Modelling studies on biological tissue properties
and mechanical responses under external stimuli

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Dedicated to my family
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Abstract

Biological tissues maintain their homeostasis by remodelling under external mechanical stimuli. In order to understand the tissue remodelling process, it is important to characterize tissue properties before detailed mechanical responses can be investigated. This project aims to develop a computational modelling framework to characterise mechanical properties of biological tissues, and to quantify tissue responses under mechanical loading.

The thesis presents, first, mechanical responses of articular cartilages under different loadings using a poroelastic model. Unique in this study, collagen fibrils are treated separately from the rest of ECM, as they only resists tension. This leads to a fibril-reinforced poroelastic model. Effects of the distribution of the collagen fibrils and their orientation on tissue mechanical responses are investigated.

Most of the effort has been on the mechanical stress distribution of the human left atrium and its correlation to electrophysiology patterns in atrial fibrillation. Detailed mechanical responses of the atrial wall to a step pressure increase in the left atrium are calculated. The geometry of the left atrium is based on patient specific images using cardio CT and incorporates variations of the atrial wall thickness as well as unique fibre orientation patterns. We hypothesize that areas of high von Mises stress are correlated to foci of abnormal electrophysiology sites which sustain cardiac arrhythmia. Results from this study show a positive correlation between them. To our
knowledge, this is the first study that establishes the relationship between the atrial wall stress distribution and the atrial abnormal electrophysiology sites.

The project also investigates hyperelastic properties of endothelial cells and the overlying endothelial glycocalyx, based on data from AFM micro-indentation. Both endothelial cells with & without the glycocalyx layer (i.e. following enzymatic digestion) are used. This is the first time that the mechanical property of the glycocalyx is estimated using an inverse biomechanical model.
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<td>AAA</td>
<td>abdominal aortic aneurysm</td>
</tr>
<tr>
<td>A-B</td>
<td>Arruda-Boyce</td>
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<td>AF</td>
<td>atrial fibrillation</td>
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<tr>
<td>AFCL</td>
<td>atrial fibrillatory cycle length</td>
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<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
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<tr>
<td>AV</td>
<td>atrio-ventricular</td>
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<tr>
<td>CFAE</td>
<td>complex fractioned atrial electrograms</td>
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<tr>
<td>DF</td>
<td>dominant frequency</td>
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<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>FE</td>
<td>fractionated electrograms</td>
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<tr>
<td>FEM</td>
<td>finite element method</td>
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<td>HUVECs</td>
<td>human umbilical venin endothelia cells</td>
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<td>LA</td>
<td>left atrium</td>
</tr>
<tr>
<td>LAA</td>
<td>left atrial appendage</td>
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<tr>
<td>LACT</td>
<td>left atrium computed tomography</td>
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<tr>
<td>LIPV</td>
<td>left inferior pulmonary vein</td>
</tr>
<tr>
<td>LSPV</td>
<td>left superior pulmonary vein</td>
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<tr>
<td>LV</td>
<td>low voltage</td>
</tr>
<tr>
<td>PGs</td>
<td>proteoglycans</td>
</tr>
<tr>
<td>PVs</td>
<td>pulmonary veins</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>RIPV</td>
<td>right inferior pulmonary vein</td>
</tr>
<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
</tr>
<tr>
<td>RSPV</td>
<td>right superior pulmonary vein</td>
</tr>
<tr>
<td>SA</td>
<td>sino-atrial</td>
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<td>STL</td>
<td>Stereolithography</td>
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</table>
List of nomenclature

Latin

$a, \alpha_j \ (j = 1 \ldots n)$ vector of model parameters and its elements (Chapter 1)

$a$ effective contact radius (Chapter 5)

$a_0, b_0$ unit vector for fibre direction

$a, a_i, a_{fs} \ (i = f \ or \ s)$ model parameters in Equation 3.1

$ared$ actual reduction

$b, b_i, b_{fs} \ (i = f \ or \ s)$ model parameters in Equation 3.1

$b$ radial distance at transition position (Chapter 5)

$C$ right Cauchy-Green deformation tensor

$C_{ij} \ (i,j = 1,2,3 \ldots)$ model parameters of polynomial strain energy function

$D_c, D_g$ reflection when indenting on cell and glass, respectively

$e$ void ratio

$E$ Young modulus

$\bar{E}$ generalized elastic modulus

$E_{A-B}$ Young’s modulus determined from Arruda-Boyce model

$E_{blunt}$ Young’s modulus determined from blunt model

$E_f$ Young’s modulus of the fibril network

$E_G$ Young’s modulus of the glycocalyx layer

$E_{sharp}$ Young’s modulus determined from sharp model

$err(a)$ error vector between measurements $y$ and predicted values $f$

$err(\alpha)_i \ (i = 1 \ldots m)$ elements of $err(\alpha)$
fibre direction

fn

fs

f

model predictions

F

reaction force

g, g_j (j = 1 ... n)

gradient vector and its elements

h

height of cartilage (Chapter 2)

height of endothelium (Chapter 5)

H, H_{jk} (j, k = 1 ... n)

Hessian matrix and its elements

I

unit matrix

I_1, I_2

1st and 2nd isotropic invariants of C

I_4, I_6

transversely isotropic invariants of C

I_{4i} (i = f or s)

transverse isotropic invariants associated with fibre direction i

I_8

coupling invariant of C

I_{8fs}

coupling invariants associated with a pair of direction f and s

J_c

Jacobian matrix of vector \Delta \sigma_s with respect to vector \Delta \varepsilon

J_{err}

Jacobian matrix of vector err(a) with respect to vector a

J_{ij} (i = 1 ... m, j = 1 ... n)

elements of J_{err}

k

permeability

k_0

intrinsic permeability

k'

hydraulic conductivity

k'_0

intrinsic hydraulic conductivity

M

constant of the nonlinear strain-dependent permeability model

n

normal direction

nf

fibre direction in the nf plane

XVII
ns   sheet direction in the ns plane

$p$   hydrostatic pressure

$pred$   predicted reduction

$r$   radius direction (Chapter 2)

radius of bead (Chapter 5)

$R$   radius of cartilage

$s$   sheet direction

sf   fibre direction in the sf plane

sn   normal direction in the sn plane

$S$   objective function

$S'$   derivative of $S$ with respect to $\delta$

$S_{ij}$ ($i, j = 1,2,3$)   elements of Jacobian matrix of material stiffness

$S_s, S_g$   separation distance when indenting on cell and glass, respectively

$t_0$   peak time point

$u_0$   displacement at peak time $t_0$

$v_s$   velocity of solid phase

$v_w$   velocity of fluid phase

$W$   strain energy potential

$W_1', W_{4f}', W_{4s}', W_{8fs}'$   first derivatives of energy function $W$ with respect to invariants $l_1, l_{4f}, l_{4s}$ and $l_{8fs}$, respectively

$W_1'', W_{4f}'', W_{4s}'', W_{8fs}''$   second derivatives of energy function $W$ with respect to invariants $l_1, l_{4f}, l_{4s}$ and $l_{8fs}$, respectively

$W_1, W_2$   first derivatives of $W$ with respect to $l_1$ and $l_2$, respectively

$x_i$ ($i = 1...m$)   m observation points

$y_i$ ($i = 1...m$)   experiment measurements at different $x_i$
\( z \)          axial direction

**Greek**

\( \alpha \)  
model parameter of Ogden’s strain energy function

\( \alpha_i, \beta \ (i = 1 \ldots 5) \)  
model parameters of Arruda-Boyce strain energy function

\( \gamma \)  
specific volume weight of the interstitial fluid (Chapter 2)

amount of shear (Chapter 3)

\( \delta \)  
small increment (Section 1.5)

indentation depth (Chapter 5)

\( \Delta \)  
trust region radius

\( \Delta \varepsilon \)  
increment in engineering strain

\( \Delta \varepsilon^r, \Delta \varepsilon^\theta, \Delta \varepsilon^z \)  
normal strain increment in radius, circumferential and axial direction, respectively

\( \Delta \varepsilon^{rz} \)  
shear strain increment

\( \Delta \sigma_s \)  
Increment in Cauchy stress of solid phase

\( \Delta \sigma^r_s, \Delta \sigma^\theta_s, \Delta \sigma^z_s \)  
solid phase normal stress increment in radius, circumferential and axial direction, respectively

\( \Delta \sigma^{rz}_s \)  
solid phase shear stress increment

\( \sigma_{xy} \ (x, y = f, s, n) \)  
shear stress

\( \varepsilon \)  
Cauchy strain

\( \varepsilon_0 \)  
Cauchy strain at peak time \( t_0 \)

\( \nu \)  
Poisson ratio

\( \sigma \)  
Cauchy stress

\( \sigma_f \)  
fibril stress

\( \sigma_m \)  
ECM stress
\( \sigma_s \) solid phase stress
\( \sigma_w \) fluid phase stress
\( \theta \) circumferential direction (Chapter 2)
\( \lambda_1, \lambda_2 \) principal stretches
\( \lambda \) Lamé's first parameter (Section 1.3.1)
\( \lambda_L \) locking stretch
\( \mu \) shear modulus or Lamé's second parameter
\( \mu_{ogden}, \mu_{A-B} \) model parameters of Ogden's and Arruda-Boyce strain energy function, respectively
\( \varphi_s \) solid volume fraction; solidity
\( \varphi_w \) fluid volume fraction; porosity
\( \phi \) approximation model function
\( \phi(D) \) indenter geometry function

**Operators**

\( \text{tr} \) trace of a matrix
\( \nabla \) gradient
\( \Sigma \) summation
\( T \) transpose of a matrix
\( \partial \) partial differential
\( \| \|_2 \) Euclidean norm
1 Introduction

1.1 Tissues background

In this section, the structure and composition of selected tissues are introduced in order to give a good understanding on the models set up in the following chapters.

1.1.1 Articular cartilage

The articular cartilage is composed of a collagen fibril network, negatively charged proteoglycans (PGs), interstitial fluid and chondrocytes (Figure 1.1) (Mow et al., 1991). The main components in the solid matrix are PGs and collagens, while the constituents of the fluid phase are water and solutes. The interstitial fluid makes up 60-89% of the wet weight of the cartilage (Setton et al., 1995). Under external mechanical loads, the porous structure of the cartilage allows fluid to flow through within the solid matrix. The water content is normally high in the superficial zone and decreases with the depth in the cartilage (Torzilli, 1985). Collagens within the articular cartilage are primarily heteromers of collagen types II, IX, and XI, approximately 90% of which is type II collagen (Eyre et al., 2006). Other types including I, III, V, VI and X have been also found (Eyre, 2002; Eyre et al., 1987), and interconnect with each other via crosslinks to stabilize the collagen fibril network (Eyre et al., 1988).
The articular cartilage has a multiple layered structure and been divided into the superficial, middle, deep and calcified zones (Jeffery et al., 1991; Mow et al., 1991). Structure and properties of the collagen network vary significantly between these zones. In the superficial layer, the concentration of collagen is the highest, the diameter of collagen fibrils is smaller than others and the orientation is parallel to the cartilage surface. In the middle zone, the collagen fibrils bend towards a perpendicular-to surface orientation. The deep zone contains the largest fibrils with diameter of 40–80 nm (Langsjo et al., 1999), which are oriented perpendicularly to the subchondral bone. The calcified layer contains the transition from the hyaline cartilage into the subchondral bone (Figure 1.1). Different zonal thicknesses have also been reported, where the superficial, middle and deep zones account for 3-24%, 1-40% and 50-94% of the total cartilage thickness respectively (Alhadlaq and Xia, 2004; Hunziker, 1992; Xia et al., 2003).

Figure 1.1 Schematic drawing of articular cartilage.

Figure shows different layer and components in articular cartilage. Adapted from Mow et al. (1991)
1.1.2 Heart and left atrium

The heart is made of 4 chambers (Figure 1.2). The two superior chambers are called the left and right atria while the two inferior chambers are called the left and right ventricles. The atria and ventricles are separated by the mitral valve and tricuspid valve in the left and right side, respectively, which is necessary to allow the chambers of the heart to fill while not pumping blood during the cardiac cycle. A thin membranous wall called the interatrial septum separates the left atrial chamber from the right while a thicker muscular wall called the interventricular septum separates the left ventricular chamber from the right (Mader, 2004)

![Figure 1.2 Anatomy of heart and conduction system.](image)

The coordinated contraction of the heart is controlled by the specialized electrical conduction system of the heart. The major parts of this conduction system
are the sino-atrial (SA) node, Bachmann’s bundle, atrio-ventricular (AV) node, the His bundle, bundle branches and Purkinje fibres (Figure 1.2). The SA node, located in the upper right atrium, is the primary pacemaker of the heart, automatically generating electrical pulses. These electrical pulses propagate over the atria, with the Bachmann’s bundle assuring fast propagation toward the left atrium, leading to atrial contraction. The atria and ventricles are electrically isolated from each other except for the region of the AV node via which the excitation passes to ventricles. The low speed of propagation in the AV node, cause a delay in the passage of the electrical wave from the atria to the ventricles, ensuring that the ventricles contract after the atria. In the ventricles the excitation spreads at a high speed from the AV node through the His bundle, left and right bundle branches and Purkinje fibres. The Purkinje fibres are electrically connected to the ventricular muscle at certain insertion sites, from which the wave enters the ventricular wall, leading to ventricular excitation and contraction (Guyton and Hall, 2006).

There are six stages in a cardiac cycle (Figure 1.3): stage 1, diastasis when both atria and ventricles are in diastole; stage 2, atrial systole while ventricular diastole; stage 3, ventricular isovolumetric contraction, atria start diastole while ventricles start systole; stage 4, ventricular ejection, atria are still diastole and ventricles enter into systole; stage 5, ventricular isovolumetric relaxation, atria are still diastole and ventricles start diastole; stage 6, atria are still diastole and ventricles enter into diastole. Most of the time during a cardiac cycle, atria are in diastole state. The AV valve are closed from stage 3 to stage 5 (Guyton and Hall, 2006).
The muscular wall of the heart is made up of three layers (Figure 1.4a). The inner layer, called the endocardium, lines the chambers of the heart. The centre layer is the myocardium, which is the thickest part and provides the contractile force for pumping. This layer of myocardium is further divided into the subendocardial area which is the inner half of the myocardium, and the subepicardial area, the outer half. The outermost layer of the heart wall overlying the myocardium is called the epicardium (Mader, 2004). The myocardium is made up of cardiac muscle, a highly specialized contractile tissue of the heart. The whole cardiac muscle is surrounded by connective tissue layer, the epimysium. Another connective tissue layer, the perimysium, divides the muscle into fascicles or muscle fibre bundles. Each individual muscle fibre in fibre bundles is separated by endomysium (Figure 1.4b). The fibrillar collagen network of the heart exists in the above mentioned three connective tissue layers. The epimysial collagen fibres are located along the epicardial and endocardial surfaces of
Figure 1.4 Layers of cardiac tissue (a) and schematic diagram of the arrangement of intramuscular connective tissue within muscle (b). Adapted from Mader (2004) (a) and Jolley and Purslow (1988) (b)
the myocardium. Perimysial collagen fibres track through the myocardium between muscle bundles.

Further information about left atrium (LA) is given here since it is one of the studied tissues. Normally, there are four pulmonary veins (PVs) connected to the LA, left superior pulmonary vein (LSPV), left inferior pulmonary vein (LIPV), right superior pulmonary vein (RSPV) and right inferior pulmonary vein (RIPV) (Figure 1.5). They have independent ostia in most cases (Marom et al., 2004). However, a number of differences have been reported in the previous publications (Hassink et al., 2003; Ho et al., 2001; Lin et al., 2000; Wittkampf et al., 2003). A common ostium for the left PVs was defined in 83% of one study, while a common ostium for the right PVs was less appearing in 40% of same study (Jongbloed et al., 2005). Ho and co-workers also shown that the frequency of a common ostium for the superior and inferior veins on the left is higher than the right (Ho et al., 2001). Another common variant, which is a separated origin for the right middle PV, was also reported in the literature. The majority of the population have the middle PV draining into the RSPV while a few have it inserted into the RIPV, with percentages of 17-23% and 3-8%, respectively, in surgical studies (Tsao et al., 2001; Yazar et al., 2002). On the other hand, separated origin is rarely seen for left-sided PVs (Ghaye et al., 2003; Jongbloed et al., 2005).
Figure 1.5 Left atrium is divided into 11 subregions (top) and dominant muscle fibre orientation on the atrium wall (bottom).

Left figures are anterior views while right figures are posterior views. Boundary of subregions and fibre orientation are based on the study from Ho and co-workers (Ho et al., 1999, 2001). LSPV: left superior pulmonary vein, LIPV: left inferior pulmonary vein, RSPV: right superior pulmonary vein and RIPV: right inferior pulmonary vein.
The walls of LA, excluding the left atrial appendage (LAA), normally can be described as anterior, superior, lateral, interatrial septum and posterior (Figure 1.5). The anterior wall is $3.3 \pm 1.2$ mm (range 1.5–4.8 mm) from epicardium to endocardium in unselected necropsy heart specimens, but this wall can become very thin at the area near the vestibule of the mitral annulus where it measures an average of 2 mm in thickness in autopsy studies. The thickness of superior wall of the LA ranges from 3.5 to 6.5 mm (mean $4.5 \pm 0.6$ mm). The thickness of the lateral wall ranges between 2.5 and 4.9 mm (mean $3.9 \pm 0.7$ mm) (Ho et al., 1999). The dominant fibres orientation in LA is longitudinal in the superior wall and circumferential paralleling to the mitral annulus in the rest of the regions. Atrial myocardium is also extended onto pulmonary veins around 10 mm (called sleeve) and the orientation is circumferential along the veins (Ho et al., 2001).

### 1.1.3 Endothelial cells and the glycocalyx layer

The luminal surface of human blood vessels and heart chambers are lined with a continuous layer of vascular endothelium (Figure 1.6) that plays an important role in maintaining cardiovascular health. The endothelial cells (endothelium) are constantly situated in a hemodynamic environment and experiencing mechanical forces exerted by the blood flow. Unlike the round red cells floating in the blood, the endothelial cells are more elliptic and attached on their substrate (Figure 1.6).
The endothelial surface glycocalyx was first identified by Luft (1966) more than forty years ago using a ruthenium red electron microscopic staining technique. Due to its sensitivity to chemical staining, the glycocalyx had not been observed \textit{in vivo} until Vink and Duking (1996). More recently Squire et al. (2001) showed that the matrix has an underlying quasi-periodic substructure. Based on the electron microscopic observation in the capillary, Weinbaum and his colleagues (Weinbaum \textit{et al}., 2003) presented the “bush-like” glycocalyx structure in which the glycocalyx matrix is 150-400 nm in thick and 20 nm in distance between molecules (Figure 1.7).

\textbf{Figure 1.6 Endothelial cell and overlying glycocalyx layer.}

Endothelial cells line the luminal side of blood vessel. All cells are coated with a thin glycocalyx layer.
1.2 Atrial fibrillation

As learned from section 1.1.2, the heart beats and pumps blood with a regular rhythm coordinated by the electrical system. The regular rhythm is conducted by the regular electrical impulses that are generated by the SA node travelling through the whole heart and causing the contraction of the heart muscle. When electrical impulses
are not only produced by the SA node but also come from other parts of the atria and adjacent parts of veins, these abnormal impulses (usually rapidly) cause the atria to quiver rather than beat as a unit. This irregular heart rhythm phenomenon is called atrial fibrillation (AF). During AF, the rapidly and abnormal electrical impulses cause ineffective contractions of the atria and reduce the ability of the atria to expel blood into the ventricles. Moreover, the irregular electrical impulses overwhelming the regular ones generated from AV node cause ventricle fibrillation which is a more severe dysfunction of the heart.

The majority of patients with AF may be asymptotic and are unaware of the abnormal heart rhythm. The most common symptom of AF is palpitation which is an unpleasant sensation of fast or strong beating of the heart. There are other symptoms of AF due to the decreased amount of blood circulation in the body, which are dizziness, fainting, weakness, fatigue, shortness of breath and angina. Even worse, blood stagnates inside the atria due to the quivering of the atria during AF and further forms blood clots along the walls of the atria. Once the blood clots travel to the brain, they can cause the stroke.

AF could be divided into varied categories according to different criteria. In terms of the episode time and termination: AF could be paroxysmal if episodes last minutes to hours and terminate spontaneously within seven days. The heart rate restores to normal between episodes. Persistent AF means the episode lasts for more than seven days and is unlikely to self-terminate. If an episode cannot be ceased by cardioversion and is ongoing for a long time, the persistent AF has converted into
permanent AF. Persistent AF and permanent AF could also refer to chronic AF (Earley and Schilling, 2006). In terms of other characteristics of patient: Lone AF, which relates to a patient who is under 60 years old and clinical or echocardiographic findings of other cardiopulmonary disease including hypertension or cardiac abnormalities such as enlargement of the LA are absent. Non-valvular AF, which applies to a patient who is without rheumatic mitral valve disease, a prosthetic heart valve, or mitral valve repair. Secondary AF, which happens to a patient who has a background of a primary condition which may be the cause of the AF, such as acute myocardial infarction, cardiac surgery, or other acute pulmonary disease (Fuster et al., 2006).

Several methodologies have been used for the diagnosis of AF due to the assortment of AF. For example, patient with chronic AF could take an electrocardiogram (ECG) recording of the heart’s electrical impulses. The abnormal ECG of AF has distinct differences from the normal one and could be distinguished easily. For paroxysmal AF, a Holter monitor which is a continuous recording of the heart’s rhythm for 24 hours is a better choice since a standard ECG test may not show AF at the time of recording. Echocardiography is an approach using ultrasound waves to generate images of the heart’s chambers, valves and the pericardium. Size of atrial chambers (an important factor in determining treatment for AF) could be measured and other possible disorders accompanying AF, such as rheumatic valve diseases, could also be detected with echocardiography. Normally, combinations of these tests and other examinations such as chest X-way are performed to detect the episode of AF (Aliot et al., 2008).
The treatment of AF could involve medication, cardioversion and procedures. If medication and cardioversion fail, the procedures will be considered. There are two kinds of procedure: open-heart surgery ablation and catheter ablation. Open-heart surgery is also called the Maze procedure, which was first performed by Cox Maze in 1987 (Cox et al., 1991). During the Maze procedure, numerous incisions are made in the atria to disturb the conduction of the electrical impulse and restore a regular rhythm to the heart. Although the Maze procedure has a high rate in correcting AF, there are significant complications during the procedure, like stroke, bleeding and

Figure 1.8 Different patterns of catheter ablation.

...
even death. On the other hand, catheter ablation is a procedure that places electrodes into the atria through a catheter inserted into a large vein in the neck, chest or groin. Firstly, these electrodes are used to detect electrical activity from inside the atria. Secondly, the electrical distribution is mapped to the atria anatomy in order to judge abnormal areas of atria based on different criteria (see next paragraph for detailed criteria. Last, electrodes emit radiofrequency energy to destroy abnormal electrical pathways in atrial tissue. According to different type of AF, varied ablation pattern is performed (Figure 1.8). It has recently been illustrated that paroxysmal AF is often initiated by focal triggers localized in one or more PVs (Haissaguerre et al., 1998), thus the strategies that target ablation of the muscular sleeves at the ostia of PVs (Haissaguerre et al., 1998) or circumferential ablation near PVs based on the anatomy of the atria (Pappone et al., 2000) have been adopted to isolate the PVs from the conduction system. In the latter approach, many operators utilize a non-fluoroscopic mapping system, such as Carto™ (Biosense Webster, Johnson & Johnson) or Ensite NavX™ (NavX™, St. Jude Medical) to guide the ablation catheter and confirm continuous lesions encircling individual or homolateral PV ostia (Schilling et al., 2009). On the contrary, the prevailing theory in chronic AF is that multiple, random wavelets of activation and circuit reentries coexist to create a chaotic cardiac rhythm (Haissaguerre et al., 2000). Therefore, different strategies, such as linear ablation to block the pathway of reentry circuits, ablation at areas of abnormal electrical conduction depending on various criteria (see next paragraph for detailed criteria) (Nademeanee et al., 2004; Rostock et al., 2006; Shah et al., 2003; Takahashi et al., 2006a; Takahashi et al., 2006b) have been tried over the last few years.
The criteria for the determination of the abnormal electrical conduction areas in atria involves complex fractioned atrial electrogram (CFAE), dominant frequency (DF), atrial fibrillatory cycle length (AFCL) and low voltage (LV). An unfractionated electrograms has a single negative deflection whereas a fractionated electrograms (FE) is composed of multiple negative deflections during one cycle. See Figure 1.9 for the detailed morphology of fractionated electrograms. CFAE was first proposed by Nademanee and co-workers in 2004 (Nademanee et al., 2004), they mentioned that the CFAE record during AF is a marker for areas with electrical wavefront breaking, changes of electrical activation front direction or slow conduction. These areas are thought to be essential for the perpetuation of small fluctuating micro-reentries sustaining AF (Nademanee et al., 2004). DF is defined as the frequency of highest-amplitude of electrograms. Since emerging experimental evidence suggests that certain cases of AF are sustained by multi-reentrant sources that cause a hierarchical distribution of frequencies of various amplitudes in the atria (Berenfeld et al., 2000; Mansour et al., 2001), the correlation between DF and ablation sites have been widely studied (Lazar et al., 2006; Ng et al., 2006; Schuessler et al., 2006). LV refers to voltage amplitude in the range of 0.05-0.25 mV in the CFAE (Takahashi et al., 2007).
Figure 1.9 Morphology of fractionated electrograms.

A single sharp deflection represents a normal electrogram. A “short double” demonstrates a biphasic deflection, while a “long double” exhibits two separate deflections. Complex FEs can have a varying number of deflections within one cardiac cycle. Adapted from Verheule et al. (2008)
1.3 Constitutive equations

In numerical modelling of mechanics of a continuous body, such as cardiac wall, the constitutive law distinguishes mechanical behaviour of sample from each other and bridges stress and strain. Some widely used constitutive equations for biological tissues are introduced in this section.

1.3.1 Linear elasticity

The Kirchhoff-St.Venant material law is also called the generalized Hooke’s law. It is a classical, widely used constitutive relation, which can well describe the mechanical response of elastic, compressible materials that are subjected to small deformations. For a homogeneous, isotropic body, only two independent parameters are needed to characterize its mechanical behaviour. In the generalized Hooke’s law, these material constants are the two coefficient of Lamé $\lambda$ and $\mu$, or the Young modulus $E$ and the Poisson ratio $\nu$, depending on the choice of the parameter. The four material properties relate as follows:

$$\lambda = \frac{E\nu}{(1 + \nu)(1 - 2\nu)},$$  \hspace{1cm} \text{Equation 1.1}

$$\mu = \frac{E}{2(1 + \nu)},$$  \hspace{1cm} \text{Equation 1.2}

in which $\lambda > 0, \mu > 0, E > 0$ and $\nu < \frac{1}{2}$ due to physical consistency.

In the Kirchhoff-St.Venant model, the total elastic strain energy function is defined as:
where $\varepsilon$ represents strain. The compressibility of a body is characterized by the Poisson ratio $\nu$. Typically, a Poisson ratio close to 0.5 represents an almost incompressible material, like rubber.

The stress $\sigma$ is given by partial derivation of $W$ over $\varepsilon$:

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

where $\mathbf{I}$ is unit matrix. It is important to notice that the stress depends linearly on the strain in the generalized Hooke’s law.

### 1.3.2 Poroelasticity

The poroelastic theory was first proposed by Terzaghi (1943) for the analysis of soil consolidation and later extended by Biot (Biot, 1962, 1972). It has been successfully applied to soft tissue such as blood vessel (Kenyon, 1979) and intervertebral disc (Simon et al., 1985). Independently the biphasic theory was initially developed specifically for articular cartilages by Mow et al. (1980). Both biphasic theory and poroelastic theory are proven to be essentially synonymous (Goldsmith et al., 1996). In this thesis, either ‘biphasic’ or ‘poroelastic’ refers to the same theory.

The poroelastic constitutive equation is defined as an addition of interstitial fluid stress $\sigma_w$ to the solid phase stress $\sigma_s$. Within the small deformation,
\[ \sigma = \sigma_s + \sigma_w \]  \hspace{1cm} \text{Equation 1.5}

\[ \sigma_s = -\varphi_s p \mathbf{I} + \lambda \text{tr}(\varepsilon) \mathbf{I} + 2\mu \varepsilon \]  \hspace{1cm} \text{Equation 1.6}

\[ \sigma_w = -\varphi_w p \mathbf{I}, \]  \hspace{1cm} \text{Equation 1.7}

where \( \varphi_s \) and \( \varphi_w \) are the solid and fluid volume fractions, respectively. \( p \) represents the hydrostatic pressure which is determined from the Darcy’s law:

\[ k \nabla p = -\varphi_w (v_w - v_s). \]  \hspace{1cm} \text{Equation 1.8}

where \( k \) is the permeability indicating how easily interstitial fluid goes through the porous medium. \( v_s \) and \( v_w \) are the velocity of solid and fluid phases, respectively.

### 1.3.3 Hyperelasticity

Most hyperelastic material models were initially proposed for rubber. They have been widely used for the biological tissues when the samples were considered as incompressible. Classic isotropic incompressible hyperelastic equations are listed below:

Polynomial model in terms of 1st and 2nd invariants \((I_1 \text{ and } I_2)\) of Cauchy-Green deformation tensor \( \mathbf{C} \) (Holzapfel, 2000):

\[ W = \sum_{i,j=0}^{n} C_{ij}(I_1 - 3)^i (I_2 - 3)^j \]  \hspace{1cm} \text{Equation 1.9}

Ogden’s model in terms of principal stretches \( \lambda_1 \) and \( \lambda_2 \) (Ogden, 1972):

\[ W = \sum_{p=1}^{n} \frac{\mu_{\text{Ogden}}}{a} (\lambda_1^{a} + \lambda_2^{a} + \lambda_1^{-a} \lambda_2^{-a} - 3) \]  \hspace{1cm} \text{Equation 1.10}
Arruda-Boyce (A-B) model in terms of 1\textsuperscript{st} invariants $I_1$ of Cauchy-Green deformation tensor $\mathbf{C}$ (Arruda and Boyce, 1993):

$$ W = \mu_{A-B} \sum_{i=1}^{5} \alpha_i \beta^{i-1}(I_1^i - 3^i), \quad \text{Equation 1.11} $$

where $C_{ij}, \mu_{\text{ogden}}, \mu_{A-B}$ and $\alpha$ are the model parameters in each model, respectively. $\alpha_i$ and $\beta$ are the model constants in Equation 1.11. The polynomial model could reduce to Neo-Hookean model, Mooney-Rivlin model and Yeoh model with specified model constants. Ogden’s book is recommended for further details (Ogden, 1984).

The 1\textsuperscript{st} and 2\textsuperscript{nd} invariants $I_1$ and $I_2$ are defined as:

$$ I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \quad \text{Equation 1.12} $$

$$ I_2 = \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_3^2 \lambda_1^2. \quad \text{Equation 1.13} $$

For an anisotropic material which has two preferred directions denoted by the unit vectors $a_0$ and $b_0$, there are transverse isotropic invariant $I_4, I_6$ and coupling invariant $I_8$ defined by:

$$ I_4 = a_0 \cdot (\mathbf{C} a_0) \quad \text{Equation 1.14} $$

$$ I_6 = b_0 \cdot (\mathbf{C} b_0) \quad \text{Equation 1.15} $$

$$ I_8 = a_0 \cdot (\mathbf{C} b_0). \quad \text{Equation 1.16} $$

Refer to Spencer (1984) for background information on the invariant theory of anisotropic materials.

Selection of the theoretical model depends on the specific research purpose and the availability of material properties for the corresponding model. In this study,
cartilage was modelled as either poroelastic medium (Equation 1.5-1.8) or fibril reinforced poroelastic medium (Equation 2.1). The incompressible anisotropic hyperelastic model (Equation 3.1) was chosen for the atrial wall while the endothelium and the glycocalyx layer were modelled as Arruda-Boyce model (Equation 5.6).

1.4 A brief review of theoretical models

Theoretical analyses play an important role in the study of biological issues. Different theoretical models have been proposed to interpret mechanical responses of biological tissues observed experimentally. These models have been improved and refined gradually by comparing analytical and/or numerical predictions to experimental measurement. This section gives a brief review of theoretical study on selected biological tissues.

1.4.1 Articular cartilage

Articular cartilages were initially assumed to be of a single phase, isotropic, homogeneous, elastic (Hayes et al., 1972) or viscoelastic material (Fung, 1981). These models worked at some particular time points or were able to describe the overall behaviour, but they didn’t consider the interstitial fluid movement during
tissue deformation, which is an important component in soft tissues. Thus these analyses are inadequate for biomechanical studies. They have been gradually superseded by more sophisticated biphasic models which consider the fluid-solid interaction (Mow et al., 1980). When attentions are drawn to the electrochemical phenomena, a triphasic model that separates ions from the fluid phase has been developed to encompass the physicochemical and swelling properties and mechanical behaviours (Frijns et al., 1997; Lai et al., 1991).

Whereas analytical approaches have been limited to simplified constitutive laws, idealised boundary conditions and simple regular geometries, numerical simulation provides an alternative for complex deformation and mechanical responses due to nonlinearity in tissue properties and realistic boundary conditions. Another advantage of numerical methods is its ability to provide detailed distribution of variables in tissues which are difficult to measure in experiments.

Since the biphasic conception was proposed for the cartilage in 1980 (Mow et al., 1980), linear biphasic theory has been the simplest but most frequently adopted biphasic model. It assumes that the solid ECM is linear elastic and isotropic, and the hydraulic permeability for the fluid phase in the ECM is constant during loading (Spilker and Suh, 1990). Considering material symmetries, Cohen et al. studied the transversely isotropic biphasic model in 1992 (Cohen et al., 1992). To account for the intrinsic viscoelasticity of the ECM, linear biphasic theory was extended to poroviscoelastic theory (Suh and Bai, 1998) in numerical simulation. The articular cartilage exhibits tension-compression nonlinearity in loading, Soulhat et al. (1999)
proposed a fibril-network reinforced model for the solid phase of the biphasic cartilage that incorporates tension-compression nonlinearity, where collagen fibrils can only sustain tensile stresses. Recently, Wilson et al. hypothesized the fibril-reinforced poroviscoelastic swelling model to describe the swelling properties of the cartilage caused by the fixed-change densities of the proteoglycans and anisotropic collagen structure (Wilson et al., 2004; Wilson et al., 2005c).

1.4.2 Cardiac tissue

Mechanical properties of cardiac tissues could be distinguished into passive-state and total-state due to the muscle fibres contraction which is a result of the length change of sarcomere units within the muscle fibres. Passive-state is when the muscle fibres are fully relaxed. On the contrary, total-state is when the muscle fibres are fully contracted. Total-state stress is the sum of passive-state stress and active stress which is contributed by the fully contracted muscle fibres.

Some investigators tried to derive resting mechanical properties from relationship between end-diastolic blood pressure and chamber volume in the earlier studies (Ghista et al., 1975). In their studies, left ventricle was represented as a homogeneous isotropic shell of muscle, effective Young’s modulus was determined using a mathematical model of the left ventricle.
Figure 1.10 Defined fibre orientation in an element for FEM analysis.

f is the fibre direction, s is the sheet direction and n is the normal direction. Adapted from Holzapfel and Ogden (2009).

Although the pressure-volume approach could determine the overall stiffness of the chamber, it is not useful in studying regional phenomena such as stress-strain behaviour defined in terms of a strain energy density function (Demer and Yin, 1983; Demiray, 1976). The uniaxial tension was adopted by Demiray (1976) to study the mechanical properties of myocardium. He proposed a simple isotropic model in which the constitutive equation is an exponential function of the invariant $I_2$. The first biaxial tests of excised passive myocardium done by Demer and Yin (1983) revealed that myocardium is an anisotropic, highly nonlinear material. To understand mechanical response in different directions, a lot of investigators used the transverse isotropic model to describe the myocardium. Humphrey and Yin (1987) developed the first anisotropic invariant-based model that took account of the fibre structure. There are two exponential components representing extracellular matrix and fibre...
separately in their model. Later, Humphrey and co-workers (Humphrey et al., 1990) developed another transverse isotropic model which couples the effects of extracellular and fibre. Some other researchers also proposed transverse isotropic models based on the Green-Lagrange strain tensor (Costa et al., 1996; Guccione et al., 1991). From studies on cardiac structure, muscle fibres show three characteristic directions (Figure 1.10) in myocardium (Legrice et al., 1997; Legrice et al., 1995). These three directions are defined as fibre (f), sheet (s) and sheet-normal (n). Pairs of the abbreviation fs, fn and sn are used to refer to the fibre-sheet, fibre-normal and sheet-normal planes, respectively, for the convenience of modelling studies (Hunter et al., 1997; Nash and Hunter, 2000). Hunter and co-workers (Hunter et al., 1997) defined the first orthotropic model using a pole-zero strain energy function. Another two orthotropic models were developed by Costa and his colleague (Costa et al., 2001), and Schmid and co-workers (Schmid et al., 2006), respectively. The former one has one exponential form which couples the effects of the material parameters in contrast to later one which consists of separate exponential terms for each component of Green strain. All the above orthotropic models were generated based on the biaxial experimental data but may not fit for the shear data. Therefore, Holzapfel & Ogden (2009) proposed an invariant based anisotropic constitutive law according to shear tests data, which Dokos et al. (2002) obtained from cube-shaped specimens of porcine heart in different orientations.

All the models mentioned above are designed for passive myocardium. For active myocardium, two types of models have been proposed: one considered active force in the fibre direction only (Guccione et al., 1993; Nash and Hunter, 2000; Usyk
et al., 2000) while the other incorporated transverse active force development (Lin and Yin, 1998; Usyk et al., 2000).

1.4.3 Endothelial cells and the glycocalyx layer

1.4.3.1 Endothelial cells

Most theoretical modelling for endothelial cells are continuum models. Endothelial cells have been modelled as linear elastic solid, viscoelastic solid and hyperelastic solid. Currently, most modelling researches are mainly serving for the experimental measurement of cell mechanical properties. Until a relatively complete set of cell parameters is ready, sophisticated modelling can start. Atomic force microscopy is a technique not only to provide high-resolution topographical imaging but also to measure the force-displacement curve (Radmacher, 1997). This has been increasingly used for the biological samples including endothelial cells (Kang et al., 2008; Sato et al., 2001). Traditional analyses of AFM micro-indentation on endothelial cells are based on the assumptions that cells are linear elastic half-space (Kataoka et al., 2002; Sato et al., 2001; Sato et al., 2000). Following these simplifications, analytical solutions to this type of frictionless contact problem are available for different tip geometries, like sharp conical tip or a microsphere bead attached to the tip (Sneddon, 1965), sharp pyramid tip (Bilodeau, 1992), blunt conical tip (Briscoe et al., 1994) and blunt pyramid tip (Rico et al., 2005).
Costa and Lin (1999) proposed a general form of the force-indentation relationship based on the linear hyperelastic (hyperelastic constitution but in small strain range) material for the AFM micro-indentation and compared the effects of different tip geometry. Na and co-workers generated a theoretical framework incorporating the hyperelastic material properties and finite thickness of the cells for sphere bead indenter (Na et al., 2004). Most recently, Kang et al. (2008) used the finite element method (FEM) incorporating the Arruda-Boyce model and finite thickness of endothelia cells.

1.4.3.2 The glycocalyx layer

For the modelling of the glycocalyx layer, the number of reported works is very limited and mainly focuses on estimation of its mechanical properties. Vink et al. (1999) reported a time-dependent restoration of the glycocalyx after the passage of a white blood cell in capillaries. Based on these experimental observations, Weinbaum et al. (2003) used an indirect approach to estimate the flexural rigidity of the layer and elastic properties of the glycocalyx layer were derived. Han et al. (2006) advanced the model to consider large deformation of the glycocalyx fibres. An electrochemical model was proposed by Stace and co-workers (Stace and Damiano, 2001) to study the transport of charged molecules through the glycocalyx matrix. Wang and Parker (1995) applied asymptotic analysis of the poroelastic theory to study the interaction between cell and the glycocalyx layer, and Wang (2007) later extended the analysis to estimate the actual force which endothelial cells receives.
from fluid shear stress. Other hemodynamic models look into flow in the glyco-
calyx layer (Damiano and Stace, 2005), restoring mechanism (Pries et al., 1998;
Secomb et al., 1998; Weinbaum et al., 2007), and permeability studies (Michel, 1997). A
comprehensive review on recent studies on the glyco-
calyx layer was written by
Weinbaum and his colleagues (Weinbaum et al., 2007).

1.5 Parameter estimation

Parameter values need to be determined from experimental measurements
after a theoretical model is decided. This section describes the algorithms used in the
inverse analysis for the parameter value estimation of model function \( f(x_i, \alpha) \) from
experiment measurements \( y_i \). Here \( \alpha \) denotes a set of model parameter values which
are material parameter values in the study. \( x_i \ (i = 1, 2 \ldots m) \) represents different
observation points. The error between measurements \( y \) and predicted values \( f(x_i, \alpha) \)
is defined as:

\[
err(\alpha)_i = y_i - f(x_i, \alpha).
\]

Equation 1.17

The estimated parameters values could be optimized using nonlinear least square
method (Fletcher, 1987) which is the minimization of the sum of error squares (also
called objective function):

\[
S(\alpha) = \sum_{i=1}^{m} [y_i - f(x_i, \alpha)]^2 = \sum_{i=1}^{m} err(\alpha)_i^2.
\]

Equation 1.18
The minimization (optimization) algorithms are actually iterative procedures. An initial guess of the parameter values is given by users to start a minimization. In each iteration step, the parameter values $\alpha$ are replaced by new estimated values $\alpha + \delta$, where $\delta$ is a small step. The sum of square errors $S(\alpha + \delta)$ could be approximated by the second order Taylor’s series:

$$S(\alpha + \delta) \approx S(\alpha) + g\delta + \frac{1}{2}\delta^T H \delta,$$  \hspace{1cm} \text{Equation 1.19}

where $g$ denotes the gradient vector of $S$ and $H$ denotes the Hessian matrix of $S$. Superscript $T$ means transpose of a matrix. Then the procedure is to find the proper step $\delta$ to minimize the sum of square errors $S$. Thus the derivative with respect to $\delta$ equals to zero, i.e.:

$$S'(\alpha + \delta) = g + H\delta = 0,$$  \hspace{1cm} \text{Equation 1.20}

then,

$$H\delta = -g.$$  \hspace{1cm} \text{Equation 1.21}

From Equation 1.18 the gradient $g$ is given by:

$$g_j = 2 \sum_{i=1}^{m} \text{err}(\alpha)_i \frac{\partial \text{err}(\alpha)_i}{\partial \alpha_j},$$  \hspace{1cm} \text{Equation 1.22}

and the elements of Hessian matrix $H$ is:

$$H_{jk} = 2 \sum_{i=1}^{m} \left( \frac{\partial \text{err}(\alpha)_i}{\partial \alpha_j} \frac{\partial \text{err}(\alpha)_i}{\partial \alpha_k} + \text{err}(\alpha)_i \frac{\partial^2 \text{err}(\alpha)_i}{\partial \alpha_j \partial \alpha_k} \right).$$  \hspace{1cm} \text{Equation 1.23}

\subsection*{1.5.1 Gauss-Newton Algorithm}
Since the Hessian matrix is difficult to achieve in some cases, it is approximated by ignoring the second-order derivative terms in Gauss-Newton method (Björck, 1996):

\[ H_{jk} \approx 2 \sum_{i=1}^{m} \left( \frac{\partial err(\alpha)_i}{\partial \alpha_j} \frac{\partial err(\alpha)_i}{\partial \alpha_k} \right). \]  \hspace{1cm} \text{Equation 1.24}

The elements of Jacobian matrix \( J_{\text{err}} \) of vector \( err(\alpha) \) are given by:

\[ J_{ij} = \frac{\partial err(\alpha)_i}{\partial \alpha_j}, \]  \hspace{1cm} \text{Equation 1.25}

thus, the gradient and the approximate Hessian matrix can be written as:

\[ g = 2 J_{\text{err}}^T err(\alpha), \]  \hspace{1cm} \text{Equation 1.26}

\[ H \approx 2 J_{\text{err}}^T J_{\text{err}}. \]  \hspace{1cm} \text{Equation 1.27}

Now, it is easier to get \( \delta \) from vector \( err(\alpha) \) and its Jacobian:

\[ J_{\text{err}}^T J_{\text{err}} \delta = -J_{\text{err}}^T err(\alpha). \]  \hspace{1cm} \text{Equation 1.28}

### 1.5.2 Levenberg-Marquardt Algorithm

If the initial guess is far away from the final minimum, the Gauss-Newton method may not be able to find the correct solution. Thus, the Levenberg-Marquardt algorithm (Levenberg, 1944; Marquardt, 1963) was proposed, in which the term \( J_{\text{err}}^T J_{\text{err}} \) in Equation 1.28 is replaced by term \( J_{\text{err}}^T J_{\text{err}} + \lambda \text{diag}(J_{\text{err}}^T J_{\text{err}}) \), i.e.:

\[ (J_{\text{err}}^T J_{\text{err}} + \lambda \text{diag}(J_{\text{err}}^T J_{\text{err}})) \delta = -J_{\text{err}}^T err(\alpha), \]  \hspace{1cm} \text{Equation 1.29}

where \( \lambda \) is non-negative damping factor and it is adjusted at each iteration.
1.5.3 Trust Region Algorithm

Since the general nonlinear functions local approximate models (such as Taylor’s expansion) can only fit the original function well locally, this well local approximation is thought to be trust approximation. The local region is thus called the trust region (Yuan, 2000). The trust region is adjusted from iteration to iteration. The approximation model function $\phi(\delta)$ could be obtained from Equation 1.19:

$$\phi(\delta) = g\delta + \frac{1}{2}\delta^T H\delta,$$

with trust region constraint:

$$\|\delta\|_2 \leq \Delta,$$

where $\|\|_2$ is Euclidean norm and $\Delta$ is the trust region radius. The predicted reduction determined from the approximate model is:

$$pred = \phi(0) - \phi(\delta),$$

and the actual reduction is defined as the reduction in the objective function $S$:

$$ared = S(\alpha) - S(\alpha + \delta)$$

The ratio between the actual reduction $ared$ and the predicted $pred$, $ratio = \frac{ared}{pred}$, is used to decide whether the step is acceptable and to adjust the new trust region radius. The trust region approach always gives a good approximation model during the iteration to make sure the solution to the objective function is reasonable and best optimized.
1.5.4 Inverse FEM

For experiments of linear elastic samples undergoing small deformation, analytical model function (analytical solution) is available to identify mechanical parameter values. This can be easily implemented into commercial software, like Igor Pro (Wavemetrics, Lake Oswego, OR), to perform optimization analysis (Ledoux and Blevins, 2007). However, nearly no biological tissues are linear elastic and most experimental tests involve the finite deformation which invalidates the infinitesimal strain analysis (Costa and Yin, 1999; Natali et al., 2010; Nguyen et al., 2010). Such complicated problems are not amenable to theoretical analysis. FEM is a cost-effective alternative. The commercial finite element software ABAQUS™ (Dassault Systemes Simulia Corp., Providence, RI) has been broadly employed in both academic and industrial world. Furthermore, the optimization algorithm can be linked to FEM to characterize material parameters, forming so-called inverse FEM (Lei and Szeri, 2007; Zhang et al., 1997).

Since the optimization algorithms mentioned above are available in Matlab™ (The MathWorks, Inc., Natick, MA), the interconnection of experimental data and FEM result can be achieved on the Matlab platform. The self-developed optimization program includes two Matlab files (main.m and objfunction.m, see Appendix F1) and two Python coded files (modifyInput.py and predictedData.py, see Appendix F2). Matlab file main.m provides an interface for the user to set the initial guess, coefficient of evaluated parameters. It also calls Matlab built-in nonlinear least square function lsqnonlin() for the parameters evaluation. The other one links to the software
ABAQUS and two Python coded files. The Python coded file *modifyInput.py* generates a new ABAQUS input file including the new guess of parameters. While the other one exports predicted data from ABAQUS odb file. The programme generates input file, runs FE model, processes the results, and compares the experimental data to the current FEM result. A new group of estimated parameters are then generated by the least square algorithm. The iteration process continues running until a predefined tolerance of increment in each parameter or squared residual between experimental data and predicted values is reached. Detailed optimization flowchart is shown in Figure 1.11.

**Figure 1.11 Flowchart of the inverse FEM.**
1.6 Aim and objectives of the study

This PhD project aims to develop a computational modelling framework that can be applied to characterise mechanical properties of biological tissues. Furthermore, it can be used to quantitively tissue mechanical responses under different conditions. For this purpose, a finite element method (FEM) has been used to study the ‘forward and inverse problem’, i.e. from the tissue mechanical response to stimuli with known parameters to the determination of its material properties using measured response. Three selective biological tissues from cell, tissue to organ level will be used as examples in the study due to close association with clinical and experimental researchers in the laboratory.

In order to build up the computational framework and apply it to biological tissues, the objectives of the present study are:

- Set up an inverse FEM procedure to characterize biological tissue mechanical properties from experiment data.
- Implement the selected biological tissues models utilizing ABAQUS interface subroutines.
- Make proper Python scripts for pre-process and post-process of current study and future analysis.
Exam mechanical response of articular cartilages under external loadings using a fibril-reinforced poroelastic model, where the solid extracellular matrix (ECM) composes mainly of the collagen and proteoglycans, and the fluid phase is mainly composed of water and the dissolved ions. Unique in this study, the collagen fibril is treated separately from the rest of ECM, as it only resists tension. Effects of the distribution of the collagen fibrils and their orientation on tissue mechanical responses are investigated.

Predict the mechanical stress distribution of the patient left atrium and exam its correlation to electrophysiology patterns in atrial fibrillation. Detailed mechanical responses of the atrial wall to a step pressure increase in the left atrium are calculated. The geometry of the left atrium is based on patient specific images using cardio CT and incorporates variations of the atrial wall thickness as well as dominant fibre orientation patterns.

Determine the hyperelastic properties of the cultured endothelial cell and the overlying layer on the cell membrane – the endothelial glycocalyx, based on experimental data from cell micro-indentation using an AFM (atomic force microscopy) probe. Both endothelial cells with the glycocalyx layer and ones without the glycocalyx layer (i.e. following enzymatic digestion of the glycocalyx) are used. This is the first time that the hyperelastic properties of the glycocalyx are extracted from AFM tests using an inverse biomechanical model.
2 Effects of collagen fibrils on mechanical responses of the articular cartilage

2.1 Introduction

The articular cartilage is an inhomogeneous, anisotropic and multiphase tissue. It is a mixture of solid extracellular matrix and interstitial fluid. Collagen fibril in the solid matrix is good at resisting tension but not compression due to its slenderness. It behaves totally different from the other major component – proteoglycan, which is strong in resisting compression owing to fixed negative charges. When viewed perpendicularly to the surface, collagen fibrils show a dominant orientation which varies systematically across the whole thickness. In line with the zonal variation across the cartilage layer, collagen fibrils are usually organized in parallel to cartilage surface in the superficial zone. In the middle zone collagen fibrils are more randomly oriented, while in the deep zone collagen fibrils become perpendicular to the subchondral bone. Benninghoff (1925) described the 3D collagen network as the Arcade model. Bundles of primary fibrils extend perpendicularly from the subchondral bone, splitting up close to the articular surfaces into fibrils (Wilson et al., 2004). Since the specialized structure is essential for the mechanical function of cartilage, any damage to the collagen network becomes one of the initiators of cartilage pathogenesis.
Collagen fibril reinforcement has been found to influence the cartilage’s mechanical responses from theoretical analysis and numerical simulation. Poroelastic theory has become well accepted to study mechanical responses of articular cartilage which are mainly attributed to the fluid-solid interaction. Recent advances employing higher levels of tissue material complexity have been theoretically modelled, including inhomogeneities in material properties and fixed charge density, material symmetries, and matrix viscoelasticity (Mow and Guo, 2002). Another important step is the development of the fibril-reinforced poroelastic model to focus on the distinct role of collagen fibrils (Soulhat et al., 1999). According to this model, a non-fibrillar matrix representing the proteoglycan network is mechanically reinforced by a fibrillar matrix representing the collagen network. Very few theoretical solutions were sought due to the complexity in the fibril-reinforced poroelastic model. Stress relaxation responses in the ideal unconfined compression are available for cartilage which either adopts homogeneous material properties or assumes transverse isotropy. These solutions not only provide insight into effects of collagen fibrils but also act as valuable validation for numerical simulation.

Most detailed roles of collagen fibrils are obtained via numerical simulations. Numerical simulations enable us to study different inhomogeneities stemming from material properties, collagen fibril strain-dependent stiffness and varying orientation, strain-dependence of permeability, and finite deformation (Li et al., 2003b; Li and Herzog, 2004b). Those simulations refine the prediction of mechanical response in the unconfined compression configuration (Li et al., 2003a; Li et al., 2000). Collagen fibrils successfully addressed observed asymmetry of the mechanical response in
loading vs unloading phase (Li et al., 2001), strain-rate dependence of cartilage stiffness (Li and Herzog, 2004b) and the interaction between collagen fibril and fluid pressurization (Li et al., 2003a). This model has been recently extended to incorporate the viscoelasticity of collagen fibrils (Li and Herzog, 2004a; Wilson et al., 2004, 2005b). It has been used to explain and correct the uncertainties in indentation testing of articular cartilage. When a term representing the effect of Donnan’s osmotic pressure is added into the non-fibrillar matrix, it led to the fibril-reinforced poroviscoelastic swelling model (Wilson et al., 2005b). According to this model, the depth-dependent compressive equilibrium properties of articular cartilage can be purely explained by its composition (Wilson et al., 2007).

Apart from the refinement in the theoretical models, the fibril-reinforced poroelastic model is also applied to more realistic applications. Mechanical responses of a homogenous cartilage to indentation test are simulated using the fibril-reinforced poroviscoelastic model (Wilson et al., 2004) and fibril-reinforced poroviscoelastic swelling model (Wilson et al., 2005a) respectively. Li investigated three-dimensional fibril-reinforced finite element model of an articular cartilage subjected to indentation and observed a significant fibril orientation dependence in the displacement, fluid pressure and stress (Li et al., 2009). Shirazi and Shirazi-Adl (2008) applied the fibril-reinforced poroelastic model to investigate the role of fibril networks in knee joint mechanics. The deep vertical fibrils network in cartilage was found to play a crucial role in stiffening global response (Shirazi and Shirazi-Adl, 2008).
All numerical simulation using fibril-reinforced poroelastic models have been, surprisingly, conducted using the software ABAQUS. ABAQUS is one of limited number of software offering the module of soil consolidation analysis, which follows the poroelastic theory. The flexibility in extension of user-subroutine and wide choice in the type of finite element are other important reasons to gain popularity. Membrane element type was initially chosen to represent collagen fibrils orientated in one direction such as horizontally or vertically (Shirazi and Shirazi-Adl, 2005, 2008). Spring element type was also suggested to represent collagen fibrils but is awkward to implement for randomly oriented collagen fibrils (Li et al., 2003a; Li et al., 2001). This was overcome by creating a user-defined continuum element type. Alternative method is to redefine the stress-strain relationship via the user-subroutine UMAT. The advantage of this approach is to offer more direct control and is thus able to incorporate any sophisticated constitutive relationship. This choice has prevailed in recent fibril-reinforced poroelastic modelling works using ABAQUS. The primitive fibril-reinforced poroelastic model may suffice for many research studies, however almost all studies used simplified testing configurations. These configurations are useful to interpret the experimental measurement but far from the realistic loading situation. Recently, a new configuration – confined indentation was proposed. The lateral confining adjacent tissues and underlying subchondral bone acts as impermeable rigid wall restricting the cartilage movement while loading is applied in the centre of top surface. This configuration is simple yet but more representative of physiological situation.
This chapter focuses on a generic approach to consider fibril reinforcement and fibril orientations with modified material constitutive equations. This extends previous work in our research group (Lu and Wang, 2008) to quantify the interaction between the interstitial fluid and the matrix in confined indentation under a ramp load. To this aim, a fibril-reinforced model will be superimposed to the poroelastic model for the soft tissue. The solid phase becomes an overlapping structure of the fibrillar matrix and the non-fibrillar matrix. The fibrillar matrix further considers of different fibril orientations. ABAQUS (Pawtucket, RI) based on a finite element method is employed to simulate mechanical responses. Effects of the collagen fibril orientation and inhomogeneous property of the articular cartilage are investigated in confined indentation.

To address effects of tissue inhomogeneity and anisotropy caused by the collagen fibrillar matrix, physiological parameters determined from unconfined compression experimental data are then applied to the confined indentation configuration. Effects of the collagen fibril inhomogeneity and orientation in each layer of the articular cartilage are investigated on the whole tissue mechanical response.

### 2.2 Theoretical models and numerical methods

#### 2.2.1 Theoretical models
In traditional poroelastic models, the cartilage is treated as a biphasic mixture including solid and fluid phases. Each phase is assumed to be intrinsically incompressible. In the solid phase, ECM consists of proteoglycans and collagen fibrils, which are considered to be bonded together. The stress for the solid matrix and fluid phases is expressed in Equation 1.5-1.7. In the fibril-reinforced poroelastic model, the solid phase is divided into two parts: fibrillar matrix and non-fibrillar matrix. The fibril distribution was assumed to be equally distributed in the radial, circumferential and axial directions (Soulhat et al., 1999) (Figure 2.1). No shear stress occurred while strains were assumed identical in the fibril network and solid matrix. Then the total stress of the solid phase is changed to the addition of ECM stress $\sigma_m$ and fibril stress $\sigma_f$ (Equation 2.1) while fluid phase remains the same.

$$\sigma_s = \sigma_m + \sigma_f,$$

where components of fibril stress $\sigma_f$ only exist in 3 directions ($r$, $\theta$ and $z$).

**Figure 2.1 Collagen fibrils distribution in fibril-reinforced model.**

Figure is adapted from Soulhat et al. (1999).
Assuming the Young’s modulus of the fibril network is $E_f$ in any of the three mutually orthogonal directions and fibril had tensile resistance only, i.e. the Young’s modulus of fibril for compression was assumed to be zero. The stress and strain relationship of fibril-reinforced composite models in $r$, $\theta$ and $z$ directions are:

$$\sigma_s = \lambda \text{tr}(\varepsilon)I + 2\mu \varepsilon + E_f \varepsilon \ I$$  \hspace{1cm} \text{Equation 2.2}

Cartilage is assumed to be fully saturated therefore the solidity $\varphi_s$ and the porosity $\varphi_w$ satisfy:

$$\varphi_s + \varphi_w = 1,$$  \hspace{1cm} \text{Equation 2.3}

The nonlinear strain-dependent permeability $k$ (Lai et al., 1991; Li et al., 1999) is represented in the analysis:

$$k = k_0 \exp(M\varepsilon),$$  \hspace{1cm} \text{Equation 2.4}

where $M$ is a constant and $k_0$ denotes the intrinsic permeability.

### 2.2.2 Boundary

In the unconfined compression, a thin cylindrical cartilage layer is placed between two impermeable, rigid plates in the solution as shown in Figure 2.2. There is no restriction on the lateral deformation. The bottom plate is fixed and the top one is allowed to move on the axial direction only. Specimen slides freely between the plates while top plate is compressing following the displacement controlled ramp loading (Figure 2.3). In the confined indentation configuration, the specimen is placed in a rigid, impermeable and confined chamber (Figure 2.2). External loads are applied through a porous semi-spherical end indenter tethered to the central region of
the top surface of the specimen. Subjected to the same loading, sample is assumed to slide freely along the indenter surface.

*Figure 2.2 Hypothesized experimental configurations to determine mechanical properties of soft tissues.*

Figure 2.3 Displacement controlled ramp loading.

$u_0$ and $\varepsilon_0$ is the displacement and strain at peak time $t_0$.

2.2.3 Numerical methods

The poroelastic modelling was carried out using the soil consolidation analysis with consideration of nonlinear geometry effects in ABAQUS. The first and the second Lamé’s constants of the solid phase, $\lambda$ and $\mu$, used in the biphasic model (Equation 1.6), were converted into Young’s modulus $E$ and the Poisson ratio $\nu$:

$$
\begin{align*}
E &= \frac{\mu(3\lambda + 2\mu)}{\lambda + \mu}, \\
\nu &= \frac{\lambda}{2(\lambda + \mu)}.
\end{align*}
$$

Equation 2.5

The permeability $k$ was defined in the biphasic model (Mow et al., 1980). However in the ABAQUS, only the hydraulic conductivity $k'$ is used, which can be converted from $k$ by multiplying the specific volume weight of the interstitial fluid $\gamma$ ($\gamma = 9.81 \times 10^{-6} Nm^{-3}$):
Void ratio $e$ is used in ABAQUS which is defined as the ratio between the solid $\varphi_s$ and fluid $\varphi_w$ volume fraction:

$$e = \frac{\varphi_w}{\varphi_s} = \frac{1 - \varphi_s}{\varphi_s}. \quad \text{Equation 2.7}$$

The fibril-reinforced poroelastic model is not directly available in ABAQUS. To incorporate the fibril-reinforced poroelastic model, the constitutive equation for the solid phase needs to be redefined. To achieve this, a user-subroutine UMAT is called to define the stress-strain relationship (Equation 2.2) for the solid extracellular matrix (Appendix A). In the subroutine UMAT, increment in Cauchy stress $\Delta \sigma_s$ in Equation 2.8 is updated during each step of the calculation. The consistent Jacobian matrix $J_c$ is defined as in Equation 2.9.

$$\Delta \sigma_s = \lambda \text{tr}(\Delta \varepsilon) \mathbf{I} + 2\mu \Delta \varepsilon + E_f \Delta \varepsilon \mathbf{I}, \quad \text{Equation 2.8}$$

$$J_c = \frac{\partial \Delta \sigma_s}{\partial \Delta \varepsilon}. \quad \text{Equation 2.9}$$

Considering the symmetry of the problem, there are four components in the increment in Cauchy stress $\Delta \sigma_s$:

$$\begin{pmatrix} \Delta \sigma^r_s \\ \Delta \sigma^\theta_s \\ \Delta \sigma^z_s \\ \Delta \sigma^{rz}_s \end{pmatrix} = \begin{pmatrix} S_{11} & S_{12} & S_{13} & S_{14} \\ S_{21} & S_{22} & S_{23} & S_{24} \\ S_{31} & S_{32} & S_{33} & S_{34} \\ S_{41} & S_{42} & S_{43} & S_{44} \end{pmatrix} \begin{pmatrix} \Delta \varepsilon^r \\ \Delta \varepsilon^\theta \\ \Delta \varepsilon^z \\ \Delta \varepsilon^{rz} \end{pmatrix}. \quad \text{Equation 2.10}$$

From Equation 2.5 and Equation 2.8, the elements of Jacobian matrix of material stiffness $S_{ij}$ are:
\[
\begin{align*}
S_{11} &= S_{22} = S_{33} = \frac{(E_f + E)(1 - \nu) - 2E_f\nu^2}{(1 + \nu)(1 - 2\nu)}, \\
S_{12} &= S_{21} = S_{13} = S_{31} = S_{21} = S_{32} = \frac{E\nu}{(1 + \nu)(1 - 2\nu)}, \\
S_{14} &= S_{24} = S_{34} = S_{41} = S_{24} = S_{34} = 0, \\
S_{14} &= \frac{E}{(1 + \nu)}. 
\end{align*}
\]

Equation 2.11

for the fibril would be zero if the strain is negative.

Additional modifications are required for the default ABAQUS input to carry the simulation. The subroutine is called through keyword *User Material in the ABAQUS input file. Keyword *orientation is used to represent fibril orientation during the deformation of cartilage. The element type has to be changed to CAX4P to include the fluid effect.

2.3 Results

2.3.1 Validation

To validate the development of fibril-reinforced poroelastic modelling, a stress relaxation in the unconfined compression configuration is used. When subjected to frictionless contact between the loading platen and samples, the mechanical response of soft tissue is reduced to one-dimensional problem based on the radial position and has analytical solutions. Analytical solutions of fibril reinforced poroelastic model were derived by Soulhat et al. (1999) which extended
the previous theoretical analysis of unconfined compression of poroelastic sample (Armstrong et al., 1984). These analytical solutions serve as ideal examples for validation of our development in numerical modelling.

Using the same parameters listed in Table 2.1, the numerical prediction using UMAT subroutine matches the published analytical solution satisfactorily for both averaged surface stress and pore pressure evolution in the sample centre. The error was found no more than 1% (Figure 2.4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of cartilage ($h$)</td>
<td>0.77 mm</td>
</tr>
<tr>
<td>radius of cartilage ($R$)</td>
<td>2.39 mm</td>
</tr>
<tr>
<td>void ratio ($e$)</td>
<td>4.0</td>
</tr>
<tr>
<td>Young's modulus ($E$)</td>
<td>0.65 MPa</td>
</tr>
<tr>
<td>Poisson’s ratio ($\nu$)</td>
<td>0.36</td>
</tr>
<tr>
<td>fibril Young's modulus ($E_f$)</td>
<td>10 Mpa</td>
</tr>
<tr>
<td>permeability ($k'$)</td>
<td>1x10^{-15} m^4/Ns</td>
</tr>
</tbody>
</table>

Table 2.1 Values of parameters used in the validation.

Values are based on experimental data of the articular cartilage in our laboratory and other studies (Lu and Wang, 2008; Soulhat et al., 1999).
Figure 2.4 Validation of fibril-reinforced poroelastic modelling implemented in ABAQUS for stress relaxation in the unconfined compression.

a) Normalized average surface stress vs. time. b) Normalized pore pressure (at centre disk) vs. time. Analytical solution is plotted as symbol while numerical result is solid line. Values are normalized by equilibrium unconfined modulus.
2.3.2 Parameter estimation

The good match between numerical results and analytical solution demonstrates not only a correct mastery of ABAQUS poroelastic model but also provides the validity of user subroutines developed for the general fibril-reinforced poroelastic model. Next the parameter values of fibril-reinforced poroelastic model are determined through the inverse FEM methods (Section 1.5). Soltz and Ateshian (2000) measured the average surface stress and pore pressure history from 6-month-old bovine cartilage sample (0.77 mm in height and 2.39 mm in radius) in the unconfined compression at strain of 10% in 300 seconds. The arbitrary initial guess are 0.267 MPa, 0.442, 14.579 MPa and $6.42 \times 10^{-9}$ m$^4$/Ns for Young’s modulus $E$, Poisson’s ratio $\nu$, fibril modulus $E_f$ and initial permeability $k'_0$, respectively. The converging criteria was set as the increment in each parameter or squared residual between experimental data and predicted values is less than $1 \times 10^{-7}$. In Figure 2.5, at the same original data, the prediction of finally accepted parameters are presented to demonstrate a good agreement, with coefficient of determination ($R^2$) for the average stress fitting (top) and pore pressure at the center line of the cartilage (bottom) fitting are 0.993 and 0.996, respectively. From the convergence history of all parameters shown in Figure 2.6, their values start to converge soon after 5 iterations. They are normalized by the optimized values in order to put parameters in a single graph.
Figure 2.5 Determination of parameters from experimental data using the inverse FEM.

Parameter values (0.25 MPa, 0.25, 10 MPa and $1.07 \times 10^{-9}$ m$^4$/Ns for $E$, $v$, $E_f$ and $k'_0$, respectively) for the fibril-reinforced poroelastic model are obtained from fitting the unconfined compression stress-relaxation data of average stress (a) and pore pressure (b) from (Soltz and Ateshian, 2000) using the inverse FEM.
2.3.3 Mechanical responses in the confined indentation configuration

To illustrate the contribution of collagen fibrils to cartilage’s mechanical response, poroelastic response was first compared when collagen fibril is absent or is present in a prescribed direction. Then different collagen fibril orientations are examined and their effects on the mechanical response are checked. Finally, mechanical responses of cartilages using physiologically relevant collagen fibril orientations are studied using a layered model for cartilage. All studies are stress
relaxation tests via controlled ramp displacement in the configuration of unconfined compression.

2.3.3.1 Fibril-reinforced poroelastic model vs poroelastic model

To test the effect of collagen fibrils, samples of the same geometry and same material properties for fluid phase and non-fibrillar matrix are used in numerical simulation. When collagen fibrils are assumed randomly distributed inside cartilage, mechanical response is calculated from the fibril-reinforced poroelastic model. If collagen fibril not separated from the rest of extracellular matrix, the poroelastic model is directly called. Under an external load, cartilage sample develops high fluid pressure beneath the indenter, which help solid matrix undertake the load. The highest pressure is in the top-centre close to indentation site. Loading causes a local compaction of cartilage which fluid pressure builds up quickly due to large friction resistance to fluid escape by solid matrix. Due to the impermeable bottom wall, the pressure in the deep zone is also relative high. The pressure level near the lateral confining wall is low as they are far from the indenter region but not far from the free surface where pressure is set zero. Although the overall pressure pattern look similar for both models, the incorporation of collagen fibril augments the pressurization level, with the peak pressure 0.235 MPa higher than 0.073 MPa of poroelastic model. The pore pressure difference reflects the contribution of fluid pressurization to undertake load. Moreover, the large pressure zone nearly spans across the whole thickness of cartilage sample in the sample reinforced by collagen fibril. Hence when
the collagen fibrillar matrix is not distinguished with non-fibrillar matrix, the poroelastic modelling will underestimate the load-sharing role of interstitial fluid.

It is noteworthy to examine the radial stress distribution. In confined indentation, the extracellular matrix will undergo extension only along the radial direction. Thus collagen fibrils are anticipated to produce a notable difference in the radial stress pattern. From the comparison in Figure 2.8, high radial stress, which confirms the radial extension, is noted in both models. However the level of radial stress existing in the central region is one order of magnitude higher in the fibril-reinforced poroelastic model. As cartilage deformation is more or less similar under the same load, non-fibrillar matrix experiences similar stress. The difference of total radial stress between these two cases reflects the contribution of collagen fibrillar matrix. A small extension in radial strain will induce a higher elastic stress along the radial direction due to exceptionally high elastic modulus of collagen fibril compared with the non-fibrillar component. During the loading phase, a significant amount of fluid is expelled from the top-centre just below the indenter. The fluid flow render stretching of collagen fibril. As a result, a high radial stress is found in the fibril-reinforced poroelastic model but not purely poroelastic model. The deformed cartilage and fibril orientation are shown in Figure 2.9. The fibril orientation is assigned to be parallel to the edges of elements. Therefore it can be changing as long as the cartilage deformation. When induced deformation is large in these two cases, change in collagen fibril orientation near the indenter will cause a considerable solid radial stress component towards the total vertical reaction force.
Figure 2.7 Comparison of the pore pressure distribution at the peak load.

collagen fibrils are considered explicitly using the random fibril-reinforced poroelastic model. b) collagen fibrils are not considered using the poroelastic model.
Figure 2.8 Comparison of the radial stress distribution at the peak load.

Radial stress is normalized by its own peak stress. a) collagen fibrils are considered explicitly using fibril-reinforced poroelastic model. b) the poroelastic model.
2.3.3.2 Effects of collagen fibril orientation

From the above studies, collagen fibrils are important to cartilage’s mechanical response. Each collagen fibril has a unique directionality. It will be interesting to examine if the collagen fibril orientation has an effect. Three collagen fibril orientations -- horizontal, random and vertical - are compared, all of which can be found in different layers of articular cartilage. For the convenience of comparison, the same model is studied using the fibril reinforced poroelastic modelling except that collagen fibril assumes different orientations. At the peak load, the pressure distribution is similar in horizontal case and random case but differs markedly from the vertical case. Compared with the high pressure zone formed in both horizontal
case and random case, the vertical case has a much lower pressurization, which is actually closer to poroelastic modelling results where no collagen fibril is considered. In other words, horizontal collagen fibrils play an essential role while vertical fibrils least important. This is not difficult to understand in confined indentation. The axial loading in the central region will compress the sample in the axial direction and squeeze out the interstitial fluid, which in turns extends the central part in the radial direction. The peripheral region is less constrained so that a slight extension in the axial direction is expected. According to pressure contour, a high pressure zone is found for the cartilage sample reinforced by horizontal fibrils. In the presence of randomly organized fibrils, the peak pressure zone occurs to similar position but at a lower range. The second high pressure zone occurs to the deep central region as it is far from free surface. In the cartilage with vertically organized collagen fibrils, the pressurization level is lowest with two high pressure zones closer to each other in the pressure level (Figure 2.10).

Radial stress contour exhibits different patterns (Figure 2.11). In both horizontal case and random case, the contour pattern is similar, just like the fluid pressure distribution. Following the loading, a severe radial stress is experienced near the indenter edge in the superficial area after equilibrium. The positive stress also confirms a certain extent of solid matrix extension, which yields high elastic stress. Although this tensile stress is not as significant as compressive counterpart shown in the top-centre of cartilage for this mild loading, collagen tension may be critical to protect tissue from excessive loading.
**Figure 2.10 Pressure contours at the peak load.**

All parameters take the same values for these cases except that collagen fibrils are oriented differently, a) horizontal direction, b) random, and c) vertical direction.
Figure 2.11 Radial stress contours at the equilibrium.

Radial stress is normalized by its own equilibrium stress. All parameters take the same values for these cases except that collagen fibrils are oriented differently a) horizontal direction, b) random, and c) vertical direction.
2.3.3.3 Mechanical response of multiple layered cartilage models

The articular cartilage has a typical multi-layered structure. Accurate prediction of cartilage mechanical response must consider the three-layers: top, mid and bottom layers. To represent realistic articular cartilage, physiological fibril orientation is chosen for each layer. In the top layer, fibril lies on the horizontal direction only. Middle layer has random directions. Fibril is mainly on the vertical direction in the bottom layer (see Figure 1.1). Percentage of thickness of each layer was the same as previous study (i.e. 12%, 19% and 69% of the whole thickness in top, random and bottom layer, respectively) (Shirazi and Shirazi-Adl, 2008). When those representative fibril orientations are incorporated, the model is referred to as the three-layer model. Pore pressure and normalized radial stress are shown in Figure 2.12.
Figure 2.12 Mechanical responses of the three layered articular cartilage to ramp loading.

a) pressure contour at the peak load. b) radial stress contour at the equilibrium.

The articular cartilage is susceptible to excessive usage, where abnormal high stress develops and causes cartilage failure. This tendency can be predicted from the information on the maximum von Mises stress. The comparison of normalized (by the peak stress) maximum von Mises stress in each zone at the same transient time point (1s) are shown in Figure 2.13. When the fibril orientation is horizontal, vertical or random, the normalized maximum von Mises stress in top zone is greater than in middle zone and bottom zone. In the three layered cartilage, the normalized maximum von Mises stress in middle zone is higher than in top and bottom zone. The normalized maximum von Mises stress in bottom zone is the lowest compare with other two zones in each case. The normalized maximum von Mises stress in middle zone in the three-layer mode is the highest whereas lowest in the top and the bottom zones when compares with corresponding zone in other cases.

The comparison of normalized (by the equilibrium stress) maximum von Mises stress in each zone at the end of loading time point are shown in Figure 2.14. The results are found similar to that in Figure 2.13.
Figure 2.13 Normalized maximum von Mises stress in each zone at transient time.

The top zone is in blue, middle zone in red and the bottom zone in green.

Figure 2.14 Normalized maximum von Mises stress of each zone at the equilibrium state.

The top zone is in blue, middle zone in red and the bottom zone in green.
Comparison of normalized (by the peak stress) maximum pore pressure in the whole cartilage where fibrils are considered is shown in Figure 2.15. The normalized maximum pore pressure is the highest (i.e. 0.086) when all the fibrils are horizontal. The value is lowered by 42% when all the fibrils are vertical. The maximum pore pressures in other 2 cases (when the fibril orientation is random and physiological pattern) are about the same in magnitude and are reduced by 11% in comparison to the horizontal case. In Figure 2.16, we give the size of the cross sectional area (in percentage) where the normalised pore pressure is greater than 10% in four cases. Here, 10% of the highest normalized maximum pore pressure is chosen arbitrarily as the pore pressure below that value is thought to be less than effective. In contrast to the rank of maximum pore pressure, the size of the area is greatest (i.e. 77%) when the fibril is vertically orientated, whereas the area is the least (i.e. 42%) when the fibril orientation is horizontal. The area is 54% and 46% respectively when fibril orientation is random and physiological pattern.

Figure 2.15 The normalized maximum pore pressure in different configurations.
Figure 2.16 Area of effective pore pressure.

The reaction forces in five different cases are show in Figure 2.17. When the fibril orientation is random, the peak reaction force (0.217 N) is the highest among the five cases, whereas without fibrils, the peak reaction force (0.063 N) is the lowest. The order of the peak reaction force for the rest 3 cases from high to low is when the fibril orientation is horizontal, random and vertical, with values of 0.19 N, 0.12 N and 0.075 N, respectively. All the five cases reach the equilibrium after 900 seconds and the reaction forces decrease to between 0.045 N and 0.07 N.
The fibril reinforced poroelastic model used in the study was initially proposed by (Soulhat et al., 1999). It was implemented in commercial software ABAQUS using subroutine UMAT. This approach is easier to apply to different configurations, for example, indentations or different geometrical aspect ratios, for future study. The other methods employed different element type (Li et al., 1999; Shirazi and Shirazi-Adl, 2008) to implement Soulhat’s model require specific techniques during the configurations setup. Thus it is inconvenient to apply them to variant configurations. There are some other complicated models incorporating nonlinear elasticity of proteoglycan (Li and Herzog, 2004b), swelling effects of proteoglycan (Wilson et al., 2005a) and different fibril properties such as viscoelastic (Li and Herzog, 2004a;
Wilson et al., 2004) and strain dependent fibril modulus (Li et al., 2003b). Benefits of these models are, however, compromised by the difficulty in obtaining a complete set of consistent parameters. This study was focused on the effects of the different fibril orientations on the cartilage mechanical response to the external loading.

To highlight the effect of collagen fibril network in cartilages, a homogeneous matrix without fibril-reinforcement is considered first as a reference. When the collagen fibril is considered, four collagen fibril orientations are considered in this study as examples: horizontal, vertical, random and a combination of these three orientations. The reaction force plot shown in Figure 2.17 indicates how much external force needed to reach a certain strain of cartilage. The higher reaction force needed in a configuration the less nominal strain will be caused on the cartilage when subject to external loading during daily activities. There is the least nominal strain in the cartilage when the fibrils are randomly distributed through the whole cartilage.

The maximum von Mises stress is analysed at each zone in each case at both transition and equilibrium (Figure 2.13 and Figure 2.14). The von Mises stress is normalized by the contact surface stress at both transition and equilibrium, respectively. Thus it is independent of external force or nominal strain. There is a lower maximum von Mises stress in both the top and bottom zones in the physiological case, although it has the highest maximum von Mises stress in the middle zone. The reduction percentage from least to highest is around 25%, 42% and 45% in top, middle and bottom zone, respectively. These numbers suggest that the physiological case is an advanced configuration of fibril orientation in terms of maximum von Mises stress.
The pore pressure is another indicator of how much the cartilage matrix could benefit from the interstitial fluid. The higher pore pressure or the larger pore pressure area the more protection cartilage matrix will obtain. In terms of pore pressure value, there is best mechanical protection when the fibrils are horizontal distributed through the whole cartilage while least when the fibril orientation is vertical (Figure 2.15). In contrast, in terms of pore pressure area, there is best mechanical protection when the fibrils are vertical distributed through the whole cartilage while least when the fibril orientation is horizontal (Figure 2.16). They are totally converse results, thus it is better to consider both factors to evaluate the mechanical protection. The physiological case is in the 2nd place in both maximum pore pressure value and effective pore pressure area. The difference to the 1st place is not very big, 17% and 30%, respectively. As a result, the physiological pattern is an optimized configuration in the light of factor of pore pressure for this loading.

The mechanical function of articular cartilage is complicated as a result of its complex structure. It is not wise to evaluate mechanical function of cartilage fibril orientation by considering any single factor. The physiological fibril orientation can be viewed as having the best mechanical protective function by accounting for the combination of the reaction force, maximum von Mises stress and the pore pressure. Previous study proposed a mechanical model for the prediction of fibril orientation and found the fibril orientation would eventually lead to the physiological configuration in the unconfined configuration test whatever the initial orientation was (Wilson et al., 2006). My study could also be used to explain the phenomena that the fibril orientation changes from mainly horizontal to distinct three layers during the
process of cartilage maturation (Julkunen et al., 2009) in the need of cartilage protection.

2.5 Summary

A numerical modelling framework has been successfully developed. Based on the commercial FEM software ABAQUS, the constitutive equation specific to the fibril-reinforced poroelastic model is introduced by a user-subroutine UMAT. With this, collagen fibrillar matrix is found to play an important role to tissue mechanical response. The presence of the collagen fibrillar matrix increases the level of interstitial fluid pressure. Moreover, the randomly organized fibrils produce a maximal stress in confined indentation. von Mises stress analysis shows that the three layered cartilage with physiological fibril orientation in each layer represents an optimal combination, with nearly frictionless surface properties as well as a reasonable mechanical strength and secure attachment to the underlying subchondral bone.
3 Stress distribution of the left atrium using patient specific geometries

3.1 Introduction

Stress analysis of the cardiac chamber inflated by internal pressure has been studied for over one hundred years (Woods, 1892). Analytical approaches based on simplified geometries and linear elastic constitutive relation provided insight into a number of cardiac mechanics and advanced our knowledge of the mechanics of the ventricle (Ghista and Sandler, 1969; Mirsky, 1970; Wong and Rautaharju, 1968; Yin, 1981). With the development of the computational technology, finite element method (FEM) shows great potential to predict detailed stress distribution on the ventricle.

The effect of the fibre orientation on local myocardial mechanics was examined by Bovendeerd et al. (1992). In their study, the left ventricle was constructed by a thick-walled ellipsoid based on the experimental data of canine left ventricle measured by Streeter (1979) and modelled as three dimensional transversely isotropic nonlinear elastic. They found that the largest passive stress on the myocardial wall is hardly affected by the variation of the fibre orientation. Schmid and his colleagues (Schmid et al., 1997) also studied the effects of fibre orientation in a biventricular model and found similar results. Their model geometry was constructed from MRI
data. Nash and Hunter (2000) proposed a pole-zero strain energy function for myocardial wall and predicted thinning of the left ventricular wall during diastole in a biventricular dog heart model (Nash and Hunter, 2000). It was found that the calculated thinning of the wall was close to the experimental data. Recently, Goktepe et al. (2011) implemented the anisotropic model proposed by Holzapfel and Ogden (2009) and performed a FEM analysis of generic biventricular heart model.

Despite numerous earlier investigations, none of them has investigated the stress distribution of left atrium (LA) or applied the current mechanical models (Section 1.4.2) to the study of LA. Thus, an anisotropic hyperelastic model was developed to predict the passive stress distribution of patient specific LA (in this chapter, LA includes LA body and pulmonary veins (PVs), where LA body refers to atrium only and PVs refers to pulmonary veins only). Single layer shell element was employed. The thickness and dominant fibre orientation were taken from the literature (Section 1.1.2) since there is no related information available in the CT scanned data. Effects of wall thickness and fibre orientation were examined.

### 3.2 Methods

#### 3.2.1 Geometry

The patient LA geometry (Figure 1.5) was reconstructed based on LACT (left atrium computed tomography). LACT was segmented on proprietary software (Ensite
Verismo, St Jude, CA, USA) in a 3D mapping system (Ensite NavX, St Jude, CA, USA) and exported as Stereolithography (STL) file. A small portion of the length of PVs was retained. The geometry needed to be smoothed as some artificial ripples caused by peripheral blood vessel motion during CT image taking period, which affected the stress distribution significantly. Thus Laplacian smoothing was utilized to reduce the artificial roughness. The Laplacian smoothing is an iterative approach that changes the position of nodes without modifying the topology of the mesh. The Laplacian smoothing reduces the volume of the body slightly. Iteration numbers were chosen to balance between the smoothing effects and the amount of volume change.
In this study, one iteration was found to be sufficient to reduce the artificial roughness and causes insignificant changes in volume. A cost-effective mesh density was determined after mesh independence test, which was used for the rest of simulation in ABAQUS.

3.2.2 Model

An anisotropic hyperelastic constitutive equation (Equation 3.1) was used for the myocardium in the study.

\[
W = \frac{a}{2b} \exp[b(l_1 - 3)] + \sum_{i=1,s}^{2} \frac{a_i}{2b_i} [\exp[b_i(l_{4,i} - 1)^2] - 1] + \frac{a_{fs}}{2b_{fs}} [\exp(b_{fs}l_{8,fs}^2) - 1],
\]

\text{Equation 3.1}

where \(a, b, a_i, b_i, a_{fs}\) and \(b_{fs}\) are model parameters. \(l_{4,i}\) are the transverse isotropic invariants associated with fibre direction \(i\). \(l_{8,fs}\) is the coupling invariants associated
with a pair of direction fibre (f) and sheet (s). The constitutive equation was proposed by Holzapfel and Ogden (2009) using experimental data from simple shear tests conducted on passive ventricular myocardium of pig hearts (Dokos et al., 2002). The model is intrinsically incompressible and anisotropic as it is able to distinguish the shear tests in 6 directions. Human LA simulation takes the parameters determined from Dokos et al. (2002) experimental data.

The quasi-static elastic analysis was employed in the study. A constant transmural pressure loading 0.2 mmHg was imposed internally which determined by constraining the atrial fibrillation (AF) patient LA volume changes in the range of 10-30 ml during diastole (Figure 3.5). Fixation of mitral valve (MV) and PVs was chosen as the boundary conditions BC throughout the analysis, excluding the mesh independent test. CT images used in this study cannot provide the information on LA thickness and fibre orientation since the resolution of CT is approximately 1 mm. Based on the variation of LA wall thickness reported by other researchers (Section 1.1.2), 2 mm was chosen as the thickness of atrial wall while 1 mm was applied for the thickness of PVs. In addition, 3 mm was chosen for both septum and appendage. A linear transition of thickness between regions that have different thickness was applied. The dominant fibre orientation described by Ho et al. (2002) was applied in the study.

The reference case is defined as: length of all the veins is over 10 mm that covers the sleeve (Section 1.1.2). Thickness is 1 mm, 3 mm and 2 mm in PVs, septum and appendage, and the rest of the LA, respectively. Fibre orientation is in the ideal
dominant pattern (Figure 1.5 and Figure 3.2). Both MV and PVs were fixed as BCs. The effect of stress distribution was studied by comparing reference case with that thickness is uniform at 2mm. The effect of different fibre orientation was investigated by the comparison among the following cases: 1. Dominant fibre orientation (Figure 1.5). 2. Fibre orientation in each zone is rotated +15° from the case 1. 3. Fibre orientation in each region is rotated -15° from the case 1.

### 3.2.3 Numerical method

The anisotropic hyperelastic model was implemented in ABAQUS using subroutine `uanisohyper_inv`. The derivatives and second derivatives of strain energy function $W$ with respect to the scalar invariants $I$ need to be defined in the subroutine. The first derivatives of strain energy function $W$ are:

\[
\begin{align*}
W_1' &= \frac{a}{2}\exp[b(I_1 - 3)], \\
W_{4f}' &= a_f(I_{4f} - 1)\exp[b_f(I_{4f} - 1)^2], \\
W_{4s}' &= a_s(I_{4s} - 1)\exp[b_s(I_{4s} - 1)^2], \\
W_{8fs}' &= a_{fs}I_{8fs}\exp(b_{fs}I_{8fs}^2),
\end{align*}
\]

Equation 3.2

where $W_1'$, $W_{4f}'$, $W_{4s}'$ and $W_{8fs}'$ are the first derivatives of energy function $W$ over invariants $I_1$, $I_{4f}$, $I_{4s}$ and $I_{8fs}$, respectively. The second derivatives of strain energy function $W$ are:

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where $W_{1''}$, $W_{4f''}$, $W_{4s''}$ and $W_{8fs''}$ are the second derivatives of energy function $W$ over invariants $I_1, I_{4f}, I_{4s}$ and $I_{8fs}$, respectively. Detailed source code can be found in Appendix B.

The fibre orientation was specified in subroutine orient (Appendix C) based on the dominant fibre orientation (Figure 1.5). Element type S3R was chosen in ABAQUS as the geometry data in the STL file were actually composed of many triangles. Thickness of each region was defined through keywords *Shell section.

### 3.3 Results

The anisotropic model was implemented using the subroutine uanisohyper_inv. It passed validation against analytical solution of shear stress ($\sigma_{xy}$) in six directions (fs, sf, fn, sn, nf and ns) in simple shear test (Equation 3.4). The values of parameters $a, b, a_f, b_f, a_s, b_s, a_{fs}$ and $b_{fs}$ in this model (Equation 3.1) are listed in Table 3.1:

<table>
<thead>
<tr>
<th>$a$ (kPa)</th>
<th>$b$</th>
<th>$a_f$ (kPa)</th>
<th>$b_f$</th>
<th>$a_s$ (kPa)</th>
<th>$b_s$</th>
<th>$a_{fs}$ (kPa)</th>
<th>$b_{fs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.059</td>
<td>8.023</td>
<td>18.472</td>
<td>16.026</td>
<td>2.481</td>
<td>11.120</td>
<td>0.216</td>
<td>11.436</td>
</tr>
</tbody>
</table>

| Table 3.1 Parameter values in the model of Holzapfel and Ogden (2009) |
\[
\begin{align*}
\sigma_{fs} &= 2(W_1' + W_{4s}')\gamma + W_{8fs}', \\
\sigma_{sf} &= 2(W_1' + W_{4s}')\gamma + W_{8fs}', \\
\sigma_{fn} &= 2(W_1' + W_{4s}')\gamma , \\
\sigma_{sn} &= 2(W_1' + W_{4s}')\gamma , \\
\sigma_{nf} &= 2W_1'\gamma , \\
\sigma_{ns} &= 2W_1'\gamma , \\
\end{align*}
\]

where $\gamma$ is the amount of shear.

In Figure 3.1, a good agreement is seen between the solid line (the simulation result) and the symbol (the analytical solution).

Figure 3.1 Validation of the implemented anisotropic hyperelastic model proposed by Holzapfel and Ogden (2009).

Solid line represents the simulation predictions while symbol mean the analytical solutions. The material parameters used are given in Table 3.1.
In another subroutine *orient*, the dominate fibre orientation in LA are specified. The result of predefined ideal fibre orientation is indicated as short black lines in Figure 3.2. The fibre orientation is assigned according to the anatomy are described in Section 1.1.2. Black lines are not seen in some regions of LA in figure as they are behind LA wall.

**Figure 3.2** Predefined ideal fibre orientation in FEM.

*Anterior view (left). Posterior view (right).*

**Figure 3.3** Selected 6 lines for the von Mises stress analysis.
Mesh independent test was performed by comparing the von Mises stress distribution on the 6 selected lines (Figure 3.3) that cover all the 11 regions described in Figure 1.5 between current mesh and fine mesh. The element number of current mesh was around 22,000 and it varied slightly with the patient geometry. Compared with an assumed fine mesh (51,000), the mesh becomes acceptable when the stress difference was less than 3%. The comparison results were shown in Figure 3.4, in which the stress difference is less than 1% in the majority of regions and around 3% in very few regions. The boundary of MV region will be slightly different among cases with different mesh density. To exemplify the difference purely caused by the mesh density, only PVs were fixed in the mesh independent test. The thickness was assumed to be uniform at 2 mm.

The relation between the volume change and the transmural pressure is shown in Figure 3.5. As transmural pressure increases, the atrium is inflated. When the transmural pressure is 0.2 mmHg, the volume change is around 10 ml which is consistent with patient with AF during diastole.

The von Mises stress contour of the reference case is show in Figure 3.6 and the stress distribution on the selected 6 lines are shown in Figure 3.7. The legend level for the stress contour (Figure 3.6) is 90th, 70th, 50th, 30th and 10th percentile. As seen from Figure 3.6 and Figure 3.7, wall stress distribution was not uniformly distributed. Wall stress varied widely from region to region. It was raised at ‘saddle points’ where invagination of the LA surface occurred, for example the PV ostia and the appendage ridge, and at other regions like the high posterior wall.
Figure 3.4 Comparison of von Mises stress distribution on the selected 6 cut-lines.

Positions of all cut-lines are shown in Figure 3.3
Figure 3.5 The atrium volume change at different transmural pressure.

Figure 3.6 Contours of the von Mises stress distribution of the reference case.

a) anterior view, b) posterior view.
Figure 3.7 von Mises stress distribution on the selected 6 lines of reference case.

Positions of all cut-lines are shown in Figure 3.3.
To compare the effect of regional thickness and fibre orientation, a self-written Python script (Appendix D1) was used to process data from ABAQUS database file in addition to the comparison between the stress distributions on the 6 lines. The data includes area of stress over median value of each region, maximum stress in each region and distance offset of maximum stress point in each region from control case to other cases. In order to make the area comparable, the median value is calculated from the reference case only.

### 3.3.1 Effect of regional thickness

The von Mises stress contour of the uniform thickness case is shown in Figure 3.8. The stress pattern is similar to the pattern in reference case, except some areas in the PVs and septum.

![Figure 3.8 Contours of the von Mises stress of the uniform case.](image)

a) anterior view, b) posterior view
Figure 3.9 Comparison of the von Mises stress along the selected 6 lines between the reference and uniform thickness cases.
Comparison of the stress along the selected 6 lines between the reference and uniform thickness cases are shown in Figure 3.9. Stress variations are found in the area of PVs, appendage, septum and ridge, in which the thickness is higher the stress is lower and vice versa. Little stress changing is found in the regions of same thickness, i.e. the anterior, posterior and lateral wall, except in superior wall which is close to the PVs.

![Figure 3.10](image)

**Figure 3.10** Comparison of the size of the area with stress above the median value between the control and uniform thickness cases.

The effect of regional thickness was also examined by comparing area of stress over median, maximum stress value and its distance offset in each region between the reference case and uniform thickness case. The difference of area of
stress over median is shown in Figure 3.10. In all PVs the area of stress over median dramatically decreased by from 38.36% (RSPV) to 58.37% (RIPV) in the uniform case, in which thickness in PVs (2 mm) is higher than in the reference case (1 mm). On the other hand, the area of stress over median increased by 42.43% and 21.09% in septum and LAA, respectively, in the uniform case where thickness (2 mm) is lower than in the reference case (3 mm). In the rest of the regions, the area of stress over median is mild varied less than 4% when the thickness is identical in both cases, except no variation in superior wall.

Figure 3.11 Comparison of the maximum stress between the control and uniform thickness cases.

The variation of maximum von Mises stress value between control and uniform thickness cases is shown in Figure 3.11. There is decrease in maximum stress in the
PVs from 3.27% to 42.01%. In LAA and septum, the maximum stress is increased by 4.97% and 16.12%, respectively. There are slight variations (less than 4%) in maximum stress in the rest of the regions. The highest stresses are in anterior wall in both cases, at 1.95 kPa in reference case and at 1.92 kPa in uniform thickness case, respectively. On the other hand, the lowest stress is around 0.23 kPa in lateral wall in both cases.

The offset of maximum value position in each region from reference to uniform thickness case is calculated. The offset is near 12.5 mm and 30 mm in LIPV and RIPV, respectively, while zero offset is found in all the other regions. Not only the magnitude of maximum stress but also the location of maximum stress has been affected by thickness variation in some regions.

### 3.3.2 Effect of fibre orientation

The von Mises stress contour of the varied fibre orientation cases are shown in Figure 3.12 and Figure 3.13. The stress patterns are similar to the pattern in the reference case as well. The high stress regions appear in the PVs, roof of PVs, ridge, low superior wall, high posterior wall and saddle area in the anterior wall.

Comparison of the stress along the selected 6 lines between the reference and the varied fibre orientation cases are shown in Figure 3.14. The stress distribution
changes randomly. It is inconclusive that whether fibre rotation of 15° or -15° from the reference increases or decreases the stress.

Figure 3.12 Contours of the von Mises stress of the 15° rotation case. a) anterior view, b) posterior view.

Figure 3.13 Contours of the von Mises stress of the -15° rotation case. a) anterior view, b) posterior view.
Figure 3.14 Comparison of the von Mises stress along the selected 6 lines between the reference and varied fibre orientation cases.
The difference in the area with stress over the median is shown in Figure 3.15. In LSPV, RIPV, LAA, superior and posterior, the area increases in both rotation directions. The area decreases when the rotation direction was positive while increased when direction was negative in LIPV, lateral and anterior. On the other hand, the area increased when the rotation direction was positive while dropped when the direction was negative in RSPV, ridge and septum.

![Graph](image)

**Figure 3.15 Comparison of the area with stress over the median value between the control and different fibre orientation cases.**

The variation of maximum stress value between control and varied fibre orientation cases is shown in Figure 3.16. In LIPV, RIPV, LAA, ridge, septum, superior and posterior wall, decrease in maximum stress in both rotation directions.
were found while increase in lateral wall. In the rest regions, the fibre rotation direction led to opposite influence, i.e., one direction caused an increase in maximum stress while the other one caused a decrease. The greatest variation in the maximum stress (129%) was found in RSPV when fibres orientated -15° from the reference case while the smallest variation (i.e. less than 0.3%) was found in the ridge when fibres orientated -15° from the reference case.

Figure 3.16 Comparison of the maximum stress between the reference and different fibre orientation cases.
Figure 3.17 Offset of the maximum value position in each region from the reference to different fibre orientation cases.

The offset of the maximum value position from the reference to varied fibre orientation cases in each region is shown in Figure 3.17. The maximum offset is approximately 33 mm and 30 mm in posterior with fibre orientation of $-15^0$ and $15^0$ from the reference pattern, respectively. There is no change in LAA and ridge when fibres are orientated by $-15^0$. In RSPV, RIPV, superior, posterior and septum, there is higher offset when fibres are rotated by $-15^0$ than by $15^0$, which is contrary to the rest regions.

3.4 Discussion
In previous stress analysis on ventricles, constant spring stiffness was applied on the external surface of chamber in addition to the internal cavity pressure to constrain the volume change (Bettendorff-Bakman et al., 2006; Goktepe et al., 2011). In this study, a constant transmural pressure was applied directly onto the internal surface and it was determined from the volume changes during diastole of AF patient (Figure 3.5). These two methods are actually equivalent as both of them aims to constrain the chamber volume change. The stress magnitude is in the same order as previous studies on ventricles (Choi et al., 2010; Goktepe et al., 2011; Guccione et al., 1995), although it is a little higher which is caused by the smaller thickness than ventricle. There are some local stress changes caused by the variations of thickness and fibre orientation. The increased thickness in appendage and septum causes the decrease in stress magnitude and in areas over median value. On the other hand, the decreased thickness in PVs leads to increase in stress in both magnitude and areas (Figure 3.10 and Figure 3.11). Few positions of maximum value are changed as the thickness variation in some regions.

There are no apparent rules to follow in case of the different fibre orientation (Figure 3.16) as positive/negative offset of fibre orientation will randomly increase/decrease stress in magnitude in any region. The change of the position of the maximum value caused by the thickness seems to be less than that caused by the fibre orientation (Figure 3.17). However, peaks in the wall stress were particularly around the anterior wall, the PVs and their ostia, the LA appendage ridge, the high posterior and low superior wall (Figure 3.6), even in the case of uniform thickness and varied fibre orientation (Figure 3.11 and Figure 3.16). The global stress pattern and the
maximum stress were not affected much by the variations of atrium wall thickness and fibre orientation. The comparison indicates that the LA natural shape and the local curvature are major factors for the relative higher stress in these areas, especially in the ridge, PVs and their ostia. Furthermore, the thickness and the fibre orientation affect the position and the magnitude of maximum stress in each region. Similar results are also found in other two patient geometries.

In the FEM stress analysis of LA, a few assumptions and simplifications were made. Firstly, a sophisticated constitutive equation for the cardiac tissue derived by Holzapfel and Ogden (2009) was adopted. The shell element was employed due to geometry profile generated from the 3D mapping system. As a consequence, there is no stress in the normal direction and might affect the stress in magnitude slightly. Moreover, the parameters used in the stress analysis were from the experiments performed on pig left ventricle by Dokos and co-workers (Dokos et al., 2002). This is a compromising approach when human cardiac myocardium properties are unavailable (Bovendeerd et al., 1992; Choi et al., 2010; Costa et al., 1996; Goktepe et al., 2011; Schmid et al., 1997). Mechanical properties of PVs were assumed to be the same as the LA, since left atrial myocardium is extended around 10 mm proximal segment of pulmonary veins (Ho et al., 2001). Secondly, since it is the first study on stress distribution of atrium wall and there is limited information available from literature and CT data, the atrium wall thickness and fibre orientation were based on the dominant thickness distribution and fibre orientation from literature (Ho et al., 2002; Ho et al., 2001; Ho et al., 1999). Thirdly, a static transmural pressure was applied and the viscous effect was ignored. Although viscoelasticity has been
demonstrated in myocardium tests, the relaxation time of the viscous effect is much longer than the time scale of the cardiac cycle (Holzapfel and Ogden, 2009), so the quasi-static hyperelastic response of the myocardium is physiologically significant. Fourthly, in a cardiac cycle, active state of LA only lasts for 0.1s while passive state takes up most time of the cycle (Figure 1.3). In addition, difference between the active and passive wall stresses is believed to be much less significant than that in the ventricle, and only passive state of atrial wall was considered.

In some patients the pulmonary artery or the aorta might lie on the LA and may affect the total deformation and stress distribution. Due to difficulties to quantify this effect, its effect was not considered in the current study. The septum was allowed to move freely but it is not the case in reality, the reason is that there would not be any stress on septum if the septum was not allowed to move. This could be solved by employing right atrium to compose a bi-atria model. As the geometry is constructed from patients having AF, the physiological pressure waveform remains largely steady and would not drop to zero at the end of systole. The residual stress on the atrial wall is ignored since it is not measureable. Only one layer of the atrial wall was considered in the analysis due to the limit information in the geometry data.

3.5 Summary

In this study, wall stress distribution on the patient specific LAs was analyzed. Anisotropic hyperelastic model was adopted to describe the mechanical behaviour of
atrium wall. A constant transmural pressure was applied onto the internal surface of
the chamber. Effects of the atrial wall thickness and the fibre orientation were
examined. It is found that the global stress pattern are similar and the maximum stress
does not change much, although there are some local variations due to the difference
of wall thickness and fibre orientation. Results from the study support later
investigations into the correlation between the LA wall stress and electrophysiologic
data in the next chapter.
4 Correlation between the left atrial wall stress and electrophysiologic remodeling in persistent atrial fibrillation

4.1 Introduction

The atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice (Nattel and Opie, 2006) and is an important public health problem causing significant morbidity and mortality (Beyerbach and Zipes, 2003). Increased atrial stretch is an aetiological factor in the majority of patients with AF (Kannel et al., 1982). Chronic stretch causes atrial dilatation, and changes in atrial architecture including myocyte hypertrophy and fibrosis (Allessie et al., 2005; Kalman et al., 2006; Kalman et al., 2003). Heterogeneity in structural remodeling of the atria may partly explain the increased vulnerability to AF in this setting (Nattel et al., 1999; Olgin et al., 2004; Olgin et al., 2003). Furthermore, atrial stretch has also been shown to cause abnormal electrophysiology, including slow conduction, prolonged effective refractory period, areas of low voltage and electrical scar, double potentials andfractionated electrograms, and increased inducibility of AF (Kalman et al., 2009; Kalman et al., 1999; Sanders et al., 2010; Sanders et al., 2008; Sanders et al., 2009).
Although pulmonary vein (PV) isolation is a successful treatment for paroxysmal AF (Hunter et al., 2010; Natale et al., 2008; Oral et al., 2002), additional substrate modification in the form of linear lesions and/or targeting of complex fractionated atrial electrograms (CFAE) improves outcomes for persistent AF (Section 1.2). Greater understanding of how atrial remodeling supports AF may allow refinement of substrate modification and improve outcomes. Computer modelling has been used to better understand complex processes such as excitation contraction coupling. Cellular electrical activity based on caricature models of atrial geometry has been studied by previous investigators (Blanc et al., 2001; Luo and Rudy, 1991; Virag et al., 1998). Seemann and other researchers extended this study to 3D realistic atrial anatomical model (Reumann et al., 2007; Seemann et al., 2006). Kharche et al. integrated more advanced cell model for human atrial action potential (Courtemanche et al., 1998) into the realistic geometry (Kharche and Zhang, 2008). However, to my knowledge, none of the studies has examined the mechanical function on the unique atrium geometry, which may help understand how stretch/stress is distributed in the walls of the LA and how this impacts on atrial remodeling.

It is not surprising that stress is correlated to the soft tissue disease. Previous studies have found high stress distribution in the specific areas of abdominal aortic aneurysm (AAA) and the localization of high stress was proposed as an indicator for the determination of probability of rupture of an AAA (Elger et al., 1996; Thubrikar et al., 2001). Pressure-induced stress concentration has been studied on the topography of atherosclerotic lesions that mainly occurs in the artery bifurcation (Salzar et al., 1995; Thubrikar and Robicsek, 1995). It has been also found that the
wall stress has an association with a rapidly expanding AAA in a recently study (Li et al., 2010). Therefore, we hypothesized that peaks in LA wall stress are associated with focal electrophysiological remodeling which maintains AF. To address this, computer modelling was used to predict wall stress in 3D reconstructions of the LA from patient’s CTs, and simulated data compared to electrophysiologic data recorded in the same patients at the time of their ablation for persistent AF. The importance of regions with high wall stress in maintaining AF was evaluated by examining how wall stress impacts on the response to CFAE ablation, as determined by change in AF cycle length (AFCL).

4.2 Methods

4.2.1 Study population

The study population was comprised of 19 patients who underwent first time catheter ablation of persistent AF at a single institution. This study was approved by East London and The City Research Ethics Committee, UK (reference number 09/H0703/6). All patients gave written informed consent.

4.2.2 Electrophysiology data

The electrophysiology data were collected before the catheter ablation of AF of each patient. In brief, after a 14 pole deflectable PV mapping catheter (Orbiter PV,
Bard EP, MA, USA) and a 3.5 mm irrigated ablation catheter (Thermo-Cool Celsius, Biosense Webster, CA, USA) were introduced to the LA through femoral artery, the LA geometry was created using a 3D mapping system (Ensite NavX, St Jude, CA, USA) prior to any ablation. All patients underwent a gated 128 slice CT scan of the LA, which was segmented on proprietary software (Ensite Verismo, St Jude, CA, USA) to create a 3D reconstruction of the LA (Richmond et al., 2008). The PV mapping catheter was moved around the LA to acquire electrograms at evenly spaced points, creating a map of electrophysiologic data (Hunter et al., 2009) (Figure 4.1).

Five seconds electrograms were recorded for analysis, since this has been shown to produce consistent results (Stiles et al., 2008). The Ensite NavX software recognizes deflections in the waveform based on a number of criteria which can be varied by the user. Each deflection must have a minimum width to exclude noise and a blanking period to prevent double counting (20 ms and 30 ms respectively have been shown to correlate with visual assessment) (Hunter et al., 2009). A minimum of 0.05mV was used. The software tags deflections meeting these criteria on-screen, and uses algorithms to generate a score for: 1.) Electrogram voltage amplitude - the mean of the largest ‘peak to peak’ deflection in each electrogram complex (see example in Figure 1.9). 2.) CFAE mean – the mean interval between deflections, or mean cycle length. This is a continuous variable with shorter mean cycle length taken to mean greater electrogram fractionation. However, for assessment of CFAE distribution < 120 ms was considered a CFAE.
Therefore, for each electrophysiologic data point where a waveform was obtained, the mapping system ascribed a coordinate (in the same 3D space as the LA reconstruction) and calculated a value for these 2 parameters.

**4.2.3 Stress analysis**

The stress of each LA was calculated using the method described in Chapter 3. To describe regional distribution of peaks in wall stress, an area with von Mises stress $\geq$ the 90th percentile of each individual was considered to be a peak in wall stress. The distribution of peaks in wall stress was assessed using a 11 segment model of the LA (as shown in Figure 1.5). To assess the relationship between LA electrophysiology and wall stress, the values derived for each electrophysiologic data point (electrogram voltage amplitude and CFAE mean) were compared to simulated wall stress at the nearest point, which is obtained from self-written Python scripts (Appendix D2 & 3), on the LA reconstruction.

**4.2.4 Statistics**

Continuous variables are reported as mean ± standard deviation, or median (range) if not normally distributed. Correlation is inevitably affected by the small proportion of points which have erroneous electrophysiologic data, for example due to poor contact. The electrophysiologic data points for each patient were therefore
divided into quartiles based on wall stress at their location, with the median value taken as representative of each quartile to reduce the impact of outlying data. The changes in electrophysiological parameters (voltage amplitude and CFAE mean) were therefore assessed across quartiles of wall stress for each patient, and this was compared for all patients using repeated measures analysis of variance (MANOVA). To examine the relationship between electrogram voltage amplitude and CFAE (i.e. independent of wall stress), the effect on CFAE mean across quartiles of electrogram voltage for each patient was assessed in the same fashion. To assess any interaction between the effect of LA volume and wall stress on electrophysiologic parameters, LA volume was included as a covariate in the MANOVA design.

Receiver operating characteristic (ROC) analysis was used to assess whether high wall stress predicted the occurrence of certain defined electrophysiologic abnormalities:

a. Fractionated electrograms (a CFAE mean <120ms) (Hunter et al., 2009),

b. Low voltage areas suggestive of abnormal conduction (<0.5mV) (John et al., 2010),

c. Very low voltage areas suggestive of scar (<0.05mV) (Roberts-Thomson et al., 2009),

To compare the distribution of peaks in wall stress and the above electrophysiologic abnormalities, their presence or absence (and their concordance) was assessed in each region of the 11 segment model shown in Figure 1.5.
4.3 Results

The characteristics of the 19 patients recruited are shown in Table 4.1. All patients had persistent AF, and 84% of these were long lasting persistent AF (i.e. ≥ 1 year).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>84% (16 out of 19)</td>
</tr>
<tr>
<td>Age</td>
<td>64 ± 7 yrs</td>
</tr>
<tr>
<td>Months of continuous AF</td>
<td>23 ± 16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>53% (10 out of 19)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>32% (6 out of 19)</td>
</tr>
<tr>
<td>Left atrial diameter</td>
<td>4.5 ± 0.7 cm</td>
</tr>
</tbody>
</table>

Table 4.1 Patient characteristics.

Data is presented as percentage of patients, or mean ± standard deviation.

A total of 8214 electrical data points were acquired. After removing points >5 mm from the LA shell, there were 6770 points remaining for analysis, 356 ± 80 per patient.
Figure 4.1 An example of the electric anatomic mapping system.

Colour purple means normal electrical signal while rest colours represent abnormal with white colour in highest level. (Left) anterior view, (right) posterior view.

Figure 4.2 shows examples of wall stress distribution. Wall stress distribution was not uniformly distributed. Peaks in wall stress were defined as areas with wall stress values ≥ 90\textsuperscript{th} percentile of each individual, which was in the range of 0.35-0.60 kPa. Figure 4.3 shows the proportion of patients who had peaks in wall stress over the different regions shown in Figure 1.5. Peaks in wall stress were particularly common around the ostia of the PVs (left superior pulmonary vein (LSPV) 100%, left inferior pulmonary vein (LIPV) 89%, right superior pulmonary vein (RSPV) 79% and right superior pulmonary vein (RIPV) 89%), the LA appendage ridge (100%), the superior wall (79%), both anterior and posterior wall regions (100%). There was no significant correlation between LA volume and median wall stress (p = 0.451). The distribution of peaks in wall stress did not differ when comparing the 9 most dilated LAs to the 9 smallest.
Figure 4.2 Left atrial wall stress distribution.

Contours of the left atrium showing wall stress distribution in 3 patients (a-c).
The proportion of patients with peaks in wall stress (>90\textsuperscript{th} percentile) and CFAE (CFAE mean < 120 ms) in each region of the left atrium.

Electrogram amplitude showed a linear inverse relationship across quartiles for wall stress meaning lower electrogram amplitude at sites of higher wall stress, with a 40\% difference between the highest and lowest quartiles for wall stress (p = 0.00012, Figure 4.4a). There was a trend towards higher CFAE mean (meaning less fractionated electrograms) at higher wall stress, with a 73\% difference between the highest and lowest quartiles for wall stress (p = 0.006, Figure 4.4b).
Figure 4.4 Relationship between the electrophysiology and the atrial wall stress.

Figures show the effect on electrophysiologic parameters (mean and 95% confidence interval) across quartiles for wall stress (1 being lowest and 4 being highest), a) electrogram voltage amplitude and b) CFAE mean. Significance was tested using repeated measures ANOVA.
There was a significant decrease in CFAE mean across quartiles of voltage amplitude (Figure 4.5; \( p < 0.0001 \)). The lowest quartile for electrogram voltage had a markedly higher CFAE mean value (meaning less fractionated electrograms). The lowest quartile for electrogram voltage likely contained the most points with poor contact, and the absence of detected deflections at these points may therefore have artificially increased the CFAE mean score. However, even if the lowest quartile of electrogram voltage is discarded, the decrease across the remaining 3 quartiles was still significant (\( p < 0.0001 \)).

**Figure 4.5 Relationship between CFAE and voltage amplitude.**

Figure shows the effect on CFAE mean (mean and 95% confidence interval) across quartiles of Voltage amplitude (1 being lowest and 4 being highest). Note a higher score reflects a longer cycle length and hence less fractionated electrograms. Significance was tested using repeated measures ANOVA.
Figure 4.6 Relationship between high wall stress and electrophysiologic abnormalities.

Receiver operating characteristic curves demonstrating the relationship between high wall stress and electrophysiologic abnormalities: a) electrical scar (defined as voltage amplitude < 0.05 mV; b) low voltage (defined as < 0.5 mV), and c) CFAE (defined as CFAE mean < 120ms). Area under curve and confidence intervals (CI) are shown.
There was an association between high wall stress and electrical scar: A wall stress value ≥ 0.39 kPa had a sensitivity of 56.1% and specificity 57.0% for predicting electrical scar (area under curve 0.58, p < 0.0001; Figure 4.6a). There was a modest association between high wall stress and low voltage, with a wall stress value ≥ 0.36 kPa there was a sensitivity and specificity both of 54.0% for predicting low voltage (area under curve 0.577, p < 0.0001; Figure 4.6b). High wall stress was associated with absence of CFAE (area under curve 0.442, p < 0.0001; Figure 4.6c).

4.4 Discussion

Increasingly complex ‘multi-scale models’ are being used to further understanding of complex interacting processes, such as those examining excitation contraction coupling and mechanical function on ventricles (Kerckhoffs et al., 2009; Nickerson et al., 2006). Others have explored the role of stretch and stretch activated ion channels on electrophysiology and arrhythmia in the context of commotio cordis (Trayanova et al., 2010). This numerical model predicted wall stress based on LA anatomy by assuming the dominant fibre orientation and wall thickness. This is the first step towards understanding LA wall stress distribution from a bioengineering perspective, and it has allowed us to study the relationship between wall stress and electrophysiology.

There was an inverse relationship between LA wall stress and electrogram amplitude, with a highly significant 40% reduction in electrogram amplitude across...
quartiles of wall stress (Figure 4.4a). ROC analysis demonstrated that high wall stress was associated with low electrograms amplitude and electrical scar (Figure 4.6a & b).

Increased atrial stretch is a consistent aetiologic factor in the development of AF (John et al., 2010; Kannel et al., 1982; Sanders et al., 2003; Sparks et al., 1999). Chronic stretch causes LA dilatation, with heterogeneous remodeling of atrial architecture including myocyte hypertrophy, fibrosis, and gap junction remodelling (Sanders et al., 2003; Takeuchi et al., 2006; Verheule et al., 2004; Verheule et al., 2003). Electrophysiologic effects include conduction heterogeneity and anisotropy, areas of low voltage and electrical scar, prolonged effective refractory period, a greater proportion of double potentials and CFAE, and greater inducibility of AF (John et al., 2010; Neuberger et al., 2005; Verheule et al., 2004). These changes could be explained by heterogeneous structural remodeling, whereby foci of high wall stress directly activate signaling pathways such as cyclic AMP, angiotensin II, and others (Saffitz and Kleber, 2004). However, the observation that correcting elevated intra-atrial pressure causes an acute increase in electrogram amplitude and conduction velocity associated with reduced AF inducibility, suggests a role for focal activation of stretch activated ion channels (Ghista et al., 1975; John et al., 2010).

There was a significant effect of an increasing CFAE mean across quartiles of wall stress (Figure 4.4b), suggesting more organized and less rapid electrical activity at higher wall stress. Similarly, the ROC analysis demonstrated that high wall stress was associated with absence of CFAE (Figure 4.6c). It is likely that there are multiple mechanisms for CFAE. Although remodeling associated with peaks in wall stress
could feasibly contribute to zones of slow conduction, pivot points, or block (Konings et al., 1994), and resultant micro- or macro-reentry (Niu et al., 2009), wall stress is less likely to bear any relationship to rotors or rapidly discharging foci (Kalifa et al., 2006) which may be more dependent on autonomic drive and proximity to ganglionated plexi (Lin et al., 2007). Since we do not completely understand the mechanisms underlying CFAE and cannot differentiate between them, it is not possible to discern whether wall stress is associated with any one mechanism. Since high wall stress was associated with absence of CFAE, this suggests that the remodeling process precludes some mechanisms of CFAE, possibly due to the lengthening of atrial refractoriness that has been demonstrated in the context of increased stretch.

Peaks in wall stress and CFAE were found to coexist at the PV ostia. Stretch has been shown to increase the frequency of depolarisation at the PV ostia without affecting the body of the LA (Kalifa et al., 2003). This may owe to activation of stretch activated ion channels causing membrane depolarisation (Li et al., 2004), although it is unclear why the PV ostia should respond differently to the rest of the LA. Since the PV ostia can dilate in response to chronic atrial stretch (Herweg et al., 2005) this may alter wall stress distribution and exacerbate stretch at the PV ostia. This provides a rationale for the association between acutely and chronically elevated LA pressure and increased PV excitability, and hence why this might cause PV ectopy and initiation of AF.
It is difficult to validate wall stress simulation by finite element analysis, however, it has been widely used in biomechanics and has produced results that correlate with clinical findings (Li et al., 2010), and biophysical properties when direct testing is feasible (Panagiotopoulou et al., 2010). Although patient specific and site specific data on LA material properties were not available, variation in these parameters have a modest effect on predicted wall stress (Taddei et al., 2006). Acquisition of an accurate geometry using modalities such as 128 slice CT is the most crucial step, since this is the main determinant of wall stress (Taddei et al., 2006). However, the resolution of currently available imaging modalities does not allow accurate determination of regional differences in wall thickness and fibre orientation, and this is accepted as a limitation. The pectinate muscles confined within the LA appendage produce a large contraction force (Guo et al., 1983), and thus generate a great active stress in LA appendage. Therefore, it is believed that incorporating active stress in the finite element analysis would have a better correlation in the appendage.

Although care was taken to achieve good tissue contact during recordings, a small proportion of low amplitude signals reflect poor contact. Electrogram amplitude is also affected by wall thickness, artefact from far field signals, and the direction of wave-front movement relative to the catheter. These technical limitations mean that these data likely under-represent the relationship between wall stress and electrophysiology.
4.5 Summary

LA wall stress varies widely in different regions of the same LA, and also in the same regions between subjects. Areas with high wall stress were less likely to support CFAE, although the PV ostia may be an exception in that they were consistently high stress and harboured CFAE. There was an inverse relationship between regional wall stress and electrogram voltage, and foci of high wall stress were associated with low voltage and electrical scar.
5 Mechanical properties of HUVECs and their glycocalyx in vitro

5.1 Introduction

It is well known that vascular endothelial cells change their macroscopic shape, cytoskeleton orientation and physiological functions in response to fluid shear stress. Changes in the mechanical properties of living endothelial cells accompany numerous pathophysiological processes, including inflammation and cardiovascular disease (Davies, 1995; Henry and Duling, 2000). The luminal surface of the vascular endothelium is coated with an endothelial glycocalyx layer. It is polysaccharide-protein and forms a negatively charged, complex meshwork. Both endothelium and glycocalyx form the permeability barrier regulating solute transport (Levick and Michel, 2010). Glycocalyx shedding and degradation in inflammation models lead to impaired endothelial mechanotransduction of fluid shear stress, adhesion of platelets and increase leakage of plasma proteins etc. (Weinbaum et al., 2007). Characterization of their mechanical properties not only helps establish function-property relationship of both endothelial cells and glycocalyx but also provides necessary parameters for the development of theoretical models and sophisticated modelling research work on their interaction.
With the development of measurement technique, it has become possible to investigate micro- and nano-mechanical properties of cell structures. Atomic force microscopy (AFM) indentation is one of the powerful tools which not only provides high-resolution topographical imaging but also obtains the force-displacement relationship at micro-scale of cells. AFM has been successfully used to measure the mechanical properties of endothelial cells. The earliest AFM works on endothelial cells focused on the effects of shear stress on cellular organization. Ohashi et al. (2002) reported that bovine aortic endothelial cells significantly increase the elastic modulus from $12.2 \pm 4.2$ to $18.7 \pm 0.43$ kPa when exposed to a constant wall shear stress. Pesen and Hoh (Pesen and Hoh, 2005) studied the cell cortex of bovine pulmonary artery endothelial cells. Its elastic modulus is found in the range of 0.2-2.0 kPa. Combined with confocal fluorescence microscopy, they also revealed that cell cortex is organized as a polygonal mesh at two length scales. Human umbilical vein endothelia cells (HUVECs) exhibited an inhomogeneity of elastic modulus by Mathur who also concluded that the nuclear region of the cells appears to be stiffer than the rest of cell body (Mathur et al., 2000). This inhomogeneity is also found in the pulmonary microvascular endothelial cells (Kang et al., 2008). Furthermore, cell mechanical properties will be systematically changed by the introduction of tumour necrosis factor-α. On the glycocalyx layer, only one AFM study was reported very recently. Stiffness and heterogeneity of the pulmonary endothelia glycocalyx is indirectly determined (O'Callaghan et al., 2011). In this study, the AFM indentation is carried out on long-term cultured endothelial cells. They correlated the change of material properties before and after enzymatic removal of the glycocalyx layer. Using some empirical formulation based on a two-layer composite compliance model
(Doerner and Nix, 1986), they reported the glycocalyx is much softer than the underlying endothelial cells and its elastic modulus is in the order of 0.5 kPa.

Most post-analysis of AFM data heavily relies on the Hertzian contact mechanics. The Hertz theory makes a number of simplifying assumptions including homogeneous, isotropic, linear elastic material properties, small strain and infinite sample thickness. None of these conditions are likely to be valid when cells are indented with an AFM. More and more studies suggested the AFM results depend on the cell thickness, indentation thickness and tip geometry (Dimitriadis et al., 2002). Within the small deformation, a series of analytical solutions are found for different probe tip geometries (Bilodeau, 1992; Rico et al., 2005; Sneddon, 1965). Even for a small indentation depth, a large strain is anticipated near the tip, which invalidates the small strain analysis. This is particularly the case when a large indentation depth comparable to the cell height is applied. Computational modelling can get around this limitation. By selecting proper finite deformation models, the force-displacement can be reproduced from simulation of the AFM indentation test. With the help of FEM modelling, different kinds of corrections or new empirical formulae have been derived to assist more precise determination of cell mechanical properties (Costa and Yin, 1999; Dimitriadis et al., 2002; Kang et al., 2007; Sun et al., 2004).

In this study, the inverse FEM method will be used to characterize mechanical properties of cultured HUVECs based on the experimental results from a different PhD project in our laboratory. The focus will be on the development of modelling framework to quantify HUVECs mechanical properties with and without the
5.2 Methods

5.2.1 Experimental data

HUVECs (Lonza Group Ltd, Switzerland) were plated on fibronectin-coated glass coverslips according to the procedures proposed by Shasby and Shasby (1986). Once HUVECs became confluent after two weeks’ culture, they were divided into two groups. One group was used as the control cells and the other group was subjected to enzymatic treatment to remove the glycocalyx layer.

AFM deflection-separation curves of HUVECs were obtained using a scanning probe microscopy solver NTEGRA (NT-MDT Company, Moscow, Russia). A rectangular silicon nitride (Si$_3$N$_4$) cantilever (OMCL-RC800PSA-W, OLYMPUS, Japan) was used, which has a length of 100 µm, width of 20 µm and spring constant of 0.39 N/m. The blunted probe is four-sided pyramidal in shape which has a spherical tip cap of radius 15 nm. The height of the probe and the diagonal length of the probe base is 2.9 µm and 5.21 µm, respectively.
Cantilever spring stiffness constant was first calibrated by thermal fluctuations method (Hutter and Bechhoefer, 1993) before each group of measurements. The nominal stiffness constants were in the range of 0.3-0.45 N/m. Three-dimensional topography of cells was obtained using AFM at the semi-contact mode. A scanning rate of 1 Hz was controlled by the piezoelectric element and the maximum scanning area was set at $165 \times 165 \mu m^2$ in the X-Y plane (resolution was $256 \times 256$). Central regions of cells which are the highest in the topographical image were selected for the AFM indentation measurement in contact mode. The cantilever deflection was controlled to be in the range of 0-1000 nm and the indentation speed was set to be 5-6 µm/s to minimize the viscous effect (A-Hassan et al., 1998). The data acquisition rate was 100 Hz. The study was performed on a total of 20 control cells and 20 cells with enzyme treatment.

The deflection-separation curve of soft biological samples has a smooth transition region from non-contact to contact status (Figure 5.1). It is not like other hard samples, e.g. glass, which has a distinct difference in the two states. Thus, it is impossible to determine the initial contact point visually. The method utilised to determine the initial contact point in the study was based on the fact that separation distance $S_g$ in deflection-separation curve of indentation on coverslip (glass) in addition to cell height $h$ determined from tapping model equals the separation distance of indentation on soft sample $S_s$, thus the initial contact point is at the point where the separation distance equals $S_s$ in the deflection-separation curve of indentation on soft sample. Consequently, the indentation depth $\delta$ equals the
difference between the deflection $D_g$ of indentation on coverslip and deflection $D_c$ of indentation on soft sample after shift cell height $h$ (Figure 5.1)

**Figure 5.1 Deflection-separation curve obtained from AFM microindentation.**

Indentation on glass (Line B), indentation on cell (Line C) and indentation on cell before shifting of cell height (Line B’). Separation means the distance between cell and indenter. When read from right to left, the separation is decreasing. The indentation depth is the difference of deflection between $D_g$ and $D_c$ ($\delta = D_g - D_c$). $h$ is the cell height.
5.2.2 Analytical solutions to AFM microindentation

Most analyses of AFM data used the traditional Hertz’s and Sneddon’s studies where the cell is assumed linear elastic and deforms within the regime of small deformation. For the indentation to half-infinite solid, the relationship between force (F) and indentation depth is derived for different types of indenter tips.

For a sharp conical tip (Sneddon, 1965):

\[ F = \frac{2Etan\theta}{\pi(1 - v^2)} \delta^2, \]

where \( \theta \) is half angle of the indenter, E is the Young’s modulus, and \( v \) the Poisson’s ratio.

For a sharp pyramid tip (Bilodeau, 1992):

\[ F = \frac{1.4906Etan\theta}{2(1 - v^2)} \delta^2; \]

For a microsphere bead attached to the tip (Sneddon, 1965):

\[ F = \frac{4E}{3(1 - v^2)} r^{1/2} \delta^{3/2}, \]

where \( r \) is the bead radius.

In most practical applications, the indenter tip is not sharp but blunt. Analytical solutions for the blunt tip within the small deformation were developed by Briscoe and co-workers (Briscoe et al., 1994) and Rico and his colleagues (Rico et al., 2005) for conical and pyramidal tip, respectively.

When \( \delta \leq b^2/r \), the spherical model applies.
When $\delta \geq b^2/r$,

$$F = \frac{2E}{(1 - v^2)} \left\{ a\delta - m \frac{a^2}{\tan \theta} \left[ \frac{\pi}{2} - \arcsin \left( \frac{b}{a} \right) \right] - \frac{a^3}{3r} ight\} \quad \text{Equation 5.4}$$

$$+ (a^2 - b^2)^{\frac{1}{2}} \left[ m \frac{b}{\tan \theta} + \frac{a^2 - b^2}{3r} \right],$$

where $b$ is the radial distance corresponding to the transition from spherical cap to pyramidal or conical face. $a$ is the effective contact radius and it is related to indentation depth $\delta$ as follows

$$\delta + \frac{a}{r} \left[ (a^2 - b^2)^{\frac{1}{2}} - a \right] - n \frac{a}{\tan \theta} \left[ \frac{\pi}{2} - \arcsin \left( \frac{b}{a} \right) \right] = 0, \quad \text{Equation 5.5}$$

with $n = 1$ for the cone and $n = 2^{3/2}/\pi$ for the pyramid.

By curve fitting any chosen model to measured experimental data, mechanical properties of biological samples are determined. However, the accuracy is compromised by the finite deformation concentrating on the indenter region.

### 5.2.3 Numerical model

To better describe the AFM micro-indentation process, a finite strain constitutive law is required. The eight-chain hyperelastic model developed by Arruda and Boyce (Arruda and Boyce, 1993) was chosen in this study. The A-B model was initially proposed to describe the mechanical response of incompressible rubber. It has been successfully used to study mechanical response of soft biological tissues.
such as arterial walls (Horgan and Saccomandi, 2003), human skin tissue (Bischoff et al., 2000) and endothelial cell (Kang et al., 2007). The free energy expression for the A-B model is

\[
W = \mu_{A-B} \left[ \frac{1}{2} (I_1 - 3) + \frac{1}{20\lambda_L^2} (I_2^2 - 9) + \frac{11}{1050\lambda_L^4} (I_1^3 - 27) \right. \\
\left. + \frac{19}{7000\lambda_L^6} (I_1^4 - 81) + \frac{519}{673750\lambda_L^8} (I_1^5 - 243) \right],
\]

Equation 5.6

where \(\mu_{A-B}\) is the shear modulus and \(\lambda_L\) is locking stretch.

Mechanical response of HUVECs under the AFM micro-indentation was studied using ABAQUS. The silicon nitride probe was considered as a rigid body since it is by far stiffer than the endothelial cell. The half-open angle of the blunted conical probe tip is 46.44° (Equation 5.2), which was converted from 41.93° of pyramidal probe using Equation 5.1 and Equation 5.2. During AFM test, the contact area between probe tip and cell was much smaller than apical surface of the cell, thus compression are mainly restricted to local region near the indentation site and are not affected by the edge of the cell. An axisymmetric model was thus applied with the axis of revolution coinciding with the central axis of the AFM probe tip. Cell was modelled as incompressible, isotropic and homogeneous cylinder with a radius of 30 \(\mu\)m. Cell thickness varies between 2 and 6 \(\mu\)m at measurement sites which was extracted from corresponding topographical image.

Hybrid element CAX4H was used for the ECs in the finite element analysis. The contact region underneath the rigid indenter used a higher mesh density. The
maximum aspect ratio of all elements was set no more than 1:10. Mesh independence test was performed to assure the accuracy of numerical solutions. The total number of elements used for the whole cell (no less than 4,000) varied with cell thickness. Cell basal membrane in Figure 5.2 was fixed to represent firm adhesion of cell to the substrate. The axisymmetric axis and the indenter were constrained in all other directions except in the axial direction. The interaction between the probe and the cell was modelled as frictionless, surface-based finite-sliding contact problem.

**Figure 5.2 Finite element model of AFM indentation on HUVECs.**

The blunt conical indenter has a spherical cap of 15 nm radius and 46.44° in half-open angle. For single layer studies, top layer and bottom layer were treated as one layer which had same mechanical properties. For the bilayer study, top layer represented the glycocalyx layer and bottom layer was the same as the layer in single
The model has two components: the glycocalyx layer and the underlying cells. In the model, cell membrane, cytoplasm, cytoskeleton and nucleus were treated as one component. In the FEM, this corresponds to the top layer and the bottom layer (representing the glycocalyx layer and the cell substrate, respectively). The average thickness of the glycocalyx was assumed to be between 500 and 1000 nm.

Mechanical properties of the cell and its covering layer were determined through the inverse analysis system (section 1.5). The converging criteria was set as the increment in each parameter or squared residual between experimental data and predicted values is less than $1 \times 10^{-7}$.

### 5.2.4 Comparison between two models

The A-B model is difficult to be directly compared with the Hertz’s model as it works for finite deformation problem. Costa and Lin (1999) developed a general form of solution to the indentation perpendicular to the hyperelastic half-space:

$$F = 2\pi \bar{E} \phi(D) = \frac{4}{3} E \pi \phi(D)$$  \hspace{1cm} \text{Equation 5.7}

where $\phi(D)$ is indenter geometry function. $\bar{E}$ and $E$ are generalized and apparent elastic modulus for any isotropic, incompressible, hyperelastic material, respectively.
\( \bar{E} \) can be derived from Equation 5.8, and where \( W_1 \) and \( W_2 \) are derivatives of \( W \) with respect to \( I_1 \) and \( I_2 \), respectively.

\[
\bar{E} = 4(W_1 + W_2)
\]

Equation 5.8

From Equation 5.7 and Equation 5.8, we have

\[
E = 6(W_1 + W_2)
\]

Equation 5.9

Thus, we could compare the Young’s moduli determined for Hertz’s model and A-B hyperelastic model, respectively.

## 5.3 Results and discussion

### 5.3.1 Validation

The FE simulation was validated by repeating previously published work (Kang *et al.*, 2008) (Figure 5.3). The parameters used for the validation are listed in Table 5.1. There was a good agreement of force-indentation curve between published result (dotted line) and the present result (solid line). A slight deviation was observed at the region of large indentation depth, which is believed to be attributed to the difference in mesh density and distribution.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>thickness ((h))</td>
<td>2 (\mu)m</td>
</tr>
<tr>
<td>half open angle ((\theta))</td>
<td>37.5°</td>
</tr>
<tr>
<td>tip radius ((r))</td>
<td>100 nm</td>
</tr>
<tr>
<td>shear modulus ((u_{A-B}))</td>
<td>0.1 kPa</td>
</tr>
<tr>
<td>lock stretch ((\lambda_i))</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Table 5.1 Values of parameters used in the validation.
Figure 5.3 Comparison of force-displacement curves.

Published result (Kang et al., 2008) (dot) and the single layer simulation result (line).

The values of parameters are listed in Table 5.1.

5.3.2 Mechanical properties of enzyme treated cell

To illustrate the necessity of finite strain model in the determination of cell mechanical properties, the effect of the cell thickness was examined as shown in Figure 5.4. At the same indentation depth, the reaction force decreases from 0.56 nN to 0.42 nN when the cell thickness is increased from 2 μm to 6 μm. As the indentation depth is much smaller than the cell thickness, the difference in reaction force is small. However, when the indentation depth becomes comparable to cell
thickness, a marked deviation is shown for cells of varying thicknesses. Influence of the cell thickness is greater as the thickness of the cell decreases. This result demonstrates that the thickness factor needs to be considered in determining the cell mechanical properties when the cell height is less than 4 μm. Figure 5.5 and Figure 5.6, compared the stress and strain distribution near contact region, respectively. For a thinner cell, the same indentation depth leads to a larger strain thus a higher stress. Concomitantly a larger reaction force develops (Figure 5.4). The strain near the contact region can reach 120% in both cases, confirming that the finite strain theory is compulsory to achieve accurate modelling of the AFM indentation (Figure 5.6).

Figure 5.4 Force-indentation curves

Figure showing the effects of different cells thickness from 2 μm to 6 μm while the μ_{A-B} and \( \lambda_L \) were fixed at 0.1 kPa and 1.05, respectively. Lower force needed for the higher thickness to maintain the compression at same indentation depth.
Figure 5.5 Stress distribution near contact area

Stress contour near contact area (zoomed in). The indentation depth is 200 nm and the cell thickness is 6 μm (left) and 2 μm (right).

Figure 5.6 Strain distribution near contact area.

Strain contour near contact area (zoomed in). The indentation depth 200 nm when the cell thickness is 6 μm (left) and 2 μm (right)
The mechanical property of HUVECs without the glycocalyx layer (i.e. enzyme treated group) was determined using both the Hertz’s and A-B hyperelastic models. Based on experimental data (shown in Appendix E1), Hertz’s Young’s moduli $E_{\text{sharp}}$ and $E_{\text{blunt}}$ of each cell were determined from Equation 5.1 and Equation 5.4. The apparent Young’s modulus $E_{A-B}$ of A-B model of same cell was converted by Equation 5.9. The average value and standard deviation was calculated over all measurements for each model (Figure 5.7). The average Hertz’s Young’s modulus $E_{\text{sharp}}$ value was $9.20 \pm 9.99$ kPa. The average Hertz’s Young’s modulus $E_{\text{blunt}}$ value was $6.02 \pm 6.53$ kPa, which was near 1.5-fold smaller than $E_{\text{sharp}}$. This agrees to results in a previous study that Young’s modulus determined from Hertz’s sharp model gave an overestimation (Costa and Yin, 1999). The average apparent A-B Young’s modulus $E_{A-B}$ was $4.32 \pm 3.63$ kPa, which was lower than Hertz’s Young’s moduli but agrees with the results reported by Kang et al. (2008).

### 5.3.3 Mechanical properties of the glycocalyx layer

To demonstrate effects of a substrate on the determination of mechanical properties of a top layer, force-displacement curves of three different sets of parameters for the substrate were compared. The moduli $\mu_{A-B}$ and locking stretch $\lambda_L$ were either mean value or mean value ± standard deviation from the single layer study, i.e. $\mu_{A-B}$ equals 1.26, 1.86 and 0.66 kPa while $\lambda_L$ is 2.2, 2.6 and 1.8 correspondingly. These results are shown in Figure 5.8. At higher shear modulus and locking stretch of the substrate, the force was greater. It shows that different substrate
mechanical properties will change the measured force. Therefore, it is essential to consider the mechanical property of the bottom layer when characterising properties for the top layer. As it is impossible to perform indentation experiments on the same cell before and after the removal of glycocalyx, mechanical properties of the bottom layer remain unknown before the application of the inverse FEM to the bilayer model. To avoid the non-uniqueness of solution and minimize the computational time, the mechanical property of the bottom layer assumes the same as that determined in the single-layer model (i.e. enzyme-treated endothelial cells are free of glycocalyx).

---

**Figure 5.7** Estimation of the average Young’s modulus using different models.

Analtical solutions to sharp cone and blunted cone are used to determine elastic modulus. Inverse FEM analysis based on A-B hyperelastic models is used to determine another elastic moduluse for the same experimental data (see Appendix E).
Forward FE simulation on effects of different substrate mechanical properties on the overall reaction force. Inset graph shows that the difference of predicted reaction force using two different sets of parameters (mean + deviation and mean - deviation) could reach up to 22% even at indentation depth of 200 nm.

Using the inverse FEM programme described in Section 1.5, the average $\mu_G$ and $\lambda_G$ values of the glycocalyx layer were calculated to be $0.46 \pm 0.11$ kPa and $2.60 \pm 0.19$, respectively, and the average apparent Young’s modulus $E_G$ was $1.51 \pm 0.56$ kPa based on the experimental data from our laboratory (see Appendix E2). It was found that the glycocalyx layer is approximately 3 times softer than the underlying cell substrate layer. In Addition, the mechanical property of the glycocalyx layer was extracted by using the Hertz’s model. Comparison of values
from the two models shows (Figure 5.9) that parameters calculated from the Hertz’s model gave an overestimation than that from the bilayer model. Clearly, this is due to the fact that stiffer underlying endothelial cell contributed to the overall analysis.

Figure 5.9 The mechanical property of the glycocalyx layer determined from the bilayer model and the Hertz’s model.

5.4 Summary

In this study, the inverse FEM was used to determine the mechanical properties of the endothelium and the glycocalyx layer. Firstly, the forward FEM was validated against previous published result (Kang et al., 2008), then auto search method is employed to find the optimized parameter values. Differences in parameter estimation from the traditional Hertz’s model and the hyperelastic FEM were presented. A further bilayer model was proposed in the study which distinguished the glycocalyx layer and the endothelial substrate. In the bilayer model, effects of the substrate were excluded from the properties characterization of the glycocalyx layer.
6 Conclusion and future work

In this thesis, I have developed computational models to study mechanical properties of biological tissues and their responses under external loading. In the model approach, an inverse analysis method that combines MATLAB optimization tools and finite element solver ABAQUS is developed to characterize the mechanical property of the tissues. ABAQUS is a well recognized package with suitable accuracy and expandability, and I have built a number of self-written FORTRAN subroutines for constitutive equations and Python scripts for post-processing into the ABAQUS for mechanical analysis. Three examples are given that represent testing samples of different anatomic levels, i.e. the left atrium of the heart (organ), the articular cartilage (tissue), the endothelial cell & its glycocalyx layer (cell).

In studies on the fibril-reinforced cartilage indentation test in Chapter 2, I have used the modified material constitutive equation for fibrils mechanical properties rather than the traditional fibril-reinforced model which employs the combination of two different types of elements. Satisfactory agreement between our model results and available analytical solution is seen in simplified geometries, validating our model. This enables us to apply the model to study the protective effects of fibrils in articular cartilage. Mechanical properties of the cartilage are adopted from published unconfined compression experimental data using the inverse FEM, and are used in the confined indentation study in the thesis.
Collagen fibril matrix is found to play an important role in tissue mechanical responses. The presence of a collagen fibril matrix will increase the levels of the interstitial fluid pressure. Furthermore, a randomly organized fibre orientation produces the maximal stress in the confined indentation. von Mises stress analysis reveals that three layered cartilage model with physiological fibril orientation in each layer may represent an optimal arrangement. It produces a close to frictionless surface, a reasonable mechanical strength and a secure attachment to the underlying subchondral bone.

Based on this study, the contribution of collagen fibrils is very important to the cartilage’s property and functions, and can dominant the fluid-solid frictional interaction effect in the articular cartilage. The three layered zones in the cartilage with unique collagen fibril orientation accounts for the depth-dependent mechanical properties of the articular cartilage. There are a number of limitations in the current study, including the lack of consideration on the inhomogeneity in the non-fibril matrix’s compressive modulus. This information is difficult to achieve from the experimental results. Moreover, the collagen fibril differs in both size and mechanical properties from zone to zone. More experimental studies are needed to determine the fibril matrix property in each zone. Once the detailed mechanical properties become available, they can be readily incorporated in the present numerical framework to improve the fibril-reinforced poroelastic model, and to provide more realistic mechanical responses of the articular cartilage. This can be one of the future work that, in my view is key to establish the accurate structure-function relationship for articular cartilages.
In the stress analysis of the atrial wall in Chapter 3, the wall stress contours based on patient specific images (using cardio CT) are calculated. The dominant fibre orientation, varied thickness and the anisotropic constitutive law proposed by Holzapfel and Ogden (2009) are incorporated in the model study. Implementation of the anisotropic model is validated in a simple shear test. The effect of the length of the vein, regional variation in wall thickness and fibre orientation are all examined. Peaks in the wall stress are found to be around the anterior wall, the PVs and their ostia, the LA appendage ridge, the high posterior and superior wall due to its intrinsic shape and curvature. The thickness and fibre orientation are found to contribute to the location and the magnitude of the maximum stress in each region. It is also found that the global stress pattern and the maximum stress does not change much, although there are some local variations due to the difference of wall thickness and fibre orientation.

In chapter 4, I extended above results to study the relationship between the regional wall stress and atrial remodelling. Wall stress varies widely in different regions of the same LA, and also in the same regions between different subjects. Peaks in LA wall stress are observed to associate with areas of low voltage and electrical scars in patients with persistent AF. Regional differences in wall stress may explain the heterogeneous remodelling that results from elevated intra-atrial pressure and promotes AF. The observation that the PV ostia has consistently high wall stress and harboured CFAE is compatible with the observations by others that stretch may elicit an excitatory response at the PV ostia without doing so elsewhere in the LA (Chen et al., 2007; Kalifa et al., 2003), and suggests a potential role for peaks in wall
stress at the PV ostia facilitating initiation of AF. This study adds to the rationale for lowering intra-atrial pressure in those at risk of AF to limit this remodelling process.

There are a number of limiting factors in the current study. Acquisition of an accurate geometry using modalities such as 128 slice CT is the most crucial step to determine the wall stress distribution (Taddei et al., 2006). However, the resolution of the current available imaging modalities does not allow accurate determination of regional differences in wall thickness and fibre orientation. Further studies can use the geometry from the MRI or DTMRI data for better resolution to retrieve thickness and different layer structure of the atrium. Further studies can also utilise information on the fibre orientation that is available in the DTMRI data (Wu et al., 2006). The movement of the septum shall be treated more realistically rather than being allowed to move freely. A dual chamber model which has both the left and the right atria can avoid the problem raised in the boundary condition for the septum. The study uses parameters based on porcine ventricle mechanical properties, since there are no experimental data on human atrium. When the atrium specific data are available, they can be incorporated in the present numerical framework for anisotropic hyperelastic modelling of the atrial wall stress.

It’s also noted that the high wall stress does not appear to be as much as the abnormal electrical signals in the LA appendage, where the pectinate muscles are confined to the inner surface. It is possible that the pectinate muscles produce a large contraction force (Guo et al., 1983), and generate an active stress in LA appendage.
Future studies may be able to address this problem by considering active stresses in order to achieve a better correlation in the appendage.

In chapter 5, I reported model results on the mechanical property of HUVECs and their overlying layer of the glycocalyx. Results are based on experimental force-displacement measurements in AFM micro-indentation tests on cultured HUVECs, with or without the glycocalyx layer being removed by the enzyme treatment. The inverse FEM programme is used to calculate values of the optimized mechanical model parameters. The traditional Hertz’s model and the hyperelastic model are employed in the parameter estimation. It is found that the Hertz’s model leads to a significant overestimation in comparison to the hyperelastic model. Finite element analysis has shown the influence of different mechanical properties of the cell substrate on the overall reaction force and stress distribution. A bilayer hyperelastic model is used in the study to determine the mechanical property of the glycocalyx layer. This model distinguishes the glycocalyx layer and the cell substrate, therefore excludes the influence of the cell substrate from the property characterization of the glycocalyx layer. Results show that the glycocalyx layer is approximately 3 times softer than the endothelial substrate. It needs to be noted that in the current confocal observation, the thickness of the glycocalyx layer cannot be determined accurately. This will affect our results. Once the thickness of the glycocalyx layer is available, it can be readily incorporated in the present numerical framework.

In summary, the computational framework presented in the thesis is versatile and can be readily improved with available experimental information in future studies.
It can be used to study mechanical properties of other biological tissues, such as chondrocytes and Achilles tendons, both of which are currently studied experimentally in our laboratory.
Appendices

Appendix A. Fibril-reinforced poroelastic model

SUBROUTINE UMAT(STRESS, STATEV, DDSDDE, SSE, SPD, SCD, RPL,
1 DDSDDT, DRPLDE, DRPLDT, STRAN, DSTRAN, TIME, DTIME, TEMP, DTEMP,
2 PREDEF, DPRED, CMNAME, NDI, NSHR, NTENS, NSTATV, PROPS, NPROPS,
3 COORDS, DROT, PNEWDT, CELENT, DFGRD0, DFGRD1, NOEL, NPT, LAYER,
4 KSPT, KSTEP, KINC)
C
INCLUDE 'ABA_PARAM.INC'
C
CHARACTER*8 CMNAME
C
DIMENSION STRESS(NTENS), STATEV(NSTATV), DDSDDE(NTENS, NTENS),
1 DDSDDT(NTENS), DRPLDE(NTENS), STRAN(NTENS), DSTRAN(NTENS),
2 PREDEF(1), DPRED(1), PROPS(NPROPS), COORDS(3), DROT(3, 3),
3 DFGRD0(3, 3), DFGRD1(3, 3)
DIMENSION ddsdde2(NTENS,NTENS)
C
PARAMETER(ZERO=0.D0, ONE=1.D0, TWO=2.D0, THREE=3.D0, SIX=6.D0,
1 ENUMAX=.4999D0, NEWTON=10, TOLER=1.0D-6)
C
IF (NDI.NE.3) THEN
WRITE (7, *) 'THIS UMAT MAY ONLY BE USED FOR Axis Symmetrical'
CALL XIT
ENDIF
C
ELASTIC PROPERTIES

EMOD=PROPS(1)
ENU=PROPS(2)
EF0=PROPS(3)
EFe=PROPS(4)

EBULK3=EMOD/(ONE-TWO*ENU)
EG2=EMOD/(ONE+ENU)
EG=EG2/TWO
EG3=THREE*EG
ELAM=(EBULK3-EG2)/THREE
EX=ONE-ENU
EF1=0
EF2=0
EF3=0

if(STRAN(2)>0.0)THEN
    EF3=EF0+2*EFe*STRAN(2)
ELSE
EF3=0
ENDIF

S11 = ((EF1 + EMOD) * EX - 2 * EF1 * ENU * ENU) / (ONE + ENU) / (ONE - TWO * ENU)
S22 = ((EF2 + EMOD) * EX - 2 * EF2 * ENU * ENU) / (ONE + ENU) / (ONE - TWO * ENU)
S33 = ((EF3 + EMOD) * EX - 2 * EF3 * ENU * ENU) / (ONE + ENU) / (ONE - TWO * ENU)
S1_1 = EMOD * EX / (ONE + ENU) / (ONE - TWO * ENU)
S2_2 = EMOD * EX / (ONE + ENU) / (ONE - TWO * ENU)
S3_3 = EMOD * EX / (ONE + ENU) / (ONE - TWO * ENU)

C
C ELASTIC STIFFNESS
C
DO K1 = 1, NTENS
DO K2 = 1, NTENS
DDSDDE(K2, K1) = 0.0
ddsdde2(K2, K1) = 0.0
END DO
END DO

DO K1 = 1, NDI
DO K2 = 1, NDI
DDSDDE(K2, K1) = ELAM
ddsdde2(K2, K1) = ELAM
END DO
END DO

C AXIAL DIRECTION
DDSDDE(2,2) = S33

C CIRCUMFERENTIAL DIRECTION
DDSDDE(3,3) = S22
DO K1 = NDI + 1, NTENS
DDSDDE(K1 , K1) = EG
ddsdde2(K1, K1) = EG
END DO

C CALCULATE STRESS
C
DO K1 = 1, NTENS
DO K2 = 1, NTENS
STRESS(K2) = STRESS(K2) + DDSDDE(K2, K1) * DSTRAN(K1)
STATEV(K2) = STATEV(K2) + ddsdde2(K2, K1) * DSTRAN(K1)
STATEV(K2+4) = STRESS(K2) - STATEV(K2)
STATEV(9) = EF1
STATEV(10) = EF2
STATEV(11) = EF3
STATEV(12) = sqrt(((STRESS(1) - STRESS(2)) * (STRESS(1) - STRESS(2)))
1 + (STRESS(2) - STRESS(3)) * (STRESS(2) - STRESS(3))
2 + (STRESS(1) - STRESS(3)) * (STRESS(1) - STRESS(3))
3 + 6 * (STRESS(4)**2) / 2.0)
STATEV(13) = sqrt(((STATEV(1) - STATEV(2)) * (STATEV(1) - STATEV(2)))
1 + (STATEV(2) - STATEV(3)) * (STATEV(2) - STATEV(3))
2 + (STATEV(1) - STATEV(3)) * (STATEV(1) - STATEV(3))
3 + 6 * (STATEV(4)**2) / 2.0)
END DO
END DO

RETURN
END
Appendix B. Anisotropic atrium

Anisotropic

subroutine uanisohyper_inv (ainv, ua, zeta, nfibers, ninv,
$     ui1, ui2, ui3, temp, noel, cmname, incmpflag, ihybflag,
$     numstatev, statev, numfieldv, fieldv, fieldvinc,
$     numprops, props)

include 'aba_param.inc'

class cmname

dimension ua(2), ainv(ninv), ui1(ninv),
$     ui2(ninv*(ninv+1)/2), ui3(ninv*(ninv+1)/2),
$     statev(numstatev), fieldv(numfieldv),
$     fieldvinc(numfieldv), props(numprops)

ainv: invariants
ua : energies ua(1): utot, ua(2); udev
ui1 : dUdI
ui2 : d2U/dIdJ
ui3 : d3U/dIdJdJ, not used for regular elements
f : local direction 1  fiber direction
s : local direction 2  sheet direction
n : local direction 3 not used

parameter ( half = 0.5d0,
*     zero = 0.d0,
*     one = 1.d0,
*     two = 2.d0,
*     three= 3.d0,
*     four = 4.d0,
*     five = 5.d0,
*     six  = 6.d0,

*     index_1 = 1,
*     index_2 = 2,
*     index_3 = 3,
*     index_J = 3 )

Anisotropic model

a_iso = props(1)
b_iso = props(2)
a_f = props(3)
b_f = props(4)
a_s = props(5)
b_s = props(6)
a_fs = props(7)
b_fs = props(8)

index_inv_1=index_1
index_inv_f=indxInv4(index_1,index_1)
index_inv_s=indxInv4(index_2,index_2)
index_inv_fs=indxInv4(index_1,index_2)
index_ui2_1=indx(index_1,index_1)
index_ui2_f=indx(index_inv_f,index_inv_f)
index_ui2_s=indx(index_inv_s,index_inv_s)
index_ui2_fs=indx(index_inv_fs,index_inv_fs)

v_iso = exp(b_iso * (ainv(index_inv_1) - three))
if (ainv(index_inv_f) .le. 1.0) then
  v_f_1 = 0.0
else
  v_f_1 = ainv(index_inv_f) - one
end if
v_f_2 = exp(b_f * v_f_1 * v_f_1)
if (ainv(index_inv_s) .le. 1.0) then
  v_s_1 = 0.0
else
  v_s_1 = ainv(index_inv_s) - one
end if
v_s_2 = exp(b_s * v_s_1 * v_s_1)

v_iso = exp(b_iso * (ainv(index_inv_1) - three))
if (ainv(index_inv_f) .le. 1.0) then
  v_f_1 = 0.0
else
  v_f_1 = ainv(index_inv_f) - one
end if
v_f_2 = exp(b_f * v_f_1 * v_f_1)
if (ainv(index_inv_s) .le. 1.0) then
  v_s_1 = 0.0
else
  v_s_1 = ainv(index_inv_s) - one
end if
v_s_2 = exp(b_s * v_s_1 * v_s_1)

c
deviatoric energy
c
ua(2) = a_iso/two/b_iso * v_iso + a_f/two/b_f * (v_f_2 * one) + a_s/two/b_s * (v_s_2 * one) + a_fs/two/b_fs * (v_fs_2 * one)

c
compute derivatives
c
ui1(index_inv_1) = a_iso/two * v_iso
ui1(index_inv_f) = a_f * v_f_1 * v_f_2
ui1(index_inv_s) = a_s * v_s_1 * v_s_2
ui1(index_inv_fs) = a_fs * v_fs_1 * v_fs_2

ui2(index_ui2_1) = a_iso * b_iso/two * v_iso
if (ainv(index_inv_f) .le. 1.0) then
  ui2(index_ui2_f)=0.0
else
  ui2(index_ui2_f) = two * a_f * b_f * v_f_1 * v_f_1 * v_f_2 + a_f * v_f_2
derend if
if (ainv(index_inv_s) .le. 1.0) then
  ui2(index_ui2_s)=0.0
else
  ui2(index_ui2_s) = two * a_s * b_s * v_s_1 * v_s_1 * v_s_2 + a_s * v_s_2
derend if
ui2(index_ui2_fs)=two * a_fs * b_fs * v_fs_1 * v_fs_1 * v_fs_2 + a_fs * v_fs_2
return
end
of symmetric matrix

integer function indx( i, j )
include 'aba_param.inc'
ii = min(i,j)
jj = max(i,j)
indx = ii + jj*(jj-1)/2
return
end

-------------------------------------------------------------

c Function to generate enumeration of scalar
Pseudo-Invariants of type 4

integer function indxInv4( i, j )
include 'aba_param.inc'
ii = min(i,j)
jj = max(i,j)
indxInv4 = 4 + jj*(jj-1) + 2*(ii-1)
return
end

-------------------------------------------------------------

c Function to generate enumeration of scalar
Pseudo-Invariants of type 5

integer function indxInv5( i, j )
include 'aba_param.inc'
ii = min(i,j)
jj = max(i,j)
indxInv5 = 5 + jj*(jj-1) + 2*(ii-1)
return
end
Appendix C. Fibre orientation

SUBROUTINE ORIENT(T,NOEL,NPT, LAYER, KSPT, COORDS, BASIS,  
1 ORNAME, NNODES, CNODES, JNUM)
C
INCLUDE 'ABA_PARAM.INC'
C
CHARACTER*80 ORNAME
C
DIMENSION T(3,3),COORDS(3),BASIS(3,3),CNODES(3,NNODES)
DIMENSION JNUM(NNODES)
C
real normal(3), vectors(3,2), xdir(3),ydir(3),normalx(3)
real length1, length2, lengthNormal,theta
C     Points on the plane of local coordinates system
real normal(3), vectors(3,2), xdir(3),ydir(3),normalx(3)
real length1, length2, lengthNormal,theta
real, parameter :: pi=3.1415926
C     vectors in each element
C     normal vector, local 3/z direction
C     normal vector of x plane. change this array for different angle
C     angle offset from horizontal line is pi/2-theta
if (orname.eq.'VEIN1') then
  theta=60.0/180.0*pi
write(7,*) 'vein1'
elseif (orname.eq.'VEIN2') then
  theta=90.0/180.0*pi
elseif (orname.eq.'VEIN3') then
  theta=90.0/180.0*pi
elseif (orname.eq.'VEIN4') then
  theta=135.0/180.0*pi
elseif (orname.eq.'VEIN5') then
  theta=60.0/180.0*pi
write(7,*) theta
elseif (orname.eq.'ORITOP') then
   theta=60.0/180.0*pi
elseif (orname.eq.'ORIBOTTOM') then
   theta=-30.0/180.0*pi
else
   theta=90.0/180.0*pi
endif

C write(7,*') 'bottom'
else
   theta=0.0/180.0*pi
endif
C normalx(1)= sin(theta)
   normalx(2)= 0.0
   normalx(3)= cos(theta)

write(7,*') normalx
C local 1/x direction = normal direction of element normal and
C z=n plane normal, so it is cross product of the two normals.
C
   xdir(1)=normal(2)*normalx(3)-normal(3)*normalx(2)
   xdir(2)=-(normal(1)*normalx(3)-normal(3)*normalx(1))
   xdir(3)=normal(1)*normalx(2)-normal(2)*normalx(1)

C local 2/y direction = normal direction of local 1 and 2
C
   ydir(1)=xdir(2)*normal(3)
   ydir(2)=-xdir(1)*normal(3)
   ydir(3)=xdir(1)*normal(2)-xdir(2)*normal(1)

c write(7,*') 'normal'
c write(7,*') normal

write(7,*') 'length 1,2 normal'
write(7,*') length1
write(7,*') length2
write(7,*') lengthNormal

c direction cosines of preferred orientation in terms of the
c default basis direction. '-' means opposite direction
T(1,1)=-xdir(2)/length1
T(2,1)=-xdir(3)/length1
T(3,1)=-xdir(3)/length1
T(1,2)=ydir(1)/length2
T(2,2)=ydir(2)/length2
T(3,2)=ydir(3)/length3
T(1,3)=-normal(1)/lengthNormal
T(2,3)=-normal(2)/lengthNormal
T(3,3)=-normal(3)/lengthNormal

c write(7,*') 'done'

RETURN
END
Appendix D. Post-process

D1. Maximum value

from abaqus import *
from abaqusConstants import *

#~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
def getCurrentViewport():
    vpName = session.currentViewportName
    return session.viewports[vpName]
#~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
def getCurrentOdb():
    vpName = session.currentViewportName
    vp = session.viewports[vpName]
    odbName = vp.odbDisplay.name
    if odbName:
        return session.odbs[dbName]
    else:
        print 'ERROR: odb file is empty.
        return
#~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
def SD(pickedElements):
    # Get odb
    vp = getCurrentViewport()
    odb = getCurrentOdb()
    # Test if the odb is read-only.
    #if odb.isReadOnly:
    #    print 'WARNING: odb is read only. reload odb without read-only.
    #    return
    # Get all the nodes
    # using index 0 to get all the nodes. if there is more than one
    # instance, make sure the first one contains all the nodes.
    instance = odb.rootAssembly.instances.values()[0]

    # Create another elementSet contain all the elements you selected under instance
    name1 = 'elements' + str(len(pickedElements))
    instance.ElementSet(name=name1, elements=pickedElements)
    # update list not working

    # Create another field output for the standard deviation in current frame
    curStepKey = vp.odbDisplay.fieldFrame[0]
    curStepName = odb.steps.keys()[curStepKey]
    curFrameKey = vp.odbDisplay.fieldFrame[1]
    curFrame = odb.steps[curStepName].frames[curFrameKey]

    # Calculate standard deviation
    # Get fieldOutput with variable 'S' from a frame
    fieldOutput1 = curFrame.fieldOutputs['S']
# Get von Mises
fieldOutput2 = fieldOutput1.getScalarField(invariant=MISES)
allStressValues = fieldOutput2.values
allStressData = [i.data for i in allStressValues]
# print allStressData[16169]

# Get subset of fieldOutput
elementSet = instance.elementSets[name1]
subFieldOutput = fieldOutput2.getSubset(region=elementSet)
subValue = subFieldOutput.values
subData = [i.data for i in subValue]

# Calculate standard deviation
totalCount = 0.0
averageValue = 0.0
totalSquareValue = 0.0
# print '1'
for i in subData:
    totalValue = totalValue + i
averageValue = totalValue / len(pickedElements)

# print '2'
squareSubData = [(i - averageValue)**2 for i in subData]
for i in squareSubData:
    totalSquareValue = totalSquareValue + i

# print '3'
standardDe = sqrt(totalSquareValue / len(pickedElements))
print 'standard deviation for the selected region is :'
print standardDe
print '
#

# Add fieldOutput into frame
name2 = 'SD' + '.' + name1
uField = curFrame.FieldOutput(name=name2, description='Standard Deviation at integration points', type=SCALAR)
elementLabels=[i.label for i in pickedElements]
elementData=[]
elementsAll = instance.elements
elementAllLabels = [i.label for i in elementsAll]
for i in elementsAll:
    if (elementLabels.count(i.label) == 0):
        elementData.append([allStressData[i.label-1]])
    elif (elementLabels.count(i.label) == 1):
        elementData.append([standardDe])
# print elementData[16169]
uField.addData(position=INTEGRATION_POINT, instance=instance, labels=elementAllLabels, data=elementData)
D2. Interpolation

# interpolation, used for compare two contour

```python
import os

def dist(u, v):
    return sqrt((float(u.split()[0]) - float(v.split()[0]))**2 + (float(u.split()[1]) - float(v.split()[1]))**2 + (float(u.split()[2]) - float(v.split()[2]))**2)

cwd = os.getcwd()

sourceFilePath = os.path.join(cwd, 'source.plt')
targetFilePath = os.path.join(cwd, 'target.plt')

sourceFileHandler = open(sourceFilePath, 'r')
targetFileHandler = open(targetFilePath, 'r')

sourceNodes = []
sourceEles = []
targetNodes = []
newValues = []
difference = []

temp = []
tempD = []

for i in range(0, 2):
    sourceLine = sourceFileHandler.readline()
    targetLine = targetFileHandler.readline()
    sourceLine = sourceFileHandler.readline()
    targetLine = targetFileHandler.readline()
    data = sourceLine.split(' ')
    nodeNum = data[3].split('=')
    sourceNodeNum = int(nodeNum[1])
    eleNum = data[4].split('=')
    sourceEleNum = int(eleNum[1].split()[0])

    nodeNum = data[3].split('=')
    targetNodeNum = int(nodeNum[1])

    for i in range(0, sourceNodeNum):
        sourceLine = sourceFileHandler.readline()
        #data = sourceLine.split(' ')
        sourceNodes.append(sourceLine)

        if i < targetNodeNum:
            targetLine = targetFileHandler.readline()
            #data = targetLine.split(' ')
            targetNodes.append(targetLine)

    targetFileHandler.close()
```

for i in range(0, sourceEleNum):
    sourceLine = sourceFileHandler.readline()
sourceEles.append(sourceLine)
sourceFileHandler.close()

#################################################################
###########          Interpolation                  #############
#################################################################

aa=1
for i in sourceNodes:
    aa=aa+1
    smallestD = 5.0
    temp=[]
    tempD=[]
    for j in targetNodes:
        dd=dist(i,j)
        if dd <= smallestD:
            temp.append(j)
            tempD.append(dd)
    newlist=tempD
    newlist.sort()
    indices=[0]*3
    indices[0]=tempD.index(newlist[0])
    indices[1]=tempD.index(newlist[1])
    indices[2]=tempD.index(newlist[2])
    # inverse distance weighted interpolation
    totalweight = 0.0
    newValue=0.0
    weight=[0]*3
    for j in range(0,3):
        weight[j]=1/ tempD[indices[j]]
        totalweight = totalweight + weight[j]
    for j in range(0,3):
        weight[j]=weight[j]/totalweight
    for j in range(0,3):
        newValue = newValue + weight[j]*float(temp[indices[j]].split()[3])
    newValues.append(newValue)
difference.append(newValue-float(i.split()[3]))
print aa

#################################################################
###########          TO TECPLLOT                     #############
#################################################################

fileName = 'interpolation' + '.plt'
variables = 'VARIABLES = X Y Z ' + 'stress ' 
f = open(fileName,'w')
f.write('TITLE = stress interpolation value\n')
f.write(variables + '\n')
#f.write('zone f=fepoint, et=quadrilateral, n=' + str(len(allNodes)) + ' e=' + str(len(allElements)))
f.write('zone f=fepoint, et=TRIANGLE, n=' + str(sourceNodeNum) + ' e=' + str(sourceEleNum))
f.write('n')
for i in range(0,sourceNodeNum):
    f.write(sourceNodes[i].split()[0])
f.write('t')
f.write('t')
f.write('t')
f.write(sourceNodes[i].split()[1])
for i in range(0,sourceNodeNum):
    f.write(sourceNodes[i].split()[0])
    f.write('	')
    f.write(sourceNodes[i].split()[1])
    f.write('	')
    f.write(sourceNodes[i].split()[2])
    f.write('	')
    f.write(str(difference[i]))
    f.write('	')
    f.write('
')
for i in sourceEles:
    f.write(i)
    f.close()
D3. Point-to-point analysis

from abaqus import *
from abaqusConstants import *
import string
import math

#point = []
points = []
partions = []
veinLabels = []
position = []
distances = []
mvLabels = []
triangles = []
stress = []
value = []
p2p = []
CFEMean = []
stddev = []
freq = []

#--------------------------------------------------
def dotProduct(u,v):
#--------------------------------------------------
def crossProduct(u,v):
   return [u[1]*v[2]-u[2]*v[1],u[2]*v[0]-u[0]*v[2],u[0]*v[1]-u[1]*v[0]]
#--------------------------------------------------
def vectorMinus(u,v):
   return [u[0]-v[0],u[1]-v[1],u[2]-v[2]]
#--------------------------------------------------
def vectorAdd(u,v):
   return [u[0]+v[0],u[1]+v[1],u[2]+v[2]]
#--------------------------------------------------
def vectorScaleTimes(u,v):
   return [u*v[0],u*v[1],u*v[2]]
# length of vector---------------------------------
def norm(v):
   return sqrt(dotProduct(v,v))
#--------------------------------------------------
def intersectRayTriangle(R,T):
   # T = [[T0X,T0Y,T0Z],[T1X,T1Y,T1Z],[T2X,T2Y,T2Z]] R,only two elements [[x,y,z],[x,y,z]]
   # global point u = vectorMinus(T[1],T[0])
   # u,v are vector T[1]-T[0]= [v1X-v0X,v1Y-v0Y,v1Z-v0Z], v means vertice
   # v = vectorMinus(T[2],T[0])
   # n = crossProduct(u,v)

---

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if(n==0):
    return -1;
    print 'you will not see this statement.'
direction = vectorMinus(R[1],R[0])

#dis = pow(distance,1/2)
w0 = vectorMinus(R[0],T[0])
a = -dotProduct(n,w0)
b = dotProduct(n,direction)
if(math.fabs(b)<0.000001):                  # ray is parallel to triangle plane
    if(a==0):                          # ray lies in triangle plane
        #print 'ray lines in triangle plane. b<0.0001, a=0.'
        return [-2,0]
    else:
        #print 'ray disjoint from triangle plane b<0.0001, a!!!=0.'
        return [-3,0]                     # ray disjoint from triangle plane
r = a/b
#if(r<0.0):                            # ray goes away from triangle
    #print 'ray goes away from triangle.'
    # return -4;
point = vectorAdd(R[0],vectorScaleTimes(r,direction))
direc = vectorMinus(point,R[1])
distance = sqrt(direc[0]*direc[0]+direc[1]*direc[1]+direc[2]*direc[2])
# test if point is inside triangle plane.
uu = dotProduct(u,u)
uv = dotProduct(u,v)
vv = dotProduct(v,v)
w = vectorMinus(point,T[0])
wu = dotProduct(w,u)
wv = dotProduct(w,v)
D = uv*uv-vv*vv
s = (uv*wv-vv*wu)/D
if(s<0.0 or s>1.0):
    #print 'point is outside triangle.'
    return [-4,0]
t = (uv*wu-uu*wv)/D
if(t<0.0 or (s+t)>1.0):
    #print 'point is outside triangle.'
    return [-4,0]
returnData=[]
returnData.append(distance)
returnData.append(point)
return returnData

def getRay():
    import os
    global p2p
    global CFEMean
    global stddev
    global freq
    global position
    #cwd=os.getcwd()
    #filePath=os.path.join(cwd,'1.csv')
    vpName = session.currentViewportName
vp = session.viewports[vpName]
odbName = vp.odbDisplay.name
filePathArray = odbName.split('\\')
fileName="
for i in range(0,len(filePathArray)-1):
    fileName = fileName + filePathArray[i] +\\
fileName = fileName+'3.csv'
fileHandler = open(fileName, 'r')
#outputHandler = open('output.dat','w')
dataStart = 0
while (1):
    line = fileHandler.readline()
    if line == "EOF\n":
        break
    if line == "Begin data\n":
        dataStart = 1
    elif dataStart == 1:
        data = line.split(',
        if data[0].find("rov trace")!=-1:
            position=data
            position[len(position)-1]=position[len(position)-1].split()[0]
        elif data[0].find("peak2peak:"!)!=-1:
            p2p=data
            p2p[len(p2p)-1]=p2p[len(p2p)-1].split()[0]
        elif data[0].find("CFE mean:"!)!=-1:
            CFEMean=data
            CFEMean[len(CFEMean)-1]=CFEMean[len(CFEMean)-1].split()[0]
        elif data[0].find("CFE stddev:"!)!=-1:
            stddev=data
            stddev[len(stddev)-1]=stddev[len(stddev)-1].split()[0]
        elif data[0].find("dom freq:"!)!=-1:
            freq=data
            freq[len(freq)-1]=freq[len(freq)-1].split()[0]
        elif data[0].find("roving x")!=-1:
            x_O=data
            x_O[len(x_O)-1]=x_O[len(x_O)-1].split()[0]
        elif data[0].find("roving y")!=-1:
            y_O=data
            y_O[len(y_O)-1]=y_O[len(y_O)-1].split()[0]
        elif data[0].find("roving z")!=-1:
            z_O=data
            z_O[len(z_O)-1]=z_O[len(z_O)-1].split()[0]
        elif data[0].find("surfPt x")!=-1:
            x=data
            x[len(x)-1]=x[len(x)-1].split()[0] #delete \n in the last string
        elif data[0].find("surfPt y")!=-1:
            y=data
            y[len(y)-1]=y[len(y)-1].split()[0]
        elif data[0].find("surfPt z")!=-1:
            z=data
            z[len(z)-1]=z[len(z)-1].split()[0]
fileHandler.close()
rays=[]
for i in range(1,len(x_O)):
    origin=[float(x_O[i]),float(y_O[i]),float(z_O[i])]
surface=[float(x[i]),float(y[i]),float(z[i])]
ray=[origin,surface]
rays.append(ray)
return rays

def reverseDis(point,coords,stress):
    if len(point) == len(coords) == len(stress):
        weight = []
        totalWeight = 0.0
        pointV = 0.0
        for v in coords:
            d = 0.0
            for i in range(0,len(v)):
                d = d+(v[i]-point[i])*(v[i]-point[i])
            dis = sqrt(d)
            weight.append(1/dis)
            totalWeight = totalWeight + 1/dis
        for i in range(0,len(stress)):
            pointV = pointV+weight[i]/totalWeight*stress[i]
        return pointV
    else:
        print 'error in array point, coords, stress'
        return 0

def getCurrentViewport():
    vpName = session.currentViewportName
    return session.viewports[vpname]

def getCurrentOdb():
    vpName = session.currentViewportName
    vp = session.viewports[vpname]
    odbName = vp.odbDisplay.name
    if odbName:
        return session.odbs[odbName]
    else:
        print 'ERROR: odb file is empty.'
        return

instanceName='PART-1-1'
nodeSetName='NALL'
variableName='S'
#componentName='E11'
invariantName=MISES
# Get odb
vp = getCurrentViewport()
odb = getCurrentOdb()

# Get file path
vpName = session.currentViewportName
vp = session.viewports[vpName]
odbName = vp.odbDisplay.name
filePathArray = odbName.split('\\')
filePath = Path('\\'.join(filePathArray[0:-1]), filePathArray[-1])
for i in range(len(filePathArray)-1):
    filePath = filePath + filePathArray[i] + '\'

# Get all the nodes
instance = odb.rootAssembly.instances[instanceName]
allNodes = instance.nodeSets[nodeSetName].nodes

# Get all elements and connectivity list and centroid coordinates
allElements = instance.elements

# Get all vein elements and label
veinElements = instance.elementSets['VEINS'].elements
for i in veinElements:
    veinLabels.append(i.label)

# Get all mitral valve elements and labels
mvElements = instance.elementSets['MV'].elements
for i in mvElements:
    mvLabels.append(i.label)

#elementData=[i.label*0 for i in allElements]

# Get current frame
curStepKey = vp.odbDisplay.fieldFrame[0]
curStepName = odb.steps.keys()[curStepKey]
curFrameKey = vp.odbDisplay.fieldFrame[1]
curFrame = odb.steps[curStepName].frames[curFrameKey]
fieldOutput = curFrame.fieldOutputs['U']
displacementValues = fieldOutput.values

if(variableName == 'S'):
    fieldOutput1 = curFrame.fieldOutputs[variableName]
    fieldOutput2 = fieldOutput1.getScalarField(invariant=invariantName)
    fieldOutput3 = fieldOutput2.getSubset(position=ELEMENT_NODAL)
    fieldOutputValues = fieldOutput3.values
length = len(fieldOutputValues)/2  # shell elements have two sides. size of data list is twice of element numbers
i = 0
for i in range(length):
    nodalData[fieldOutputValues[i].nodeLabel - 1] = fieldOutputValues[i].data
for i in range(0, len(nodalData)):
    averageData = 0.0
    for j in range(0, len(nodalData[i])):
        averageData = averageData + nodalData[i][j]
    averageData = averageData / len(nodalData[i])
    nodalData[i] = averageData
for j in allElements:
    v1 = allNodes[j.connectivity[0]-1].coordinates
    v2 = allNodes[j.connectivity[1]-1].coordinates
    v3 = allNodes[j.connectivity[2]-1].coordinates
    T = [v1,v2,v3]
    triangles.append(T)
    S=[]
    for j2 in j.connectivity:
        s = nodalData[j2-1]
        S.append(s)
    stress.append(S)

rays = getRay()
print (len(rays))

for i in rays:
    #print (i[1][0])
    if norm(vectorMinus(i[0],i[1])) >= rTos:
        points.append([0.0,0.0,0.0])
        partitions.append(-1)
        value.append(0.0)
        distances.append(rTos)
        continue
    unFound = 1
    if(i[1][0]!=0.0 or i[1][1]!=0.0 or i[1][2]!=0.0):
        tempD=100000.0
        tempP=0.0
        for j in range(0,len(triangles)):
            data=intersectRayTriangle(i,triangles[j])
            if(data[0]>0.0 and data[0]<tempD and data[0]<tol):
                tempP=data[1]
                tempD=data[0]
                tempJ=triangles[j]
                tempIndex=j+1
                unFound=0
                print (tempP)
                print (rays.index(i),triangles.index(tempJ))
                print '
    if(unFound==0):
        print (tempP)
        print (rays.index(i),triangles.index(tempJ))
        print 'n 'n'
        points.append(tempP)
        partitions.append(tempIndex)
        distances.append(tempD)
        value.append(reverseDis(tempP,tempJ,stress[triangles.index(tempJ)]))
    if(unFound):
        print 'not found,missing that point due to smooth,or increase tol value.
        print (rays.index(i))
        print 'n 'n'
        points.append([0.0,0.0,0.0])
        partitions.append(-1)
        value.append(0.0)
        distances.append(0.0)
    elif(i[1][0]==0.0 and i[1][1]==0.0 and i[1][2]==0.0):
        print 'found: 0.0 0.0 0.0'
        print (rays.index(i))
        print 'n 'n'
points.append(i[1])
partions.append(-1)
value.append(0.0)
distances.append(0.0)
fileName = filePath + 'ray.inp'
output = open(fileName,'w')
m=0
n=0
print (len(points))
print 'n'
print (len(rays))
print 'n'
print (len(value))
print 'n'
for i in range(0,len(points)):
    nset = '*Node,nset=test'+str(n)
    output.write(nset)
    output.write('
')
    output.write(str(m+900000))
    output.write(',
    output.write(rays[i][0][0])
    output.write(','
    output.write(rays[i][0][1])
    output.write(','
    output.write(rays[i][0][2])
    output.write('
')
    output.close()

fileName = filePath + 'projectPoints.inp'
output = open(fileName,'w')
output.write('*Node,nset=testProject')
output.write('
')
m=0
fileName = filePath + 'result.xls'
output = open(fileName,'w')
output.write('ID	 position 	 x_o 	 y_o 	 z_o 	 x_s 	 y_s 	 z_s 	 p2p 	 mean 	 stddev 	 freq 	 stress 	 distance	 partion
')
m=1
for i in range(0,len(points)):
    output.write(str(m)+'	'+position[i+1]+'	'+str(rays[i][1][0])+'	'+str(rays[i][1][1])+'	'+str(rays[i][1][2])
    output.write('
')
    output.write(points[i][0])
    output.write(str(points[i][1])
    output.write(str(points[i][2])
    output.write(if partions[i]==-1:
        ifVein=0
    elif partions[i]!=-1:
        if veinLabels.count(partions[i])!=0:
            ifVein=1
        elif mvLabels.count(partions[i])!=0:
            ifVein=2
        else:
            ifVein=3
    output.write(p2p[i+1]+',CFEMean[i+1]+',stddev[i+1]+',freq[i+1]+',value[i]+',distances[i]+',m=m+1
    output.close()
Appendix E. AFM micro-indentation data

E1. Force-displacement data on HUVECs without the glycocalyx

Figure A.1 Examples of AFM experimental data on endothelial cells without the glycocalyx.
E2. Force-displacement data on HUVECs with the glycocalyx

Figure A.2 Examples of AFM experimental data on endothelial cells with the glycocalyx.
Appendix F. Inverse FEM

F1. Matlab Code

%MATLAB code for inverse analysis. The code is adapted from the Appendix A by Lei and Szeri (2007).
%1
%The following is the code for “main.m”.
clear; close all;
% Declaration of global variables
global Iter FunEvals ParamHistory c1 c2 c3 c4
global nob nop E niu Ef K results exp_x exp_y
load initial.txt
% Initial parameters
E=initial(1,1); niu=initial(1,2);Ef=initial(1,3);K=initial(1,4);
% The total number of optimizing parameters
nop=4;
% store the experimental data
load test.txt
% load points.txt
exp_x=10.^test(:,1);
exp_y=test(:,2);
observed_points=10.^test(:,1);
exp_points=spline(exp_x,exp_y,observed_points);
results=exp_points;
% The scale coefficients for corresponding parameters
% c1=1.0e-06; c2=1.0e-6;c3=1.0e-6;
c1=1.0e-04; c2=1.0e-04;c3=1.0e-5; c4=1.0e-8;
% Scale initial parameters
IniGuess=[E*c1, niu*c2, Ef*c3, K*c4];
% Initialize counters
Iter=0; FunEvals=0; ParamHistory=[];
% Set optimization options.
options=optimset('TolFun',1e-10, 'TolX',1e-7, 'MaxIter',100,'Algorithm','levenberg-marquardt')
options=optimset('TolFun',1e-10, 'TolX',1e-7, 'MaxIter',100)
%Solve the nonlinear least squares problem. It needs "objfunction.m"
[x, ErrNorm,residual,exitflag,output]=lsqnonlin(@objfunction,IniGuess,[0 0 0 0], [inf 0.5*c2 inf inf],options)

% Scale the optimized parameters to normal values
x1=x(1)/c1; x2=x(2)/c2; x3=x(3)/c3; x4=x(4)/c4;
OptParam=[x1 x2 x3 x4];
% Save the optimized parameters in "param.txt".
save param.txt OptParam -ascii
% Save the parameter evolution history in "history.txt".
ParamHistory=[ParamHistory; [Iter FunEvals x1 x2 x3 x4 ErrNorm]];
save history.txt ParamHistory -ascii
% Call external programs to solve direct problem and get "result.txt".
! abaqus python modifyInput.py interactive
! abaqus job=unconfinedComp user=fiber-std.obj interactive
! abaqus viewer noGUI=predictedData.py
load result.txt
TimeCom=result(:,1); ForceCom=result(:,2);
% Plot predicted response
plot(TimeCom,ForceCom,'b')

% The following is the code for "objfunction.m".
function err=objfunction(params)
global Iter FunEvals ParamHistory c1 c2 c3 c4
global nob nop results exp_x exp_y
% Scale the parameters to normal values
x1=params(1)/c1; x2=params(2)/c2;x4=params(4)/c4; x3=params(3)/c3;
x5=params(5)/c5; x6=params(6)/c6;
OptParam=[x1 x2 x3 x4];
% Save the parameters in "param.txt".
save param.txt OptParam -ascii
% Read experimental data from "test.txt"
load test.txt
observed_points=10.^test(:,1);
exp_points=spline(exp_x,exp_y,observed_points);
% TimeExp=test(:,1); ForceExp=test(:,2);
% The total number of observations
nob=length(observed_points);
% Plot experimental response
plot(observed_points,exp_points,'r*'), hold on
Iter
! abaqus python modifyInput.py interactive
! abaqus job=unconfinedComp user=fiber-std.obj interactive
! abaqus viewer noGUI=predictedData.py
load result.txt
TimeCom=result(:,1); ForceCom=result(:,2);
cal_points=spline(TimeCom,ForceCom,observed_points);
% save speed.txt ForceCom -ascii
% Plot predicted response
plot(observed_points,cal_points,'gx'), hold on
plotResult=111111
% Error vector is difference between experimental and predicted forces
err=exp_points-cal_points;
% Calculate the norm of error vector
ErrNorm=sum(err.^2);
% Save the parameter evolution history in "history.txt".
ParamHistory=[ParamHistory; [Iter FunEvals x1 x2 x3 x4 ErrNorm]];
save history.txt ParamHistory -ascii
results=[results,[cal_points]];
save results.txt results -ascii
% Update the counters
FunEvals=FunEvals+1;
if rem(FunEvals,nop)==0
Iter=Iter+1;
End
F2. Python Code

```python
#1
import os

cwd=os.getcwd()
filePath=os.path.join(cwd,'unconfinedComp.inp')
paramPath=os.path.join(cwd,'param.txt')
paramHandler = open(paramPath, 'r')
params=paramHandler.read()
param=params.split()
hyper=param[0]+','+param[1]+','+param[2]+','+'0.0'+'
visco=param[1]+','+'0.0'+','+param[2]+'

i=0
newValue = 'new value
fileHandler = open(filePath, 'r+')
lines = fileHandler.readlines()

while (fileHandler.readline() != ''):
    i=i+1
    newLine=str(fileHandler.readline())
    if newLine.find('*Material') != -1 or newLine.find('*material') != -1:
        print newLine
        #fileHandler.next()
        newLine=str(lines[i+1])
        fileHandler.seek(-11,1)
        fileHandler.write("dddd")
        break

while (fileHandler.readline() != ' '):
    i=i+1
    newLine=str(lines[i])
    if newLine.find('*user material') != -1 or newLine.find("*User material") != -1:
        #print newLine
        lines[i+1]=str(hyper)
        break
    elif newLine.find('*Permeability,') != -1 or newLine.find("*permeability,") != -1:
        lines[i+1]=str(p)

fileHandler.close()

fileHandler = open(filePath, "w")
for i in range(0,length):
    fileHandler.write(lines[i])
fileHandler.close()
```

# write new input file

```
#1
import os

cwd=os.getcwd()
filePath=os.path.join(cwd,'unconfinedComp.inp')
paramPath=os.path.join(cwd,'param.txt')
paramHandler = open(paramPath, 'r')
params=paramHandler.read()
param=params.split()
hyper=param[0]+','+param[1]+','+'0.0'+'
hyper=param[0]+','+param[1]+','+param[2]+','+'0.0'+'
p=param[3]+','+'4.0'+'
#visco=param[1]+','+'0.0'+','+param[2]+'
i=0
#newValue = 'new value
'
fileHandler = open(filePath, 'r+')
lines = fileHandler.readlines()
length=len(lines)
#while (fileHandler.readline() != ''):
#fileHandler.seek(0)
#for i in range(0,length):
#    newLine=str(fileHandler.readline())
#    if newLine.find("*Material") != -1 or newLine.find("*material") != -1:
#        print newLine
#        #fileHandler.next()
#        newLine=str(lines[i+1])
#        fileHandler.seek(-11,1)
#        fileHandler.write("ddddd")
#        break
#fileHandler.close()

#while (fileHandler.readline() != ''):
#fileHandler.seek(0)
for i in range(0,length):
    newLine=str(lines[i])
    if newLine.find("*user material") != -1 or newLine.find("*User material") != -1:
        #print newLine
        #fileHandler.next()
        newLine=str(lines[i+1])
        fileHandler.seek(-11,1)
        fileHandler.write("ddddd")
        break
fileHandler.close()

#-----------------------
# write new input file
#-----------------------
fileHandler = open(filePath, "w")
for i in range(0,length):
    fileHandler.write(lines[i])
fileHandler.close()


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References


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