tissue, which is presented in Chapter 6.
In Chapter 2, approaches for modelling the interaction between blood and tissue were reviewed. It was determined that poroelasticity provides a suitable method for whole-organ scale modelling of coupled blood flow and tissue mechanics. However, the use of poroelasticity for modelling pressure-driven stiffening in tissues has not been thoroughly investigated. Previous studies that have modelled vascularised tissue as a poroelastic material have typically focused on the influence of solid mechanics on fluid dynamics, in order to determine the effect of deformation on the transport of various molecules through the vasculature.

This chapter considers the influence of fluid pressure on tissue mechanics in a poroelastic model. In Section 5.1, a static poroelastic model for investigating the influence of fluid pressure on tissue mechanics is presented. Then in Section 5.2, several constitutive relations are applied to modelling myocardium. The behaviour of these relations is used to explain how the form of a constitutive relation controls a material’s response to perfusion pressure, and determine which relations are suitable for reproducing the pressure-driven stiffening behaviour demonstrated experimentally by May-Newman et al. (1994).

It was found that for the stiffness under a particular mode of deformation to increase with perfusion pressure, the strain energy must exhibit strain-stiffening for this deformation mode, and the strain-stiffening terms must be volume dependent. The process of swelling also causes an effective dilution of strain energy, such that the strain-stiffening of the tissue must overcome this dilution in order for the tissue to exhibit pressure-driven stiffening.
5.1 STATIC PoroELASTICITY

The poroelastic model used in this study is based on the work of Coussy (2004), to which readers are directed for detailed derivations of the governing equations of poroelasticity. The present investigation considered only the steady-state behaviour of vascularised tissue. Therefore, a static form of the governing equations for poroelasticity was implemented using the finite element method, which is described in Section 5.1.4.

5.1.1 Deformation and Porosity

Vascularised biological tissue can be considered to be a fully saturated porous material, consisting of a solid skeleton with fluid completely filling its pore space. The fluid component contains only blood within the vasculature; other fluids, such as cytosol or extracellular fluid, are considered to be part of the solid skeleton.

Poroelasticity is a mixture method, in which the discrete nature of the solid skeleton and fluid is not considered. Instead, both the solid and fluid are represented by superimposed continuous fields. Values relating to the solid and fluid components will be referred to by the subscripts $s$ and $f$, respectively, with $\alpha$ used to represent either component. Points within the mixture are identified by their position in a reference configuration, with coordinates $X_\alpha$. Under loading, the fluid and solid components undergo different deformation and points originally at $X_\alpha$ in the reference state move to $x_\alpha$ in the deformed state.

The deformation of the skeleton is described in the same manner as for standard finite deformation elasticity. The deformation gradient tensor is defined as:

$$
F = \nabla_{X=x_s}, \quad F_{ij} = \frac{\partial (x_s)_i}{\partial X_j}
$$

(5.1)

where $\nabla_X$ denotes the gradient with respect to the reference geometry.

An infinitesimal volume in the reference configuration, $d\Omega_0$, becomes $d\Omega$ in the deformed state, where $d\Omega = J d\Omega_0$. $J$ is the Jacobian of the deformation, which is the determinant of the deformation gradient tensor:

$$
J = \det(F)
$$

(5.2)

This volume change includes both changes in the solid component volume and changes in the pore space due to fluid flux.
Two useful measures of deformation are the right Cauchy–Green deformation tensor, defined as $\mathbf{C} = \mathbf{F}^T \mathbf{F}$, and the Green–Lagrange strain tensor: $\mathbf{E} = \frac{1}{2} (\mathbf{C} - \mathbf{I})$, where $\mathbf{I}$ is the identity tensor.

Values in terms of the reference configuration are referred to as Lagrangian, and those with respect to the current deformed configuration are Eulerian. It is generally useful to refer to solid deformation in Lagrangian terms, and fluid flow using Eulerian terms.

The proportion of fluid within a volume $d\Omega$ is the Eulerian porosity, $n$. The Lagrangian porosity, $\phi$, is the current fluid volume per unit total volume in the reference state. The Eulerian and Lagrangian porosity are related through the equation: $\phi = J n$.

### 5.1.2 Governing Equations

The governing equations of poroelasticity can be derived using multiple approaches. A microstructural approach explicitly considers the separate solid skeleton and fluid structure (Buhan et al., 1998). Alternatively, mixture theory can be used, where the solid and fluid are represented by two superimposed continua (Bowen, 1980).

#### 5.1.2.1 Mass Conservation

The velocity field of component $\alpha$ is denoted $\mathbf{v}_\alpha$. Fluid flow is more naturally described by the Eulerian relative fluid mass velocity, which is the velocity of fluid mass relative to the surrounding solid, and is defined as:

$$\mathbf{w} = \rho_f \mathbf{l} = \rho_f n (\mathbf{v}_f - \mathbf{v}_s)$$

(5.3)

where $\rho_f$ is the fluid-specific density and $\mathbf{l}$ is known as the filtration vector. The corresponding Lagrangian mass flow vector is given by:

$$\mathbf{M} = \mathbf{J F}^{-1} \mathbf{w} = \mathbf{J F}^{-1} \rho_f n (\mathbf{v}_f - \mathbf{v}_s)$$

(5.4)

Assuming that no mass is transferred between the solid and fluid components, the Eulerian mass balances for the fluid and solid components are:

$$\frac{\partial (\rho_f n)}{\partial t} + \nabla \cdot (\rho_f n \mathbf{v}_f) = 0$$

(5.5)

$$\frac{\partial (\rho_s (1 - n))}{\partial t} + \nabla \cdot (\rho_s (1 - n) \mathbf{v}_s) = 0$$

(5.6)
Using Equations 5.4 and 5.5, the Lagrangian fluid mass balance equation can be derived:

\[
\frac{dm_f}{dt} + \nabla \cdot \mathbf{M} = 0
\]  
(5.7)

where \(m_f\) is the Lagrangian fluid mass per unit initial volume, \(m_f = \rho_f \phi\). Equivalently, the Lagrangian solid mass per unit initial volume is \(m_s = \rho_s(J - \phi)\), which is constant due to conservation of mass.

### 5.1.2.2 Momentum Conservation

Forces in the current configuration per unit current area are described by the Cauchy stress tensor, \(\boldsymbol{\sigma}\). The second Piola–Kirchhoff stress, \(\mathbf{S}\), describes forces in the reference configuration per unit reference area. These stress measures are related through the equations:

\[
\begin{align*}
\boldsymbol{\sigma} &= \frac{1}{J} \mathbf{F} \mathbf{S} \mathbf{F}^T, \\
\mathbf{S} &= J \mathbf{F}^{-1} \boldsymbol{\sigma} \mathbf{F}^{-T}
\end{align*}
\]  
(5.8)

From mixture theory, the total stress is found to be a volume average of the stresses in the solid and fluid components, such that the Cauchy stress can be partitioned into the fluid and solid Cauchy stress tensors:

\[
\boldsymbol{\sigma} = (1 - n) \boldsymbol{\sigma}_s + n \boldsymbol{\sigma}_f
\]  
(5.9)

The stress within the fluid component can be assumed to be an isotropic perfusion pressure \(p\), giving:

\[
\boldsymbol{\sigma} = (1 - n) \boldsymbol{\sigma}_s - np \mathbf{I}
\]  
(5.10)

Assuming no momentum interaction between the components, the momentum balance equation for the mixture is:

\[
\nabla \cdot (\mathbf{f} \mathbf{S}) + m_s^0 (\mathbf{f} - \mathbf{\gamma}_s) + m_f (\mathbf{f} - \mathbf{\gamma}_f) = 0
\]  
(5.11)

where \(\mathbf{S}\) is the second Piola-Kirchhoff stress of the mixture, \(\mathbf{f}\) is the body force per unit mass, which is assumed to be equal for the solid and fluid components, and \(\mathbf{\gamma}_s\) and \(\mathbf{\gamma}_f\) are the accelerations of the solid and fluid components, respectively. It is also assumed that there is no moment of momentum interaction between components, such that \(\boldsymbol{\sigma}_s\) and \(\boldsymbol{\sigma}_f\) are symmetric.
5.1.3 Constitutive Relations

5.1.3.1 Thermodynamics

The governing equations of poroelasticity are not sufficient to solve a poroelastic problem. A constitutive relation giving the mixture stress as a function of fluid pressure and skeleton deformation is required, as well as a relation between fluid pressure and flow. These relations depend on the properties of the solid and fluid materials, so can not usually be derived analytically. However, thermodynamics can be used to provide restrictions on the form of these constitutive relations, and empirical observations can then be used to deduce an appropriate form of constitutive relation that satisfies thermodynamic principles.

The first law of thermodynamics states that energy is conserved, meaning that the rate of change of internal energy of the poroelastic mixture is equal to the sum of external work performed and the movement of energy in and out of a volume due to fluid flux. The second law of thermodynamics states that the entropy within a closed system cannot decrease, such that the system will tend towards thermal equilibrium. Combining these two laws leads to the separated Clausius-Duhem inequalities for the skeleton and fluid (Coussy, 2004):

\[
\Phi_s = S : \frac{dE}{dt} + p \frac{d\varphi}{dt} - \frac{d\Psi_s}{dt} \geq 0
\]

\[
\varphi_f = ( -\nabla X p + \rho_f (\mathbf{f} - g_f)) \cdot \mathbf{l} \geq 0
\]

where \( \Phi_s \) is the Lagrangian dissipation density of the skeleton and \( \varphi_f \) is the Eulerian dissipation density of the fluid, which is related to the Lagrangian fluid dissipation density through: \( \varphi_f d\Omega = \Phi_f d\Omega_0 \). Dissipation is the rate of internal entropy production multiplied by the absolute temperature, and represents the conversion of energy to a form no longer usable for performing work. Dissipation due to heat flux was not considered in this study as temperature is generally constant in biological tissues.

\( \Psi_s \) is the Lagrangian Helmholtz free energy density of the skeleton. The Helmholtz free energy is a measure of the internal energy that can be converted to work in a system at a constant temperature. Compared to standard finite deformation elasticity, the dissipation of the skeleton contains an additional \( p \frac{d\varphi}{dt} \) term, which accounts for work done by the fluid pressure acting on the solid skeleton to increase the pore volume.
5.1.3.2 Fluid Flow

The constitutive behaviour for fluid flow can be derived by assuming that the filtration vector is linearly related to the force producing the fluid dissipation in Equation 5.13, which leads to Darcy’s equation (Darcy, 1856). In the Lagrangian reference frame, Darcy’s equation is:

\[
\frac{\mathbf{F}_\text{M}}{\rho_f} = J \mathbf{K} \mathbf{F}^T \left( -\nabla p + \rho_f \mathbf{F}^T (\mathbf{f} - \gamma_f) \right)
\]  (5.14)

where \( \mathbf{K} \) is the permeability tensor, which depends on both the skeleton pore structure and the fluid viscosity, and has units of length per time.

5.1.3.3 Skeleton Deformation

In order to derive constitutive relations for the skeleton deformation, it is assumed that the skeleton Helmholtz free energy density is a function of the Green–Lagrange strain and Lagrangian porosity:

\[
\Psi_s = \Psi_s(E, \phi)
\]  (5.15)

Equation 5.12 can then be rewritten as:

\[
\Phi_s = \left( S - \frac{\partial \Psi_s}{\partial E} \right) : \frac{dE}{dt} + \left( p - \frac{\partial \Psi_s}{\partial \phi} \right) \frac{d\phi}{dt} \geq 0
\]  (5.16)

Since \( E \) and \( \phi \) can vary independently, and Equation 5.16 must hold for positive and negative values of \( \frac{dE}{dt} \) and \( \frac{d\phi}{dt} \), the following relations must be satisfied:

\[
S = \frac{\partial \Psi_s}{\partial E}, \quad p = \frac{\partial \Psi_s}{\partial \phi}
\]  (5.17)

Specifying the form of the Helmholtz free energy density results in a fully defined system of equations that can be solved. Specific forms of the Helmholtz free energy density for describing isotropic poroelastic materials are discussed in Section 5.2.1, and Chapter 6 investigates more complex constitutive relations for anisotropic poroelastic materials.

An alternative form for the constitutive equations can be obtained by defining the overall Helmholtz free energy density of the mixture, \( \Psi \), as a function of \( E \) and \( m_f \), giving:

\[
S = \frac{\partial \Psi}{\partial E}, \quad \gamma_f = \frac{\partial \Psi}{\partial m_f}
\]  (5.18)
where \( g_f \) is the fluid free enthalpy, which is also known as the Gibbs potential and relates the fluid density and pressure through:

\[
g_f = g_f(p, T), \quad \frac{1}{\rho_f} = \frac{\partial g_f}{\partial p}
\]  

(5.19)

### 5.1.3.4 Effective Stress

The effective stress principle was originally developed by Terzaghi (1943) to describe the effect of fluid pressure on soil mechanics. The effective stress principle assumes that there is an effective stress that directly determines the solid skeleton deformation, and is independent of fluid pressure. Using an effective stress formulation, the total Cauchy stress is given by:

\[
\sigma = \sigma_{\text{eff}} - pI
\]

(5.20)

where \( \sigma_{\text{eff}} \) is the Cauchy effective stress tensor. Comparing Equation 5.20 to the stress partition (Equation 5.10) results in:

\[
\sigma_s = \frac{1}{1-n} \sigma_{\text{eff}} - pI
\]

(5.21)

This shows that with an effective stress formulation, the fluid contributes an isotropic stress to the solid skeleton equal to the fluid pressure.

When applied to soil mechanics, an effective stress model explains the reduced compressive load on soil particles as fluid pressure increases. Although the microstructure of biological tissue is much different to soil, the effective stress formulation has been shown to accurately model cartilage (Lai et al., 1991; Wilson et al., 2005). An effective stress formulation has also been used to model numerous other tissue types such as skeletal muscle and myocardium (Vankan, Huyghe, Slaaf, et al., 1997), but experimental validation with other tissue types has been limited.

Buhan et al. (1998) showed that an effective stress formulation can be derived by assuming the solid component is incompressible. In this situation, the Lagrangian porosity is a function of the Jacobian:

\[
\phi = J - 1 + \phi_0
\]

(5.22)

The solid dissipation (Equation 5.12) can then be reformulated as:

\[
\Phi_s = (S + p\mathbf{C}^{-T}) : \frac{d\mathbf{E}}{dt} - \frac{d\Psi_s}{dt} \geq 0
\]

(5.23)
where $\Psi_e$ is the Helmholtz free energy density of the skeleton for a material modelled with the effective stress principle. $\Psi_e$ is dependent only on the Green–Lagrange strain, as the porosity is no longer independent of the skeleton deformation. The constitutive equations are then defined by:

\[
S_{\text{eff}} = \frac{\partial \Psi_e}{\partial \mathbf{E}} \tag{5.24}
\]

where $S_{\text{eff}}$ is the second Piola–Kirchhoff effective stress, defined as:

\[
S_{\text{eff}} = S + pJC^{-T} \tag{5.25}
\]

which is related to the Cauchy effective stress through:

\[
S_{\text{eff}} = JF^{-1}\sigma_{\text{eff}}F^{-T} \tag{5.26}
\]

Note that since $C$ is symmetric, $C^{-T} = C^{-1}$.

Chapelle and Moireau (2014) showed that an effective stress formulation can also be derived by assuming that the Helmholtz free energy density of the skeleton can be decomposed into a function of the skeleton deformation and a function of $J - \phi$, the Lagrangian solid volume fraction. The skeleton Helmholtz free energy density can then be represented as:

\[
\Psi_s = W_{\text{skel}}(\mathbf{E}) + W_{\text{bulk}}(J - \phi) \tag{5.27}
\]

where $W_{\text{skel}}$ is the strain energy density associated with skeleton deformation and $W_{\text{bulk}}$ is the energy density associated with changes in the solid component volume. From Equation 5.17, the second Piola-Kirchhoff stress is:

\[
S = \frac{\partial W_{\text{skel}}}{\partial \mathbf{E}} + (W_{\text{bulk}})'JC^{-T} \tag{5.28}
\]

\[
p = -(W_{\text{bulk}})' \tag{5.29}
\]

where $(W_{\text{bulk}})'$ denotes the derivative of $W_{\text{bulk}}$ with respect to $J - \phi$. Substituting for $(W_{\text{bulk}})'$ in the equation for $S$ gives:

\[
S = \frac{\partial W_{\text{skel}}}{\partial \mathbf{E}} - pJC^{-T} \tag{5.30}
\]

With $\frac{\partial W_{\text{skel}}}{\partial \mathbf{E}} = S_{\text{eff}}$, this matches the second Piola–Kirchhoff effective stress definition in Equation 5.25.
### 5.1.3.5 Swelling

When fluid mass content increases and causes an overall swelling of tissue, the pressure required to drive this volume increase can be calculated from the change in the effective Helmholtz free energy of the skeleton. The total work done to swell the material is given by:

\[
\Delta W = p\Delta V = (\Delta \Psi_e) V_0 + \Delta W_e
\]  

(5.31)

where \(\Delta V\) is the volume change, \(V_0\) is the initial volume, and \(W_e\) is the external work applied.

With no externally applied forces, the external work done is zero and the pressure can then be determined by:

\[
p = \frac{d\Psi_e}{dJ}
\]  

(5.32)

### 5.1.4 Static Formulation

For investigating the influence of perfusion pressure on tissue mechanics, it is most straightforward to consider only the static, steady-state response of perfused tissue.

For steady state problems with negligible body forces, the equation of motion (Equation 5.11) simplifies to:

\[
\nabla X (FS) = 0
\]  

(5.33)

and the fluid mass balance (Equation 5.7) simplifies to:

\[
\nabla X \cdot M = 0
\]  

(5.34)

Using Darcy’s equation (Equation 5.14) to define the Lagrangian mass flow vector \(M\) in the fluid mass balance gives:

\[
\nabla X \cdot \left( -\rho_f F^{-1} K F^{-T} \nabla X p \right) = 0
\]  

(5.35)

which is the generalised Laplace’s equation. Solving this equation rather than the two separate equations for pressure and velocity is less computationally demanding, however, it leads to a weaker enforcement of mass conservation and lower accuracy in the velocity solution (Badia and Codina, 2010).
5.1.5 Finite Element Implementation

Equations 5.33 and 5.35 were solved as a single system of coupled nonlinear equations using the finite element method with OpenCMISS-Iron (Bradley et al., 2011). OpenCMISS-Iron is the numerical computation component in OpenCMISS (www.opencmiss.org), which is a computational modelling environment for bioengineering.

OpenCMISS-Iron was used in this study due to its extensibility, which allows experimentation with new constitutive relations. OpenCMISS-Iron also has strong support for finite deformation elasticity and high-order basis interpolation, both of which are important for modelling the mechanics of biological tissues. OpenCMISS-Iron has also been designed for solving problems on distributed systems using the message passing interface (MPI) standard, which allows simulating much larger problems than those that are tractable on a single workstation or shared-memory computer.

OpenCMISS-Iron has been designed to support multi-physics modelling so is well suited to modelling the coupled fluid and solid mechanics in poroelasticity. A new multi-physics problem type was added to OpenCMISS-Iron that couples the nonlinear, generalised Laplace equation for fluid pressure to the existing finite deformation elasticity equations. When setting up the coupled finite element problem, OpenCMISS-Iron assembles the element equations for elasticity and fluid pressure into a single system of nonlinear equations. This monolithic coupling approach is more robust than segregated approaches that iterate between solving the solid and fluid equations (Heil et al., 2008).

5.2 Constitutive Relations for Pressure-Driven Stiffening

The way in which a poroelastic material responds to increases in fluid pressure is controlled by the material’s constitutive relation, defined by the Helmholtz free energy density function. This is generally an empirical relation based on experimental results or assumptions about the material microstructure, with parameters that must be fitted to experimental data. In such cases, the constitutive relation may only be suitable for modelling particular types of deformation, so it is important to understand what constitutive relations are suitable for describing the behaviour of biological tissue that has swollen due to perfusion pressure.

The response to swelling with a number of isotropic constitutive relations was
analysed by applying a swelling pressure, followed by homogeneous uniaxial or simple-shear deformation.

5.2.1 Analysed Constitutive Relations

A variety of constitutive relations have been used to model poroelastic materials. Some have been developed specifically for use with a poroelastic model, while others are based on relations traditionally used with pure finite deformation elasticity models. When developing a constitutive relation for a poroelastic material, it is important to consider that the volume of the material may change due to fluid movement in or out of a body, so an incompressible constitutive relation is unsuitable. The solid material that makes up the skeleton may be considered incompressible, but the skeleton itself must allow some bulk volume change if fluid volume may change.

Many constitutive relations developed for finite deformation elasticity depend on an incompressible formulation and may exhibit a non-zero stress at zero strain that would be balanced by a non-zero Lagrangian multiplier representing hydrostatic pressure. In a dynamic simulation, this can be overcome by enforcing the incompressibility of the solid component through a solid specific Lagrangian multiplier. Otherwise, this non-zero hydrostatic pressure offset must be accounted for by introducing a non-zero hydrostatic pressure at the reference configuration, \( p_0 \). With an effective stress formulation, the total Cauchy stress then becomes:

\[
\sigma = \sigma_{\text{eff}} - (p + p_0)I
\]  

(5.36)

This avoids the non-physical result of a volume change without the influence of any external force or internal fluid pressure.

Although many biological tissues are anisotropic, and incorporating this anisotropy into mechanical models is important for increasing model fidelity, the addition of anisotropy to a model adds significant complexity. This study therefore focussed only on the behaviour of isotropic poroelastic materials without considering the effect of anisotropy. The conclusions presented here may be applied to anisotropic materials. However, further investigation incorporating anisotropy may uncover more interesting and complicated effects.

The first relation considered in this study was a compressible form of the Mooney–Rivlin relation (Mooney, 1940; Rivlin, 1947), with the skeleton Helmholtz free energy
density given by:

\[
\Psi_e = c_1 (I_1 - 3) + c_2 (I_2 - 3) + K (J - 1 - \ln J) \tag{5.37}
\]

where \(c_1, c_2\) and \(K\) are material parameters, and \(I_1\) and \(I_2\) are the first and second invariants of \(C\), respectively. Although \(I_1\) and \(I_2\) both have some dependence on volume, terms in \(K\) are used to provide additional control over the material’s bulk behaviour and oppose net fluid volume changes. For this Mooney–Rivlin relation, \(p_0 = 2c_1 + 4c_2\).

Next, an exponential relation in \(I_1\) was considered, with skeleton free energy density given by:

\[
\Psi_e = \frac{c_1 c_2}{c_2} (e^{c_2(I_1 - 3)} - 1) + K (J - 1 - \ln J) \tag{5.38}
\]

where \(c_1, c_2\) and \(K\) are again material parameters, and \(p_0 = 2c_1\). Similar exponential relations have been used throughout the literature to represent the strain-stiffening behaviour of myocardium and many other tissues. Many anisotropic relations have been developed based on the basic exponential form originally proposed by Fung (1967) (Chuong and Fung, 1983; Costa et al., 2001; Guccione et al., 1991). These exponential relations have also been adapted for use in poroelastic models. Vankan, Huyghe, Drost, et al. (1997) used a Guccione based constitutive relation to describe the gastrocnemius muscle with a two dimensional biphasic model, and May-Newman and McCulloch (1998) also used a similar relation for their model of swelling tissue. Holmes and Mow (1990) developed a simple isotropic exponential relation for poroelastic modelling of cartilage. In their relation, the exponential strain energy is inversely related to \(I_3\), such that strain energy tends to infinity as volume tends to zero, and the hydrostatic pressure in the reference state can be set to zero by an appropriate choice of parameters.

The third type of constitutive relation investigated was a power-law based on \(I_1\):

\[
\Psi_e = c_1 (I_1 - 3) + \frac{c_2}{c_3} (I_1 - 3)^{c_3} + K (J - 1 - \ln J) \tag{5.39}
\]

where \(c_1, c_2, c_3\) and \(K\) are material parameters and \(p_0 = 2c_1\). The \(c_1\) term gives a non-zero initial stress-strain gradient in the reference state, while \(c_2\) and \(c_3\) together control the strain-stiffening of the material, with \(c_3 > 1\).

When using a compressible constitutive relation it is common to decompose a material’s strain energy into its dilatational behaviour and behaviour under distortional, volume independent deformation. Doing so is useful as the dilatational and distortional behaviours of a material are usually very different, and this allows
the parameters controlling these two behaviours to be decoupled and determined independently (Holzapfel, 2000). The hydrostatic pressure offset at zero strain is also avoided in such a formulation. For isotropic materials, this is achieved by basing the volume independent strain energy on a set of modified invariants, which are invariants of the volume-independent right Cauchy–Green deformation tensor, \( \overline{\mathbf{C}} = \mathbf{J}^{-\frac{2}{3}} \mathbf{C} \), where \( \det(\overline{\mathbf{C}}) = 1 \). These modified invariants can be defined in terms of the standard invariants:

\[
\overline{I}_1 = I_3^{-\frac{1}{3}} I_1 = \mathbf{J}^{-\frac{2}{3}} I_1 \tag{5.40}
\]

\[
\overline{I}_2 = I_3^{-\frac{2}{3}} I_2 = \mathbf{J}^{-\frac{4}{3}} I_2 \tag{5.41}
\]

Although these equations appear to be functions of \( \mathbf{J} \) and a standard invariant, the \( \mathbf{J} \) terms cancel the dependence of the standard invariants on \( \mathbf{J} \), such that the modified invariants are independent of \( \mathbf{J} \).

Chapelle et al. (2010), for example, used a Mooney–Rivlin based constitutive relation in terms of these modified invariants, with separate dilatational strain energy terms. In the present study, the modified Mooney–Rivlin, exponential and power-law constitutive relations that are based on these modified invariants were also considered, by substituting \( I_1 \) with \( \overline{I}_1 \) and \( I_2 \) with \( \overline{I}_2 \). For example, the skeleton free energy of the modified exponential relation is given by:

\[
\Psi_e = \frac{c_1}{c_2} \left( e^{c_2(\overline{I}_1 - 3) - 1} \right) + K(\mathbf{J} - 1 - \ln \mathbf{J}) \tag{5.42}
\]

### 5.2.2 Constitutive Parameters

LeGrice et al. (1995) showed that myocardium has a complex laminar structure. This structure leads to mechanical behaviour that requires an orthotropic constitutive relation and a rich set of experimental data to parameterise (Dokos et al., 2002). The aim of this study was not to present an accurate poroelastic model of myocardium, but rather to investigate the effect of the form of the material free energy density function on its swelling behaviour, without introducing additional modelling complexity related to anisotropy. For each of the isotropic constitutive relations considered, the parameters were set in order to produce a swelling of 15% at 15 kPa based on the results of May-Newman et al. (1994), and, based on the results of Demer and Yin (1983), a uniaxial stiffness of 50 kPa was selected as an approximation of the initial tangent stiffness of myocardium.

As only two pieces of data were used for parameter estimation and the constitutive
relations studied have more than two parameters, certain parameters were selected to be estimated and others were constrained based on the estimated parameters. For the Mooney–Rivlin constitutive relation, $c_2$ was constrained to be $\frac{1}{4} c_1$. For rubber, the ratio of $c_2$ to $c_1$ is approximately $\frac{1}{7}$ (Rivlin, 1947), but ratios of close to 1 have been used to model some tissues (Bilston, 2002). The resulting parameters were $c_1 = 7.01$ kPa, $c_2 = 1.75$ kPa, and $K = 117$ kPa. For the exponential relation, $c_2$ was fixed at 2 to produce a significant level of strain-stiffening, and the estimated parameters were then $c_1 = 8.93$ kPa, $c_2 = 2$ and $K = 17.1$ kPa. Since only the case of constant perfusion pressure was considered, the permeability tensor had no influence on the solution and was set to the identity tensor.

5.2.3 Simulation Protocols

The swelling and then uniaxial extension and simple shear behaviours of a block of tissue were considered. As only homogeneous deformations were applied, a single element model was used, with dimensions of 10 mm $\times$ 10 mm $\times$ 10 mm.

The simulation procedure for the uniaxial extension simulations is illustrated in Figure 5.1. The $x = 0$, $y = 0$ and $z = 0$ faces were fixed to remain in their respective planes. In the first simulation step, a constant perfusion pressure constraint was applied and the material was allowed to swell. Next, a uniaxial extension was applied by prescribing a set of homogeneous uniaxial displacements in the $x$ direction over a

![Figure 5.1. Uniaxial deformation of an isotropic, pressurised cube. The dotted lines indicate the initial, unperfused geometry; dashed lines indicate the perfused, swollen geometry; and solid lines indicate the deformed geometry under a homogeneous uniaxial extension along the $x$ axis.](image-url)
range of values up to an extension ratio of 1.3, and the stress at each extension ratio was recorded. The applied extension ratios were based on the perfused, swollen geometry, such that a ratio of 1 corresponds to no externally applied force, and a ratio of 1.3 means all points are extended to 1.3 times their position in the perfused state. Basing the extension ratio on the perfused state in this way allows for more straightforward comparison of the material behaviour between the perfused and unperfused states. The tangent stiffness was measured as a function of pressure at extension ratios of 1, 1.1, 1.2 and 1.3. The tangent stiffness at a particular pressure and extension ratio was calculated by first perfusing and extending the tissue, then applying an extension increment of $1 \times 10^{-4}$ and recording the change in Cauchy stress normal to the extension direction. All stiffness values are given as the change in Cauchy stress divided by the extension ratio increment. The tangent stiffness at an extension ratio of 1 is referred to as the initial tangent stiffness. This approach was repeated with a simple shear deformation by displacing the $z = 10$ mm face in the $x$ direction, with shear deformation ratios from 0 to 0.3.

The volume was free to vary after swelling, when applying external boundary conditions. This represents a quasistatic, drained situation where solid deformations are slow enough to allow fluid mass to shift and equilibrate. This is in contrast to a quasistatic, undrained problem, in which fluid flow is treated as slow in comparison to the skeleton deformation, so that the fluid volume retains the same value as in the purely perfused state.

5.2.4 Results

5.2.4.1 Comparison of Mooney–Rivlin and Exponential Models

Figure 5.2 plots stress against extension ratio under uniaxial deformation at a range of perfusion pressures for the Mooney–Rivlin and exponential constitutive relations. Although both relations have parameters specified to give the same initial tangent stiffness, their behaviour becomes significantly divergent at larger extension ratios and with increased perfusion pressure. The Mooney–Rivlin relation shows an approximately linear increase in stress with extension, with a slight decrease in gradient at higher pressures. The exponential relation shows strain-stiffening behaviour and an increase in tangent stiffness as perfusion pressure increases.

It can be seen in Figure 5.3 (a) that both relations swell with an increase in perfusion pressure. The Mooney–Rivlin relation shows an increase in the rate of volume change with respect to pressure at greater pressures, whereas the exponential
Figure 5.2. First principal Cauchy stress versus extension ratio for uniaxial extension at a range of perfusion pressures with (a) the Mooney–Rivlin constitutive relation (Equation 5.37) and (b) the exponential constitutive relation (Equation 5.38). Note that the stress scales are different for each plot.

Figure 5.3. (a) Swollen volume ratio against perfusion pressure and (b) initial tangent stiffness against perfusion pressure for the Mooney–Rivlin (Equation 5.37) and exponential (Equation 5.38) constitutive relations.
relationship shows the opposite effect, with further volume change being opposed more strongly at higher pressures.

This difference in the pressure–volume behaviour is a consequence of the change in stiffness with swelling exhibited by each relation. Figure 5.3 (b) illustrates the initial tangent stiffness as a function of perfusion pressure. Although both relations responded to the perfusion pressure by swelling, this swelling has affected their behaviour differently. The Mooney–Rivlin relation shows a small decrease in stiffness, whereas the exponential relationship shows a large increase in tangent stiffness with perfusion pressure.

The decrease in stiffness with swelling exhibited by the Mooney–Rivlin relation can be explained by considering the “dilution” of the skeleton free energy density under swelling. When \( \Psi_e \) is linearly related to some strain measure, the same increment in this strain measure applied to both the swollen and unswollen states results in the same increase in \( \Psi_e \). However, as \( \Psi_e \) is defined per unit volume in the reference state, the increase in Helmholtz free energy per unit Eulerian volume is lower in the swollen state by a factor of \( J^{-1} \). Physically, this process can be thought of as an influx of non-elastic fluid causing a dilution of the elastic components (Bogen, 1987).

For the constitutive relations considered in this study, the tangent stiffness at large extension ratios responds to increased perfusion pressure in the same way as for smaller extensions. As an example, Figure 5.4 shows the change in tangent stiffness with perfusion pressure when using the exponential relation at extension ratios of 1, 1.1, 1.2 and 1.3.

Figure 5.5 shows how the tangent stiffness with shear deformation changes as perfusion pressure increases for the Mooney–Rivlin and exponential constitutive relations. Comparing this plot with Figure 5.3 (b), it can be seen that the change in tangent stiffness for simple shear deformation is very similar to the tangent stiffness for uniaxial deformation. Similar behaviour was observed for all other relations investigated in this study. This result is not surprising given that these relations all have simple forms with no explicit dependence on shear deformations.

5.2.4.2 Mooney–Rivlin and Exponential Parameter Variations

The results presented above for the Mooney–Rivlin relation show a decrease in tangent stiffness with perfusion pressure. However, this response is dependent on the choice of parameters. If \( c_2 \) is chosen to be large compared to \( c_1 \), the behaviour reverses, as shown in Figure 5.6. In this simulation, the tangent stiffness was
Figure 5.4. Tangent stiffness against perfusion pressure at a range of extension ratios ($\lambda$) when using the exponential constitutive relation (Equation 5.38).

Figure 5.5. Initial tangent stiffness for shear deformation against perfusion pressure for the Mooney–Rivlin (Equation 5.37) and exponential (Equation 5.38) constitutive relations. The response to perfusion is similar to that for uniaxial extension (Figure 5.3 (b)).
compared with \( c_2 = \frac{1}{4} c_1 \) and \( c_2 = 2c_1 \). For the second case, the estimated parameters that gave an initial tangent stiffness of 50 kPa and 15% swelling at 15 kPa perfusion pressure were \( c_1 = 2.92 \text{ kPa} \), \( c_2 = 5.84 \text{ kPa} \), and \( K = 108 \text{ kPa} \).

As this constitutive relation is isotropic, applying a constant perfusion pressure causes an isotropic swelling. If the deformation gradient under swelling is \( \lambda_s \mathbf{I} \), where \( \lambda_s \) is referred to as the swelling stretch, stretches based on the swollen geometry can be defined as \( \Lambda_i = \frac{\lambda_i}{\lambda_s} \) (Bogen, 1987), where \( \lambda_i \) is the stretch ratio in the \( i \)-th direction, and the Jacobian in the swollen state is defined as \( J = \lambda_s^3 \). Determining stress as a function of \( \Lambda_i \) demonstrates how the swollen material would behave if the swollen state was taken as the material’s reference state, allowing comparison of its mechanical response across different levels of swelling.

Although \( c_1 (I_1 - 3) \) and \( c_2 (I_2 - 3) \) are linear in their respective invariants, they are nonlinear in terms of stretches. When aligned with the principal stretch directions, \( I_1 \) and \( I_2 \) are:

\[
I_1 = \text{trace } (C) = \lambda_1^2 + \lambda_2^2 + \lambda_3^2
\]  
(5.43)

\[
I_2 = \frac{1}{2} \left( (\text{trace } (C))^2 - \text{trace } (C^2) \right) = \lambda_2^2 \lambda_3^2 + \lambda_1^2 \lambda_3^2 + \lambda_1^2 \lambda_2^2
\]  
(5.44)

\( I_1 \) has terms proportional to squared stretch and \( I_2 \) has terms proportional to stretch to the power of four. The rate of increase of \( I_1 \) with swelling is lower than the rate

![Figure 5.6. Initial tangent stiffness against perfusion pressure for the Mooney–Rivlin relation (Equation 5.37) with two sets of parameters. In the first, \( c_2 = \frac{c_1}{4} \), and in the second, \( c_2 = 2c_1 \).](image-url)
of free energy density decrease due to dilution, therefore, a free energy density function linear in \( I_1 \) shows decreasing stiffness with swelling. However, the \( I_2 \) based term causes an increase in free energy density with swelling that overcomes the dilution effect and can lead to an overall increase in stresses at swollen volumes.

This can be seen algebraically by considering the first principal stress when applying a uniaxial extension in the \( x \) direction. The tangent stiffness of the swollen material is defined as the rate of change of \( \sigma_{11} \) with respect to \( \Lambda_1 \). The Cauchy stress in this direction is found to be:

\[
\sigma_{11} = (\sigma_{\text{eff}} - p I)_{11} \\
= \frac{2}{J^{\lambda_2^2}} \lambda_1^2 \lambda_2 \left( \frac{\partial \Psi}{\partial C} \right)_{11} - p
\]

where \((...)_ij\) denotes the value in the \( i \)th row and \( j \)th column of the tensor expression within the parentheses.

Using the Helmholtz free energy function for the Mooney–Rivlin relation (Equation 5.37) and making use of the derivatives of \( I_1, I_2 \) and \( J \) with respect to \( C \) (Holzapfel, 2000) gives the result:

\[
\frac{\partial \Psi}{\partial C} = c_1 \frac{\partial I_1}{\partial C} + c_2 \frac{\partial I_2}{\partial C} + K \left( 1 - \frac{1}{J} \right) \frac{\partial J}{\partial C} \\
\sigma_{11} = \frac{2}{J^{\lambda_2^2}} \lambda_1^2 \lambda_2 \left( c_1 + c_2 \lambda_2^2 (\Lambda_2^2 + \Lambda_3^2) \right) + K \left( 1 - \frac{1}{J} \right) - p
\]

By assuming that volume changes are negligible for small deformations about the swollen geometry, \( J = \lambda_3^3 \), giving:

\[
\sigma_{11} = 2\lambda_2^{-1} \lambda_1^2 \left( c_1 + c_2 \lambda_2^2 (\Lambda_2^2 + \Lambda_3^2) \right) + K \left( 1 - \lambda_3^{-3} \right) - p
\]

By considering the stress as a function of \( \Lambda_i \) as the tissue swells and \( \lambda_s \) increases, it can be seen that the dilution of strain energy leads to a scaling of uniaxial stress by a factor of \( \lambda_s^{-1} \) when compared to the reference state. The free energy terms that increase with stretch may counteract this dilution. As the derivative of \( I_1 \) with respect to \( C \) has no volume dependence, the term linear in \( I_1 \) cannot overcome the dilution. However, \( I_2 \) terms in the skeleton free energy lead to \( \lambda_2^2 \) terms that increase faster than the \( \lambda_3^{-1} \) scaling, leading to an overall increase in the \( I_2 \) based stress terms. Therefore, for large \( c_2 \), an overall increase in stress with swelling is observed, but for small \( c_2 \), the \( c_1 \) terms dominate and stresses decrease.
The stiffening with perfusion pressure exhibited by the exponential relation is also
dependent on the choice of parameters. The rate of change of the strain energy
density is low for small values of the exponent, \(c_2(1_1 - 3)\), such that for small
values of \(c_2\), there may be an initial decrease in stiffness observed for small increases
in pressure, followed by a subsequent recovery and then increase in stiffness as
pressure and hence swelling increases further. This is illustrated in Figure 5.7,
where the behaviours for \(c_2\) values of 2 and 0.1 are compared. For the case with
\(c_2 = 0.1, c_1\) and \(K\) were kept at the same values as those fitted to the case with \(c_2 = 2,\)
as these parameters better demonstrate pressure-softening behaviour compared
to parameters obtained when matching the initial tangent stiffness and swollen
volume at 15 kPa. In general, fitting an exponential relation to experimental results
from biological tissue yields exponent parameters much larger than those that
demonstrate this softening behaviour (Chuong and Fung, 1983; Schmid et al., 2006).

5.2.4.3 Power–Law Relation

The power-law relation in Equation 5.39 was then considered. When selecting
parameters, \(c_2\) was first constrained to be equal to \(c_1\) and \(c_3\) was set at 2.0, 2.5 and 3.0.
Next, \(c_3\) was held constant at 2.0, and \(c_2\) was set to 0 kPa, then equal to \(c_1\) and \(2c_1\).
The parameters fitted following the approach in Section 5.2.2 are given in Table 5.1.

![Figure 5.7](image-url)  

Figure 5.7. Initial tangent stiffness against perfusion pressure for the exponential relation
(Equation 5.38) with two sets of parameters. In the first, \(c_2 = 2.0\), and in the second, \(c_2 = 0.1\).
For both simulations, \(c_1\) and \(K\) were set to 8.93 kPa and 17.1 kPa, respectively.
Table 5.1. Constitutive parameters used for the power-law relation.

<table>
<thead>
<tr>
<th>Constraint</th>
<th>$c_1$ (kPa)</th>
<th>$c_2$ (kPa)</th>
<th>$c_3$</th>
<th>$K$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_3 = 2.0$</td>
<td>8.76</td>
<td>8.76</td>
<td>2.0</td>
<td>83.5</td>
</tr>
<tr>
<td>$c_3 = 2.5$</td>
<td>8.85</td>
<td>8.85</td>
<td>2.5</td>
<td>101</td>
</tr>
<tr>
<td>$c_3 = 3.0$</td>
<td>8.80</td>
<td>8.80</td>
<td>3.0</td>
<td>110</td>
</tr>
<tr>
<td>$c_2 = 0$ kPa</td>
<td>8.76</td>
<td>0.0</td>
<td>2.0</td>
<td>121</td>
</tr>
<tr>
<td>$c_2 = c_1$</td>
<td>8.76</td>
<td>8.76</td>
<td>2.0</td>
<td>83.5</td>
</tr>
<tr>
<td>$c_2 = 2c_1$</td>
<td>8.77</td>
<td>17.5</td>
<td>2.0</td>
<td>45.9</td>
</tr>
</tbody>
</table>

Plots of the tangent stiffness against perfusion pressure for variations in $c_3$ are shown in Figure 5.8 (a), and for variations in $c_2$ in Figure 5.8 (b). Figure 5.8 (a) shows an increase in tangent stiffness with perfusion pressure for all tested values of $c_3$, and the stiffness increase is greater for lower values of $c_3$. Although increasing $c_3$ increases the nonlinearity of the stress–strain relation, the gradient of stress against extension for larger values of $c_3$ is lower at small extensions, leading to the observed response. As pressure and swollen volume increases further this trend begins to reverse, albeit incompletely within the physiological pressure range considered here. Figure 5.8 (b) shows a more intuitive response. With $c_2 = 0$ kPa, the relation is purely neo-Hookean and shows a decrease in stiffness as it swells. As $c_2$ increases relative to $c_1$, stiffness increases more rapidly with perfusion pressure.

![Figure 5.8](image-url)
5.2.4.4 Modified Invariants

Figure 5.9 (a) plots stress against extension ratio for uniaxial extension at a range of perfusion pressures, when using the modified form of the invariants with volume-independent terms in the exponential constitutive relation. The skeleton free energy density for this relation is defined by Equation 5.42. The parameters estimated for the modified exponential relation were $c_1 = 8.76$ kPa, $c_2 = 2.0$ and $K = 115$ kPa. By comparing the results for the modified versus standard exponential relations in Figure 5.9 (a) and Figure 5.2 (b), respectively, it can be seen that the uniaxial behaviour is similar for the zero-pressure state, but stiffness decreases rather than increases as pressure increases. Figure 5.9 (b) shows a decrease in initial tangent stiffness as perfusion pressure increases, and compares the response of the modified exponential relation to the standard exponential relation (Equation 5.38). Both the modified Mooney–Rivlin relation and the modified power-law relation showed the same change in initial tangent stiffness with perfusion pressure as the modified exponential relation. Unlike the Mooney–Rivlin relation where stiffening with perfusion pressure can be produced depending on the material parameters, these relations based on the modified invariants with separate volumetric terms will always show a decrease in stiffness with pressure. This behaviour can be explained
by algebraically considering the change in stress as a material swells.

Again making use of the swelling stretch, $\lambda_s$, and stretches based on the swollen geometry, $\Lambda_i$, the Cauchy principal stress along the direction of extension is given by Equation 5.45. When the skeleton free energy is decoupled into dilatational terms based on $J$ and volume independent terms in $\tilde{I}_1$ and $\tilde{I}_2$, this stress is found to be:

$$\sigma_{11} = \frac{2}{J} \lambda_s^2 \Lambda_1^2 \left( \frac{\partial \Psi_e}{\partial \tilde{I}_1} \frac{\partial \tilde{I}_1}{\partial \mathbf{C}} + \frac{\partial \Psi_e}{\partial \tilde{I}_2} \frac{\partial \tilde{I}_2}{\partial \mathbf{C}} + \frac{\partial \Psi_e}{\partial J} \frac{\partial J}{\partial \mathbf{C}} \right)_{11} - p$$

$$= \frac{2}{J} \lambda_s^2 \Lambda_1^2 \left( \frac{\partial \Psi_e}{\partial \tilde{I}_1} \frac{\partial \tilde{I}_1}{\partial \mathbf{C}} + \frac{\partial \Psi_e}{\partial \tilde{I}_2} \frac{\partial \tilde{I}_2}{\partial \mathbf{C}} \right)_{11} + \frac{\partial \Psi_e}{\partial J} \frac{\partial J}{\partial \mathbf{C}} - p$$

(5.50)

When considering the initial tangent stiffness of the swollen material, only small deformations are applied in which the volume change is negligible. Therefore it can be assumed that $J = \lambda_s^3$. In the initial swelling phase where external forces are absent, $p = \frac{\partial \Psi_e}{\partial J}$ (Equation 5.32), and as $\tilde{I}_1$ and $\tilde{I}_2$ are independent of volume, $\frac{\partial \Psi_e}{\partial J} = \frac{\partial \Psi_e}{\partial J}$. As volume is assumed to remain constant, and $\frac{\partial \Psi_e}{\partial J}$ is a function of $J$ only, $\frac{\partial \Psi_e}{\partial J}$ remains equal to $p$. Using these results, the stress in the extension direction is found to be:

$$\sigma_{11} = 2 \lambda_s^{-1} \Lambda_1^2 \left( \frac{\partial \Psi_e}{\partial \tilde{I}_1} \frac{\partial \tilde{I}_1}{\partial \mathbf{C}} + \frac{\partial \Psi_e}{\partial \tilde{I}_2} \frac{\partial \tilde{I}_2}{\partial \mathbf{C}} \right)_{11}$$

(5.51)

From this equation it can be seen that the same relative deformation applied to the swollen material produces a stress that is scaled by $\lambda_s^{-1}$ when compared to the reference geometry. This scaling is independent of the form of the dilatational and volume independent terms, so applies to all constitutive relations where the dilatational and volume independent deformations are additively decoupled.

### 5.3 Discussion

These results show that for a strain-stiffening poroelastic constitutive relation, an increase in perfusion pressure will result in increased uniaxial and shear stiffness. However, the strain-stiffening terms must be volume dependent and must increase with stretch at a rate greater than the decrease in stress due to the swelling itself (the dilution effect). The presence of a pressure-stiffening response also depends critically on the chosen parameters.

For a linear constitutive relation or a strain-stiffening relation where the strain-stiffening terms are volume independent, the stiffness will always decrease with
increased perfusion pressure. This may seem counterintuitive, but many people will be familiar with a similar effect when inflating a balloon: it is initially stiff and difficult to inflate, but it becomes more compliant as volume and hence stretch increases (Bogen and McMahon, 1979). A poroelastic material is a three-dimensional solid rather than a thin membrane, but the effect of increasing its volume is similar to the biaxial stretch in a balloon. Bogen (1987) explains this decrease in stiffness as an effective “dilution” of the strain energy density in the swollen state, which is scaled by a factor of $\lambda^{-3}$ when compared with the reference state. The same strain resisting elements in the original volume are now dispersed over a larger volume.

In this study, stiffness and extension ratios were calculated based on the swollen geometry. If extensions were instead based on the zero-pressure reference geometry, then the same physical deformation applied to a swollen geometry would result in larger changes in the extension ratio, so that the calculated stiffnesses would have lower values. The softening seen with linear relations would increase and the stiffening seen in other relations would decrease. However, the overall conclusions would not change. Using extension ratios based on the swollen geometry appears to be the most natural approach when trying to understand the mechanical behaviour of swollen tissues, as the swollen configuration is an intuitive reference state. This approach also seems appropriate when considering the difference in the mechanical behaviour of unperfused tissues in-vitro, and perfused, in-vivo tissues.

Numerous investigators, including Demer and Yin (1983), have shown that the stiffness of myocardium increases with strain, demonstrating that linear constitutive relations are not suitable when modelling myocardium with finite deformation elasticity. From the results presented in this study, it can be concluded that when modelling myocardium with a poroelastic approach, a nonlinear, strain-stiffening relation is also required in order to reproduce the increase in stiffness with perfusion pressure observed experimentally by May-Newman et al. (1994). By using a poroelastic modelling approach and selecting a constitutive relation that accurately models a tissue’s response to passive stretches at large strains, pressure-driven stiffening behaviour naturally arises as a direct consequence of the strain-stiffening of the tissue.

These results also show that additively decoupling the dilatational and volume independent behaviour of the solid skeleton is not appropriate when modelling myocardium as a poroelastic material. Although this provides useful mathematic simplifications, for example, ensuring the solid hydrostatic pressure is zero in the undeformed state, it is not consistent with the mechanical response at the microstructural scale in most tissues. The nonlinearity of the stress–strain relationship
in tissues is due to the straightening of collagen and muscle fibres as strain increases; a slack, wavy fibre initially presents negligible stiffness but once it is straightened it contributes to the tissue stiffness. The gradual recruitment of multiple fibres in this way leads to an overall smoothly continuous stress–strain relationship, with stiffness increasing as strain increases (Horowitz et al., 1988). This same fibre stretching process takes place under volumetric deformation so it is appropriate that the strain energy contains coupled terms that depend on volumetric and non-volumetric changes, as these more accurately reflect the actual length changes of fibres within the tissue. Basing the strain energy function on components of the strain tensor aligned with respect to the material coordinate system is a common approach for modelling anisotropic tissues, and this approach preserves the contribution of dilatation to fibre stretch (Costa et al., 2001; Guccione et al., 1991).

Sansour (2008) considered the decoupling of volumetric and isochoric contributions to strain energy in finite deformation elasticity and showed that this approach is not valid for anisotropic, fibrous materials, as it results in fibre stresses that are not one-dimensional. Nolan et al. (2014) also investigated the effect of decoupling the volumetric and isochoric behaviour of tissue in the context of modelling arterial walls as a compressible material. They provide strong evidence that this decoupling is not appropriate for modelling anisotropic, compressible materials; non-physical behaviour was observed for a range of deformation modes. The present study shows that even for isotropic materials, it is inappropriate to use an additive decomposition of strain energy into volumetric and distortional terms if the material’s constitutive behaviour is nonlinear.

In some poroelastic models, the skeleton Helmholtz free energy density is decomposed into the energy due to skeleton deformation, which contains volumetric contributions, and energy due to solid component volume changes (Chapelle and Moireau, 2014). As the solid component can be considered incompressible or nearly incompressible, this decoupling is valid.

Constitutive relations based on the modified invariants of \( \mathbf{C} \) can produce pressure-driven stiffening, provided these terms are coupled to volumetric terms. For example, Cookson et al. (2012) developed an isotropic, exponential multi-compartment model of cardiac tissue that describes the hierarchical flow of blood through vessels of differing dimensions. This model used the modified invariants in its strain energy function, but they were coupled to the fluid mass content. Because of this, Cookson et al. observed increased stiffness with fluid volume when applying their model to a whole heart simulation.
The interaction between swelling and the stiffening due to swelling is important when considering the influence of perfusion pressure on mechanics. For the tissue to stiffen significantly with perfusion pressure, volumetric swelling must be allowed, but the swollen tissue must also stiffen and oppose further deformation. May-Newman et al. (1994) observed volume changes of up to 15% in myocardium at physiological perfusion pressure levels. If a tissue stiffens significantly when swelling but does not allow sufficient swelling at physiological perfusion pressures, then no increase in stiffness with perfusion pressure will be observed. When formulating a constitutive relation and fitting material parameters, it is therefore important to consider not only the strain-stiffening behaviour of the tissue, but also its dilatational behaviour, and how this interacts with the strain-stiffening terms in the relation. This requires mechanical testing of tissues at a range of perfusion pressures.

In this study, only isotropic power-law, Mooney–Rivlin and exponential constitutive relations were considered. Many relations used to describe myocardium have been based on one of these forms (one notable exception is the pole-zero relation developed by Hunter et al. (1992)). When an anisotropic constitutive relation is based on one of the isotropic forms considered in this study, the anisotropic relation often uses a linear combination of components of the Green–Lagrange strain tensor, \( \mathbf{E} \), in the place of strain invariants. Such relations will behave similarly to an isotropic relation in terms of \( I_1 \), as both \( I_1 \) and components of \( \mathbf{E} \) are proportional to squared stretch ratios. On the other hand, many anisotropic relations incorporate products of strain components or other higher order terms, which will alter the material’s response to swelling. The same approach used in this study may be applied to these anisotropic relations to analyse their behaviour. The swelling deformation will be anisotropic, and the behaviour of the swollen material may be determined with respect to this anisotropic swollen state. Incorporating the anisotropic properties of tissue will be important for increasing the predictive ability of poroelastic simulations, and this is a natural next step in developing further cardiac poroelastic constitutive relations.

In the simulations presented in this study, a quasistatic poroelastic formulation was used that describes the steady-state behaviour of the tissue once fluid has been allowed to equilibrate to a constant pressure. This model also represents quasistatic situations in which blood flow mechanics operate on a much faster timescale than tissue deformations. For some physiological deformations, such as the beating of the heart or rapid skeletal muscle contraction, the rate of tissue deformation is of a similar magnitude to the blood velocity. In such situations,
the resistance of blood vessels means that tissue compression or expansion will cause an increase or decrease in vascular pressure, respectively, and this will in turn affect the mechanics of the tissue. Modelling these situations would therefore require a dynamic poroelastic formulation. For the purpose of analysing the change in material behaviour in response to perfusion pressure, the static formulation presented here provides a useful simplification and is acceptable as a first approach. The simulations in this study could be repeated assuming an undrained situation where fluid cannot flow, or using a time-dependent formulation that allows various strain rates to be analysed.

5.4 CONCLUSION

When modelling perfused tissue with poroelasticity, it is important to consider the influence of perfusion pressure on the mechanics of the tissue. This is particularly important when modelling myocardium, where it has been shown that increasing perfusion pressure increases stiffness. In order to reproduce this behaviour, a strain-stiffening constitutive relation is required. Therefore, linear constitutive relations are not suitable for modelling cardiac tissue. Exponential constitutive relations are commonly used to model myocardium, as they reproduce the strain-stiffening behaviour of the tissue. These relations are also generally suitable for reproducing pressure-driven stiffening behaviour, provided the exponential terms are volume dependent.

This study only considered isotropic constitutive relations, which do not accurately represent vascularised myocardium or skeletal muscle. In Chapter 6, poroelastic models that account for anisotropy in the vasculature are presented. An anisotropic poroelastic model is then used to model passive inflation of the left ventricle of the heart in Chapter 7, and simulation results are compared to results from canine heart experiments.
ANISOTROPIC Poroelasticity for Vascularised Tissues

Aspects of this chapter were presented at the 12th International Symposium on Computer Methods in Biomechanics and Biomedical Engineering (2014).

In Chapter 5, it was demonstrated that a poroelastic model can produce stiffening of tissues with an increase in perfusion pressure. In that chapter, only isotropic constitutive relations based on an effective stress formulation were considered. However, May-Newman et al. (1994) showed that myocardium swells and stiffens anisotropically when perfused. In Chapter 4, a representative volume element (RVE) model was used to demonstrate that the anisotropic swelling and stiffening of myocardium is mainly due to anisotropy in the constitutive behaviour of the solid component. However, anisotropy in the vascular structure of the RVE did have a significant influence on how the RVE deformed as vascular fluid pressure increased.

In this chapter, approaches for poroelastic modelling of tissues with anisotropic vasculature are investigated. The responses of anisotropic poroelastic models to perfusion pressure changes were compared to results from RVE models, to determine which poroelastic models are most suitable for describing myocardium and other vascularised tissues.

6.1 Validity of an Effective Stress Formulation

The validity of an effective stress formulation for modelling tissues with an incompressible matrix material and anisotropic vascular structure was verified using the RVE model developed in Chapter 4. The effective stress assumption states that the mixture stress is equal to an effective stress that depends only on deformation, minus an isotropic stress tensor equal to the fluid pressure (Equation 5.20):

\[ \sigma = \sigma_{\text{eff}} - p \mathbf{I} \]
In Chapter 4, it was shown that an RVE model with anisotropic vascular structure swells anisotropically as pressure increases. Therefore, one might assume that an effective stress formulation is not appropriate for representing tissues that have anisotropic vascular structure. On the other hand, Buhan et al. (1998) demonstrated that an effective stress formulation is valid for the case of an incompressible solid component. The derivation of the effective stress equation made no assumptions about the pore geometry. As the solid component of a poroelastic tissue model consists mostly of cells that contain incompressible cytosol, the matrix material can be assumed to be incompressible. Therefore, an effective stress assumption should be valid for representing biological tissues with anisotropic vasculature. If the RVE model of vascularised tissue does match an effective stress formulation, then constraining the external geometry of the RVE and increasing fluid pressure should lead to stresses on the external faces that are equal to a constant offset due to deformation, minus the fluid pressure, based on Equation 5.20.

Figure 6.1 presents results from a constrained pressurisation simulation using the RVE model with geometry based on myocardial vasculature and the anisotropic

![Graph](image_url)

Figure 6.1. External face stress against internal pressure during constrained pressurisation of the RVE model with anisotropic vascular structure. The external face normal to the $x$ direction was extended by a ratio of 1.05, the face normal to the $y$ direction was held in plane, and the face normal to the $z$ direction was compressed by a ratio of 1.05$^{-1}$. The face stresses are an area weighted sum of the solid and fluid stresses. At zero fluid pressure, this deformation resulted in tensile stress in the $x$ direction and compressive stresses in the $y$ and $z$ directions. The decreases in face stresses as fluid pressure increases are equal to the fluid pressure, demonstrating that the RVE model matches an effective stress formulation.
Holzapfel–Ogden (2009) constitutive relation for the matrix material. The RVE geometry for this model had a dimension ratio of 0.652, representing a structure with vessels predominantly aligned in the $x$ direction but with some vessels oriented transversely. Parameters for the matrix constitutive relation were based on experimental data from shear experiments, as described in Section 4.3. The RVE was deformed by fixing the external faces at defined extension ratios. The fluid pressure was then increased from 0 kPa to 3 kPa. Stresses on the external faces were calculated as an area weighted average of the solid and fluid stresses. The results show that the RVE model exactly produced an effective stress response, as stresses on the three external faces were all equal to a constant offset caused by the deformation, minus the isotropic fluid pressure. This behaviour was verified using RVE models with a range of dimension ratios, and all RVE models exhibited an effective stress response when using an incompressible constitutive relation for the solid component.

From this result, it can be concluded that poroelastic models of vascularised tissue should use an effective stress formulation when the tissue surrounding the vessels is incompressible. Therefore, the anisotropic swelling response of tissues should be modelled using the Helmholtz free energy density function for the skeleton, which, due to the effective stress assumption, is only a function of the skeleton deformation. To validate the assumption of an incompressible solid component for specific tissue types, constrained pressurisation tests can be performed on tissue samples.

This result also demonstrates that it is not appropriate to consider that vascular fluid pressure acting normal to vessel wall surfaces imparts an anisotropic stress on the overall tissue. Instead, anisotropic vascular structure modulates the constitutive behaviour of a tissue, such that isotropic pressure increases cause anisotropic swelling deformations.

### 6.2 Constitutive Relations for Anisotropic Poroelasticity

Poroelastic constitutive relations for an effective stress model generally have the form:

$$
Ψ_{e}(E) = Ψ_{d}(E) + Ψ_{b}(J)
$$

where $Ψ_{e}$ is the effective Helmholtz free energy density of the skeleton, $Ψ_{d}$ is the distortional strain energy, $Ψ_{b}$ is the bulk strain energy, $E$ is the Green–Lagrange strain tensor in the material coordinate system, and $J$ is the determinant of the deformation gradient tensor. $Ψ_{d}$ may be anisotropic to describe materials where the response to stress is direction dependent, and may be based on strain energy func-
tions developed for finite deformation elasticity models, such as the constitutive relations of Guccione et al. (1991) and Holzapfel and Ogden (2009). $\Psi_b$ determines how strongly the material opposes volume changes. Common forms of $\Psi_b$ are $\Psi_b = K(J - 1)^2$, and $\Psi_b = K(J - 1 - \ln J)$, where $K$ is a bulk modulus parameter. These isotropic forms of $\Psi_b$ have a minimum strain energy of zero at $J = 1$, where volume in the deformed (current) configuration is equal to the volume in the reference state.

Chapter 5 showed that the form of $\Psi_d$ is important for determining how the mechanical behaviour of a poroelastic model changes as a material swells. Strain stiffening forms of $\Psi_d$ result in increased stiffness with an increase in fluid pressure.

A combination of an anisotropic form of $\Psi_d$ and an isotropic $\Psi_b$ is suitable for modelling tissues with anisotropic stress–strain behaviour, but cannot accurately model the behaviour of tissues with anisotropic vascular structure. For example, Figure 6.2 compares the response to uniaxial extension at zero fluid pressure to the deformation with increasing fluid pressure, using the RVE model developed in Chapter 4. The RVE model contains three orthogonal vessels, with one vessel oriented in the axial direction, and two transverse vessels. The anisotropy of the RVE model was prescribed by setting the RVE dimension ratio ($r_d$), where $r_d = 1$ represents an isotropic structure, and $r_d = 0$ represents a fully anisotropic structure,

![Figure 6.2](image.png)

Figure 6.2. Comparison of the response to uniaxial extension and internal fluid pressure for the RVE model with anisotropic vascular structure ($r_d = 0.652$) and isotropic constitutive behaviour (Equation 6.2). On the left, stress against extension ratio is plotted for uniaxial extension at zero fluid pressure, for extensions in the axial and transverse directions. Both directions show a very similar response to extension. On the right, the stretch ratios in the axial and transverse directions are plotted against fluid pressure, for swelling simulations where vascular fluid pressure was increased without applying external boundary conditions. In this case, the RVE model’s response was much more anisotropic.
containing a single vessel oriented in the axial direction. The simulations presented in Figure 6.2 were based on an RVE geometry with \( r_d = 0.652 \) and an isotropic exponential constitutive relation for the matrix material, defined as:

\[
\Psi = \frac{c_1}{2c_2} \left( e^{c_2(\sigma_1-3)} - 1 \right)
\]  

(6.2)

The parameters \( c_1 \) and \( c_2 \) were estimated based on shear data from myocardium, as described in Section 4.3. The estimated parameters were \( c_1 = 4.32 \) kPa, and \( c_2 = 5.84 \).

The uniaxial stress–strain response of this RVE model is highly isotropic. The anisotropic internal vascular structure does not significantly affect its response to uniaxial extension, and the axial direction is only slightly stiffer than the transverse direction. However, the deformation with increasing fluid pressure is anisotropic; transverse directions swell significantly more than the axial direction. Figure 4.10 showed that for models with a higher degree of vascular anisotropy, an increase in perfusion pressure could result in a contraction in the axial direction.

In order to model this difference in swelling and distortional behaviour, new forms of constitutive relations were investigated. \( \Psi_b \) was considered to be a function of \( \mathbf{E} \), rather than \( J \), to allow the incorporation of anisotropy in the swelling behaviour of a material:

\[
\Psi_e(\mathbf{E}) = \Psi_d(\mathbf{E}) + \Psi_b(\mathbf{E})
\]  

(6.3)

To ensure physically appropriate and stable mechanical behaviour, \( \Psi_b \) should be a convex function of \( \mathbf{E} \), with a minimum at \( \mathbf{E} = 0 \). In this study, three different forms of \( \Psi_b \) were considered, which were labelled A, B and C, and are shown in Equations 6.4, 6.5 and 6.6, respectively. These three constitutive relations are based on the idea that the anisotropic vascular structure of tissue leads to differences in the strength of coupling between particular directions.

Relation A considers:

\[
\Psi_b = b_1 \left( C_{22}C_{33} - \frac{1}{C_{11}} \right)^2 + b_2 \left( C_{11}C_{33} - \frac{1}{C_{22}} \right)^2 + b_3 \left( C_{11}C_{22} - \frac{1}{C_{33}} \right)^2
\]  

(6.4)

where \( b_1, b_2, \) and \( b_3 \) are constitutive parameters with units of stiffness, and \( \mathbf{C} \) is the right-Cauchy deformation tensor aligned with the material coordinate system. This strain energy relation has three terms, where each term represents the coupling between two orthogonal directions in the material coordinate system. For example, the first term is minimised when an extension or contraction in the 2 direction is
balanced by contraction or extension, respectively, in the 3 direction.

To show how these terms relate to volume changes, the case where the material coordinate system is aligned with the principal directions of deformation can be considered. In this situation, off-diagonal shear components are zero and the Jacobian is given by \((C_{11} C_{22} C_{33})^{\frac{1}{2}}\). The three terms in Relation A are then minimised when \(J = 1\). A limitation of this relation is that it does not consider the contribution of off-diagonal, shear components of the strain tensor to volume changes.

Relation B is defined as:

\[
\Psi_b = K (J - 1)^2 + b_2 (C_{11} C_{33} (J - 1))^2 + b_3 (C_{11} C_{22} (J - 1))^2
\]  

(6.5)

where \(K\), \(b_2\), and \(b_3\) are constitutive parameters with units of stiffness. This relation is based on a similar approach to that of Relation A, but includes a \(J - 1\) term, which accounts for the contribution of shear strain components to volume change. This also means that the \(C_{11} C_{33}\) and \(C_{11} C_{22}\) based terms only contribute to the strain energy when \(J \neq 1\), so may have benefits for parameter estimation by decoupling \(\Psi_d\) and \(\Psi_b\) parameters. The first term is an isotropic term based on \(J\), rather than using a \(b_1\) term, such that this constitutive relation can reduce to a single isotropic term for isotropically swelling materials. This form is designed to represent materials where the coupling between the 2 and 3 directions is weakest, such that the \(b_2\) and \(b_3\) terms allow for additional coupling strength between the 1 direction and the 3 and 2 directions, respectively.

Relation C is defined as:

\[
\Psi_b = K (J_a - 1)^2 \\
J_a = (1 + 2b_1 E_{11})(1 + 2b_2 E_{22})(1 + 2b_3 E_{33})
\]  

(6.6)

where \(K\) is a bulk modulus parameter with units of stiffness and \(b_1\), \(b_2\), and \(b_3\) are dimensionless constitutive parameters between zero and one. Relation C has a very different form to the other two relations, and is based on a decomposition of the Jacobian. For cases where the material coordinate system is aligned with the principal directions of the deformation, the squared Jacobian is given by:

\[
J^2 = C_{11} C_{22} C_{33} = (1 + 2E_{11})(1 + 2E_{22})(1 + 2E_{33})
\]  

(6.7)

In an isotropic form of \(\Psi_b\), extension in any direction is balanced by contraction of the other two directions, and vice-versa, in order to minimise volume changes.
Relation C uses an anisotropic form of \( J \), denoted \( J_\alpha \), that introduces direction coupling parameters \( b_1, b_2 \) and \( b_3 \). When \( b_1 = b_2 = b_3 = 1 \), and off-diagonal strain terms are zero, this model is equivalent to \( \Psi_b = K (J^2 - 1)^2 \). Setting a parameter \( b_i \) to less than one reduces the strength of coupling between the \( i \) direction and the two directions orthogonal to the \( i \) direction.

### 6.3 Model Comparison Methods

Relations A, B and C were evaluated on their ability to reproduce the behaviour of the RVE model from Chapter 4. Although there is some experimental data available, such as that of May-Newman et al. (1994), comparison to these data is made complicated by the complex geometry and anisotropic constitutive behaviour of tissue. Therefore, the RVE data provide a reasonable alternative for determining the suitability of these continuum level constitutive models for modelling anisotropically swelling materials. Chapter 7 investigates whether an anisotropic poroelastic model is able to accurately reproduce the behaviour of perfused myocardium.

The RVE model used for poroelastic model evaluation had a transversely isotropic structure, with a dimension ratio of \( r_d = 0.652 \), and used the isotropic exponential constitutive relation (Equation 6.2) to model the matrix material.

For each form of \( \Psi_b \) tested, \( \Psi_d \) was set to the same isotropic exponential form used to represent the solid component of the RVE model (Equation 6.2). Because this choice for \( \Psi_d \) results in an isotropic stress at zero strain, this was accounted for by setting the hydrostatic pressure to be non-zero in the reference configuration, \( p_0 = c_1 \), and modifying the effective stress formulation according to Equation 5.36:

\[
\sigma = \sigma_{\text{eff}} - (p + p_0)I
\]

Parameters for both \( \Psi_d \) and \( \Psi_b \) were estimated for each form of \( \Psi_b \) based on three sets of simulation data from the RVE model. The first set of simulation data was the deformation with an increase in fluid pressure from 0 kPa to 10 kPa, in 50 increments. This evaluated the ability of the constitutive relation to produce anisotropic swelling deformations.

The next set of data was stress against strain for uniaxial extension, in both the axial and transverse directions, at pressures from 0 kPa to 10 kPa in increments of 2 kPa. The axial direction refers to the preferred vessel direction of the RVE. For each pressure and direction, extensions up to a ratio of 1.1 based on lengths in
The swollen geometry were applied in 20 increments. This evaluated how well the constitutive relation could reproduce the pressure-driven stiffening behaviour of the RVE model.

The third set of data considered was the deformation in directions orthogonal to the extension direction, when applying uniaxial extension in the axial and transverse directions. This was used to characterise how well the volume conservation of the poroelastic model matched that of the RVE model under extension. Because the RVE model contained vessels, which could change their volume, the overall RVE was compressible. The directions orthogonal to the extension direction are the two transverse directions for extension in the axial direction, and the axial direction and the other transverse direction for extension in one of the transverse directions.

The three sets of simulation data from the RVE model all represent homogeneous deformations at the continuum level, so simulations using the poroelastic models were performed with a single element, finite element model, with linear interpolation of the geometric field and a constant fluid pressure.

Because the RVE geometry was transversely isotropic, the constitutive parameters for each form of $\Psi_b$ were constrained so that $b_2 = b_3$. For Relation C (Equation 6.6), the use of a bulk modulus parameter $K$ and three $b_i$ parameters introduces redundancy, so $b_1$ was fixed at a value of 1. Lower bounds of zero were applied on all parameters.

In order to combine the three sets of simulation data for parameter estimation without biasing results towards a particular simulation, the errors in the fit for each simulation were calculated using stretch values. Therefore, for the uniaxial extension simulations, tensile stress boundary conditions were specified in the extension direction, and the resulting extension ratio was compared to that produced by the RVE model.

When comparing the orthogonal stretches under uniaxial extension, however, displacement boundary conditions were applied in the extension direction. This was done to decouple the orthogonal stretch for a particular extension simulation from the stress–strain behaviour of the poroelastic model.

Because each set of simulation data contained a different number of data points, parameters were estimated by minimising a combined residual, which was the sum of squares of the root mean square (RMS) residual from each set of simulation data. This ensured that each type of deformation had equal weighting in the parameter.
estimation process. The sum of squares was defined as:

\[ S = \sum_{i=1}^{3} r_i^2 \]

\[ r_i = \sqrt{\frac{1}{N_i \sum_{j=1}^{N_i} Z_{ij}^2}} \]

(6.8)

where \( r_i \) is the RMS error for the \( i \)th set of simulation data, \( N_i \) is the number of data points in simulation \( i \), and \( Z_{ij} \) is the difference between the RVE simulation and poroelastic simulation for experiment \( i \) and data point \( j \).

### 6.4 Results

Parameters estimated for each of the constitutive relations are given in Table 6.1, and the RMS errors for each set of simulation data are shown in Table 6.2. The parameters estimated for \( c_1 \) and \( c_2 \) from the isotropic exponential constitutive relation used for \( \Psi_d \) slightly differ for each form of \( \Psi_b \), due to interaction with the bulk terms. For Relation A, the value estimated for \( b_1 \) was zero, while \( b_2 \) and \( b_3 \) were positive. Similarly for Relation B, the value estimated for \( K \) was zero, with positive \( b_2 \) and \( b_3 \) values. This indicates that coupling between the axial direction and each of the transverse directions is stronger than the coupling between the two transverse directions, and that the coupling between the transverse directions is accounted for by the form of \( \Psi_d \) and the \( b_2 \) and \( b_3 \) terms, without the \( b_1 \) and \( K \) terms being required for Relation A and Relation B, respectively. For Relation C, the value for \( b_2 \) and \( b_3 \) that best reproduced the RVE data was 0.897, meaning that the transverse directions (2 and 3) are less tightly coupled to their orthogonal directions than the axial direction, as \( b_1 \) was fixed at 1.0.

Table 6.2 shows that Relation B best reproduced the swelling deformation and uni-axial extension behaviour of the RVE model, but did not reproduce the orthogonal deformations as well as Relation C. Relation A performed the most poorly for all

<table>
<thead>
<tr>
<th>Relation</th>
<th>( c_1 ) (kPa)</th>
<th>( c_2 )</th>
<th>( K ) (kPa)</th>
<th>( b_1 )</th>
<th>( b_2, b_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.17</td>
<td>3.06</td>
<td>0.0 kPa</td>
<td>1.93 kPa</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.26</td>
<td>2.87</td>
<td>0.0</td>
<td>4.89 kPa</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.37</td>
<td>2.76</td>
<td>3.85</td>
<td>1.0</td>
<td>0.897</td>
</tr>
</tbody>
</table>
Table 6.2. Comparison of dimensionless RMS errors in stretch ratios when comparing simulation results from poroelastic models to results from the RVE model.

<table>
<thead>
<tr>
<th>Relation</th>
<th>Swelling deformation</th>
<th>Uniaxial extension</th>
<th>Orthogonal deformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$6.22 \times 10^{-3}$</td>
<td>$2.64 \times 10^{-3}$</td>
<td>$2.65 \times 10^{-3}$</td>
</tr>
<tr>
<td>B</td>
<td>$5.02 \times 10^{-3}$</td>
<td>$1.46 \times 10^{-3}$</td>
<td>$2.36 \times 10^{-3}$</td>
</tr>
<tr>
<td>C</td>
<td>$5.28 \times 10^{-3}$</td>
<td>$2.06 \times 10^{-3}$</td>
<td>$1.89 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

three simulation measures. Figures 6.3, 6.4 and 6.5 compare the RVE behaviour to simulations using Relations A, B and C, respectively.

All three relations produced anisotropic deformation with increasing perfusion pressure, and greater stiffening in the transverse directions than the axial direction. However, none of the relations accurately captured the shape of the perfusion deformation.

All three constitutive relations produced greater orthogonal direction contraction under uniaxial extension than the RVE model. This means that they overestimated the bulk stiffness of the porous material, and predicted less volume change with uniaxial extension.

For extension in the axial direction, both transverse directions responded the same. However, for extension in either of the transverse directions, the transversely isotropic behaviour of the model means that the response in the two directions orthogonal to extension can differ. Despite this anisotropy, the RVE simulations and all three poroelastic relations showed very similar contractions in the orthogonal transverse and axial directions for extension in a transverse direction.

The identifiability of parameters for each constitutive relation was assessed using the Hessian matrix at the optimal solution ($\mathbf{H}_0$), which is a matrix of second-order derivatives with respect to the estimated constitutive parameters, and describes the curvature of the residual function. The condition number of the Hessian is the ratio of its smallest and largest eigenvalues, and describes the eccentricity of the residual function with respect to the constitutive parameters. The scaled Hessian matrix ($\tilde{\mathbf{H}}_0$) describes how parameters interact (Nathanson and Saidel, 1985), and is defined as:

$$\tilde{H}_{ij} = \frac{H_{ij}}{\sqrt{H_{ii}H_{jj}}} \quad \text{(no implied summation)} \quad (6.9)$$

Large off-diagonal terms in this scaled Hessian matrix indicate coupling between parameters. The determinant of the scaled Hessian, known as the M-optimality,
Figure 6.3. Comparison between poroelastic Relation A and RVE simulation results. The top plot shows deformation with increasing fluid pressure. The two centre plots compare uniaxial extension at a range of fluid pressures, in both the axial and transverse directions. The lower two plots show the contraction in directions orthogonal to the extension direction for extensions in the axial direction (left) and a transverse direction (right), at zero vascular perfusion pressure.
Figure 6.4. Comparison between poroelastic Relation B and RVE simulation results. The top plot shows deformation with increasing fluid pressure. The two centre plots compare uniaxial extension at a range of fluid pressures, in both the axial and transverse directions. The lower two plots show the contraction in directions orthogonal to the extension direction for extensions in the axial direction (left) and a transverse direction (right), at zero vascular perfusion pressure.
Figure 6.5. Comparison between poroelastic Relation C and RVE simulation results. The top plot shows deformation with increasing fluid pressure. The two centre plots compare uniaxial extension at a range of fluid pressures, in both the axial and transverse directions. The lower two plots show the contraction in directions orthogonal to the extension direction for extensions in the axial direction (left) and a transverse direction (right), at zero vascular perfusion pressure.
provides a scalar measure of the parameter coupling. A determinant of one represents no coupling, while determinants closer to zero indicate a large degree of parameter interaction.

The Hessian condition numbers, scaled Hessians, and determinants of the scaled Hessians are given in Table 6.3 for each form of constitutive relation. Relation A is the most well conditioned and has the greatest M-optimality value, which means there is low interaction between parameters. All three relations show strong interactions between \( c_1 \) and \( c_2 \), by the large value in the first row and second column of the scaled Hessian. The parameters of \( \Psi_b \) also interact with \( \Psi_d \) in each constitutive relation. Relation A shows low interaction between \( b_1 \) and \( b_2 \) (row 3, column 4), while Relation C shows low interaction between \( K \) and \( b_2 \) (row 3, column 4). This indicates that the anisotropy of the bulk behaviour is well identified by the bulk parameters and the simulation data used for parameter estimation. However, Relation B shows strong interaction between \( K \) and \( b_2 \) (row 3, column 4), which is likely due to both of these parameters multiplying terms containing \( J^{-1} \).

Because the RVE results in Chapter 4 predicted that highly anisotropic vascular geometries could produce contraction in the axial direction with increasing fluid pressure, it was of interest to determine whether the three poroelastic constitutive relations investigated in this study could also produce this behaviour. Therefore, the parameter estimation procedure was repeated using simulation results from the fully anisotropic RVE model where \( r_d = 0.0 \), which contains only a single vessel oriented in the axial direction and represents tissue with parallel vessels.

Table 6.3. Comparison of parameter identifiability criteria for the three anisotropic poroelastic constitutive relations.

<table>
<thead>
<tr>
<th>Relation</th>
<th>( \text{cond}(\mathbf{H}_0) )</th>
<th>( \text{det}(\mathbf{H}_0) )</th>
<th>( \mathbf{H}_0 )</th>
</tr>
</thead>
</table>
| A        | \( 4.13 \times 10^{-3} \)    | \( 19.8 \times 10^{-3} \)    | \[ 
1.0 0.960 0.220 0.360
1.0 0.418 0.214 0.206
1.0 0.206 1.0
\] |
| B        | \( 0.145 \times 10^{-3} \)   | \( 8.48 \times 10^{-3} \)    | \[ 
1.0 0.971 0.277 0.408
1.0 0.123 0.308 0.818
1.0 0.818 1.0
\] |
| C        | \( 0.235 \times 10^{-3} \)   | \( 8.17 \times 10^{-3} \)    | \[ 
1.0 0.984 0.367 0.00640
1.0 0.263 0.105 0.0216
1.0 0.105 1.0
\] |
Table 6.4 lists the parameters estimated based on the fully anisotropic RVE simulations, and Table 6.5 lists the RMS residuals for each set of simulation data. Plots of the perfusion deformation predicted by each poroelastic constitutive relation are shown in Figure 6.6.

For Relations A and B, the estimated values of $b_1$ and $K$, respectively, were again zero. Figure 6.6 shows that both these relations underestimate the degree of axial direction contraction. This indicates that the coupling between the axial direction and transverse directions is too strong, even without including the $b_1$ or $K$ terms. Relation A can be seen to have terms similar to $J^2 - 1$, and Relation B includes $J - 1$ terms. These terms introduce additional coupling between all directions and are the likely cause of this response. The isotropic exponential in $I_1$ used for $\Psi_d$ also introduces some coupling between orthogonal directions. Relations A and B also do a poor job of capturing the initial shape of the deformation against pressure curves. The RVE simulations showed different initial gradients in the axial and transverse deformations. However, for Relations A and B, the axial and transverse directions both initially deform at the same rate as pressure increases from zero. The approach used by Relation C, with an anisotropic decomposition of $J$, appears to be much more suitable for reproducing highly anisotropic perfusion deformations.

Table 6.4. Parameters estimated for each of the anisotropic poroelastic models, based on the fully anisotropic RVE model.

<table>
<thead>
<tr>
<th>Relation</th>
<th>$c_1$ (kPa)</th>
<th>$c_2$</th>
<th>$K$ (kPa)</th>
<th>$b_1$</th>
<th>$b_2, b_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.02</td>
<td>0.865</td>
<td>0.0 kPa</td>
<td>2.72 kPa</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.2</td>
<td>0.451</td>
<td>0.0</td>
<td>6.04 kPa</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.27</td>
<td>2.45</td>
<td>10.1</td>
<td>1.0</td>
<td>0.721</td>
</tr>
</tbody>
</table>

Table 6.5. Comparison of dimensionless RMS errors for each constitutive relation when comparing simulation results from poroelastic models to results from the fully anisotropic RVE model.

<table>
<thead>
<tr>
<th>Relation</th>
<th>Swelling deformation</th>
<th>Uniaxial extension</th>
<th>Orthogonal deformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$30.0 \times 10^{-3}$</td>
<td>$16.1 \times 10^{-3}$</td>
<td>$2.14 \times 10^{-3}$</td>
</tr>
<tr>
<td>B</td>
<td>$30.5 \times 10^{-3}$</td>
<td>$19.0 \times 10^{-3}$</td>
<td>$2.66 \times 10^{-3}$</td>
</tr>
<tr>
<td>C</td>
<td>$5.19 \times 10^{-3}$</td>
<td>$11.4 \times 10^{-3}$</td>
<td>$5.58 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
Figure 6.6. Comparison of the perfusion deformation behaviour of the three poroelastic constitutive relations to the RVE model with fully anisotropic geometry. The RVE model showed extension in the transverse directions and contraction in the axial direction with increasing fluid pressure. Only Relation C was able to reproduce this response with reasonable accuracy.

6.5 DISCUSSION

Poroelastic constitutive relations for modelling anisotropic swelling behaviour in vascularised tissues were investigated. Three relations were considered, and all three could produce anisotropic swelling and stiffening behaviour. Of the three relations studied, Relation B (Equation 6.5) was best able to reproduce the behaviour of an RVE model of tissue microstructure that had vascular anisotropy representing myocardium. This constitutive relation also has the advantage of reducing to a
standard isotropic relation in terms of $J$ for isotropic materials. However, Relation B did exhibit slightly greater coupling between parameters, which was evident in the low M-optimality value and was likely due to the use of similar terms based on $J−1$. This relation was also unable to reproduce the behaviour of a fully anisotropic RVE model representing tissue with parallel vessels, which only Relation C could reproduce with reasonable accuracy.

In this study, isotropic forms of $\Psi_d$, describing the distortional behaviour of a material, were combined with anisotropic forms of $\Psi_b$ that describe the bulk behaviour of a material. Many tissues have both anisotropic vascular structure and an anisotropic stress-strain response. For such tissues, it would be appropriate for both $\Psi_d$ and $\Psi_b$ to be anisotropic. $\Psi_d$ would then also contribute to the anisotropic swelling response of a tissue, as stiffer directions would oppose swelling more strongly. This was demonstrated with the RVE model in Chapter 4.

It is important to note that the form of $\Psi_d$ used in the poroelastic models did not necessarily need to match the constitutive relation used to describe the solid component of the RVE, as this distortional strain energy at the continuum level is influenced by the microstructural geometry. In this study, it was found that the same isotropic exponential relation used to define the constitutive behaviour of the RVE model solid component (Equation 6.2) reproduced the behaviour of the porous skeleton at a continuum level reasonably well. However, the presence of vessels in the RVE geometry reduces the coupling between directions, and the isotropic exponential relation in $I_1$ has strong coupling between directions, which was evident in the estimated values of 0 for the $b_1$ and $K$ parameters in Relations A and B, respectively. Therefore, other forms of constitutive relation could also be considered, such as separate exponential terms for the strain in each orthogonal direction.

No previous study has investigated how to incorporate anisotropic swelling behaviour in a poroelastic constitutive relation. An anisotropic form of linear poroelasticity was developed by Biot (1955), but this model represents compressible materials so is not suitable for modelling most tissues. Poroelastic models are currently being used for modelling myocardium and other vascularised tissues, but rely on isotropic bulk constitutive behaviour. As experimental studies have shown anisotropic swelling and stiffening of myocardium, it is important to be able to accurately account for this behaviour in poroelastic models of myocardium.

An RVE model could be used to directly model tissues at a continuum scale by using a homogenisation approach, such as in Rohan et al. (2006). However, this approach
has very high computational cost, so it is desirable to investigate constitutive relations that can approximate the behaviour of tissue with anisotropic vasculature. The constitutive relations presented in this study provide a promising first approach for modelling anisotropic bulk behaviour of tissues. Further study into constitutive modelling of bulk tissue behaviour is necessary to improve the ability of these models to represent tissues with highly anisotropic vascular structure.

The relations in this study were evaluated based on simulation results from an RVE model. It is debatable how accurately this RVE model represents the microstructural behaviour of vascularised tissue, as the geometric structure of this model is greatly simplified compared to actual tissue microstructure. The tissue components were lumped into a single solid component, and the vasculature was represented by a set of three orthogonal vessels. For simplicity, the RVE model used to evaluate anisotropic poroelastic relations used an isotropic constitutive relation for the solid component. This avoided conflating the effect of constitutive anisotropy with geometric anisotropy. Although parameters for the isotropic RVE constitutive relation were estimated from experimental data, only shear data at zero pressure was used, so it is uncertain how accurately the RVE represents the swelling behaviour of tissue. Therefore, it can only be concluded from this study that these anisotropic poroelastic models can reproduce the behaviour of the RVE model, and further study is required to determine if these models are capable of describing tissues with anisotropic vasculature. Chapter 7 applies an anisotropic poroelastic constitutive relation to modelling perfusion experiments in the left ventricle of the heart.

6.6 Conclusion

Constitutive relations for modelling anisotropic swelling behaviour in vascularised tissues were investigated. Relation B (Equation 6.5) was identified as a promising approach, but has limitations for modelling highly anisotropic structures. Nevertheless, this relation may be useful for modelling the anisotropic swelling and stiffening behaviour of myocardium. This is investigated further in Chapter 7 and Appendix C, using a static poroelastic model of the left ventricle of the heart during passive inflation.
This chapter presents an investigation into whether a poroelastic model can reproduce the effect of perfusion pressure on the passive mechanics of the left ventricle.

May-Newman et al. (1994) found that myocardium swells and stiffens in directions transverse to the muscle fibre direction when perfused. It was hypothesised that this anisotropic swelling and stiffening response was due to anisotropy in vascular structure, which was quantified in a histological study by (May-Newman et al., 1995). May-Newman and McCulloch (1998) then developed an approach for modelling the swelling and stiffening of myocardium using finite deformation elasticity, with a pre-stretch based on perfusion pressure and vascular compliance (see Section 2.3.3 for a more detailed description). However, this model did not account for the influence of surrounding tissue on vessel inflation, which may play a significant role in tissue swelling behaviour as discussed in Chapter 4. Poroelastic models of cardiac tissue have been shown to be useful for modelling flow through the microcirculation, and can be coupled to explicit-geometry models of flow in larger vessels (Cookson et al., 2012; Michler et al., 2013). They can also produce experimentally observed flow features such as the greater degree of systolic flow inhibition in the subendocardium compared to the subepicardium (Chapelle et al., 2010). It has not yet been demonstrated that such models can reproduce the anisotropic swelling and stiffening behaviour observed by May-Newman et al. (1994).

Huyghe et al. (1991) presented a poroelastic model of the left ventricle using a viscoelastic and anisotropic constitutive relation, and showed that increased blood volume in the coronary vasculature stiffened the ventricle, which led to an increase in the ventricular pressure required to fill the ventricle to a given volume. However, the local deformation due to perfusion and local stiffness changes within the ventricular wall were not considered. Yang and Taber (1991) found that poroelasticity can reproduce viscoelastic behaviours exhibited by myocardium, and Yang et al. (1994) developed a poroelastic model of the embryonic heart.
More recently, Chapelle et al. (2010) developed a poroelastic model of the left ventricle that included the active contraction of muscle fibres. The isotropic Mooney–Rivlin constitutive relation (Mooney, 1940; Rivlin, 1947) was used to describe the passive mechanics of myocardium, and fibre contraction contributed a directional stress along the myocardial fibre axes. Arterial inflow and outflow were modelled with distributed sources and sinks, respectively. Despite the simplified geometry and constitutive behaviour used in this model, it was able to reproduce complex flow features such as the increased arterial flow during diastole, increased venous flow during systole, and the greater effect of contraction on flow in the subendocardium compared to the subepicardium.

Cookson et al. (2012) developed a multi-compartment, poroelastic model of the whole heart, which accounted for the separate perfusion regions in the heart and flow in separate levels of the vascular hierarchy. This model produced a stiffening of the ventricle as perfusion pressure was increased, but the perfusion-induced deformation was found to be largely in the longitudinal (apex to base) direction, in contrast with the predominantly radial swelling observed experimentally, which corresponds to a thickening of the ventricular wall (May-Newman et al., 1994). It was suggested that this discrepancy between the model and experimental results could be due to the isotropic constitutive relation used to represent the myocardium.

The results presented in Chapter 4 suggested that the constitutive anisotropy of microstructural components was the most significant factor controlling the anisotropic swelling and stiffening behaviour of myocardium. It was also found, however, that anisotropy in vascular structure can influence swelling behaviour. Chapter 6 presented poroelastic models of vascularised tissue that were developed to describe anisotropic swelling behaviour independent of stress–strain anisotropy. For the present chapter, the left ventricle was modelled using a poroelastic constitutive relation with anisotropic distortional strain energy and isotropic bulk strain energy. A model using both anisotropic distortional strain energy and anisotropic bulk strain energy was also investigated to determine whether this approach could better reproduce the swelling behaviour of myocardium, compared to a model using a constitutive relation that only accounts for anisotropy in the distortional strain energy.

7.1 ISOLATED PERFUSED HEART EXPERIMENTS

May-Newman et al. (1994) used an isolated, perfused dog heart preparation to investigate the influence of perfusion pressure on the passive mechanics of my-
ocardiurn. Figure 7.1 illustrates their experimental setup. Columns of radiopaque beads were embedded within the free wall of the left ventricle, up to a depth of 70% through the ventricular wall. These beads were imaged in two planes with an x-ray imaging system. From the bead positions, the full Green–Lagrange strain tensor was calculated at a range of depths. Fibre orientations were measured from fixed tissue samples taken after the experiments were completed. This enabled estimates of strain tensor components with respect to the fibre direction, the radial direction, and a cross-fibre direction orthogonal to both the fibre and radial directions.

The perfusion pressure within the myocardium was altered by controlling the fluid pressure in the aorta at the level of the coronary ostia, off of which the coronary arteries branch. The left ventricular cavity volume was controlled with a volume infusion pump, and the cavity pressure was measured.

Ventricular cavity inflation cycles were performed at vascular perfusion pressures of 0 kPa, 7 kPa, 11 kPa, and 15 kPa. The perfusion strain was quantified as the

![Diagram of isolated perfused heart setup](image-url)

Figure 7.1. Isolated perfused heart setup used by May-Newman et al. (1994). Perfusate was pumped into the coronary arteries at a controlled pressure and the left ventricular volume was controlled with an infusion pump. A bi-plane x-ray imaging system was used to measure the positions of the radiopaque lead beads in anterior–posterior (AP) and lateral (LAT) views. Adapted with permission from May-Newman et al. (1994).
strain from zero to 15 kPa vascular perfusion pressure, while the ventricular cavity pressure was held at zero. The inflation strain is defined as the strain caused by inflating the left ventricular cavity while maintaining a constant perfusion pressure. The inflation strains at perfusion pressures of 0 kPa and 15 kPa were reported for a left ventricular inflation pressure of 1.3 kPa. For the perfused inflation experiment, inflation strains were quantified with respect to the ventricular geometry in the perfused state at zero ventricular pressure.

Figure 7.2 plots the $E_{ff}$, $E_{cc}$, and $E_{rr}$ components of the Green–Lagrange strain tensor across the myocardial wall, when inflating the left ventricular cavity to a pressure of 1.3 kPa. The response with a perfusion pressure of 15 kPa is compared to that of the unperfused heart. The fibre strain did not substantially change between the perfused and unperfused states. However, strains in both the cross-fibre and radial directions were decreased for the perfused state, suggesting increased stiffness in these directions.

Figure 7.2. Green–Lagrange strains across the ventricular wall for left ventricle inflation, comparing the response of the unperfused heart to the heart perfused at a perfusion pressure of 15 kPa (May-Newman et al., 1994). Axial strains are given for the fibre ($ff$), cross-fibre ($cc$) and radial ($rr$) directions. A depth of 0% corresponds to the epicardium, and 100% corresponds to the endocardium. Strain values are the mean value from eight experiments. Decreased strain is apparent for the perfused left ventricle in the cross-fibre and radial directions.
7.2 Methods

7.2.1 Geometry

An axisymmetric prolate spheroid model was used to represent the left ventricular geometry. This prolate spheroidal geometry can be described using prolate spheroidal coordinates, with components $\lambda$, $\mu$ and $\theta$. These correspond to positions in a Cartesian coordinate system through the equations:

$$
x = d \sinh(\lambda) \sin(\mu) \cos(\theta)
$$

$$
y = d \sinh(\lambda) \sin(\mu) \sin(\theta)
$$

$$
z = d \cosh(\lambda) \cos(\mu)
$$

where $d$ is the focus (a property of the coordinate system), $\lambda$ is a non-dimensional coordinate representing distance from the origin, $\mu$ is a longitudinal angle in the interval $[0, \pi]$ and $\theta$ is a circumferential angle in the interval $[0, 2\pi)$. The $z$ Cartesian coordinate is oriented along the ventricular long axis, and the $x$ and $y$ coordinates are oriented radially and define the short axis plane. Figure 7.3 illustrates how these prolate spheroidal coordinates vary on the left ventricle model.

Following Costa et al. (1996), the focal distance for the ventricular model was set to 37.5 mm, the ventricular base was represented by truncating $\mu$ at $\frac{3\pi}{2}$, the

![Figure 7.3](image_url)

Figure 7.3. The prolate spheroidal coordinate system used for the left ventricular geometry, showing how the prolate spheroidal coordinates $\lambda$, $\mu$ and $\theta$ vary in the ventricular model compared to Cartesian coordinates $x$, $y$ and $z$. The focus, $d$, is shown at the square marker as a distance from the origin.
endocardial surface was positioned at $\lambda = 0.38$, and the epicardial surface was at $\lambda = 0.69$.

The finite element mesh for the reference geometry is shown in Figure 7.4. Cubic Hermite interpolation was used to represent the geometric fields for the reference and deformed configurations. Linear Lagrange interpolation was used to represent the perfusion pressure field, as well as the variations in fibre angles and constitutive parameters. The use of cubic Hermite interpolation for the geometric field ensures that strains are continuous across element boundaries. Elements at the apex of the heart had degrees of freedom constrained to collapse the faces at the apex.

The fibre angle was assumed to vary from $-45^\circ$ at the epicardium to $90^\circ$ at the endocardium, based on the measurements of myocardial fibre orientation reported...
by May-Newman et al. (1994). Strains were calculated across the ventricular wall at \( \mu = 1.16 \) and \( \theta = 0 \), to match the location at which strains were measured experimentally (Figure 7.4, green spheres).

This is a simplified geometric model of the left ventricle, and more accurate models of the cardiac geometry and fibrous structure have been developed, for example, see Nielsen et al. (1991). The present model does not aim to accurately reproduce the mechanics of the entire heart, but rather the general anisotropic swelling and stiffening behaviour of myocardium. May-Newman and McCulloch (1998) showed that a model with a similar geometry could be used to reproduce this behaviour. The present study aims to improve on that model by using a poroelastic approach that accounts for the effect of surrounding tissue on the swelling deformation.

### 7.2.2 Boundary Conditions

For all simulations, the epicardial nodes at the base of the heart were fixed in position and had derivatives with respect to the circumferential element coordinate fixed. This boundary condition represents the effect of the stiff anulus fibrosus cordis, which anchors the valves that separate the left ventricle from the left atria and aorta.

For perfused simulations, fluid pressure was applied as a constant, fixed pressure across the entire cardiac domain. This approach does not account for the pressure gradient from arteries through to veins, which would require a multi-compartment poroelastic model to reproduce. The fluid pressures applied were set to match the coronary artery pressure prescribed in experiments, and are thus likely to overestimate the actual pressures within the vasculature.

For ventricular cavity inflation simulations, left ventricular pressure was applied using pressure boundary conditions on the endocardial surface in the surface normal direction.

### 7.2.3 Constitutive Properties

The constitutive behaviour of myocardium was described using a compressible form of the transversely isotropic relation developed by Guccione et al. (1991), with an effective stress assumption used to define the effect of fluid pressure on the mixture
stress. The strain energy density of the poroelastic skeleton is given by:

\[
\psi_e = \frac{c_0}{2} (e^Q - 1) + K (J - 1)^2 \\
Q = 2c_1 (E_{ff} + E_{cc} + E_{rr}) + c_2 E_{ff}^2 \\
+ c_3 (E_{cc}^2 + E_{rr}^2 + E_{cr}^2 + E_{rc}^2) + c_4 (E_{fc}^2 + E_{cf}^2 + E_{fr}^2 + E_{rf}^2)
\]

(7.2)

where \( c_0, c_1, c_2, c_3, c_4 \) and \( K \) are material parameters to be determined, and \( E \) is the Green–Lagrange strain tensor aligned with the myocardial fibre (f), cross-fibre (c) and radial (r) directions. Chapter 5 demonstrated that a strain-stiffening strain energy function is required for pressure-driven stiffening to occur, and that the strain-stiffening terms must be volume dependent. The Guccione (1991) strain energy function meets these requirements as the gradients with respect to individual strain components will increase as the material swells and these strain components increase.

The bulk modulus parameter, \( K \), was defined to vary linearly across the ventricular wall, from \( K_{epi} \) at the epicardium to \( K_{endo} \) at the endocardium. A transmural variation in bulk modulus was required to enable the experimentally observed difference in capillary volume fraction between the epicardium and endocardium to be represented (May-Newman et al., 1995). Similarly, May-Newman and McCulloch (1998) applied a variation in vessel compliance across the myocardial wall in their left ventricle model. In that approach, an explicit vessel compliance term was used, whereas in this poroelastic model, the swelling deformation is controlled by the strain energy function for the mixture.

### 7.2.4 Mesh Convergence

A mesh convergence analysis was performed to determine a computationally efficient finite element mesh size. Inflation simulations at a myocardial perfusion pressure of 15 kPa and a ventricular cavity pressure of 1.3 kPa were performed using the set of constitutive parameters listed in Table 7.1, which were identified using the approach described in Section 7.2.5. The inflation strains at the ventricular mid-wall with a series of refined meshes were compared to the strain in the most refined mesh, which had 2060 degrees of freedom, including geometric and fluid pressure degrees of freedom. For the selected mesh, all strain components were within \( 2 \times 10^{-3} \) of the most refined mesh. The selected mesh contained 1324 degrees of freedom in total, and is shown in Figure 7.4. This mesh had three elements across the ventricular wall, four elements around the circumference, and three elements in the longitudinal direction.
7.2.5 Parameter Estimation

Parameters for the strain energy density function were estimated using the lsqnonlin routine from Matlab’s (version R2012b, The MathWorks, Inc) optimisation toolbox, which minimises the sum of squares of an objective function using a trust-region reflective algorithm (Nocedal and Wright, 2006).

Three sets of transmural strain measurements from May-Newman et al. (1994) were used to form the objective function. These were the strain due to increasing perfusion pressure from zero to 15 kPa with zero ventricular pressure, the strain due to applying a ventricular cavity pressure of 1.3 kPa with no perfusion pressure, and the strain due to inflating the ventricle to a ventricular cavity pressure of 1.3 kPa with a perfusion pressure of 15 kPa.

Each set of strain measurements included the six unique components of the Green–Lagrange strain tensor at eight positions, from 1% to 70% transmural depth below the epicardial surface, as shown in Figure 7.4.

Because only static perfusion was considered, the permeability tensor had no influence on the solution and was set to the identity tensor.

7.3 RESULTS

The constitutive parameters estimated for the strain energy function are shown in Table 7.1. The estimated value for $K_{\text{endo}}$ was less than that for $K_{\text{epi}}$, which matches the expected increase in vascular compliance towards the endocardium.

Figure 7.5 plots the simulated perfusion deformation strains compared to those measured experimentally. The perfusion deformation is the deformation caused by increasing perfusion pressure from zero to 15 kPa, with zero ventricular cavity pressure. Perfusion caused stretch predominantly in the myocardial radial direction, which corresponds to a thickening of the ventricular wall. The experimentally measured radial strain increased from the epicardium to the endocardium. This

Table 7.1. Constitutive parameters estimated for the myocardial strain energy function (Equation 7.2).

<table>
<thead>
<tr>
<th>$c_0$ (kPa)</th>
<th>$c_1$</th>
<th>$c_2$</th>
<th>$c_3$</th>
<th>$c_4$</th>
<th>$K_{\text{epi}}$ (kPa)</th>
<th>$K_{\text{endo}}$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0358</td>
<td>0.240</td>
<td>239</td>
<td>8.45</td>
<td>19.3</td>
<td>78.6</td>
<td>46.0</td>
</tr>
</tbody>
</table>
Figure 7.5. Simulated perfusion strain compared to experimental data. Perfusion strain is the strain caused by increasing perfusion pressure from zero to 15 kPa at zero ventricular pressure. Axial strains in the myocardial fibre, cross-fibre and radial directions were well reproduced, but the smaller magnitude shear strains were not. Small discontinuities in the strain gradient are evident at element boundaries.
strain gradient was well reproduced by the poroelastic simulation due to the linear variation in the bulk modulus parameter.

The cross-fibre perfusion strain was predicted to vary from positive to negative towards the endocardium. However, there was no experimental data measured near the endocardium to confirm this behaviour. Interestingly, the experimental results showed similar perfusion strains in the myocardial fibre and cross-fibre directions, whereas the simulation showed less perfusion strain in the fibre direction due to its greater stiffness.

The poroelastic simulation poorly reproduced the shear strains caused by perfusion. However, these were relatively small compared to the axial strains. The experiments showed a significant, positive $E_{fr}$ strain, whereas the simulations predicted a smaller, negative $E_{fr}$ strain. May-Newman et al. (1994) hypothesised that this shear strain is caused by perfusion increasing vessel diameters, thereby unloading fibrous interconnections between vessels and muscle fibres, and releasing residual stress. This complex behaviour is not accounted for by the strain energy density function used in the present model (Equation 7.2), so the positive $E_{fr}$ strain was not produced. Models that account for the stress-free state of the left ventricle may be able to model this behaviour with an appropriate strain energy density function.

Figure 7.6 plots the simulated ventricular inflation strains across the myocardial wall, for unperfused inflation and inflation at a vascular perfusion pressure of 15 kPa. The simulated results are compared to measurements made by May-Newman et al. (1994). The poroelastic simulation generally reproduced the behaviour of the left ventricular wall, including the trends in strain across the myocardial wall. Decreased inflation strain with increased perfusion pressure is evident for all strain components, indicating stiffening in all directions. The changes in strain in the myocardial cross-fibre and radial directions were less than the experimentally observed strain changes, but the fibre direction showed a greater reduction in strain than the experiments. The relative changes in average strain for the eight measurement points are compared in Table 7.2. These show that although the poroelastic model did a reasonable job of reproducing the anisotropic perfusion deformation of myocardium, the relative changes in stiffness caused by the perfusion were not accurately reproduced. The experimental results showed very little change in fibre direction strain, while strain changes in the myocardial cross-fibre and radial directions were over 20%. In contrast, the poroelastic model predicted a more isotropic reduction in strains.

Despite the fibre direction perfusion strains predicted by the poroelastic simulation
Figure 7.6. Comparison of perfused and unperfused left ventricular inflation strains, at a ventricular cavity pressure of 1.3 kPa. For the experimental perfused data, perfusion pressure was 15 kPa. For perfused simulations, a homogeneous perfusion pressure of 15 kPa was applied. Perfused inflation strains were quantified with respect to the perfused geometry at zero ventricular pressure.
Table 7.2. Comparison of the change in inflation strains with perfusion pressure between the experimental measurements and poroelastic simulation. Values were calculated as the mean percentage change in strain component values for the eight measurement positions, ± the standard deviation, when comparing perfused inflation to unperfused inflation.

<table>
<thead>
<tr>
<th></th>
<th>$E_{tt}$</th>
<th>$E_{cc}$</th>
<th>$E_{rr}$</th>
<th>$E_{fc}$</th>
<th>$E_{fr}$</th>
<th>$E_{cr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>$-3.6 \pm 7.1$</td>
<td>$-25.9 \pm 8.3$</td>
<td>$-24.6 \pm 5.6$</td>
<td>$21.5 \pm 101$</td>
<td>$-20.1 \pm 16.5$</td>
<td>$-17.3 \pm 110$</td>
</tr>
<tr>
<td>Simulation</td>
<td>$-14.6 \pm 3.3$</td>
<td>$-18.3 \pm 3.2$</td>
<td>$-12.0 \pm 3.2$</td>
<td>$-16.6 \pm 7.2$</td>
<td>$-25.8 \pm 5.6$</td>
<td>$-52.4 \pm 39.1$</td>
</tr>
</tbody>
</table>

being lower than those measured experimentally (Figure 7.5), the change in fibre direction inflation strains predicted by the model was much greater than the experimentally observed reduction. This indicates that the stress-strain response of the fibre direction may be more linear in the low-strain region than is predicted by the exponential strain energy relation (Equation 7.2).

The same approach used to estimate parameters for the transversely isotropic constitutive relation with an isotropic bulk term in Equation 7.2 was applied to a constitutive relation with the same distorsional strain energy, but an anisotropic bulk term, defined by Relation B from Chapter 6 (Equation 6.5). It was found that the set of constitutive parameters that best fits the combination of inflation strains and perfusion strains in a least-squares manner had values of zero for $b_2$ and $b_3$. These parameters multiply the anisotropic bulk terms, such that the model reduces to the case of an isotropic bulk term when these parameters are zero. The constitutive relation with an isotropic bulk term already predicted lower fibre strains for the perfusion deformation than the experimental results, and introducing the anisotropic bulk terms would reduce the fibre strains further, resulting in a poorer fit to the experimental data. The behaviour of the left ventricle model with the anisotropic bulk constitutive relation is investigated further in Appendix C.

7.4 Discussion

A poroelastic model of the left ventricle was developed and shown to reproduce the anisotropic swelling response of myocardium measured by May-Newman et al. (1994). This model also exhibited an increase in stiffness with perfusion pressure, but the proportional changes in inflation strain in each direction did not accurately match the strain changes measured experimentally. This indicates that the strain energy density function does not accurately describe how stiffness changes with swelling deformation.
Because the exponential strain energy density function (Equation 7.2) couples the response of strain in any direction to the strain in other directions, perfusion strains in one direction increase the stiffness in orthogonal directions. A separated exponential form of the strain energy function, such as that used by Schmid et al. (2006), reduces coupling between directions, and might better reproduce the anisotropic stiffening behaviour of myocardium. However, initial simulations have shown that a model using a separated exponential strain energy density function performs similarly to the model used in this study (see Appendix D). Therefore, it is apparent that further research into poroelastic constitutive relations for myocardium is necessary. The present constitutive model is an improvement over previously published poroelastic constitutive relations for myocardium, such as those applied by Cookson et al. (2012) and Chapelle et al. (2010), which both used isotropic strain energy functions.

A constitutive relation with anisotropic bulk strain energy terms based on Relation B from Chapter 6 was tested, but did not improve the ability of the model to reproduce the experimental results. Although RVE simulations in Chapter 4 suggested that the anisotropic swelling and stiffening behaviour of myocardium is predominantly due to anisotropy in the constitutive behaviour of microstructural components such as muscle fibres, it also showed that the anisotropic vascular structure has an additional effect on swelling behaviour. It may be the case that the separate effects of microstructural constitutive anisotropy and anisotropic vascular structure are both accounted for by the anisotropic distortional strain energy function that was used to describe myocardium, such that additional anisotropic bulk terms were not required. However, Chapter 6 showed that a material may show highly isotropic stress–strain behaviour in certain deformation modes, but swell anisotropically. Therefore the use of an anisotropic bulk strain energy term may be required for materials other than myocardium. The behaviour of the model with anisotropic bulk strain energy is investigated further in Appendix C.

The behaviour of this poroelastic model compares favourably to the model presented by May-Newman and McCulloch (1998), although that model better reproduced the relatively low change in fibre direction strain with ventricular inflation when increasing perfusion pressure. In contrast to the model developed by May-Newman and McCulloch (1998), the present poroelastic model does not define the perfusion deformation in terms of pressure explicitly. Instead, anisotropic swelling behaviour arises from the anisotropy of the constitutive relation for the poroelastic material. The RVE model presented in Chapter 4 suggested that the anisotropic swelling of myocardium is mainly due to the anisotropic constitutive behaviour of
its microstructural components, rather than its vascular structure, indicating that using the constitutive anisotropy of myocardium to control its swelling deformation is most appropriate. Further experimental studies into the swelling behaviour of different tissue types are necessary to provide a better understanding of the different effects of stress–strain anisotropy and anisotropy of vascular structure.

In this model, the solid component of the poroelastic mixture represents a variety of structures including myocytes, blood vessel walls, and collagen struts between myocytes and vessels. It is not yet clear which microstructural components are responsible for the pressure-driven stiffening behaviour of myocardium. It is well known that individual myocytes increase in stiffness as they are stretched axially (Fish et al., 1984). Weizsäcker et al. (1983) showed that blood vessels also exhibit strain-stiffening behaviour in the axial direction, and increase in axial stiffness as perfusion pressure increases. Blood vessels also stiffen in the transverse direction as their diameter increases (Bergel, 1961). However, it is uncertain whether the transverse stiffness of vessels contributes significantly to the overall stiffness of myocardium in response to ventricular inflation. Inflation of blood vessels may unload some collagen struts between vessels and fibres, but it is likely that inflation of vessels stretches and loads struts between nearby muscle fibres. Therefore, it is most likely that the transverse stiffening behaviour of myocardium with an increase in perfusion pressure is due to the stretching of transversely oriented collagen struts between muscle fibres.

In the constitutive relation applied in this model (Equation 7.2), the different microstructural components are not considered separately but are represented as a homogeneous material with a transversely isotropic strain energy density function. More advanced constitutive relations may be able to better reproduce the behaviour of myocardium by accounting for the different microstructural components, but such models are likely to be difficult to parameterise and validate experimentally.

The parameter estimation procedure found that a significant transmural gradient in the bulk modulus parameter, \( K \), was required to accurately reproduce the experimentally observed ventricular strains due to increased perfusion pressure. The bulk modulus at the epicardium was almost twice that at the endocardium. May-Newman et al. (1995) performed histological studies of myocardial capillaries and found a significant transmural gradient in capillary diameter, which increased from the epicardium to the endocardium. However, there was no statistically significant transmural trend in the change in capillary diameter with perfusion pressure. Perfusion strains were only measured to a depth of 70% by May-Newman et al. (1994), so it is not known whether perfusion strain continues to increase towards
the endocardium, as predicted by the poroelastic model in Figure 7.5. Therefore, the model results beyond a ventricular wall depth of 70% should not be assumed to represent the actual behaviour of myocardium.

The distortional strain energy function used to represent myocardium’s constitutive behaviour was based on a transversely isotropic relation developed by Guccione et al. (1991). More recent studies have found that myocardium is orthotropic, rather than transversely isotropic (Dokos et al., 2002), and that the myocardial sheet direction is stiffer than the myocardial normal direction. The inflation strains in the myocardial cross-fibre direction (Figure 7.6), which corresponds to the myocardial normal direction at the ventricular mid-wall, were underestimated with the poroelastic model. Therefore, an orthotropic constitutive relation may be able to better reproduce the ventricular inflation. However, the perfusion strains in the myocardial cross-fibre direction were greater at the epicardium than those measured experimentally (Figure 7.5). Using the orthotropic constitutive relation developed by Holzapfel and Ogden (2009) for the distortional strain energy did not improve the model fit (see Appendix D), but it is possible that this constitutive relation may be more appropriate with a more realistic sheet angle distribution.

One area where the poroelastic model did not perform well was in reproducing the shear response of the ventricular wall to cavity inflation and increased perfusion pressure. In particular, a large $E_{fr}$ strain was observed experimentally when perfusing the myocardium, but this was not predicted by the model. Reproducing this behaviour may require more complex constitutive relations to be developed that account for the interaction between vessels and muscle fibres, as well as accounting for the residual stress present in the reference state. However, the magnitudes of the shear strains measured experimentally were small compared to the axial strains, so accounting for this complex behaviour may not be necessary for many modelling applications.

One large simplification applied in this model was the use of a constant perfusion pressure field. In reality, the coronary circulation consists of a hierarchy of vessels, and pressure decreases from arteries through to veins. In-vivo cardiac perfusion pressures are cyclic and vary with the beating of the heart. In the experimental study conducted by May-Newman et al. (1994), perfusion pressure was held constant at the coronary arteries, but venous pressure was not controlled. Therefore, there was a static gradient of pressure from the coronary arteries through to the veins, and the average pressure within the coronary vasculature would have been lower than the arterial pressure. This means that the constant perfusion pressure applied in
this model overestimated the average experimental pressure, and the bulk modulus parameter will have been overestimated.

More detailed mechanical models of the perfused left ventricle could account for the vascular hierarchy using, for example, the method presented by Cookson et al. (2012), which defines multiple fluid compartments. This approach also allows the separate perfusion regions of the heart to be modelled. Dynamic changes in perfusion pressure throughout the cardiac cycle could also be modelled. This would provide more insight into whether in-vivo dynamic perfusion pressure changes may have a significant effect on cardiac mechanics. Chapter 4 showed that changes in mechanics are most significant at low perfusion pressures, and as perfusion pressure increases into the physiological range, the effect of pressure on stiffness and swelling becomes less significant due to the strain-stiffening behaviour of myocardium. This implies that the effect of normal perfusion pressure changes during the cardiac cycle may not be significant, but confirming this requires further experimental research.

A poroelastic model using a transversely isotropic distortional strain energy function and an isotropic bulk strain energy term was found to reproduce the swelling deformation of myocardium. This model also reproduced changes in stiffness in the myocardial cross-fibre and radial directions, but overestimated the increase in stiffness in the fibre direction.

Introducing anisotropic bulk strain energy terms did not improve the model’s ability to reproduce experimental results. The shortcomings in these poroelastic models indicate that further development of poroelastic constitutive relations for myocardium is required. Such developments may improve the predictive ability of cardiac models if these constitutive relations are experimentally validated.

Changes in stiffness with perfusion pressure were only indirectly observed in the experimental study of May-Newman et al. (1994), through the decrease in ventricular inflation strains for the perfused ventricle compared to the unperfused ventricle. Chapter 8 presents experiments where more direct stiffness measurements were performed on the rat tibialis anterior muscle.
8

MECHANICS OF THE PERFUSED
RAT TIBIALIS ANTERIOR

Aspects of this chapter were presented at the 7th World Congress of Biomechanics (2014).

8.1 INTRODUCTION

The experimental data available to study the influence of perfusion on tissue mechanics is currently limited. Gefen and Margulies (2004) performed compression experiments on perfused and unperfused porcine brain tissue, and Wildhaber et al. (1998) studied the effect of perfusion in lung tissue. These are both isotropic and relatively linear tissues, so although these studies provide valuable insight into the mechanics of perfused tissue, they do not cover the more complex case of an anisotropic tissue with anisotropic vasculature. Physical phantoms provide a complementary approach that can provide additional experimental data. However, as demonstrated in Chapter 3, producing a phantom that is suitable for mechanical testing and behaves similarly to tissue when perfused is a difficult task.

May-Newman et al. (1994) carried out mechanical experiments on perfused dog hearts and found that myocardium swells and stiffens anisotropically. Chapter 7 showed that a poroelastic model that accounts for myocardium’s stress–strain anisotropy can reproduce the anisotropic swelling behaviour. However, the ability to interpret these experimental results is limited by the complex geometry and microstructure of the heart. Experiments on tissues with a simpler geometry that maintain the nonlinearity and anisotropy present in myocardium may provide more insight into the behaviour of vascularised tissue.

The tibialis anterior (TA) of the rat, shown in Figure 8.1, is one such alternative experimental preparation that was considered to be useful for investigating the mechanics of perfused tissue. This muscle is positioned at the anterior of the lower hind-limb and acts to dorsiflex the foot. Its proximal end is attached to the proximal
end of the tibia and its distal end inserts at the first metatarsal bone. The TA is fusiform, meaning its fibres are aligned with the muscle axis. Therefore, anisotropy in its mechanical response with respect to the fibre orientation can be analysed. This simple fibre geometry also means that it is straightforward to construct a computational biomechanics model of the TA. Capillary orientation within the TA is highly anisotropic; its capillaries are predominantly aligned with the muscle fibre direction (Takahara et al., 1996). This high degree of anisotropy is thought to be a consequence of the large proportion of fast-twitch muscle fibres in the TA; it contains approximately 67% type-2B fibres (Takahara et al., 1996). Slow-twitch muscles have a higher demand for oxygen so require a higher capillary density, resulting in a more tortuous and isotropic arrangement of capillaries. Comparing experimental results from the TA to those obtained from muscle with more isotropic vasculature may provide insight into the influence of capillary anisotropy on the mechanics of perfused tissue.

Previous studies have shown that the TA can be tested mechanically while maintaining its connection to the vascular system. The arteries and veins transporting blood in and out of the TA enter at its proximal end, near the knee, allowing its distal end to be extracted. Bosboom et al. (2001) performed transverse compression experiments on the TA, and Shin et al. (2008) and Ramírez et al. (2010) investigated the active mechanics of the TA by stimulating it via the sciatic nerve while measuring the produced axial force. These studies used anaesthetised rats and the TA was perfused by the rat’s own heart and vascular system.

Perfused rat hind-limb preparations provide a method for controlling the perfusion pressure of the rat hind-limb muscles. A perfused whole hind-quarter preparation
was first presented by Ruderman et al. (1971) for studying muscle metabolism. In this preparation the aorta and inferior vena cava were cannulated above the common iliac vessels. This method has since been modified and extended to a wide range of perfused muscle preparations, which were reviewed by Baker and Hepple (2005).

In this study, the mechanics of the rat TA muscle were investigated while varying perfusion pressure using a perfused single hind-limb preparation. Both axial extension and transverse indentation experiments were performed.

8.2 methods

8.2.1 Experiment Preparation

All rats used in this study were male Wistar rats, obtained post-mortem from other studies. These other studies involved extraction of the heart and lungs immediately post-mortem and were conducted in accordance with protocols approved by the University of Auckland Animal Ethics Committee (approval numbers R787, R925, R939, and R1057). Prior to death, rats were anaesthetised with isoflurane and peritoneally injected with heparin (1000 IU kg$^{-1}$). After 15 minutes, the rats were killed by cervical dislocation. The right common iliac artery was immediately exposed and cannulated, as illustrated in Figure 8.2. The artery was then perfused with a modified Tyrode’s solution as described below. The solution pH was adjusted to 7.4 with the addition of Tris. Initial perfusion of the TA was generally achieved within ten minutes from the time of death.

The perfusate solution was based on a modified Tyrode’s solution (Table 8.1). Polyethylene glycol was added to act as an osmotic agent and prevent the movement of perfusate across vessel walls, limiting oedema formation. Heparin was used to prevent clot formation, and 2,3-butanedione monoxime (BDM) was used to prevent contraction of the skeletal muscle fibres. The L-type calcium channel blocker, nifedipine, was used to prevent the contraction of smooth muscle within blood vessel walls, thereby inhibiting the muscle’s myogenic contraction in response to increased perfusion pressure, and allowing vessels to expand as fluid pressure increased. Studies in myocardium have indicated that the effect of perfusion on mechanics is limited unless the myogenic contraction of vascular smooth muscle is prevented (Westerhof et al., 2006). When myogenic autoregulation is not inhibited, an increase in perfusion pressure results in the contraction of smooth-muscle cells
in the vasculature, which limits the deformation induced in surrounding the tissue. Nifedipine is insoluble in water, therefore a concentrated solution of 2.89 mM nifedipine in ethanol was prepared and mixed into the perfusate as required. Care was taken to avoid exposure of nifedipine to light due to its photosensitivity.

After cannulation of the common iliac vessels, the anterior skin of the right lower limb was removed and the epimysium sheath surrounding the TA was removed. The muscle was kept moist by dripping the perfusate solution onto its surface throughout all experiments.

Perfusion pressure was controlled using a precision pressure regulator (IR1000-01, SMC) connected to a compressed air supply to pressurise the perfusate reservoir.

Table 8.1. Concentrations of solutes in the modified Tyrode’s solution used to perfuse the rat hind limb.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Molar mass (g mol(^{-1}))</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>58.44</td>
<td>130 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>74.55</td>
<td>5.4 mM</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>95.21</td>
<td>1 mM</td>
</tr>
<tr>
<td>NaH(_2)PO(_4)</td>
<td>156.01</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>HEPES</td>
<td>238.31</td>
<td>10 mM</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>110.98</td>
<td>20 mM</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>346.34</td>
<td>0.6 µM</td>
</tr>
<tr>
<td>Heparin</td>
<td></td>
<td>5000 IU L(^{-1})</td>
</tr>
<tr>
<td>2,3-BDM</td>
<td>101.11</td>
<td>20 mM</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>≈ 20 000</td>
<td>30 g L(^{-1})</td>
</tr>
</tbody>
</table>
Pressure was measured at the arterial input using a pressure sensor (24PCBFA6G, Honeywell). This configuration was used to provide a constant perfusion pressure, in contrast to the cyclic pressure produced by standard roller pumps.

8.2.2 Mechanical Testing Device

In order to measure the mechanical properties of perfused skeletal muscle, a mechanical testing device was designed and constructed. The device was designed for both axial extension and transverse indentation experiments. Figure 8.3 illustrates the use of this device when applied to axial extension of the rat TA muscle, and Figure 8.4 demonstrates the transverse indentation configuration. Dimensioned drawings of this device are provided in Appendix E.

The testing device uses a voice coil motor (LA15-16-024A, BEI Kimco Magnetics) to provide linear actuation. This motor drives a shaft that is constrained to only move axially by two flexure bearings, which were laser cut from 0.4 mm thick Tufnol phenolic fabric (RS Components Ltd). The flexure bearings also limited the axial displacement to prevent movements that could damage the device or tissue. A potentiometer (RDC1014, ALPS Electric) was used to measure the shaft displacement and a load cell was mounted at the end of the shaft to measure force. For extension experiments, the load cell used was an Entran® ELFS-T3E-10N capable of measuring both compressive and tensile forces from −10 N to 10 N.

Transverse indentation experiments were performed by modifying the testing device as shown in Figure 8.4. The load cell mounted at the end of the shaft was replaced with a compression load cell (LLB210, Futek), which has a maximum force of 44.5 N. The load cell capable of measuring both compression and tension was used to measure the tensile force constraining the foot of the rat.

Figure 8.3. Experimental rig demonstrating an axial extension test of the rat TA muscle.
The voice coil motor was driven by a dual power operational amplifier (PA75, Cirrus Logic), wired in a parallel configuration. The motor was operated with open-loop control by prescribing a voltage input signal, rather than controlling on displacement or force. The force applied by the motor acts on both the flexure bearings and the muscle, but only the muscle force was directly measured by the load cell.

The potentiometer and load cell signals were amplified using instrumentation amplifiers in a circuit mounted on the testing device. The amplified analogue signals were transmitted to a receiver module, where they were filtered to remove high frequency noise, before being transmitted to a National Instruments USB-6211 data acquisition module connected to a laptop computer. A user interface was developed using National Instruments LabVIEW (version 2011) for controlling the device while visualising and recording the output signals.

8.2.3 Experiment Protocol

Axial extension tests were performed on six rats, numbered 1 to 6, and transverse indentation tests were performed on eight different rats, numbered 7 to 14. The mean age of the rats was 66.6 days, with a standard deviation of 8.2 days. The mean
mass of the rats was 360.6 g, with a standard deviation of 43.1 g.

For each animal experiment, the perfusion pressure was alternated between 5 kPa and 20 kPa. At each perfusion pressure cycle, twenty load cycles were performed, but only data from the last ten cycles were analysed, to ensure that the response had equilibrated after each perfusion pressure change. This protocol is illustrated in Figure 8.5. At least eight sets of load cycles were performed for each animal. In half of the animal experiments, the first set of load cycles were performed at 5 kPa perfusion, and for the remaining experiments, the first cycles were at 20 kPa. This was to avoid any potential bias in the ordering of pressures. During each perfusion pressure cycle, the change in volume of the fluid reservoir was recorded to provide an approximate measure of volume flow rate.

Transverse indentation experiments were performed with the muscle attached at both ends, as illustrated in Figure 8.4. A circular indenter with 5 mm diameter was used. The maximum voltage applied to the voice coil motor was set to provide a maximum force of 0.6 N in initial test load cycles. This maximum voltage was then maintained for all subsequent load cycles. Linear voltage ramps were applied, with the maximum voltage held for 0.5 s before beginning to linearly decrease the voltage. The voltage ramping rate was set to 1 V s⁻¹, such that the average duration for a single loading and unloading cycle was 7.9 s.

Axial extension experiments were performed by detaching the muscle at its distal end and clamping the tendon to a force transducer, as illustrated in Figure 8.3. The proximal end of the muscle remained attached to maintain its vascular supply. The maximum voltage applied was again set to produce a maximum tensile force of 0.6 N in initial test load cycles. The same voltage ramping rate of 1 V s⁻¹ was applied, producing an average loading and unloading cycle duration of 8.8 s.

![Figure 8.5. Protocol for load and pressure cycles in extension and indentation experiments. Perfusion pressure was cycled between 5 kPa and 20 kPa. At each pressure cycle, twenty load cycles were performed, and data from the last ten cycles were analysed.](image)
8.3 Results

The mean TA mass, measured after experiments were completed, was 0.695 g, with a standard deviation of 0.078 g. The mean TA length with the foot plantarflexed was 30.7 mm, with a standard deviation of 1.3 mm.

The mean flow rate at a perfusion pressure of 5 kPa was 0.52 mL min\(^{-1}\), with a standard deviation of 0.14 mL min\(^{-1}\). The mean flow rate at 20 kPa perfusion pressure was 2.21 mL min\(^{-1}\), with a standard deviation of 0.72 mL min\(^{-1}\).

8.3.1 Axial Extension

Figure 8.6 plots ten successive load–displacement loops for axial extension of the TA. Displacement values quantify the motion of the tendon clamp relative to the initial tendon clamp position. Within one set of ten load–displacement cycles, the muscle response was consistent, and separate cycles could not be distinguished. A significant amount of hysteresis can be observed by the difference between the loading and unloading curves. On the other hand, the repeatability between cycles was good, as illustrated by the overlaying curves.

Figure 8.7 compares two individual load–displacement curves at 5 kPa and 20 kPa perfusion pressure. Both loops are taken from the final set of load cycles at their

![Figure 8.6](image_url)
Figure 8.7. Load versus displacement for axial extension of the TA muscle from Rat 2 at perfusion pressures of 5 kPa and 20 kPa. For each pressure, the final load cycle at that pressure is plotted. The 5 kPa trace (green) is obscured as the two traces overlap very closely.

The tangent stiffness and displacement at 0.1 N were measured for each load cycle, using the calculation procedure illustrated in Figure 8.8. A least-squares optimisation was performed to fit a line to the loads between 0.07 N and 0.13 N, on the region of the load–displacement loop where load is increasing. The residual vector for the optimisation was weighted by a Gaussian window according the load value, such that points near 0.1 N had the greatest contribution to the residual. The equation for the line has the form:

$$f = k(d - d_0)$$  \hspace{1cm} (8.1)

where $f$ is force in N, $k$ is stiffness in N m$^{-1}$, $d$ is displacement in metres and $d_0$ is the displacement intercept (also in metres). From the fitted line, the displacement at 0.1 N was calculated as:

$$d_{0.1 \text{N}} = d_0 + \frac{0.1}{k}$$  \hspace{1cm} (8.2)

The least-squares optimisation process was used to minimise the sum of squared weighted residuals, defined as:

$$S = \sum_{i=1}^{N} w_i r_i^2$$  \hspace{1cm} (8.3)
Figure 8.8. Procedure for quantifying the tangent stiffness and displacement at 0.1 N. The weighting function was applied to residuals in a least-squares optimisation. This example shows one load–displacement loop at 20 kPa perfusion.

where \( N \) is the number of data points within the load window, and \( r_i \) is the weighted residual for the \( i^{th} \) data point, given by:

\[
r_i = w(f_i) \left(f_i - k(d_i - d_0)\right)
\]  

(8.4)

where \( f_i \) and \( d_i \) are the load and displacement measured for the \( i^{th} \) data point, respectively, and \( w \) is the Gaussian weighting function:

\[
w(f) = e^{-\frac{1}{2} \left(\frac{f-0.1}{\sigma}\right)^2}
\]  

(8.5)

where \( \sigma \) is the standard deviation of the Gaussian, which was set to 0.015 N to ensure that data points near the centre of the load window were weighted much more strongly than points towards the edges of the window.

Figures 8.9 and 8.10 show box-and-whisker plots of tangent stiffnesses and displacements, respectively, at 0.1 N for axial extension experiments from Rat 2, which was representative of the results obtained from all rats. A small increase in tangent stiffness with set number was observed, but there was no apparent effect of perfusion pressure. The displacement at 0.1 N decreases with set number. Initially, the displacement decreased rapidly, but differences in displacement between later load sets were small. The decreases in displacement at 0.1 N with set number indicate
Figure 8.9. Tangent stiffness at 0.1 N for axial extension experiments from Rat 2, at each set of load cycles. Boxes show the interquartile range, and the centre line is the median. Whiskers extend to the minimum and maximum values within 1.5 times the interquartile range from the lower and upper quartile, respectively. Outliers are plotted with a + symbol.

Figure 8.10. Displacement at 0.1 N for axial extension experiments from Rat 2, at each set of load cycles. Displacements were quantified relative to the clamp position at 0.1 N for the first load cycle of the first set. Negative displacement represents movement away from the rat knee, indicating that the muscle slackened over time.
that the muscle was becoming more slack over time, which may have been due to damage at the proximal attachment site of the muscle, or rearrangement of the internal muscle structure caused by the repeated loading cycles.

The tangent stiffnesses and displacements at 0.1 N from all animal experiments were analysed using SAS (version 9.3, SAS Institute). A generalised linear model was fitted to both the tangent stiffness and displacement data, with each of these measured variables being explained by: the rat number; perfusion pressure; the set number, which is nested within pressure; and the load cycle, which is nested within both pressure and set number. Variations in the effect of pressure, set number and cycle number between rats were also included. Listing 8.1 shows the SAS program for the generalised linear model of tangent stiffness.

Analysis of variance (ANOVA) tests were performed to test whether pressure, set number or cycle number had a statistically significant effect on tangent stiffness or displacement at 0.1 N. Listing 8.1 includes the SAS statements for performing these ANOVA tests. Each test compares whether the variance explained by one variable, for example pressure, is significant compared to the variance in the response to pressure across different animals. Table 8.2 lists the results from the ANOVA tests. The F statistic is the variance between treatments, for example, different pressure groups, divided by the variance between the error term groups. Large F statistic values indicate that a large amount of the data variance is explained by the test variable. The p-value is the probability of obtaining the calculated F statistic, given that the null hypothesis is true. For example, in the ANOVA test for set number as a predictor of tangent stiffness, the null hypothesis is that set number has no effect

Listing 8.1. SAS procedure for analysing the tangent stiffness at 0.1 N from all TA muscle experiments.

```sas
proc glm data=pressureStiffness;
  class rat pressure setNumber cycle;
  model stiffness = pressure setNumber(pressure)
    cycle(pressure setNumber)
    rat rat*pressure rat*setNumber(pressure)
    rat*cycle(pressure setNumber);
  test h = pressure
    e = rat*pressure;
  test h = setNumber(pressure)
    e = rat*setNumber(pressure);
  test h = cycle(pressure setNumber)
    e = rat*cycle(pressure setNumber);
```
8.3 Results

Table 8.2. ANOVA results for axial extension experiments using the generalised linear models of tangent stiffness and displacement at 0.1 N.

<table>
<thead>
<tr>
<th>Test variable</th>
<th>Tangent stiffness at 0.1 N F statistic</th>
<th>p-value</th>
<th>Displacement at 0.1 N F statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>0.06</td>
<td>0.8256</td>
<td>2.86</td>
<td>0.1658</td>
</tr>
<tr>
<td>Set number</td>
<td>0.23</td>
<td>0.9984</td>
<td>19.93</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cycle number</td>
<td>0.88</td>
<td>0.8414</td>
<td>1.78</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

on tangent stiffness. A small p-value means that the null-hypothesis is unlikely, suggesting that set number does affect the tangent stiffness.

The results show no evidence that pressure, set number or cycle number had an effect on tangent stiffness at 0.1 N. There is also no evidence that pressure had an effect on displacement at 0.1 N. However, there is strong evidence that both set number and cycle number affected the displacement at 0.1 N. The significant effect of cycle number is not surprising, since the muscles slackened over time, as seen in the change in displacement at 0.1 N between set numbers. Although the effect of cycle number on displacement is statistically significant, the magnitude of the effect is small; the mean difference in displacement at 0.1 N between cycle one and ten was 0.011 mm with a standard deviation of 0.022 mm.

8.3.2 Transverse Indentation

Figure 8.11 plots ten successive load cycles for transverse indentation of the TA. The load–displacement response is consistent for all ten cycles and separate cycles cannot be distinguished. Similar to the axial extension experiments, there is a substantial amount of hysteresis. Displacement values quantify the motion of the indenter probe relative to the furthest probe position from the muscle surface. The probe initially starts above the muscle and comes into contact with the muscle surface part-way through its travel. The position of this surface contact point is difficult to identify due to the nonlinear load–displacement response and shallow toe of this curve.

Figure 8.12 plots two load–displacement curves at 5 kPa and 20 kPa perfusion pressure, where both loops are from the final set of load cycles at their respective perfusion pressures from one animal. There is no observable difference between the response at 5 kPa and at 20 kPa.

The tangent stiffness and displacement at 0.1 N were calculated using the same process as for the axial extension analyses. Figures 8.13 and 8.14 show box-and-
Figure 8.11. Load versus displacement for ten successive transverse indentation cycles of the TA muscle for Rat 13 muscle at a perfusion pressure of 20 kPa. Arrows indicate the loading (upward) and unloading (downward) directions.

Figure 8.12. Load versus displacement for transverse indentation of the TA muscle for Rat 13 at perfusion pressures of 5 kPa and 20 kPa. For each pressure, the final load cycle at that pressure is plotted. The 5 kPa trace is obscured as the two traces overlap closely.
whisker plots of all measured tangent stiffnesses and displacements, respectively, at 0.1 N for Rat 13, which was representative of most rats. Figure 8.13 shows that the tangent stiffnesses at 0.1 N were similar for all set numbers and pressures. In Figure 8.14, there is no apparent effect of perfusion pressure on displacement at 0.1 N. However, there was an initial decrease in displacement over time, followed by an increase in displacement. The initial decrease in displacement is consistent with multiple indentation cycles irreversibly deforming the tissue. The subsequent increase in displacement may have been due to slip of the muscle surface with respect to the indenter, or the formation of oedema.

The plots in Figures 8.12, 8.13 and 8.14 are representative of the results from most rats. However, three out of the eight rats did exhibit some noticeable, consistent change in the displacement at 0.1 N with perfusion pressure. These rats did not exhibit any change in tangent stiffness with perfusion pressure. Figures 8.15 and 8.16 plot the tangent stiffness at 0.1 N and displacement at 0.1 N, respectively, for Rat 12. These results clearly show an effect of both time and perfusion pressure on the displacement at 0.1 N. The displacement is greater at 20 kPa than at 5 kPa, indicating that the muscle had swelled transversely and its surface was closer to the indenter. The difference in the mean displacements at 0.1 N between the final load sets at 20 kPa and 5 kPa was 0.060 mm. A comparison between load-displacement curves at 5 kPa and 20 kPa is shown in Figure 8.17.

The approximate volume change corresponding to this displacement was calculated using a simple ellipse-based model of the TA cross-section, and assuming the change in displacement at 0.1 N was representative of the TA surface displacement due to perfusion. The dimensions of the major and minor axes of the ellipse in the unperfused state were set to 10.7 mm and 6.7 mm, respectively, based on micro-computed tomography scans of a TA from another rat. Assuming homogeneous transverse deformation and no change in the axial length of the TA, a surface displacement of 0.060 mm corresponds to a volume change of 1.8 %. This is much lower than volume changes measured in the myocardium by May-Newman et al. (1994) of 7 % to 15 %.

The same generalised linear model used for the extension results was used to analyse the tangent stiffness and displacement data for transverse indentation (Listing 8.1). Table 8.3 lists the results of the ANOVA tests. There is no evidence for an effect of pressure or cycle number on the tangent stiffness at 0.1 N. However, there is weak evidence for an effect of set number on stiffness, due to a small increase in stiffness over time.
Figure 8.13. Tangent stiffness at 0.1 N for transverse indentation experiments from Rat 13 at each set of load cycles.

Figure 8.14. Displacement at 0.1 N for transverse indentation experiments from Rat 13 at each set of load cycles. Displacements were quantified relative to the probe position at 0.1 N for the first load cycle of the first set. Greater displacement values indicate that the muscle had swelled and its surface had shifted outwards.
Figure 8.15. Tangent stiffness at 0.1 N for transverse indentation experiments from Rat 12 at each set of load cycles.

Figure 8.16. Displacement at 0.1 N for transverse indentation experiments from Rat 12 at each set of load cycles. Displacements were quantified relative to the probe position at 0.1 N for the first load cycle of the first set. Greater displacement values indicate that the muscle had swelled and its surface had shifted outwards.
There is evidence for an effect of pressure on the displacement at 0.1 N. Although most rats showed no visible effect of pressure, or inconsistent changes in displacement at 0.1 N between pressure cycles, three out of eight rats displayed a small amount of measurable transverse swelling, which was consistent with changes in perfusion pressure. The reason for this difference in response between rats is unclear, but may be due to slip between the indenter and the muscle surface causing shifts in displacement and obscuring the small effect of fluid pressure. The changes in perfusion pressure may also have affected the tissue structure without causing consistent swelling behaviour. There is no evidence for an effect of set number on displacement at 0.1 N, but strong evidence for an effect of cycle number. It is surprising that cycle number had a statistically significant effect on displacement at

<table>
<thead>
<tr>
<th>Test variable</th>
<th>Tangent stiffness at 0.1 N</th>
<th>Displacement at 0.1 N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F statistic</td>
<td>p-value</td>
</tr>
<tr>
<td>Pressure</td>
<td>0.94</td>
<td>0.369</td>
</tr>
<tr>
<td>Set number</td>
<td>1.9</td>
<td>0.0505</td>
</tr>
<tr>
<td>Cycle number</td>
<td>1.01</td>
<td>0.464</td>
</tr>
</tbody>
</table>
0.1 N, whereas set number did not, as both variables are proportional to the elapsed time, but over difference scales. Although displacement at 0.1 N consistently increased by a small amount with cycle number, the inconsistent changes between pressure cycles meant that any change in displacement at 0.1 N associated with the set number was not statistically significant.

## 8.4 Discussion

May-Newman et al. (1994) showed that myocardium swells and stiffens in response to perfusion pressure, predominantly in directions transverse to the muscle fibre direction. It was expected that pressure-driven stiffening would also be evident in skeletal muscle. The TA has a highly aligned vascular structure, so it was expected that this muscle would also swell and stiffen in the transverse fibre direction, similarly to myocardium. However, no stiffening was observed in the TA between perfusion pressures of 5 kPa and 20 kPa, either in axial extension experiments or transverse indentation experiments.

A possible explanation for the difference in behaviour between myocardium and the TA is the difference in their volume fraction of capillaries. It is expected that a higher capillary volume fraction would result in a tissue with greater volumetric compliance, which would allow more swelling with a perfusion pressure increase.

No study has measured the volume fraction of capillaries in the TA. However, Torrella et al. (2000) measured the density of capillaries in sections transverse to the muscle fibre direction in various regions of the muscle. Their results from the equatorial region are compared to similar studies on myocardium and the soleus muscle in Table 8.4. The soleus is included for comparison as it is predominantly composed of slow-twitch muscle fibres, so has a higher oxygen demand. There is not a substantial difference in capillary density between the fast-twitch TA and the soleus. However, myocardium has a significantly higher density of capillaries.

Table 8.4. Comparison of capillary densities in rat muscle. Densities were calculated by counting capillaries per unit area in sections taken transverse to the muscle fibre direction. Values are given as the mean ± the standard error of the mean.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Capillary density (mm(^{-2}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior</td>
<td>1,035 ± 57</td>
<td>Torrella et al. (2000)</td>
</tr>
<tr>
<td>Soleus</td>
<td>1,303 ± 76</td>
<td>Kano et al. (2000)</td>
</tr>
<tr>
<td>Myocardium</td>
<td>4,105 ± 318</td>
<td>Poole et al. (1992)</td>
</tr>
</tbody>
</table>
than either type of skeletal muscle. This higher capillary density suggests that the volume fraction of capillaries is greater in myocardium, which could result in greater swelling of myocardium as perfusion pressure increases.

Myocardium and skeletal muscle also have different microstructures. Fibres in skeletal muscle are bound into fascicles, whereas myocardium has a laminar sheet structure with fibrous connections between sheets. Myocardium also contains much smaller fibres than skeletal muscle (Sylvén et al., 1984). This difference in structure may also contribute to the different response of these tissues to changes in perfusion pressure.

It has been shown that autoregulation of vascular smooth muscle inhibits the effect of perfusion pressure on mechanics in myocardium. This was accounted for in these experiments by the inclusion of nifedipine in the perfusate. 2-3 BDM also has a vasodilatory effect (Otun et al., 1993). May-Newman et al. (1994) used both adenosine and nifedipine to perfuse myocardium. Both adenosine and nifedipine produce vasodilation, but adenosine has been shown to be a more effective vasodilator in skeletal muscle (Hill and Meininger, 1994). Therefore, using adenosine instead of, or in addition to, nifedipine may result in greater deformation with perfusion.

Poroelasticity has been used to model skeletal muscle and allows changes in blood perfusion with contraction to be modelled. Donkelaar et al. (2001) predicted a heterogeneous effect of contraction on blood perfusion in the rat gastrocnemius using a poroelastic model. Chapter 5 demonstrated that different forms of constitutive relations for poroelasticity can result in different responses to perfusion pressure. Strain-stiffening constitutive relations generally exhibit increased stiffness with a perfusion pressure increase, depending on the choice of parameters. Because skeletal muscle is a strain-stiffening material, it is appropriate to use a strain-stiffening constitutive relation to model its mechanics. However, care must be taken when selecting parameters to ensure that changes in perfusion pressure within physiological ranges do not lead to non-physiological changes in stiffness.

One limitation of this study was the use of displacement at 0.1 N as a measure of the amount of tissue swelling. An improvement to the experimental protocol would be to incorporate a displacement transducer that could measure the position of the muscle surface. A one-dimensional displacement transducer such as a laser interferometer would still be prone to errors introduced by transverse shifts in the muscle surface. Using a multiple-camera rig to image the full three-dimensional surface of a muscle would provide richer perfusion displacement data and would improve understanding of the effect of perfusion on tissue mechanics. Parker et al.
present such a system, and apply it to imaging the surface of silicone gel phantoms. This method relies on a speckle pattern applied to the gel surface. It is expected that although skeletal muscle has some surface detail, the regularity of the muscle surface means that a speckle pattern would also be required for high accuracy tracking of the muscle surface.

A further limitation of this study was the low number of experiments performed. There was some evidence of an effect of perfusion pressure on the displacement at 0.1 N when performing transverse indentation experiments. This was due to three rats showing a small, measurable effect of perfusion pressure, while the majority did not. Performing more experiments or improving the approach for measuring swelling deformation would provide greater certainty on whether changes in perfusion pressure within a physiological range causes measurable transverse displacements.

Interpreting the experimental data from this study is made more difficult because the fluid pressure within the TA is not known; only the pressure at the arterial cannulation point and the venous return, which was at atmospheric pressure, are known. Attempts were made to control the pressure across the entire vasculature within the lower limb by cannulating and blocking flow at the common iliac vein, but this could not be reliably achieved. Instead, the resistance of blood vessels could be used to predict the blood pressure within the TA for given arterial and venous pressures.

8.5 conclusion

The tibialis anterior does not exhibit an observable change in stiffness with perfusion pressure, within physiological pressure levels. However, a measurable change in surface displacement was observed between 5 kPa and 20 kPa perfusion pressure. This response is likely to be similar for all skeletal muscles. The low density of capillaries in skeletal muscle compared to myocardium is a likely explanation for the difference in the response to perfusion pressure between these two tissue types.

When modelling skeletal muscle as a poroelastic material, it is important to carefully select an appropriate constitutive relation and parameters, so that a non-physiological change in mechanical behaviour with perfusion pressure is not produced. Poroelastic constitutive relations suitable for describing myocardium may not be suitable for modelling skeletal muscle.
If blood flow is not an important outcome from a model, then skeletal muscle can be accurately modelled using incompressible finite deformation elasticity, without accounting for the effect of perfusion on mechanics.
9

CONCLUSIONS

The aims of this thesis were to improve understanding of how a tissue’s mechanical behaviour is influenced by internal fluids such as vascular blood, and to develop methods for modelling coupled solid and fluid mechanics at the whole-organ scale.

9.1 SUMMARY OF RESULTS

9.1.1 Experimental Studies

In Chapter 3, results from experiments on vascularised silicone gel phantoms were presented. Experiments on a perfused phantom with embedded wool yarn did not produce strain-stiffening or pressure-driven stiffening behaviour. This demonstrated that the initial low stiffness region in a strain-stiffening response is essential for producing pressure-driven stiffening behaviour. This also meant that the silicone phantom data were not suitable for validating tissue mechanics models. Therefore, results from a representative volume element model of tissue microstructure (Chapter 4), and data from previously published dog heart experiments were used for model evaluation.

In Chapter 8, the response of the rat tibialis anterior muscle to perfusion pressure was investigated. Increasing perfusion pressure from 5 kPa to 20 kPa caused a small transverse swelling, corresponding to a volume increase of approximately 1.8%. However, no change in stiffness with perfusion pressure was observed. It was hypothesised that the difference between the response of the tibialis anterior and the behaviour of myocardium is due to the much lower density of capillaries in skeletal muscle, which results in a lower bulk compliance.

9.1.2 Model Development

Approaches for modelling the effect of vascular fluid on tissue mechanics were investigated in Chapter 2. Modelling tissue as a poroelastic material was identified
as a promising approach that has been applied to modelling numerous tissue types and has proven to be well suited to modelling flow through the microcirculation.

In Chapter 4, a representative volume element of myocardial microstructure was developed. This used a simplified representation of vessels within a block of tissue, where the surrounding tissue was assumed to behave as a continuum with anisotropic constitutive behaviour. This model predicted that the anisotropic swelling and stiffening behaviour of myocardium is largely due to the anisotropy in the constitutive behaviour of solid tissue components, but that anisotropy in the vascular structure also contributes to its swelling deformation. There was found to be a significant change in mechanical behaviour between the unperfused state and low perfusion pressures, but for perfusion pressures above 2 kPa, the effect of increasing pressure further was less pronounced. This was a result of the strain-stiffening constitutive behaviour of myocardium.

Chapter 5 showed that poroelastic models could produce stiffening with increasing perfusion pressure, provided that the strain energy density function is strain-stiffening, and that the strain-stiffening terms increase as the material swells. This behaviour represents the underlying microstructural mechanics: swelling causes a stretch of strain-stiffening fibrous structures, which pushes them into a stiffer region of their stress–strain curve. Importantly, strain energy density functions that additively decompose the contributions of volumetric and distortional deformations are not appropriate for modelling poroelastic materials that are anisotropic or have a nonlinear stress–strain response.

Constitutive relations for poroelastic materials that have anisotropic vascular structure were investigated in Chapter 6. These were shown to do a reasonable job of reproducing the behaviour of a representative volume element model that had a solid component with isotropic constitutive behaviour, but an anisotropic vascular structure. Changes in the stress–strain response with perfusion pressure were reproduced well, but the accuracy with which swelling deformations were reproduced could be improved, particularly for the case of a highly anisotropic vascular structure.

In Chapter 7, it was demonstrated that poroelasticity can be used to model the deformation of the left ventricle caused by an increase in perfusion pressure. This model also produced changes in stiffness with perfusion pressure, but did not accurately reproduce the anisotropy in stiffness changes; the model predicted a more isotropic reduction in ventricular inflation strains than was observed experimentally. This demonstrated that poroelasticity shows promise for reproducing
the effect of perfusion pressure on tissue mechanics, but there is a need for further research into constitutive relations for representing myocardium as a poroelastic material.

9.2 FUTURE WORK

9.2.1 Experimental Studies

May-Newman et al. (1994) performed experiments on perfused myocardium and obtained detailed measurements of local strain in response to perfusion pressure changes. Although experiments on the rat tibialis anterior did not show any change in stiffness with perfusion pressure, and only negligible volume change, there are other tissues where the effect of fluid may be more significant. For example, lung tissue has been shown to stiffen as blood pressure increases (Peták et al., 2002; Wildhaber et al., 1998). However, these experiments only measured lung elastance using the change in lung volume in response to airway pressure changes. Making more detailed measurements of strain within lung tissue may provide more insight into how fluid pressure affects the airways. Tagged magnetic resonance imaging has been used to measure strain within lungs (Napadow et al., 2001), and could be applied to measuring the strain caused by vascular pressure changes.

Investigation into the effect of fluid pressure on breast mechanics may also be important for improving the accuracy of breast mechanics models. There is evidence that milk in mammary glands can alter breast mechanics, but no study has been performed to quantify this effect. Quantifying the effect of fluid pressure on mechanical properties of the breast in-vivo may be difficult, but progress is being made in this area. Babarenda Gamage et al. (2011) used the surface deformation of a heterogeneous phantom under gravity loading to estimate material properties of components within the phantom, with the aim of eventually using this method for estimating the properties of fat, muscle and skin in the breast.

9.2.2 Poroelastic Modelling

Although May-Newman et al. (1994) demonstrated large volume and stiffness increases with an increase in perfusion pressure, it is important to note that these experiments were performed on hearts with inhibited autoregulation. Studies on hearts with intact autoregulation have not exhibited significant changes in mechanics with perfusion pressure (Westerhof et al., 2006). This means that modellers
developing poroelastic models of the heart should be careful not to introduce non-physiological volume and stiffness changes with perfusion pressure. This may require the development of models that incorporate the effect of autoregulation, which could be achieved by varying constitutive parameters such as the bulk modulus according to the vascular pressure.

In Chapter 6, constitutive relations were developed for poroelastic materials that swell anisotropically due to having an anisotropic vascular structure. This general approach showed promise for more accurately reproducing the swelling and stiffening behaviour of myocardium, but the particular constitutive relations investigated did not improve the ability of a poroelastic model to reproduce the swelling and stiffening behaviour of the left ventricle. Further development of poroelastic constitutive relations for myocardium should consider how to better represent the influence of anisotropic vascular structure on the response of myocardium to perfusion pressure.

For realistically modelling blood flows in tissue, a multi-compartment method is required, as described in Cookson et al. (2012). In this thesis, the solid mechanics of the ventricle were the primary interest, so a model with only a single fluid compartment was used for simplicity. The constitutive relation applied to a single fluid compartment model of the left ventricle in Chapter 7 could also be applied to a multi-compartment model of the heart, in order to better reproduce the influence of perfusion pressure on mechanics. However, further investigation into the constitutive behaviour of perfused myocardium may consider whether pressures in different compartments, such as the capillaries and arteries, have different effects on the mechanics of the surrounding tissue, due to differences in vessel geometry and vessel wall mechanics.

This thesis focussed on one small area of the interaction between fluid and tissue mechanics, which was the mechanical effect of fluid pressure on the passive mechanics of tissue as a mixture. More complete models could build on this work to account for other interaction mechanisms such as the myogenic contraction of vessel walls and chemical signalling pathways that respond to fluid velocity.

9.3 IMPLICATIONS

The findings in this thesis are important for patient-specific heart modelling. The results indicate that constitutive parameters for heart models should be based on perfused, in-vivo experiments, rather than using unperfused tissue samples, as
there may be a significant difference between the mechanics of perfused and unper-
fused myocardium. However, the effect of perfusion pressure within physiological
perfusion ranges is less pronounced, so it may not be necessary to account for the
effect of normal variations in vascular pressure throughout the cardiac cycle.

Understanding the influence of fluid pressure on tissue mechanics has important
clinical implications. Pathologies involving perfusion changes, such as coronary
artery blockage, may be detectable through their influence on tissue mechanics.
Furthermore, understanding the influence of inflammation on muscle mechanics is
important for sports science, and head trauma treatment may benefit by improved
understanding of the role of swelling. The results presented in this thesis may
also be important to consider when developing heart models for patients with
pathological hypertension, which is often associated with heart failure.
METHODS FOR VASCULARISED PHANTOM CONSTRUCTION

In Chapter 3, a physical model, or phantom, of vascularised tissue was presented. This model was constructed using silicone gel with embedded silicone tubes. Wool yarn was used to provide a strain-stiffening element, similar to the mechanical function of fibres in biological tissue. In the process of developing this model, multiple approaches for constructing phantoms representing vascularised tissue were investigated. This appendix discusses some of these approaches.

A.1 OPEN-CELL FOAM PHANTOM

The first approach used to investigate the mechanics of porous materials was to develop a tissue phantom using a polyurethane open-cell foam. This was enveloped in 3M™ VHB tape to seal the external surfaces of the phantom. The phantom was then filled with water and attached to a mounting plate to allow controlling the fluid pressure.

Compression experiments were performed on this phantom using an Instron 5800 series testing device, as illustrated in Figure A.1. The 40 mm high phantom was compressed by 20 mm in 2 mm increments. After each displacement increment the position was held for at least 40 seconds until the compression force equilibrated. Stress is plotted against compression ratio at a range of pressures in Figure A.2. The results demonstrate an increase in stiffness for low compression ratios that becomes less apparent as compression increases.

Although this phantom model did demonstrate some stiffening with an increase in perfusion pressure, it was not pursued further due to a number of limitations with this approach. The structure of open-cell foam is very different to vascularised tissue; it has a high porosity and an isotropic pore structure, unlike many tissues where vessels make up a small proportion of the tissue volume and are aligned.
Figure A.1. Rig for compression testing of foam phantoms using the Instron machine. Fluid pressure was controlled by adjusting the height of the fluid reservoir.

Figure A.2. Stress against compression ratio for the polyurethane foam phantom at a range of fluid pressures. The compression ratio is the ratio of the deformed phantom height to the height of the undeformed phantom at zero fluid pressure.
in a preferred direction. The pore structure of foam is also much more connected than the branching structure of blood vessels. The compression of the foam exhibited some strain-softening at high compressions, in contrast to the strain-stiffening behaviour of most tissues. The need to wrap the foam in tape also introduces additional mechanical complexity; this tape has a significant effect on the mechanics of the phantom model and would have to be accounted for in any analysis or model of the phantom.

### A.2 Silicone Phantoms

Silicone gel has previously been used to develop physical phantom models of tissue (Chung et al., 2008). Although it has a linear stress–strain relationship that does not accurately represent myocardium or skeletal muscle, it is a good model of more linear tissue types such as brain and breast tissue.

Silicone gel is not naturally porous, so phantoms representing vascularised tissue were created by moulding the silicone gel around removable cylinders. As a first approach, an array of nylon fishing line was used. This approach was not successful as the silicone gel adhered to the nylon, causing tearing when the nylon line was removed.

The next approach investigated was to embed stainless steel tubes within the gel. 304 grade stainless steel tubes were used with an outer diameter of $0.514 \text{ mm}$. The friction created when removing the steel rods also caused some tearing, but it was found that with an appropriate lubricant, a usable phantom model could be constructed. Figure A.3 illustrates the tearing of silicone when removing stainless steel rods, when using a number of different lubricants. Stoner Rapid Release A324 produced the minimum damage to the silicone gel compared to the other tested lubricants.

Figure A.4 shows a silicone gel moulded with the array of steel tubes. Some tearing is still evident. Removing the steel rods also resulted in the silicone gel adhering to itself in some areas, blocking vessels.

### A.3 Polyvinyl Alcohol Hydrogel Phantoms

As silicone gel has linear mechanical behaviour, a more nonlinear, strain-stiffening version of the phantom was also required to investigate the influence of strain-stiffening on the change in mechanics with fluid pressure. Polyvinyl alcohol (PVA)
Figure A.3. Silicone gel samples after removing an embedded stainless steel rod that had been treated with one of the tested lubricants. The lubricants used were: (a) CRC PTFE dry spray, (b) ChemZ M18 wax based corrosion protection, (c) Lubeserv graphene based lubricant, (d) CRC silicone lubricant, (e) Rocol PTFE based dry lubricant, and (f) Stoner Rapid Release A324.
hydrogel was identified as a suitable material. Wan et al. (2002) investigated PVA as a heart valve replacement material and found that it could reproduce the mechanical behaviour of porcine aortic root tissue.

PVA hydrogel can be produced using a number of cross-linking methods. One method is to mix PVA with water and apply multiple freezing and thawing cycles. This freezing process causes the formation of crystallites that physically cross-link the polymer chains (Hassan and Peppas, 2000). Hydrogels produced with this method may be useful as a biocompatible, implantable material, as they do not contain potentially toxic cross-linking agents.

The stiffness and strain-stiffening level of PVA can be varied by altering the freezing rate, the length of time the gel is held frozen during the cross-linking stage, or the number of freezing cycles (Wan et al., 2002). Decreasing the thawing rate increases stiffness and increases the nonlinearity of the stress–strain response. Increasing the time the gel is held frozen or the number of freezing cycles increases stiffness.

Dawson et al. (2009) demonstrated that PVA hydrogel with an anisotropic structure can be produced by applying a temperature gradient during the freezing process. Millon et al. (2006) also developed PVA hydrogels with anisotropic mechanical properties by applying strain to the gel during temperature cycling. This ability to create an anisotropic material has potential applications for developing more complex physical models of biological tissue.

PVA hydrogel phantoms were constructed by mixing a 15 % solution of PVA in water for three hours at 90 °C. A special mould was constructed with an end plate that could move linearly to allow for expansion of the fluid during freezing, illustrated
in Figure A.5. The PVA solution was poured into the mould and frozen for 16 hours, then thawed at room temperature in water for 8 hours. This freezing and thawing cycle was repeated four times to obtain the final PVA hydrogel phantom.

Compared to silicone gel, the PVA hydrogel surface is extremely slippery, so removing the stainless steel rods from the mould did not require the use of any lubricant.

Cyclic compression experiments were performed on these PVA gels, and plots of load against displacement are shown for one sample in Figure A.6. Both the silicone gel phantoms and PVA gel phantoms could not sustain large fluid pressures, and ruptured as pressures increased above 10 kPa. This limited their usefulness, as Figure A.6 shows no increase in stiffness of the PVA gel at this level of fluid pressure. Some swelling is evident in the shift of the load displacement curve, but no change in the gradient of the curve is present.

Figure A.5. (a) Mould used for constructing PVA hydrogels, showing the end plate that can move to allow for gel expansion during freezing. Stainless steel tubing was used to mould parallel vessels. (b) The resulting PVA gel phantom.
Figure A.6. Compression load against displacement for PVA gel at a range of pressures. Swelling can be observed as a shift in the initial increase in force, but no change in stiffness with fluid pressure was observed.
Figure B.1. Drawing of the base for mounting gel phantoms, which was machined out of aluminium. Third-angle projection is used and all dimensions are in mm.
ANISOTROPIC BULK BEHAVIOUR
IN A LEFT VENTRICLE MODEL

C.1 INTRODUCTION

Chapter 7 presented a poroelastic model of the passive mechanics of the left ventricle. An anisotropic strain energy function with an isotropic bulk deformation term was used to model myocardium’s constitutive behaviour. Chapter 6 showed that an anisotropic bulk strain energy function may be more appropriate for materials that swell anisotropically. However, using an anisotropic bulk strain energy term in the left ventricle model did not improve the model’s ability to reproduce experimental measurements. This appendix further investigates the behaviour of a constitutive relation that uses an anisotropic bulk strain energy term, demonstrating how this affects the left ventricle model’s response to perfusion pressure and ventricle inflation.

C.2 METHODS

Simulations were performed following the same procedure as in Chapter 7, but with a strain energy density function given by:

\[
\Psi_e = \Psi_d(\mathbf{E}) + \Psi_b(\mathbf{E}) \\
\Psi_d = \frac{c_0}{2} (e^Q - 1) \\
Q = 2c_1 (E_{ff} + E_{cc} + E_{rr}) + c_2 E_{ff}^2 \\
+ c_3 (E_{cc}^2 + E_{rr}^2 + E_{cr}^2) + c_4 (E_{fc}^2 + E_{cf}^2 + E_{fr}^2 + E_{rf}^2) \\
\Psi_b = K(J - 1)^2 + b_2 (C_{ff}C_{rr}(J - 1))^2 + b_2 (C_{ff}C_{cc}(J - 1))^2
\]

This strain energy density function uses the same form of \( \Psi_d \) used in Chapter 7, but with an anisotropic form of \( \Psi_b \) based on Relation B (Equation 6.5) from Chapter 6.
This alternative form of $\Psi_b$ introduces additional terms representing coupled deformation between the myocardial fibre and radial directions, and the myocardial fibre and cross-fibre directions. This accounts for swelling deformation primarily consisting of stretch in the myocardial cross-fibre and radial directions.

The constitutive parameters used are listed in Table C.1. The parameters estimated for $\Psi_d$ in Chapter 7 have been used, but the parameters for $\Psi_b$ have been chosen to demonstrate the effect of the anisotropic bulk term while producing a volume change at a perfusion pressure of 15 kPa that is similar to the model in Chapter 7. The parameters $K$ and $b_2$ were varied linearly across the ventricular wall, decreasing from epicardium to endocardium, with the ratio of endocardial parameters to epicardial parameters given by $c_{\text{endo}}$.

### C.3 Results

Figure C.1 plots the perfusion strain caused by increasing perfusion pressure from zero to 15 kPa at zero ventricular pressure. Figure C.2 plots the inflation strains caused by increasing ventricular cavity pressure from zero to 1.3 kPa, for perfusion pressures of zero and 15 kPa.

Compared to Figure 7.5, Figure C.1 shows greater swelling deformation in the myocardial radial direction, and less strain in the fibre direction. The perfusion strain in the fibre direction is negative at the ventricular mid-wall, representing a contraction in the fibre direction due to the swelling in the cross-fibre and radial directions. The magnitudes of the predicted shear strains for perfusion are greater than those measured experimentally and predicted by the model in Chapter 7.

Compared to Figure 7.6, Figure C.2 shows similar changes in inflation strain in the myocardial radial direction, but a much greater reduction in strain in the myocardial cross-fibre direction at the ventricular mid-wall. The fibre strain is relatively unchanged between the perfused and unperfused simulations, which better matches

<table>
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<tr>
<th>$c_0$ (kPa)</th>
<th>$c_1$</th>
<th>$c_2$</th>
<th>$c_3$</th>
<th>$c_4$</th>
<th>$K$ (kPa)</th>
<th>$b_2$ (kPa)</th>
<th>$c_{\text{endo}}$</th>
</tr>
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<tr>
<td>0.0358</td>
<td>0.240</td>
<td>239</td>
<td>8.45</td>
<td>19.3</td>
<td>19.6</td>
<td>19.6</td>
<td>0.586</td>
</tr>
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Table C.1. Constitutive parameters for the left ventricle strain energy function using the constitutive relation in Equation C.1, which has anisotropic bulk strain energy terms. The values listed for $K$ and $b_2$ correspond to the epicardium. At the endocardium, these values are both weighted by $c_{\text{endo}}$, and they vary linearly from the epicardium to the endocardium.
Figure C.1. Simulated perfusion strain using the constitutive relation with anisotropic bulk strain energy terms (Equation C.1), compared to experimental data from May-Newman et al. (1994). Perfusion strains are the strains at zero ventricular pressure caused by increasing perfusion pressure from zero to 15 kPa. Small discontinuities in the strain gradients can be observed at element boundaries.
Figure C.2. Comparison of perfused and unperfused left ventricular inflation strains, for simulations with the constitutive relation with anisotropic bulk strain energy terms (Equation C.1), compared to experimental data from May-Newman et al. (1994). For the experimental perfused data, perfusion pressure was set at 15 kPa. For perfused simulations, a homogeneous perfusion pressure of 15 kPa was applied. Perfused inflation strains are quantified with respect to the perfused geometry at zero ventricular pressure.
the experimental results. The model also predicts a much greater reduction in the $E_{fr}$ shear strain with perfusion than observed experimentally, and with the model from Chapter 7.

The large reduction in cross-fibre strain in the perfused state appears to be due to the bulk deformation terms from Equation C.1 introducing additional stiffness as the volume increases. Ideally, the anisotropic bulk terms would produce an anisotropic swelling deformation but not influence the behaviour of subsequent volume-preserving deformations besides the effect of volume change on the distortional strain energy terms.

C.4 CONCLUSION

These results demonstrate why the poroelastic constitutive relation with anisotropic bulk terms (Equation C.1) was not able to better reproduce the experimental swelling deformation and ventricular inflation results of May-Newman et al. (1994), compared to the model with an isotropic bulk term presented in Chapter 7. This model did produce a reduction in strains in the myocardial cross-fibre and radial directions, whilst the fibre direction strain was relatively unchanged. This response matches the behaviour observed in the experimental studies. However, this anisotropic change in stiffness required that the strains in the fibre direction caused by a perfusion pressure increase were much lower than those measured experimentally, resulting in a worse least-squares fit to the combined experimental data.

This demonstrates that by accounting for the anisotropic swelling behaviour of myocardium in the strain energy density function, a poroelastic model may be able to better reproduce experimental results, but further investigation into appropriate forms of strain energy density functions is required.
This appendix presents additional simulation results using the left ventricle model presented in Chapter 7, but with alternative constitutive relations.

**D.1 Holzapfel–Ogden Based Constitutive Relation**

The behaviour of the left ventricle model was investigated using a poroelastic strain energy density function based on the Holzapfel–Ogden (2009) constitutive relation, given by:

\[
\Psi_e = \frac{a}{2b} \exp \left( b(I_1 - 3) \right) + H(I_{4f} - 1) \frac{a_f}{2b_f} \left[ \exp \left( b_f(I_{4f} - 1)^2 \right) - 1 \right] + H(I_{4s} - 1) \frac{a_s}{2b_s} \left[ \exp \left( b_s(I_{4s} - 1)^2 \right) - 1 \right] + \frac{a_{fs}}{2b_{fs}} \left[ \exp \left( b_{fs}I_{8fs}^2 \right) - 1 \right] + K(J - 1)^2
\]  

(D.1)

where \(a, b, a_f, b_f, a_s, b_s, a_{fs}, b_{fs}\) and \(K\) are material parameters to be determined. \(H\) denotes the Heaviside step function, which is zero for negative inputs and one for inputs greater than or equal to zero. \(I_1\) is the first invariant of \(\mathbf{C}\), \(I_{4f} = C_{ff}, I_{4s} = C_{ss}\), and \(I_{8fs} = C_{fs}\), where \(\mathbf{C}\) is referred to the microstructural fibre, sheet and normal coordinates. \(K\) is the bulk modulus, and \(J\) is the Jacobian of the deformation.

This constitutive relation is orthotropic and differentiates between the myocardial sheet and normal directions, in contrast to the constitutive relation applied in Chapter 7, which is transversely isotropic and based on the constitutive relation developed by Guccione et al. (1991). Table D.1 lists the constitutive parameters estimated for the constitutive relation in Equation D.1, following the approach described in Section 7.2.5. For these simulations, the myocardial sheet direction
Table D.1. Constitutive parameters estimated for the myocardial strain energy function based on the Holzapfel–Ogden (2009) constitutive relation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1 (kPa)</th>
<th>Value 2 (kPa)</th>
<th>Value 3 (kPa)</th>
<th>Value 4 (kPa)</th>
<th>Value 5 (kPa)</th>
<th>Value 6 (kPa)</th>
<th>Value 7 (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>0.572</td>
<td>67.5</td>
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<td>42.2</td>
<td>1.48 × 10^{-4}</td>
<td>16.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$b$</td>
<td>1.17</td>
<td>1.14</td>
<td>1.14</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>67.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$a_f$</td>
<td>42.2</td>
<td>67.5</td>
<td>3.49</td>
<td>42.2</td>
<td>1.48 × 10^{-4}</td>
<td>16.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$b_f$</td>
<td>16.5</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>67.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$a_s$</td>
<td>1.48 × 10^{-4}</td>
<td>1.14</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>5.70 × 10^{-3}</td>
<td>67.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$b_s$</td>
<td>16.5</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>5.70 × 10^{-3}</td>
<td>1.14</td>
<td>67.5</td>
<td>49.5</td>
</tr>
<tr>
<td>$K_{epi}$</td>
<td>67.5</td>
<td>49.5</td>
<td>67.5</td>
<td>49.5</td>
<td>67.5</td>
<td>49.5</td>
<td>67.5</td>
</tr>
<tr>
<td>$K_{endo}$</td>
<td>49.5</td>
<td>67.5</td>
<td>49.5</td>
<td>67.5</td>
<td>49.5</td>
<td>67.5</td>
<td>67.5</td>
</tr>
</tbody>
</table>

was assumed to be oriented in the ventricular radial direction. The myocardial normal direction was therefore aligned with the cross-fibre direction. The estimated value for $a_s$ was small, such that the myocardial sheet direction was not predicted to be substantially stiffer than the myocardial normal direction. Similarly, the values estimated for $a_{fs}$ and $b_{fs}$ were low, such that the response to shear in the myocardial sheet direction was not substantially more than that in the myocardial normal direction.

Figure D.1 plots the perfusion strain caused by increasing perfusion pressure from 0 kPa to 15 kPa with zero ventricular cavity pressure, and Figure D.2 compares the inflation strains caused by inflating the ventricular cavity to 1.3 kPa between the perfused and unperfused ventricle. Simulation results are compared to the data reported by May-Newman et al. (1994).

The left ventricular model using the Holzapfel–Ogden (2009) based constitutive relation did not exhibit significantly different behaviour to the model using the Guccione (1991) based constitutive relation, which was presented in Chapter 7. This is likely due to the parameter estimation procedure identifying a set of parameters where the myocardial sheet direction was not substantially stiffer than the myocardial normal direction. Although the inflation strains in the cross-fibre direction, which corresponds to the myocardial normal direction, were underestimated in these simulations, the perfusion strains in the cross-fibre direction were mostly overestimated. Therefore, decreasing the stiffness of the myocardial normal direction relative to the sheet direction may have improved the model’s fit to the ventricular inflation strains, but this would have produced a poorer fit to the perfusion strain.
Figure D.1. Simulated perfusion strain using the Holzapfel–Ogden (2009) constitutive relation, compared to experimental data from May-Newman et al. (1994). Perfusion strains are the strain at zero ventricular pressure caused by increasing perfusion pressure from zero to 15 kPa.
Figure D.2. Comparison of perfused and unperfused left ventricular inflation strains, for simulations with the Holzapfel–Ogden (2009) constitutive relation and experimental data from May-Newman et al. (1994). For the experimental perfused data, coronary artery pressure was set at 15 kPa. For perfused simulations, a homogeneous perfusion pressure of 15 kPa was applied. Perfused inflation strains were quantified with respect to the perfused geometry at zero ventricular pressure.
The behaviour of the left ventricle model was also investigated with a separated form of the Guccione (1991) constitutive relation. This constitutive relation has a strain energy density function given by:

\[ \Psi = \frac{c_0}{2} \left[ \left( e^{c_1(E_{ff}+E_{cc}+E_{rr})} - 1 \right) + \left( e^{c_2E_{fi}} - 1 \right) + \left( e^{c_3E_{cc}} - 1 \right) + \left( e^{c_4E_{rr}} - 1 \right) + \left( e^{c_5(E_{cr}+E_{rc})} - 1 \right) + \left( e^{c_6(E_{fc}+E_{cf})} - 1 \right) + \left( e^{c_7(E_{fr}+E_{rf})} - 1 \right) + K(J - 1)^2 \right] \]  

(D.2)

where \( c_0, c_1, c_2, c_3, c_4 \) and \( K \) are material parameters to be determined, and \( E \) is the Green–Lagrange strain tensor aligned with the myocardial fibre \( (f) \), cross-fibre \( (c) \) and radial \( (r) \) directions. This constitutive relation is based on the constitutive relation given in Equation 7.2, but terms within the exponent have been separated into individual exponential terms.

Chapter 7 suggested that this constitutive relation may better reproduce anisotropy in stiffness changes between different directions, as there is a reduction in coupling between orthogonal strain components.

Table D.2 lists the constitutive parameters estimated for the constitutive relation in Equation D.2, following the approach described in Section 7.2.5.

Figure D.3 plots the perfusion strain caused by increasing perfusion pressure from 0 kPa to 15 kPa with zero ventricular cavity pressure, and Figure D.4 compares the inflation strains caused by inflating the ventricular cavity to 1.3 kPa between the perfused and unperfused ventricle. Simulation results are compared to the data reported by May-Newman et al. (1994).

This separated Guccione relation showed much greater discontinuities in the gradient of strains when compared to the standard Guccione relation (Figures 7.5 and 7.6). Additionally, this constitutive relation failed to improve the model’s ability

<table>
<thead>
<tr>
<th>( c_0 ) (kPa)</th>
<th>( c_1 )</th>
<th>( c_2 )</th>
<th>( c_3 )</th>
<th>( c_4 )</th>
<th>( K_{endo} ) (kPa)</th>
<th>( K_{epi} ) (kPa)</th>
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<td>0.200</td>
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<td>0.0</td>
<td>39.8</td>
<td>67.0</td>
<td>52.0</td>
</tr>
</tbody>
</table>
Figure D.3. Simulated perfusion strains using the separated Guccione constitutive relation (Equation D.2), compared to experimental data from May-Newman et al. (1994). Perfusion strains are the strain at zero ventricular pressure caused by increasing perfusion pressure from zero to 15 kPa. Large discontinuities in the strain gradient can be observed at element boundaries.
Figure D.4. Comparison of perfused and unperfused left ventricular inflation strains, for simulations with the separated Guccione (Equation D.2) constitutive relation and experimental data from May-Newman et al. (1994). For the experimental perfused data, perfusion pressure was set at 15 kPa. For perfused simulations, a homogeneous perfusion pressure of 15 kPa was applied. Perfused inflation strains were quantified with respect to the perfused geometry at zero ventricular pressure.
to reproduce the anisotropic reduction in inflation strain observed experimentally. The fibre direction still demonstrated a significant reduction in inflation strain, which was not observed in experiments. This reduction in fibre direction strain is likely to be due to the positive perfusion strain in the fibre direction causing an increase in the fibre direction stiffness, since the fibre direction strain energy is not coupled to strain in other directions.
Figure E.1. Drawing of the assembled mechanical testing device used for testing silicone gel phantoms and the rat tibialis anterior muscle. Third-angle projection is used and all dimensions are in mm.


Chapelle, D. and P. Moireau (2014). General coupling of porous flows and hyperelastic formulations—From thermodynamics principles to energy balance and compati-


