Effect of time and loading protocol on mechanical behavior of healthy porcine coronary arteries

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BMTE 09.13

22 April 2009

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Chapter 1

Introduction

In 2006, more than 41,000 people died of cardiovascular disease in the Netherlands [1]. Almost 10,000 people who died from cardiovascular disease did so by suffering from an acute myocardial infarction, often without previous symptoms. Atherosclerosis is the main cause of cardiovascular disease, and it is characterized by local thickening of the vessel wall, or plaque formation. Coronary arteries are among the regions most susceptible to atherosclerosis [2]. A subset of atherosclerotic plaques, called the vulnerable plaques, are characterized by lipid accumulation in the vessel wall, with a thin fibrous cap separating the lipid core from the lumen. Rupture of the cap of a vulnerable plaque and clot formation is the underlying cause of the majority of acute myocardial infarctions [3].

Rupture of the cap of a vulnerable plaque occurs when the mechanical stress the cap has to bear exceeds cap strength. The stress distribution in a plaque is determined by the loading conditions, the overall geometry of the plaque and the mechanical properties of the constituents of the plaque. Strength of the fibrous cap depends on cap thickness and the properties of the constituents. Both stresses in the cap and cap strength will vary locally. To gain more knowledge about vulnerable plaque rupture we have to learn more.
about stresses in the vulnerable plaque. How blood pressure translates to deformation of and stresses in the arterial wall and vulnerable plaque can be determined by application of the finite element method. Geometry and material properties need to be fed into a finite element program to determine deformation and stresses from its constitutive framework. Each constitutive framework and its associated set of material parameters requires detailed studies of the particular material of interest. Its reliability is strongly related to the quality and completeness of available experimental data, which may come from appropriate \textit{in vivo} tests or from \textit{in vitro} tests that mimic real loading conditions in a physiological environment. \textit{In vivo} tests seem to be preferable because the vessel is observed under real life conditions. However, \textit{in vivo} tests have major limitations because of, for example, the influence of hormones, nerve control and limitations in imaging resolution. Data sets from the complex material response of arterial walls can be measured in an \textit{in vitro} experiment without the \textit{in vivo} limitations. Recent developments in Magnetic Resonance Imaging (MRI) allow high-resolution imaging of various plaque components, including the thin fibrous cap, in human coronary arteries \textit{in vitro} [4]. However, if we want to image the arteries with sufficient resolution, imaging time increases to (approximately 10) minutes.

The mechanical behavior of coronary arteries depends on physical and chemical environmental factors. In \textit{in vitro} conditions the mechanical properties are altered due to biological degradation. Therefore \textit{in vitro}, arteries should be tested in environment that mimics the physiological conditions. But even in physiological conditions the mechanical properties of the coronary artery can change due to biological degradation or structural changes in the wall due to the loading conditions.

The mechanical properties of coronary arteries have been studied extensively. Since 1935 until now many researchers have investigated the properties of coronary arteries in animals like dogs and pigs [5], [6], [7], [8], [9], [10], [11], [12]. As experimental methods became more and more sophisticated, knowledge was gained in the coronary vessel mechanics and visualization modalities gave \textit{in vivo} measurement possibilities. Because of this, it became possible to compare the mechanical behavior of coronary arteries for human and animals \textit{in vitro} to each other and to the \textit{in vivo} situation. [13], [14], [15], [16], [17], [18], [19], [20], [21]. That showed that although, the compliance of porcine coronary arteries is approximately two to three times greater compared too human, the qualitative elastic behavior of porcine coronary arteries is similar to human. The extension and inflation test of straight artery tubes, is one of the most used mechanical tests to obtain the mechanical behavior of coronary arteries. This test is also useful to grasp the mechanical behavior and fracture mechanics of the vulnerable plaque, by inducing deformation and rupture of the vulnerable plaque. The effect of structural changes in the biological tissue, due to the \textit{in vitro} environment and mechanical testing, on the mechanical properties is poorly documented in literature and that effect is different in every setup. In MRI the visualization of a simple inflation test can take more than 2 hours. In 2 hours \textit{in vitro} testing it can be expected that the mechanical behavior changes, due to biological degradation or the testing protocol it self.
The goal of this study is to determine the influence of time and the loading protocol on the mechanical properties of the healthy coronary arteries. After a series of pilot experiments a MRI compatible setup for mechanical testing was developed. Secondly IVUS experiments were done to test the setup and the protocol for the MRI experiments. Thirdly, MRI experiments were done to visualize the mechanical behavior of the coronary artery over time. Finally, the experiments were used to fit a four-fiber model introduced by Baek [22], is used to interpret the experimental data.
Chapter 2

Composition and mechanical properties of coronary arteries

The mechanical behavior of the coronary arteries is determined by the different components in the arterial wall. That is why we need to know the composition and the mechanical properties of the components to understand the mechanical properties of the arteries. We will first discuss the composition and macroscopic behavior of coronary arteries. Next we will discuss the different components and their mechanical behavior. Finally we will summarize the consideration needed in in vitro testing of coronary arteries.

2.1 Composition coronary arteries

The heart is the organ that pumps blood through the entire body; the circulation is a simple but remarkable system. Only one pump that supplies the large skeletal muscles and at the same time delivers a delicate regulated blood flow to the organs. This is possible because there is a sophisticated infrastructure that controls and supplies the blood flow, the blood vessels. There is a huge variation in geometry and structure of the blood vessels, for example the diameter can vary from 3 cm to 10 µm. These differences make it possible to transport and control the blood to the most distant parts of the body. The structure of blood vessels also varies along the arterial tree. Arteries can be subdivided into several groups with descending diameter: elastic arteries (the aorta, brachiocephalic trunk and the carotid arteries), muscular arteries (all others, with diameter > 0.1mm) and arterioles (10-100 µm). Coronary arteries are muscular arteries; they are called muscular because the media of a muscular artery contains predominantly smooth muscle cells. The two main coronary arteries branch off from the aortic root, giving rise to the left and right main coronary artery (LMCA and RMCA). The LMCA branches off into the left anterior descending (LAD) and into the left circumflex artery (LCX) and together they supply the left ventricle with blood [23]. In the proximal part of the three main coronary arteries, the vulnerable plaque can be found most frequently [24].
Figure 2.1: Schematic representation of the heart and coronary arteries (left) and the left coronary artery (right).

The arterial wall consists of three layers which are called from inside out the intima, media and tunica adventitia (figure 2.2). These layers are composed of many microstructural components such as collagen, elastin, smooth muscle cells (SMC) and ground substances.

Figure 2.2: A schematic drawing of a muscular artery. The arterial wall consists of three layers, which are called from inside out the tunica intima, tunica media and tunica adventitia.
The intima is composed of the endothelial cells and the basal lamina (~80 nm thick). In young, healthy humans and pigs, the intima contributes negligibly to the mechanical properties of the coronary artery. Nevertheless the endothelial cells is a important sensing layer of the vessel wall through which a strong mechanical response of the SMC can be trigger, due to for example changes in shear stress. The media is made up of smooth muscle cells, elastic sheets, bundles of collagen fibrils, and a network of elastic fibrils. Its dividing line with the adventitia is a layer of elastin. Smooth muscle cells have a nearly circumferential orientation in the coronary artery [25] and, when activated, alters circumferential mechanical properties by constricting or dilating [26], [27]. Medial elastin helps to keep blood flowing by expanding with pressure, whereas medial collagen prevents excessive dilation [28], [29], [30]. The media makes up the greatest volume of the coronary artery and is responsible for most of its mechanical behavior. The adventitia consists of loose connective tissue containing collagen fibers, ground substances and some fibroblasts, macrophages, blood vessels (vaso vasorum), nerves [31]. The adventitia contributes to the mechanical properties mainly by tethering to the surrounding connective tissue [28]. The mechanical behavior of coronary artery, which is not completely surrounded by the myocardium, is barely influenced by the surrounding tissue [32]. Some investigators [33] consider the contribution of the adventitia to be signigicant due to the presence of the collagen fibers. The collagen fibers stiffen and reinforce the wall as they align, and so prevent the whole artery from overextension and rupture. However, the elastic modulus of adventitia is usually considered to be at least one order of magnitude lower than that of media and therefore contribution of adventitia to the overall behavior of the wall is smaller than of the media [33].

2.2 Macroscopic mechanical behavior of coronary arteries

The macroscopic mechanical properties of coronary arteries have been studied extensively [5-11, 13, 14, 19]. In 1935 Gregg et al. [8] did a study on coronary flow and measured the pressure-volume (P-V) relationship of the coronary arterial tree of dogs. They concluded that a linear pressure rise gave a non-lineare volume response (figure 2.3A). In 1970 Patel et al [9] determined the volume-pressure (V-P) relationship of segments of isolated left circumflex arteries of dogs. They found the hysteresis effect in the response of arteries (figure 2.3B). At the same time Douglas et al. [7] measured the dynamic P-V relation of dog coronary arteries between 70 and 120 mmHg (figure 2.3C). Douglas discovered that during pressurization the artery is also deforms in axial direction next to the obvious radial deformation of the lumen. The P-D relationship of excised coronary arteries from dogs and humans was measured by Gow et al. [5] and Gow and Hadfield [14], respectively. Gow predicted from his results that human coronary arteries have elastic properties similar to those shown for the dog. They concluded that it seems not unreasonable that human coronary arteries, like dogs coronaries, have a mean elastic modulus round $1.2 \times 10^6$ N/m² in the linear response region. In 1981, Tomoike and colleagues [11] also measured the P-D relationship of dog coronary arteries in situ using an ultrasonic dimension gauge with piezoelectric crystals (figure 2.3D). Tomoike showed this ultrasonic technique, which allows continuous measurement of the diameter of small
vessels, should provide accurate measurements of the diameter of small vessels, which provided a new tool for the study of coronary circulation.


More recently, in 2001, in the study of Kassab et al. [17] they have determined coronary surface area (CSA) response at different positions in the porcine coronary tree and the volume compliance of the porcine coronary arterial tree, using a video-densitometry technique. A cross-sectional area response curve from this study of Kassab is shown in figure 2.4A. In 2003 Andel et al. [20] quantified wall stretch the nonlinear mechanical behavior of the coronary artery in and beyond the physiologic range to compare human and porcine results. Andel showed that the elasticity of porcine coronary arteries is approximately 2 to 3 times higher than that of the human, but that the qualitative elastic behavior is similar. Two examples from this study are shown in figure 2.4B. Andel also investigated the influence of prestretch, which was later in 2008 quantified by Van Den Broek et al. [34] at 1.4 +/- 0.05.
From an engineering perspective the pressure response of a coronary artery can be expressed in terms of compliance, distensibility, stiffness or elastic modulus. Compliance is defined as the change in luminal dimension (CSA) divided by the corresponding change in pressure; stiffness is the reciprocal of compliance and distensibility is a normalized compliance. Compliance can be measured under static or dynamic loading; the latter is referred to as the dynamic compliance or capacitance. A selection of the previous mentioned work on coronary elasticity is summarized table 2.1:
<table>
<thead>
<tr>
<th>Species</th>
<th>Diameter [mm]</th>
<th>Distensibility [mmHg⁻¹ 10⁻³]</th>
<th>Pressure [mmHg]</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>4.9 +/- 0.3</td>
<td>2.2 +/- 0.53</td>
<td>70 to 110</td>
<td>In vitro, caliper</td>
<td>[12]</td>
</tr>
<tr>
<td>Dog</td>
<td>3.6</td>
<td>0.69</td>
<td>60 to 140</td>
<td>In vivo, ultrasonic</td>
<td>[10]</td>
</tr>
<tr>
<td>Dog</td>
<td>3.1</td>
<td>0.68</td>
<td>60 to 140</td>
<td>In vitro, caliper</td>
<td>[12]</td>
</tr>
<tr>
<td>Dog</td>
<td>2.6</td>
<td>0.77</td>
<td>107 to 135</td>
<td>In vitro, microscopy</td>
<td>[21]</td>
</tr>
<tr>
<td>Pig</td>
<td>2.6 +/- 0.34</td>
<td>0.68 +/- 0.21</td>
<td>60 to 140</td>
<td>In situ, angiography</td>
<td>[17]</td>
</tr>
<tr>
<td>Pig</td>
<td>1.3 +/- 0.24</td>
<td>1.2 +/- 0.39</td>
<td>60 to 140</td>
<td>In situ, angiography</td>
<td>[17]</td>
</tr>
<tr>
<td>Pig</td>
<td>0.79 +/- 0.20</td>
<td>1.6 +/- 0.73</td>
<td>60 to 140</td>
<td>In situ, angiography</td>
<td>[17]</td>
</tr>
<tr>
<td>Pig</td>
<td>3.44 +/- 0.39</td>
<td>2.1</td>
<td>0 to 300</td>
<td>In vitro, lasermicrometer</td>
<td>[17, 20]</td>
</tr>
<tr>
<td>Human</td>
<td>3.54 +/- 0.51</td>
<td>1.2</td>
<td>0 to 200</td>
<td>In vitro, lasermicrometer</td>
<td>[20]</td>
</tr>
</tbody>
</table>

Table 2.1: Selection of diameter distensibility data from literature

We see in figure 2.4 that it is impossible to quantify non-linear behavior of an artery with one parameter and that the found parameters vary over on LAD [17]. Biological tissue is complex anisotropic material, which has a non-linear pressure response and needs more sophisticated mechanics to describe its behavior. We can translate pressure response curves to stress-strain curves. Healthy coronary arteries are highly deformable composite structures and show a nonlinear stress–strain relationship with a typical exponential stiffening effect at higher pressures, as illustrated in figure 2.5 [35]. The cyclic loading and unloading, associated with stress softening effects, lead to a conditioned material which behaves (perfectly) elastically or viscoelastically (nearly repeatable cyclic behavior) – point I. Loading beyond the (visco)elastic domain up to point II leads to inelastic deformations. The thick solid line indicates the (approximate) engineering response of the material. This stiffening effect, is based on the recruitment of the embedded (load carrying) wavy collagen fibrils, which leads to the characteristic non-linear mechanical behavior of arteries; see the classical work of Roach et al. [36]. Loading beyond the (visco)elastic domain the deformation process in an arterial layer is associated with inelastic effects (elastoplastic and/or damage mechanisms) leading to significant changes in the mechanical behavior [35]. This overstretching involves dissipation, which is represented by the area between the loading and unloading curves and results in strain remaining in unloaded situation.
Due to all this research it is well known that blood vessels exhibit viscoelastic properties such as creep, relaxation, and hysteresis. Fung gives a more complete and detailed overview of the mechanical properties of arteries in his book [31]. This complex mechanical behavior of coronary arteries is derived from its microstructural components: collagen, elastin, smooth muscle cells and ground substances. To get more insight in the mechanical behavior of coronary arteries the material properties of the microstructural components must be known.

2.3 Microstructural components and their mechanical behavior

Mechanical behavior of the coronary artery wall stems not only from intrinsic mechanical properties of microstructural components, but is also dependent on how the microstructural components build up the coronary arterial wall. Orientation of the components and the interplay between the different components are important for the resulting mechanical behavior of the coronary arterial wall. A convenient way to describe the intrinsic mechanical properties of the components we use a number of functional attributes to be able to quantify the associated material properties, (table 2.2 and 2.3).
Table 2.2: Functional attributes that can be assigned to structural materials and the associated material properties and units that can be used to quantify these attributes.

These microstructural components in the arterial wall have different intrinsic mechanical properties (table 2.3).

Table 2.3: quantified attributes of the components from literature

Elastin
Elastin is primarily composed of four amino acids: glycine, valine, alanine, and proline. It is a specialized protein with a molecular weight of 64 to 66 kDa, and an irregular or random coil conformation made up of 830 amino acids (figure 2.6A). The flexible random coil molecules can easily change their shape, or conformation, when stretched. The molecules are randomly distributed in a layer in the vessel wall (figure 2.6B).
Elastin is a specialized protein with a molecular weight with an irregular or random coil conformation. Elastin is molecules are randomly organize.

The shape and orientation of the elastin molecules makes elastin a rubber-like protein with low stiffness and high extensibility. The elastin shows reversible deformation with very high resilience. In addition elastin is reaching maximal extensions in excess of 100%, with a very low modulus of elasticity [40]. Elastin is a major component of arteries, where its stretchiness and ability to store strain energy allow arteries to smooth the pulsatile flow of blood from the heart. This lowers peak blood pressure and the mechanical work of the heart and maintaining a relatively steady flow of blood through tissues. The elastic properties are strongly affected by strain rate in a mechanical test. In addition, because conformational change in elastic proteins occurs only in hydrated proteins, elastic properties can also be strongly affected by hydration level. Conformational changes are driven largely by thermal agitation, thus the properties are also influenced by temperature. Elastin is an unusual protein in that it not replaced during the lifetime of an animal [41],[42]. That is, elastin synthesized during development remains in place through the full life span of the organism. Thus, elastin must be an extremely durable material.

Collagen
Collagen molecules form fibrils, collagen fibrils found in arteries are 54–65 nm in length and have a diameter range from 16 to 500 nm. Collagen fibrils pack together to form collagen fibers, (figure 2.7B). Collagen fibers, can hardly be described as stretchy, since their extensibility, $\varepsilon_{\text{max}}$, is only 0.13. Neither is collagen soft, since its modulus is approximately 1000 times greater than that of elastin. It is also much stronger and somewhat tougher than elastin. The collagen provides a network of wavy, reinforcing fibers that become aligned in the direction of stretch (figure 2.7A). At low strains the response is low, but as extension proceeds it rises gradually and becomes constant when the collagen fibers become aligned and then finally stretched. When aligned the collagen fibers are engaged in load bearing, this network limits tissue deformation and prevents the rupture of the artery. This finding was first reported by Roach et al. [36], who used
trypsin and formic acid to digest collagen and elastin, respectively, out of blood vessels. These findings have been confirmed by Zoumi et al. [18], in intact vessels.

**Figure 2.7A:** At low strains the response is low, but as extension proceeds it rises gradually and becomes constant when the collagen fibers become aligned and then finally stretched. Adapted from presentation N. Stergiopulos 2008. 

B: Collagen fibers in aligned orientation.

The study for Zulliger et al. [43] indicates that the changes in vessel biomechanics with progressing age are not to be sought in the elastic constants of elastin and collagen or their volume fractions of the vessel wall but in alterations of the collagen mesh arrangement and the waviness of the collagen fibers. In old subjects, the collagen fiber ensemble engages in load bearing much more abruptly than in young subjects. Reasons for this change in collagen fiber dynamics may include fiber waviness remodeling or cross-linkage of fibers.

**Smooth Muscle Cells**

Smooth muscle cells have one central nucleus, and are anatomically discrete, but they must contract synchronously to function optimally. A variety of junctions between cells coordinate communication and force transmission. Contraction of smooth muscle is based on a sliding filament/crossbridge mechanism, as in skeletal muscle, although the thick (myosin) and thin (actin) filaments of smooth muscle are not organized into sacromeres. Smooth muscle cells are controlled by various systems, including autonomic nerves (both excitatory and inhibitory, involving a large number of neurotransmitters), circulating hormones, locally generated hormones or metabolites from associated cell types and electrical or chemical signals coupling cells via gap junction. Ca\(^{2+}\) regulates contraction in smooth muscle by binding to calmodulin, followed by the formation of an active myosin kinase-calmodulin-Ca\(^{2+}\) complex. Activated myosin kinase uses ATP to phosphorylate crossbridges, which enables the crossbridges to attach to the thin filament and cycle. Dephosphorylation of attached crossbridges by myosin phosphatase slows their detachment rate, reducing crossbridge cycling rates and ATP consumption in
sustained contractions. Relaxation is caused by lowering of cell Ca\(^{2+}\) to levels that inactivate myosin kinase and thus lead to cessation of myosin phosphorylation [44]. It is widely accepted that smooth muscle cells are oriented in a helical pattern in coronary artery walls with predominantly circumferential orientation. Contraction therefore occurs largely in this direction [45]. Active stresses due to smooth muscle contraction that have been reported in the literature are in the range of 0.10–0.35 MPa [46], [47]. Thus excitation of arterial smooth muscle can completely close the coronary artery [48]. This active character of the vascular smooth muscle cells make it possible to control the total peripheral resistance, arterial and venous muscle tone, and thus the distribution of blood flow throughout the body. Figure 2.8A gives a clear visualization how the arterial pressure diameter response can change due to the contribution of the vascular smooth muscle cell activation [49]. Additionally it visualizes where in the response curve the different microstructural components contribute the most to the total response of the artery.

![Figure 2.8A](image)

**Figure 2.8A:** Description of arterial pressure-diameter relations, arterial response to an inflation test, with different the smooth muscle tones: blue (contracted), green (normal) and orange (relaxed). B: Vascular smooth muscle cells.

### 2.4 *In vitro* experimental considerations

In this study, we will investigate the mechanical behavior of the porcine coronary arteries *in vitro*. From the above, it is clear that removing the coronary from the *in vivo* environment can influence the material properties. In this section we will discuss these different aspects that influence the behavior *in vitro*.

**Axial pre-stretch**

Axial pre-stretch is a factor that influences the elastic behavior of the coronary artery. *In vivo*, the change in vessel length in the cardio vascular cycle is negligible compared to the
pulsation of the diameter. *In vivo*, the length is constrained by vessel branches and surrounding tissue and the vessel is stretched longitudinally [39]. Due to this axial stretch, a coronary artery will undergo a longitudinal retraction when it is excised from its surroundings, this is the unstrained *ex vivo* state. The longitudinal stretch is known to be a major factor that affects the vessel elasticity in vitro [34] [15]. Thus it is important to do in vitro experiments at a physiological pre-stretch. Chantal van den Broek showed in an *in vitro* study, that the physiological pre-stretch can be defined as the strain of an artery at which the axial force is relatively insensitive to the pressure change inside the artery [34]. The physiological pre-stretch of the porcine LAD is 1.4. Thus the physiological pre-stretch, which is applied in this study, is 1.4 times the length of an excised and retracted LAD segment.

**Vascular Smooth Muscle tone**

The impact of SMC tone on the elastic behavior of the coronary artery is substantial: if we add a powerful vasoconstrictor in vivo, the contracting SMC’s can cause the coronary artery to contract completely. In this case stresses in the wall exceed those induced by pressure. The smooth muscles in the coronary arteries are controlled by various systems; this is why *in vivo* the smooth muscle tone is ever changing. Until now it is impossible to simulate the *in vivo* muscle tone in an *in vitro* experiment. This makes *in vitro* elasticity studies of muscular arteries a tough job, because the elasticity of muscular arteries is very dependent on the smooth muscle tone [48],[46]. Various pharmacological substances can be used to modify SMC tone *in vitro*. These substances include the powerful but short working papaverine [50], the longer working calcium blocker amlodipine [50], and the growth factor endothelin [51]. In this study we choose to not use any SMC activity influencing substances to since we had no experience in controlling the dynamic concentration of in the buffer.

**Curved geometry of the coronary artery**

Although it is not the most important factor, it does determine the fact that we use the LAD. Anatomically, the coronary arteries originate from the aortic ostia, just above the aortic valve, and continue along the surface of the heart (figure 2.1). So *in vivo* the coronary artery has a curved geometry. In the setup, the coronary artery is straightened due to the applied axial pre-stretch. This straightening influences the mechanical behavior on the sites where, *in vivo*, the coronary artery was curved. So in this study measurements will only be done on segments of the coronary artery that were as straight as possible *in vivo*.

**Perivascular support from the surroundings tissue**

*In vivo* the LAD is partly embedded by the myocardium. Close to the aortic valve the LAD lies on top of the myocardium in a fatty like tissue, the more proximal the more embedded the LAD gets. The support the LAD gets from its surrounding tissue (fatty tissue and myocardium) is called the perivascular support of the LAD. Nevertheless majority of mechanical measurements are made on vessels after the surrounding tissue are dissected away [13], [52], [14], [5], [53], [54],[15], neglecting the influence of the surrounding tissue. In the experiments of Hamza *et al.* [55] the influence of surrounding tissue on the vasodilated left anterior descending (LAD) coronary artery was quantified.
The intravascular pressure was varied in a triangular pattern, whereas the absolute cross-sectional area of each vessel and the total arterial volume were calculated using video densitometry under different intra luminal pressures. In the range of the positive pressures (0, 50, 100 and 150 mmHg) they found that the compliance of the proximal LAD artery \textit{in vivo} \((4.85 \pm 3.8 \times 10^{-3} \text{ mm}^2/\text{mmHg})\) is smaller than that of the same artery \textit{in vitro} \((16.5 \pm 6 \times 10^{-3} \text{ mm}^2/\text{mmHg}; \ P = 0.009)\). Hence the myocardium restricts the compliance of the epicardial artery under distension. This conclusion is supported by the study of Tajaddini \textit{et al.} \cite{16} where they compare \textit{in vivo} to \textit{in vitro} IVUS measurements to obtain the mechanical properties of the porcine LAD. In a recent study of Lui \textit{et al.} \cite{32} a finite element model was used to study the effects of myocardial constraint on the passive mechanical behavior of the LAD vessel wall. The results showed that the myocardial constraint is a major factor that affects vessel elasticity and wall strain. The elasticity and wall strain of partially embedded vessels are found similar to the free vessel, with higher local circumferential stretch. Reduced vessel elasticity, along with experimental observations \cite{55}, \cite{56}, emphasize the importance of myocardial constraint in coronary wall mechanics. Furthermore, suggests that the pressure-radius relation of large coronary arteries which are partially embedded can be approximated as free of myocardial constraint. Thus in this study we choose to use the part of the LAD that is partly embedded in the myocardium, so we can approximate the coronary artery as free of myocardial constraint.

\textbf{Osmotic pressure}

The mechanical behavior of coronary arteries depends on physical and chemical environmental factors, such as osmotic pressure, pH, partial pressure of carbondioxide and oxygen, ionic concentrations and monosaccharide concentration. All previous mentioned factors are stable and controlled in a Krebs buffer. The buffer is aerated with Carbogen \((95\% \text{ O}_2 + 5\% \text{ CO}_2)\) to hold the pH to 7.4

\textbf{Temperature}

The elastic behavior of coronary arteries is dependent on the temperature of the tissue and the surrounding medium. In the study of Guinea \textit{et al.} \cite{57} the thermo-mechanical behavior of human carotid arteries in the passive state is studied. The results show that the change of temperature and stress has an effect on the dilatation coefficient of the arterial wall. The stiffness of the arterial wall does not change in the range of temperatures tested \((17, 27, 37 \text{ and } 42^\circ \text{C})\). This indicates that it is necessary to do the \textit{in vitro} experiments on \textit{in vivo} temperatures \(39^\circ \text{C}, \text{ (porcine body temperature} \cite{58})\). In the study of Venkatasubramanian \textit{et al.} \cite{59}, the effects of freezing and cryopreservation on the mechanical properties of arteries are investigated. Their results suggest that freezing does have an effect on stress-strain properties, particularly in the low stress region corresponding to physiological conditions. Therefore fresh porcine coronary arteries are used in this study.

\textbf{Preconditioning}

Fung’s way of preconditioning is the most used and accepted in, \textit{in vitro} biomechanical experiments \cite{31}. After an artery is excised and installed into a testing machine to be tested with a load-elongation protocol. First cyclic loading and unloading at a constant
rate of elongation is applied. In the first three cycles, the stress-strain curves are seen to shift to the right, with an increase in strain. If the test is repeated indefinitely, the difference between successive cycles is decreased, and eventually disappears. Then the specimen is said to have been preconditioned. The reason that preconditioning occurs in a specimen is that the internal structure of the tissue changes with every cycle. By repeated cycles, eventually a steady state is reached at which no further change will occur unless the cycles are changed [31]. Preconditioning gives a reproducible but not necessarily physiological behavior. In this study we precondition before every pressure loop.

To create an environment in which we can create reproducible results of the mechanical behavior of the coronary artery, we need to take into account all the aforementioned factors. Additionally we the choose the all these factors as close to physiological values as possible to make the in vitro experimental results comparable to in vivo situations. Thus we used of the proximal part of the LAD as fresh as possible. Applied a physiological pre-stretch and did not use muscle tone inducing agents. We had a stable buffer temperature and applied preconditioning before every pressure loop.
Chapter 3

Intravascular Ultrasound experiments

3.1 Introduction

The first set of experiments involves intravascular ultrasound (IVUS) measurements of the deformation of the porcine coronary vessel wall induced by intraluminal pressure changes. The experiments are a pilot to quantify the mechanical behavior of the coronary segment in the setup during the loading protocol over 24 hours. This pilot will demonstrate the feasibility of the setup and the protocol. The results of this pilot will not be compared to literature but only to the MRI results.

3.2 Methods

In the methods section we will first discuss the design of the IVUS experiments, we will look into the design of the experimental setup, discuss the loading protocol and walk through the preparation process of the coronary arteries. This will be followed by a section in which we explain of the imaging technique that visualizes the deformed LAD during the pressure induced deformation. Finally the analysis of the experimental data will be discussed.

3.2.1 Design of IVUS experiment

*Experimental setup*

The setup is designed to visualize the wall and lumen of the LAD segment with IVUS and MRI, during deformation induced by intraluminal pressure changes. The design fulfills all the mechanical and physical aspects stated in chapter 2. The setup is shown in figure 3.1.
Figure 3.1: Schematic drawing of the setup. The LAD is pre-stretched and pressurized in a 39 °C Krebs buffer.

The cannulated LAD segment was installed into the setup. The proximal part of the LAD was connected to the piston and the distal part to the tissue bath. The position of the piston is adjustable, which gave the possibility to precisely (0.1 mm) apply an axial prestretch of 1.4 on the LAD segment. The tissue bath contained 6.3 ml Krebs buffer (115 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 1.2 mM NaH₂PO₄, 1.2 mM Na₂SO₄, 2.5 mM CaCl₂, 25 mM NaHCO₃, 10 mM glucose). The pump (Micropump, Watson-marlow, US) delivered a constant flow from the heated reservoir (MGW Lauda M3, US), through the tissue bath back to the reservoir. At a flow rate of approximately 30 [ml/min] the temperature in the setup remained stable at 39±0.5 °C. In the heated reservoir the buffer is heated to 46 °C and aerated with Carbogen (95% O₂ + 5% CO₂) to hold the pH at 7.4. To be able to keep the temperature at the appropriate values, a number of experiments were done (Appendix A). A water colon was used to apply an intraluminal pressure between 0 – 160 mmHg. The extraluminal pressure was dependent on the flow of the buffer and was during the IVUS experiments 3 mmHg. The IVUS catheter (Atlantis SR Pro 40Mhz Coronary Imaging Catheter), connected to the IVUS system (Galaxy 2 system), was introduced in the lumen and positioned at point where the catheter images a circular part of the lumen. The catheter was fixed at this position by the hemostasis valve.

Loading protocol

In future experiments, the deformation of the LAD and the plaques therein needs to be imaged with high resolution. The loading protocol is designed to image the LAD segment in every pressure step with MRI to for 10 minutes to reach this high resolution. The protocol is also useful to induce the vulnerable plaque rupture.

After the LAD segment was installed, 20 preconditioning cycles between 80 and 120 mmHg were applied. Every precondition cycle was completed within approximately 5
seconds. The preconditioned LAD segment was loaded with a static pressure of 40 mmHg for 8 minutes and the response was measured after 2, 4 and 6 minutes. Every 8 minutes ($\Delta T$) the pressure was increased with 20 mmHg ($\Delta P$) and the response was measured, until 160 mmHg was reached. At 160 mmHg the maximum pressure was reached and from there the pressure was decreased with the same $\Delta T$ and $\Delta P$ and measurement interval, until 40 mmHg was reached. This is the end of the first pressure loop. The pressure loop including the preconditioning was repeated twice with a different time interval over the next 24 hours. The interval between the pressure loops was varied to make it possible to discriminate between the influence of the time between the pressure loops and the influence of the pressure loop itself. In loading protocol 1 the first and second loops follow directly after each other and the second and third loop have 14 hours in between. In loading protocol 2 the first and second loop have 14 hours in between and the second and third follow directly after each other. An overview of these loading protocols is shown in figure 3.2. During the entire experiment the LAD segment stayed pre-stretched and submerged in buffer with the IVUS catheter at a fixed position.

Figure 3.2: Top: The loading protocol of one pressure loop. Middle: Loading protocol 1. Bottom: Loading protocol 2.
Preparation of coronary arteries

In the present study, porcine hearts (age range 3-5 months) were harvested during the slaughter process in the abattoir (Westfort v.o.f) within 30 minutes from death. Until excision, the hearts were stored in a kreb’s buffer at 4°C. Within 5 hours from death the LAD segments were excised, 5 to 10 mm distal to the ostium of the left coronary arteries, right after the first curve and side branch, as shown in figure 3.3. The LAD segments were 30 to 50 mm long with an inner diameter of 2 to 4 mm. This part of the LAD is quite straight and has usually 3 to 5 side branches, which were ligated with surgical suture. Both ends of the LAD were connected to cannules to install the LAD in the testing setup. The details of the excision procedure have been described in Appendix C. The LAD segment was then stored in kreb’s buffer at 4°C until the installation in the setup and all tests were performed within 36 hours from death.

To obtain the test specimen we collected eight hearts from the abattoir. From the eight hearts seven LAD segments were successfully installed in the setup and one ruptured during the application of the prestretch. One experiment had to be aborted, air inside the lumen of the LAD segment made it impossible to visualize the LAD segment. Two experiments had to be aborted due to failure of the IVUS catheter. The remaining four LAD segments were visualized during the complete loading protocol. Results from the second and third experiment had a discontinues character and were useless due to this unrealistic CSA response (Appendix B). Hence only two experiments could be analyzed, the first and fourth experiment.
3.2.2 Imaging procedure

To image the lumen of the LAD segment in the setup we used IVUS, a technique used in the clinic to visualize a cross-section of the lumen of an artery real time. In this section we will explain the basic fundamentals of IVUS and discuss the settings we used to visualize the LAD in this study.

**Intravascular Ultrasound**

The IVUS system employs low-level acoustic energy to image vascular structures [60]. The transducer is a single piezo-electrical element. When transmitting, it converts the electrical energy that is applied to excite the transducer into acoustic energy. When receiving, it converts reflected acoustic energy to electrical energy, which is later used to determine the grayscale intensities within the image. The transducer emits a narrow beam of acoustic energy from one point on the side of the transducer. Because IVUS requires a full 360 degree scan of the interior of the vessel, the transducer must be rotated through a full circle in order to transmit and receive acoustic energy at all points within a cross-section of that vessel. The rotating core is enclosed in a flexible housing, similar in principal to the outer housing on a bicycle brake cable. The core, and therefore the transducer, is typically rotated at about thirty rotations per second in order to develop an image. When the imaging catheter travels through extreme curves, or the hemostasis valve is too tight, rotation of the core is impeded, and in some cases, results in a smearing effect known as N.U.R.D (Non-Uniform Rotational Displacement). The image quality of the IVUS images can be described by two important factors; spatial resolution and contrast resolution. The spatial resolution can be divided into axial resolution (parallel to the beam and depends on the frequency) and the lateral resolution (perpendicular to the beam and depends on the transducer size and focusing system). The lateral resolution closer to the catheter is better than further away. If the catheter is positioned concentrically in the vessel and there are no substantial asymmetries, the morphologic structures in the image are well visible due to the high axial resolution. But as soon as the catheter is positioned non-concentrically in the vessel, the image quality decreases due to the poor lateral resolution.

**Instruments and settings for experiment**

Usually, 20- or 30-MHz IVUS catheters are suitable for vascular procedures in large peripheral (non coronary) vessels because they have a larger axial scan area than 40-MHz catheters. However, we used the 40-MHz catheter that can generate more detailed images of the vessel wall anatomy. For a 40 MHz transducer, the typical resolution is 80 microns axially and 250 microns laterally. A test phantom was imaged to quantify the effect of N.U.R.D., non-concentrically placement of the catheter and to check the calibration. The results from the phantom test showed that the calibration was correct and that neither N.U.R.D. nor non-concentrically placement of the catheter blurred the images (figure 3.4 and Appendix B). Thus the 40-Mhz IVUS catheter proved to be a good tool to visualize the coronary surface area change, during the loading protocol, and to analyze the elastic behavior of the LAD.
3.2.3 Data analysis

*Image analysis*

The LAD segment consists of several structures: lumen, intima, media, and adventitia. The lumen is identified by the region inside the interface between blood and intima. It is typically a dark, relative echo-free region adjacent to the catheter. The intima itself is a thin layer and cannot be imaged in healthy coronary arteries. An echo-lucent layer, enclosed by the internal and external elastic laminae, identifies the media. Due to the acoustic impedance mismatch, these layers can produce typical bright-dark-bright patterns. The adventitia is composed of loose collagen and elastic tissue that merges with the surrounding peri-adventitial tissue, and cannot be identified separately [61]. In IVUS measurements only two layers are normally distinguished: the lumen border represented by the leading edge of the lumen-intima interface, and the vessel border represented by the leading edge of the media-adventitia interface. Figure 3.4a shows a typical example of IVUS image from one of the experiments. The circular structure in the middle of the image is the catheter. The lumen is the dark, relative echo-free region adjacent to the catheter. The larger circular structure is the vessel wall. An echo lucent layer, enclosed by the internal and external elastic laminae, identifies the media. One can clearly see the lumen but the media-adventitia interface cannot be identified reliably. Thus it was possible to measure the lumen area of the LAD during the loading protocol, but not the wall thickness.

![Figure 3.4: A: IVUS image of the LAD segment from one of the experiments. B: The yellow line encloses the coronary surface area.](image)

Images were acquired on the Galaxy 2 system and stored on a CD. The DICOM images were imported in ImageJ, and the lumen contours manually drawn on the lumen-intima interface, (figure 3.5B). In every pressure step we did 3 measurements and in every measurement the lumen contour was drawn 3 times. So for every pressure step we have 9 contours, for every pressure loop 117 contours and thus in every experiment 351 contours. The coronary surface area (CSA) was computed in ImageJ, and subsequent analysis performed in Matlab.
Derived quantities
The lumen of the LAD is assumed to be circular during entire experiment. This makes it possible to calculate the inner radius of the LAD segment. The inner radii ($r_i$) at different pressures can by calculated with equation 3.1,

$$r_i = \sqrt{\frac{CSA}{\pi}}$$  \hspace{1cm} (3.1)

The elastic response of the coronary vessels can be expressed in terms of compliance or distensibility. Compliance is defined as the change in luminal dimension (diameter, CSA) divided by the corresponding change in pressure. Distensibility ($D$) is a normalized compliance and can be calculated with equation 3.2,

$$D = \frac{\left( \frac{\Delta d}{d_o} \right)}{\Delta P}$$  \hspace{1cm} (3.2)

where d the diameter, $d_o$ the diameter at 100 mmHg and $P$ is the pressure. With the CSA response curves and the distensibility we can visualize and characterize the behavior of the LAD.

3.3 Results

In this section the results from the first and fourth experiment will be discussed. The results will be expressed in pressure-CSA curves to analyze the response. Distensibility will be calculated from the data to make it possible to quantitatively compare the results. First we will look into the first experiment and compare the response of the first loop the consecutive loops. Then the results of the first experiment will be compared to the fourth experiment.

First loop
From the experiments we obtained CSA and pressure data at each pressure step in the loading protocol. The measured CSA induced by pressure gave us the possibility to visualize the response of the LAD as pressure-CSA graphs (figure 3.5).
Figure 3.5: The blue curve visualizes the CSA response induced by intra luminal pressure change (dotted loading, continues unloading). The four IVUS images in the graph are images of the LAD lumen and wall at 40, 80, 120 and 160 mmHg.

Taking a closer look at the response of the first loop (figure 3.5) we see a typical behavior. Increase of the pressure induces a non-linear increase of the CSA. The unloading response is also non-linear and similar to the loading curve, but follows a different path from the loading response. The difference between the loading and unloading response increases at lower pressures. The CSA at 40 mmHg at the end of the first pressure loop is 10% larger than the CSA at 40 mmHg at the start of the pressure loop. The non-linear response of the CSA seems to indicate that the LAD gets stiffer at higher pressures.

Consecutive loops
The first experiment is a result of protocol 1, (figure 3.2). The response curves of all three pressure loops of the first experiment are visualized in together in figure 3.6A. The responses to the second and third pressure loops are similar to the response to the first pressure loop. In the second and third pressure loop the end CSA at 40 mmHg is 7% and 3 % larger than the start CSA at 40 mmHg. The end-CSA at 40 mmHg of a pressure loop is similar the start-CSA at 40 mmHg of the next pressure loop. The loading response of the following pressure loop is at the lower pressures the same as the previous unloading response but becomes larger at higher pressures. Thus response at 160 mmHg increases with every pressure loop. The global trend of the distensibility is downward, but in the high-pressure ranges we see a rise of the distensibility (figure 3.6B). Thus it seems that the artery becomes stiffer with every pressure loop.
Figure 3.6: A: Response curves of the first experiment. The dotted lines are the loading responses; the continuous lines are the unloading responses. B: Distensibility of the loading curves. The blue, red and green represent the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} pressure loop.

Comparison experiments
The response curves of the fourth experiment are a result of protocol 2 (figure 3.7A). Upon visual inspection the first and fourth experiment look similar. Qualitative responses are the same in both experiments but quantitative results are different.
Figure 3.7: 

A: Response curves of the fourth experiment. The dotted lines are the loading responses; the continuous lines are the unloading responses. 

B: Distensibility of the loading curves. The blue, red and green represent the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} pressure loop.

The comparison between the start and end CSA within one pressure loop at 40 mmHg and CSA of the first and third loop at 160 mmHg. The differences within de loop are expressed as a percentage of the start CSA at 40 mmHg of that loop. The different responses of the loops at 160 mmHg are expressed as a percentage of the CSA of the first loop at 160 mmHg. In both experiments we see the same trend in relative change of response at 40 and 106 mmHg. With every loop the response is increasing at 40 mmHg and that this increase is decreasing with every loop. The response at 160 mmHg is also increasing with every loop and this increase is increasing with every loop, (table 3.1).

<table>
<thead>
<tr>
<th>Loop</th>
<th>1\textsuperscript{st} experiment 40 mmHg</th>
<th>4\textsuperscript{th} experiment 40 mmHg</th>
<th>1\textsuperscript{st} experiment 160 mmHg</th>
<th>4\textsuperscript{th} experiment 160 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10%</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7%</td>
<td>3%</td>
<td>5%</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>3%</td>
<td>3%</td>
<td>10%</td>
<td>4%</td>
</tr>
</tbody>
</table>

*Table 3.1: Differences in CSA within the loops at 40 mmHg and difference between first and third loop at 160 mmHg*

Comparing the distensibility found in the two experiments we see that the trend in distensibility change over the loops is similar, (table 3.2). The mean distensibility over
the pressure range 60 to 160 mmHg of the three pressure loops of the first and fourth experiment is $0.74 \times 10^{-3}$ and $0.84 \times 10^{-3}$ [1/mmHg]. The mean distensibility of the second and third loop are similar and lower than the first in both experiments (table 3.2).

<table>
<thead>
<tr>
<th>Loop</th>
<th>First experiment: Distensibility [1/mmHg]</th>
<th>Fourth experiment: Distensibility [1/mmHg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$0.92 \times 10^{-3}$</td>
<td>$0.96 \times 10^{-3}$</td>
</tr>
<tr>
<td>2</td>
<td>$0.69 \times 10^{-3}$</td>
<td>$0.78 \times 10^{-3}$</td>
</tr>
<tr>
<td>3</td>
<td>$0.68 \times 10^{-3}$</td>
<td>$0.78 \times 10^{-3}$</td>
</tr>
<tr>
<td>Mean</td>
<td>$0.76 \times 10^{-3}$</td>
<td>$0.84 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Table 3.2: Mean distensibility over P: 60-160 mmHg of pressure loops.

The start-CSA at the beginning of the fourth experiment is smaller than in the first experiment (8.75 vs. 9.75 mm$^2$). In the first experiment the differences between the loading and unloading curve becomes larger at lower pressures. In the fourth experiment this is effect is only visible in the first pressure loop. At higher pressure the distensibility shows unexpected increase in the first experiment that is much less dominant in the fourth experiment.

**Accuracy IVUS measurement**

To evaluate the accuracy of the experimental technique the standard deviation of the CSA measurements are calculated in each pressure step. The average standard deviation is lower than +/- 0.10 mm$^2$ and the maximum in the entire experiment is +/- 0.18 mm$^2$. Since the surface change in a pressure loop is minimal 2 mm$^2$, the standard deviation is on average equal to 5% of the measured diameter change in the experiment. Thus the experimental measurement method is accurate enough to measure the diameter change.

In every pressure step we measure the CSA of the LAD segment the 2$^{ed}$, 4$^{th}$ and 6$^{th}$ minute, to determine whether the CSA was growing during the static pressure step. The slope of the curve-fit through the three measurements in every pressure step was calculated. The mean slope in every loading and unloading ramp was calculated from the slope in every pressure step, (figure 3.8). There is a small up going trend in all up loading ramps (0.02, 0.01 and 0.04 [mm$^2$/min]), and a small down going trend in two of the three the unloading ramps (-0.03, -0.02 and 0.01 [mm$^2$/min]). The average slope in a loading step is approximately 0.02 [mm$^2$/min] and in an unloading step is approximately -0.01 [mm$^2$/min].
3.4 Discussion & Conclusion

We can conclude from the experimental results that the loading responses were different from the unloading responses in every loop. The response at the end of each loop at 40 mmHg was always higher than the start responses at 40 mmHg. The following pressure loop started with approximately the same CSA as the previous pressure loop ended, independent of the time between the pressure loops. The loading curve of the following pressure loop follows the previous unloading curve and becomes larger in the higher-pressure ranges. Therefore the elastic behavior of the coronary artery was different in every repeated pressure loop. Qualitatively results for the two different loading protocols are similar.

If we try to explain the changing behavior of the coronary artery in the IVUS experiment we could reason from the micro structural components. Davis et al.[41] and Shaprio et al.[42] showed that elastin is an extremely durable material. Thus it is not expected that during the experiment the mechanical properties of elastin change. Consequently it can be expected that elastin is not causing the changing diameter response. Sorop et al.[50] showed that the remodeling of the wavy collagen network takes more than 36 hours. However, it is reasonable to assume that the creep process may occur within the fibers themselves, or possibly at their surface connections to the matrix. The changing diameter response looks very similar to preconditioning effect described by Fung et al.[12] The specimens are preconditioned between 80-120 mmHg, this sets the SMC’s in the similar activation state as in vivo. It can be expected that the static pressure steps, in one pressure loop are changing the activation state of the SMC’s. This change of the activation state of SMC’s can cause the difference in start and end-CSA plus the growing diameter at 160 mmHg. It could explain why the differences become smaller every pressure loop as the specimen gets more conditioned to the protocol with every pressure loop. That a change in activation of the SMC is causing the changing behavior is supported by the fact that the CSA response is not chancing over time when it is left in the setup between the pressure loops. Since arteries are very complex structures with a behavior that results
from an interplay between many factors, it is very though to address the cause of the changing behavior that we observe in the experiments. Nevertheless it seems possible that the state of activation of the SMC’s or creep processes can cause this typical change of CSA response in the three pressure loops.

The results show that it was possible to visualize the in vitro behavior of the coronary artery in controlled physiological conditions with IVUS in this setup. The similarities between the first and fourth experiment show that the setup can generate reproducible results. In this study the rate of failure of the experiments was high, this is because the IVUS catheter was moving. The movements are caused due to manual pressurization via the water colon. Since a pressure pump will be applied in the future experiments, we expect that the failure rate will decrease dramatically.

The character of the protocol gives the ability to discriminate between the influence of time being in the setup and time being loaded. The applied pressure ranges could be extended to 20-160 mmHg to generate a wider response curve that visualizes a more completely the nonlinear behavior of the LAD.

In conclusion, these pilot experiments show that we can visualize the non-linear behavior of healthy porcine coronary arteries. The loading protocol significantly influences the response of the artery. This change of behavior can probably attributed to micro structural changes in the arterial wall.
Chapter 4

Magnetic Resonance Imaging experiments

4.1 Introduction

For this study, we used Magnetic Resonance Imaging (MRI) to image a healthy coronary segment. MRI can be applied to image structures in the vessel wall, which is important for plaque imaging. We demonstrate the feasibility of MRI to visualize structures in the vessel wall with high resolution. The pressure induced deformation is measured using a protocol similar to the IVUS protocol. The results are analyzed to evaluate the mechanical properties of the LAD. Since MRI gives the possibility to visualize the vessel wall, we can calculate stress response in the arterial wall. To qualitatively compare the results to literature and each other, they are expressed in incremental elastic modulus and elastic modulus next to the distensibility.

4.2 Methods

The methods section will present the design of the experiment, the imaging procedure and the data analysis of the results. First we will discuss the design of the experimental setup followed by the changes we made to the protocol and the way the coronary arteries were prepared compared to the protocol and preparation in the IVUS experiments. Secondly we will discuss the basics of MRI and the settings that we used in our experiments. Finally in the data analysis section we will discuss how the images were analyzed and explain how we derived quantities from the images.

4.2.1 Design of experiments

Experimental setup
The setup used in the IVUS experiments was slightly adjusted for the MRI experiments. Instead of introducing the IVUS catheter to image the lumen, we used an 18 mm receiver coil. The receiver coil, was positioned in the middle of the LAD segment around the tissue bath and was connected to the MRI system. The water colon used to pressurize the artery in the IVUS experiments, was replaced by a pressure pump (pressure myograph 110P, Danish Myo Technology). The extraluminal pressure was during the MRI experiments identical to the IVUS experiments, 3 mmHg. The setup is shown in figure 4.1.
The installation of the segment and the buffer regulation were identical to the IVUS experiments. To regulate the setup from outside the MRI room, the length of all the tubing from the pumps to the setup was extended to 7 m. Despite isolation of the tubing, the temperature loss in the long tubing was vast and it was impossible to obtain the temperature at 39 °C. At a flow rate of approximately 30 [ml/min] the temperature in the tissue bath remained stable at 29+/−0.5 °C.

**Loading protocol**

The loading protocols are identical to the one in the IVUS experiments. In brief, preconditioning is followed by a pressure loop with a stepwise increase and decrease of 20 mmHg in pressure. The main difference is that the pressure range was increased from 40-160 to 20-160 mmHg. A visual overview of the loading protocols are shown in figure 4.2.
Figure 4.2: Top: The loading protocol of one pressure loop. Middle: Loading protocol 1. Bottom: Loading protocol 2.

Specimen
The LAD segment used in the MRI experiments were harvested and stored in the same way as in the IVUS experiments. We collected seven hearts from the abattoir, all seven LAD segments were successfully installed in the setup. Five experiments had to be aborted because of air inside the tissue bath and lumen of the LAD segment: the air made it impossible to visualize the LAD segment. One experiment had to be aborted due to scan time schedules. The remaining LAD segment was tested with protocol 2 and visualized successful. Thus finally this resulted in the measurement of 3 first loops, 1 second and 1 third loop.

4.2.2 Imaging procedure

MRI: Basics
The hydrogen nucleus is a single proton. Since it is charged positively and spins, it generates a small magnetic field ($B_1$). These small magnetic fields align when placed in a larger magnetic field ($B_0$). Thus when the setup is placed in the magnet of the MRI scanner the hydrogen nuclei in the setup align with the magnetic field and it becomes temporarily magnetized, (figure 4.3A and B). In the magnetized state, the hydrogen
nuclei in the setup respond to exposure to radio frequency (RF) pulse at a particular frequency, (figure 4.3C).

The RF pulse tips the small magnetic fields of the hydrogen nuclei out of alignment, and when the RF pulse is turned off the nuclei precess back to the aligned position. The movement of the small magnetic fields of the hydrogen nuclei causes an electrical current in the receiver coil, which can be measured. The received signal is called the spin echo signal. This phenomenon only occurs at one frequency, the Larmor frequency, which corresponds to the specific strength of the magnetic field. The rate at which the proton precess around the external magnetic field is given by the Larmor equation: $\omega = \gamma B_0$; where $\omega$ is the angular precessional frequency of the proton and $\gamma$ is the gyromagnetic ratio which is fixed for every nucleus. Since the magnetic field in the MRI scan has three gradients in the x,y,z direction called the slice-select, phase-encoding and readout gradient, respectively, the spin echo signal is composed of multiple frequencies, reflecting different positions along the magnetic field gradient. When the signal is broken into its component frequencies, each frequency is proportional to location of that signal. The magnetic resonance signal intensity reflects the density of mobile hydrogen nuclei. Thus with the frequency and intensity of the signal we are able to constructed an image.

The parameters that are fixed in the MRI system include the field strength [T], the gradient strength [mT/m] and rise time [s]. Factors that can be controlled are: flip angle, echo time, repetition time, bandwidth and field of view. The flip angle (FA) is used to define the angle of excitation for a field echo pulse sequence. It is the angle to which the net magnetization is rotated or tipped relative to the main magnetic field direction. The FA has an effect on the contrast between different tissues and a larger FA counteracts saturation effects of flow. The echo time (TE) represents the time in milliseconds.
between the application of the RF pulse and the peak of the echo signal in spin echo. The TE has an effect on the T₂ relaxation and thereby on the contrast in the image. The repetition time (TR) is the amount of time that exists between successive pulse sequences applied to the same slice. Variations of TR have an important effect on the T₁ relaxation and thus on the image contrast characteristics. TR is a major factor in total scan time. The contrast characteristics of the image depend on TR and TE, and how they interact (table 4.1). Bandwidth (BW) is a measure of frequency range, the range between the highest and lowest frequency allowed in the signal. For analog signals, BW is the width, measured in Hertz of a frequency range in which the signal's Fourier transform is nonzero. An inverse relationship exists between BW and signal to noise ratio. If we decrease the BW, we allow less noise to come through, and the signal to noise ratio increases. Decreasing the BW by factor 2 causes the signal to noise ratio to improve by a factor of 2^{1/2}. Field of view (FOV) is defined as the size of the three dimensional spatial encoding area of the image and is usually defined in units of mm². The smaller the FOV, the higher the resolution and the smaller the voxel size but the lower the measured signal will be. The magnetic field homogeneity decreases as more tissue is imaged (greater FOV). As a result the precessional frequencies change across the imaging volume. Smaller FOV require higher gradient strength to identify the position of the accurately measured signals. A compromise between these factors is needed. The right choice of the field of view is important for MR image quality.

<table>
<thead>
<tr>
<th>TE short</th>
<th>TE long</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR short</td>
<td>T₁ weighted</td>
</tr>
<tr>
<td>TR long</td>
<td>Proton density weighted</td>
</tr>
</tbody>
</table>

*Table 4.1: TR and TE interaction on imaging contrast, [62].*

**Settings for experiment**
The MRI scan used in this study is the GE whole body scan and has an field strength of 3 T, a gradient strength of 40 mT/m and a rise time of 266 s. The FA was set at 40°, which is fairly large. This was done to reduce the effect of the flowing (Krebs buffer) medium. The TE was 6 ms and the TR was 39.3 ms, which resulted in a T₁ weighted images of the LAD. The BW was set to 8,1 kHz which results in a low noise ratio. The FOV was set to 3.1x2.5x2.5 cm to be able to image the complete artery. With these setting 52 slices at 0.6 mm distance were imaged. The imaged cross sections of 2.5 by 2.5 cm were visualized in 384 by 256 pixels, resulting in a resolution of 65 by 97 micron. The resulting scan time was approximately 10 minutes.

**4.2.3 Data analysis**

*Image analysis*  
The MR images were acquired on the GE system with 12X software, and stored on an external hard disk. The slices were interpolated to 104 slices at 0.3 mm distance. The images were reconstructed to 512 by 512 pixels with a resolution of 0.049 mm/pixel. This resulted in 104 slices with T₁ weighted cross sectional images of the coronary artery at every pressure step. An example of one slice is given in figure 4.4A. The DICOM images were imported in ImageJ. The contours on the lumen-intima interface and the adventitia-
surrounding tissue interface were manually drawn (figure 4.4B). For every pressure step we selected 3 slices. The selected slices had to be close to circular and not close to a cannule of bifurcation.

![Figure 4.4](image)

**Figure 4.4:** A: The circular structure in the middle of the image is the lumen. The bright layer surrounding the lumen is the arterial wall. The dark and bright structure around the wall is surrounding tissue. The gray surrounding is the flowing medium. The dark circular outside layer is the plexiglass wall of the tissue bath. The bright outer layer is the back flow of medium. B: The yellow lines enclose the coronary surface area and the vessel area.

**Derived quantities**

In every image of a slice the inner and outer contours were done 3 times. For every pressure step we have 9 contours, for every pressure loop 135 contours and thus in every experiment 405 contours. The coronary surface area (CSA) and the vessel area (VA) were computed in ImageJ, and subsequent analysis preformed in Matlab. A schematic drawing of the LAD is shown in figure 4.5.

![Figure 4.5](image)

**Figure 4.5:** A schematic presentation the visualized cross-section of the LAD, with the measured quantities CSA and VA.
From the pressure and area measurements, we can derive the stress-strain relationship and the incremental modulus, following a procedure introduced by Huang [63]. The analysis is based on several assumptions. First we assumed the LAD to be circular throughout the whole experiment so we can calculate the inner ($r_i$) and outer ($r_o$) radius of the LAD segment. We considered the material of the arterial wall to be homogeneous, incompressible, and orthotropic and to obey linear elasticity law. The classic theory of thin-walled elastic shells is applicable to the cylindrical layer. The major simplification was to ignore the radial stress, and radial shear, so that the arterial wall was treated as a two-dimensional shell. The distributions of the axial and circumferential strains, referred to the unloaded state, are assumed uniform. The circumferential deformation of an artery may be described by the mid wall circumferential Green strain ($E_\theta$), which is defined as in equation 4.1:

$$E_\theta = \frac{1}{2} (\lambda_\theta^2 - 1)$$  \hspace{1cm} (4.1)

with,

$$\lambda_\theta = \frac{c_m}{C_m}$$  \hspace{1cm} (4.2)

$$c_m = 2\pi (r_i + \frac{r_o - r_i}{2})$$  \hspace{1cm} (4.3)

$$C_m = 2\pi (R_i + \frac{R_o - R_i}{2})$$  \hspace{1cm} (4.4)

Where $\lambda_\theta$ is the mid wall stretch ratio as in equation 4.2, $c_m$ refers to the midwall circumference of the vessel in the loaded state and $C_m$ refers to the corresponding mid wall circumference in the unloaded state. The unloaded CSA$_i$ and CSA$_o$ are found with linear interpolation from the lower pressures in the CSA-P graph. The second Piola-Kirchhoff stresses in circumferential ($S_\theta$) directions is given by:

$$S_\theta = \frac{P \cdot r_i}{h \cdot \lambda_\theta^2}$$  \hspace{1cm} (4.5)

Where $P$ and $h$ are luminal pressure and the wall thickness, respectively. The incremental theory is developed under the assumption of linear elasticity. This makes it possible to describe a non-linear response with an incremental linear model. If we consider a small perturbation of stress and strain from a homeostatic no load state, defined by stress $S_{ij}$ and strains $E_{ij}$, then the perturbations may be written as:

$$S_{ij} = S'_{ij} + \delta S_{ij}$$  \hspace{1cm} (4.6)

$$E_{ij} = E'_{ij} + \delta E_{ij}$$  \hspace{1cm} (4.7)
in which \( \delta E_{ij} \) and \( \delta S_{ij} \) are infinitesimal and quantities with a superscript o are homeostatic values. We assume that there is no stress and strain in the no-load state. We can write the result in the following form to introduce the definitions of the incremental elastic moduli:

\[
\delta S_{11} = \partial Y_{11} \cdot \delta E_{11} + \partial Y_{12} \delta E_{22} \tag{4.8}
\]

\( \partial Y_{11} \) is the classic incremental Young’s modulus in the circumferential direction; \( Y_{12} \) has no equivalent in classic mechanics and was denoted as cross-modulus by Fung and Liu [64]. These equation is Hookean but not isotropic. For an incremental analysis, we assume a linear stress-strain relation as given by equations 4.8. If we further assume that the cross-modulus is significantly smaller than the circumferential and longitudinal moduli, then equation 4.8 is reduced to:

\[
\delta S_{11} = \partial Y_{11} \delta E_{11} \tag{4.9}
\]

Now reverting to the \( r, \theta, z \) notations we can write the elastic modulus \( (Y_{\theta \theta}) \) and the incremental elastic modulus \( (\delta Y_{\theta \theta}) \) in the circumferential direction as:

\[
\delta S_{\theta \theta} = \partial Y_{\theta \theta} \delta E_{\theta \theta} \tag{4.10}
\]

and,

\[
S_{\theta \theta} = Y_{\theta \theta} E_{\theta \theta} \tag{4.11}
\]

If we know \( r_{1}, r_{o} \) and the extrapolated unloaded values, we can compute the \( \delta E_{\theta \theta} \) and \( \delta E_{\theta \theta} \). Combined with the pressure measurements \( \delta S_{\theta \theta} \) and \( S_{\theta \theta} \) can be determined, and from this we can compute the \( \delta Y_{\theta \theta} \) and \( Y_{\theta \theta} \). Additionally to the distensibility we now also have the elastic modulus and incremental elastic modulus to compare the results from this study to the results found in literature.

### 4.3 Results

In this section we will evaluate the results of the MRI experiments. Frist we will take a closer look at the response of the first loop and compare the response to literature. We will translate the response to values of distensibility, to quantify and compare the response of consecutive loops. Secondly the response of first and consecutive loops will be translated to stress-strain responses and values of incremental elastic moduli and elastic moduli, to quantify and compare the response to literature and consecutive loops.

#### 4.3.1 Geometrical analysis

The measured CSA changes induced by pressure changes give us the possibility to visualize the pressure-CSA graphs (figure 4.6).
Figure 4.6: The blue curve visualizes the CSA response induced by intra luminal pressure change. The four MRI images in the graph are images of the LAD lumen and wall at 20, 80, 120 and 160 mmHg.

If we look at the response of the first loop we see that an increase of the pressure induces a non-linear increase of CSA. The unloading response is also non-linear and similar to the loading curve, but follows a different path from the loading response. The difference between the loading and unloading response increases at lower pressures. The CSA at 20 mmHg at the end of the first pressure loop is 50% larger than the CSA at 20 mmHg at the start of the pressure loop.

Since we can measure the CSA and the VA, we can derive wall thickness, (figure 4.7A). The CSA and VA are following each other in an expected way. The wall thickness decreases with increasing pressure while the surface area of the arterial wall stays constant, 3.4 +/- 0.2 mm², (figure 4.7B). This indicates that, we can conclude that when the artery expands due to pressurization, the wall becomes thinner while the wall volume stays almost constant.
Figure 4.7: A: VA and CSA response of the first loop. B: The wall thickness at every pressure step in the loading response of the first loop. C: The wall surface at every pressure step in the loading curve of the first loop.

To compare the response found in this study to in literature we normalize the response to the CSA at $p = 100$ mmHg. The normalized response of the first loading curve is plotted together with to the normalized results of Van Andel [20] and Garcia [65], (figure 4.8). From this we can see that there is a good resemblance between the normalized data from literature and this study.
Figure 4.8: The normalized CSA from: the loading response from this study, Van Andel [20] and Garcia [65].

To be able to compare the quantitative results to literature the distensibility of the unloading and loading response of the first loop are calculated and visualized (figure 4.9). The average distensibility (over P between 60 –160 mmHg) of the first loop of the MRI experiment and the average distensibility found by Kassab [17] are plotted in the dotted lines. The average of Kassab [17] is in between the average of the loading and unloading of the MRI data. The decreasing distensibility at increasing pressures is in line with the expected nonlinear behavior of blood vessels.
To be able to compare the first pressure loops from the three experiments we normalized the loops to the CSA at 100 mmHg of the loading response. Qualitatively we see that the behavior is comparable, (figure 4.10). Quantitatively they are different, two are identical and one a bit stiffer, reflecting the variability of the individual arteries.
4.3.2 stress-strain analysis

With the wall thickness and the geometrical change of the lumen we can calculate the stress in the wall, (section 4.2.3). It is important in stress calculations to take into account the extra luminal pressure (3 mmHg). Consequently we can calculate the modulus and incremental modulus from the stress and strain. If we translate the response of the first loop to a Cauchy stress-strain response, we see a nonlinear stress–strain relationship with a typical exponential stiffening effect at higher pressures, (figure 4.11).
Figure 4.11: Cauchy stress-strain curve of the first pressure loop

From these stress-strain values we calculated the elastic moduli of the unloading and loading response of the first loop at every pressure step (figure 4.12). The increasing modulus at increasing pressure is in line with the stiffening effect that is known to occur in arteries.
We take an increment of the response at 120-160 mmHg and calculate the 2nd Piolla-Kirchhoff and Green strain over this increment to compare the results to literature. We can calculate the incremental elastic modulus with the incremental stress and strain. This results in an incremental modulus of 81 kPa, at a stress of 32-43 kPa, for the first loading response, (figure 4.15).

4.3.3 Consecutive loops

The result of the complete MRI experiment with all three the pressure loops responses, is visualized in figure 4.13. The end-CSA at 20 mmHg of a pressure loop is similar to the start-CSA at 20 mmHg of the next pressure loop. The loading response of the following pressure loop is in the lower pressure-range comparable to the previous unloading response but becomes larger in the higher pressure-range. Thus the response at 160 mmHg becomes larger with every pressure loop. The CSA at 20 mmHg at the end of the first pressure loop is 50% larger than the CSA at 20 mmHg at the start of the pressure loop. In the second and third pressure loop the end CSA at 20 mmHg is 30% and 10% larger than the start CSA at 20 mmHg. The CSA response at 160 mmHg is approximately 5% higher every loop.

Figure 4.12: Elastic modulus at each pressure step of the first pressure loop.
Figure 4.13: CSA response of the three consecutive loops. The dotted lines are the CSA responses to the loading ramps, the continuous lines are the CSA responses of the unloading ramps.

If we express the response of the three consecutive loops as a Cauchy stress-strain response, we can see that all three loops are qualitatively similar to the first loop, (figure 4.14). Nevertheless we see that with every loop the amount of strain and the stress at a pressure of 160 mmHg slightly increases. But the relative strain between 20 and 160 mmHg is decreasing with every pressure loop. Thus it seems that the vessel is becoming larger and stiffer every loop.
Figure 4.14: The Cauchy stress-strain response of the three consecutive loops.

If we calculate the incremental stress and strain in every pressure loop and derive the incremental elastic moduli, we see that the incremental modulus is increasing with every pressure loop, (figure 4.15). Thus we can conclude that the response of the LAD is becoming stiffer at increasing pressure and with every pressure loop.
Since the elastic behavior is changing with every loop we want to quantify these changes and compare them to literature. To quantify the changes we compare the mean distensibility, incremental elastic moduli and mean elastic moduli of the three consecutive loops to each other (table 4.2). The mean distensibility is decreasing with every loop and the mean incremental elastic moduli and the elastic moduli are increasing with every loop.

<table>
<thead>
<tr>
<th>Loop</th>
<th>Mean Distensibility [1/mmHg]</th>
<th>Mean elastic modulus [kPa]</th>
<th>Incr. elastic modulus [kPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.7 \times 10^{-3}$</td>
<td>193</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>$1.8 \times 10^{-3}$</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>3</td>
<td>$1.5 \times 10^{-3}$</td>
<td>223</td>
<td>278</td>
</tr>
</tbody>
</table>

*Table 4.2: Mean distensibility, incremental elastic modulus and elastic modulus per pressure loop.*

The calculated mean incremental modulus of all three loops of this experiment is in line with the incremental moduli found by Lu [66] and Garcia [65], (table 4.3). Additionally the changes in behavior of the RCA in experiment of Garcia [65] resemble the changes we see between pressure loops our study.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Circ. Incr. Modulus [kPa]</th>
<th>SD +/-</th>
<th>Mean stress [kPa]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig LAD 163</td>
<td>163</td>
<td>22.9</td>
<td>45-48</td>
<td>Lu</td>
</tr>
<tr>
<td>Pig LAD 176</td>
<td>176</td>
<td>51.7</td>
<td>45-48</td>
<td>Lu</td>
</tr>
<tr>
<td>Pig RCA 110</td>
<td>110</td>
<td>25</td>
<td>32-53</td>
<td>Garcia: control</td>
</tr>
<tr>
<td>Pig RCA 250</td>
<td>250</td>
<td>30</td>
<td>42-63</td>
<td>Garcia: high flow</td>
</tr>
<tr>
<td>Pig LAD 81</td>
<td>81</td>
<td>30</td>
<td>32-43</td>
<td>This study: 1st loop</td>
</tr>
<tr>
<td>Pig LAD 210</td>
<td>210</td>
<td>30</td>
<td>29-43</td>
<td>This study: 2nd loop</td>
</tr>
<tr>
<td>Pig LAD 278</td>
<td>278</td>
<td>31-45</td>
<td></td>
<td>This study: 3rd loop</td>
</tr>
</tbody>
</table>

Table 4.3: Circumferential incremental modulus of Garcia’s[65], Lu’s[66] and our study.

4.3.4 Accuracy MRI measurement

To evaluate the accuracy of the experimental technique the standard deviations on the CSA measurements were calculated. The average standard deviation is lower than +/- 0.14 mm$^2$ and the maximum in the entire experiment is +/- 0.25 mm$^2$. Since the surface change in a pressure loop is minimal 2.5 mm$^2$, the standard deviation is on average equal to 5% of the measured CSA change in the pressure loop. In the high-pressure range where the response changes are small the SD is max 0.15 +/- . Thus on average the experimental measurement method is accurate enough to measure the diameter change in the pressure loop.

4.4 Discussion & Conclusion

4.4.1 General results

The results from the MRI experiments showed non-linear behavior of the LAD, stiffening at higher pressures. The loading response was different from the unloading response in every loop. Consequently the end response at 20 mmHg was always higher than the start response at 20 mmHg. The following pressure loop started with approximately the same response as the previous pressure loop ended, seemingly independent of the time in between the pressure loops. The loading response of the following pressure loop follows the previous unloading response at the lower pressure-range and becomes larger in the higher-pressure range. Therefore the LAD got larger with every pressure loop. The distensibility, elastic moduli and incremental elastic moduli found in this study are in line with results found in literature. The changes in distensibility, elastic moduli and incremental elastic moduli within and between the pressure loops, tells us that the LAD is becoming stiffer with increasing pressure and with every pressure loop.

We examined the change of response at 20 mmHg as a function of the time the LAD spend in the setup. We computed the relative change in response at 20 mmHg in every pressure loop and in between every pressure loop from the 3 MRI experiments.
As can be seen in figure 4.16 there does not seem to be an influence of time on the response of LAD at 20 mmHg. Since time is not influencing the behavior of the LAD, it is most likely the loading protocol that causes the changing behavior. Consequently we can conclude that the loading protocol, loading the vessel with 15 static pressure steps of 10 minutes each, is inducing changes in the vessel wall.

4.4.2 Comparison with IVUS

Qualitatively, the response of the LAD in the MRI experiments are similar to the IVUS experiments. Quantitatively however, there are some differences between the IVUS and MRI experiments. The response at the start of a pressure loop is smaller than at the end of the pressure loop, both in MRI and IVUS. In the IVUS experiments we see a relative change of: 10%, 4% and 4% for the three loops. This is smaller than what we see in the MRI experiments were we find a relative change of: 50%, 30% and 10%. The mean distensibility of the first loop of the IVUS experiment (5.3x10^{-3} 1/mmHg) is quit high compared to the distensibility of the first loop of the MRI experiment (2.7x10^{-3} 1/mmHg). These quantitavie differences can be caused by the mounting of thee LAD to the
cannules. Since the LAD segment is mounted to the cannules with suture, it is expected that the mounting influences the elastic behavior nearby the mounting site, (figure 4.16A). As shown in figure 4.16B the influence of the mounting is seen in the difference in the CSA at the beginning and end of the loop at 20 mmHg. This difference is increasing as we move further away from the cannul and stabilizes approximately 20 to 30 slices from the mounting site. We compare the normalized CSA and the Cauchy stress-strain responses at 7, 21 and 51 slices away from the cannul, (figure 4.16C and D). There is a smaller increase of the normalized CSA during pressurization closer to the cannul and the strain is smaller at the same stress values closer to the cannul. Thus the closer we are to the cannul, within the 30 slice to the mounting site, the stiffer the response. These changes resemble the differences found between IVUS and MRI.

Figure 4.16: A: Schematic deformation of a LAD segment mounted to cannules. B: CSA at the beginning and at the end of the first pressure loop at P=20 plotted to the slice position. C: Normalized CSA at the three different slice positions. D: Cauchy stress-strain curves at the three different slice positions.

It was possible to visualize the in vitro behavior of the coronary artery in controlled physiological conditions with MRI at a high resolution. In this study the rate of failure of
the experiments was high. Since the last two experiments were successful and were not aborted because of setup failure, such as air in the setup, we expect that the failure rate will decrease dramatically. Not having a physiological temperature has an effect on the behavior of the LAD but is probably small and will have no influence on the conclusions.

The character of the protocol gives the ability to discriminate between the influence of time being in the setup and time being loaded. Additionally the applied pressure ranges generate a response curve that contains the complete nonlinear behavior of the LAD. The time of static loading in every pressure step is enough to obtain high-resolution images with MR and to generate a complete three dimensional visualization of the elastic response of the LAD. Due to the length of the protocol the flexibility in scheduling is limited.

4.4.4 Conclusions

MRI and IVUS experiments show qualitative similar elastic behavior. The overall behavior found in the IVUS experiments is stiffer than that in the MRI experiments. It seems plausible that the stiffer response measured in the IVUS experiments is a consequence of the mounting effect. The distensibility and incremental modulus of the first loop resemble values found in literature ([65], [66]). The increasing distensibility and decreasing elastic modulus at increasing pressure seen in the frist loop are in line with the known stiffening effect in arteries. We see that with every pressure loop the response becomes stiffer. We conclude that not time being in the setup but the loading time has an effect on the elastic properties of the LAD in the setup. The cause of changing behavior of the LAD can not be addressed, but is seems plausible, from the aforementioned arguments in the IVUS conclusion, that change in state of SMC activation can play a major role.
Chapter 5

Mathematical model

5.1 Introduction

The MRI and IVUS data revealed a particular change in the mechanical behavior of the LAD segment during the loading protocol, and we want to model this. Recently Beak et al. [22] published the four fiber model, in which it is assumed that the arterial wall consist of a mixture of an elastin, families of collagen fibers and SMC. The model of Baek has the potential to show whether it is possible that the changing elastic behavior of the LAD segment, seen in our experiment, can be achieved by a change in SMC activation. We demonstrate the feasibility of material identification for porcine coronary artery based on in vitro MRI measurements over 3 pressure loops and we investigated whether SMC activation can cause the change in elastic behavior over the three pressure loops.

5.2 Methods

5.2.1 Kinematics

If we consider the LAD segment as thick-walled circular cylinder, the kinematics is best described using a cylindrical coordinate system relative to the basis \((e_r, e_\theta, e_z)\). The deformation field over the loading protocol can be described via two successive steps, one mapping material particles from a nearly stress-free reference configuration to an unloaded configuration and another from the unloaded configuration to loaded configuration, (figure 5.1).

![Figure 5.1: Kinematics of the arterial wall relative to the nearly stress-free reference configuration \((\Omega_0)\), the excised unloaded configuration \((\Omega_1)\), and the in vitro loaded configurations \((\Omega_2)\) having coordinates \((R, \Theta, Z)\), \((\rho, \phi, \xi)\) and \((r, \theta, z)\), respectively. Adapted from Masson [67].](image)
Let $\chi: \mathbf{X} \rightarrow \mathbf{x} = \chi(\mathbf{X})$ be the mapping of a position vector from the reference to the current configuration and $\mathbf{X}(R, \Theta, Z)$. $\chi(\rho, \varphi, z)$ and $\mathbf{x}(r, \theta, \xi)$ are the, reference, unloaded, and currently loaded positions of the material particles. The deformation gradient tensor from the reference to the loaded configuration is defined as:

$$\mathbf{F} = \frac{\partial \chi(\mathbf{X})}{\partial \mathbf{X}}$$

(5.1)

Hence we can express the coordinates of the unloaded configuration as a function of the reference configuration, and the coordinates of the loaded configuration as a function of the unload configuration (Humphrey et al. [68]). The position vectors of the different states are related through the following equations:

$$\begin{align*}
\rho &= \rho(R); \varphi = \frac{\pi}{\Theta_0} \Theta; \xi = \Lambda \cdot Z \\
r &= r(\rho, t); \theta = \varphi; z = \lambda \xi
\end{align*}$$

(5.2)

The parameter $t$ denotes time over the pressure loops whereas an opening angle ($\Theta_0$) and an axial stretch ($\Lambda$) account for residual stresses [69]. The parameter $\lambda$ accounts for the load-induced axial stretch in vivo, which is assumed to be constant over the cardiac cycle [70]. $R_i$ and $r_i(t)$ denote inner radii of the artery, in reference and loaded configurations, respectively. $R_m$ and $r_m(t)$ similarly denote radii at the medial–adventitia l interface. This relation between the reference state and the loaded state gives us the possibility to express the deformation gradient tensor as:

$$\mathbf{F} = \text{diag}\left[ \frac{\partial \mathbf{r}}{\partial R}, \frac{\pi R}{\Theta_0 R}, \lambda \Lambda \right] = \text{diag}\left[ \lambda_r, \lambda_\theta, \lambda_z \right]$$

(5.3)

Where $\lambda_r, \lambda_\theta, \lambda_z$ are the principal stretches in radial, circumferential and axial directions, respectively. The left and right Cauchy-Green tensors, denoted $\mathbf{B}$ and $\mathbf{C}$, are

$$\mathbf{B} = \mathbf{F} \mathbf{F}^T, \quad \mathbf{C} = \mathbf{F}^T \mathbf{F}$$

(5.4)

Where $\mathbf{F}^T$ is the transpose of $\mathbf{F}$. Arterial tissue is considered to be incompressible, thus the local volume ratio is:

$$J = \det(\mathbf{F}) = \lambda_r \lambda_\theta \lambda_z = 1$$

(5.5)

From equations 5.3 and 5.5, we can express the reference radius as:

$$R = \left( R_m^2 - \frac{\pi \lambda \Lambda}{\Theta_0} (r_m^2 - r^2) \right)^{1/2}$$

(5.6)
5.2.2 Constitutive relation

We use the four-fiber family model of Beak et al. [22], which is an extension of the model by Holzapfel et al. [35]. In this model we account for passive behavior by the isotropic elastin-dominated matrix and the anisotropic collagen. The associated strain energy function $W$ is

$$W = \frac{c}{2} (I_1 - 3) + \sum_{k=1}^{4} \frac{c_{1(k)}}{4c_{2(k)}} \left\{ \exp[c_{2(k)} (I_{4(k)} - 1)^2] - 1 \right\},$$

(5.7)

where $c, c_{1(k)}, c_{2(k)}$ are material parameters. The invariants $I_j$ ($j = 1, 4$) are:

$$I_1 = \text{tr}(C), \quad I_{4(k)} = M_{\alpha k}(CM_{\alpha k}), \quad (k = 1, 2),$$

(5.8)

where the fiber orientations are defined in the reference configuration by unit vectors $M_{\alpha k}$, which depend on angles $\alpha_k$ defined between the direction of the $k$th family of collagen fibers and the axial direction of the artery. These fiber directions $M_{\alpha k}$ relate to the direction $a_{\alpha k}$ in the in vivo deformed state by:

$$a_{\alpha k} = FM_{\alpha k}.$$

(5.9)

The Cauchy stress tensor $\sigma$ results from three contributions: a reaction stress that enforces incompressibility, an extra stress that models passive behaviors via strain energy functions, and an active stress due to smooth muscle tone:

$$\sigma = -p I + \sigma^p + \sigma^a,$$

(5.10)

where $p$ is a Lagrange multiplier, $I$ the identity tensor, $\sigma^p$ and $\sigma^a$ the passive and active stress contributions. Humphrey et al. [68] computed these as:

$$\sigma^p = 2F \left( \frac{\partial W}{\partial C} \right) F^T = 2W_1 B + 2W_4 FM_{\alpha k} \otimes M_{\alpha k} F^T$$

(5.11)

$$\sigma^a = T_m \lambda_q \left[ 1 - \left( \frac{\lambda_m - \lambda_0}{\lambda_m - \lambda_0} \right)^2 \right] e_0 \otimes e_0$$

(5.12)

with the strain energy function $W_j = \partial W/\partial I_j$ ($j = 1, 4$). This phenomenological form for the active stress was introduced by Rachev [71] et al. and assumes that the smooth muscle is oriented primarily in the circumferential direction. The parameter $T_m$ denotes the level of activation, $\lambda_m$ is the stretch at which the contraction is a maximum, and $\lambda_0$ is the stretch at which active force generation ceases (i.e., minimum stretch possible). Using previous equations with:

$$M_{\alpha k} = [0, \sin \alpha_k, \cos \alpha_k],$$

(5.13)
the components of the Cauchy stress tensor are:

\[
\sigma_{rr} = -p + c\lambda_r^2 \\
\sigma_{\theta\theta} = -p + c\lambda_\theta^2 + \lambda_\theta^2 \sum_{k=1}^{4} c_{4(k)} (\lambda_k^2 - 1) \cdot \exp(c_{2(k)} (\lambda_k^2 - 1)^2) \cdot \sin^2 \alpha_k + Tmax_\theta \left[ 1 - \left( \frac{\lambda_m - \lambda_\theta}{\lambda_m - \lambda_0} \right)^2 \right] (5.14) \\
\sigma_{zz} = -p + c\lambda_z^2 + \lambda_z^2 \sum_{k=1}^{4} c_{1(k)} (\lambda_k^2 - 1) \cdot \exp(c_{2(k)} (\lambda_k^2 - 1)^2) \cdot \cos^2 \alpha_k
\]

where \( I_{4(k)} = \lambda_k^2 \cdot \sin^2 \alpha_k + \lambda_z^2 \cdot \cos^2 \alpha_k \) is the square of the stretch of the \( k \)th fiber family.

5.2.3 Equilibrium

Assuming the Lagrange multiplier \( p \) depends only on radial direction and time, the equation of motion, in absence of body forces, reduces to [68],

\[
\frac{\partial \sigma_{rr}}{\partial r} + \frac{\sigma_{rr} - \sigma_{\theta\theta}}{r} = \rho \cdot a_r 
\]

(5.15)

where \( \rho \) is the mass density of the wall and \( a_r \) is the radial acceleration. The contribution of the inertial term is only \( +/\text{--}0.1\% \) of the pressure, thus the elastodynamics can be studied quasi-statically [67]. Equation 5.15 can be solved by integrating over the radius from the inner wall to the media adventitia surface (denoted by \( r_m \)). Consequently, integrating equation 5.15 between the measured inner and medial radii, the transmural pressure acting on the intimal-medial tissue is:

\[
P_i(t) = \int_{r_i(t)}^{r_m(t)} \frac{\sigma_{\theta\theta}(r,t) - \sigma_{rr}(r,t)}{r} dr
\]

(5.16)

where \( P_i(t) \) is the computed intraluminal pressure. Equation 5.16 allows the interluminal pressure to be computed given information on the kinematics, including residual stress effects and the individual structural constituents of the wall. These calculated pressures can than be fitted to experimental pressure-radius data to obtain the material properties of the tested vessel.

5.2.4 Fitting procedure

\textit{Assumptions}

Since we have only 8 data points to fit the model on with 14 fitting parameters, we need to fix some parameters to. In table 5.1 we see an overview of the fitting parameters of the model of Beak [22], the found best-fit parameters of Masson [67] who used the same
model to fit the behavior of human carotid arteries and the fixed values and constrains on the fitting parameters in our study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Approximation this study</th>
<th>Human carotid (Masson)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_m$</td>
<td>2.39</td>
<td>4.26 (mm)</td>
</tr>
<tr>
<td>$\Theta_o$</td>
<td>110</td>
<td>128.7 ($^\circ$)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>1.4</td>
<td>1.11</td>
</tr>
<tr>
<td>$c$</td>
<td>fitted ($c \geq 0$)</td>
<td>29.82 (kPa)</td>
</tr>
<tr>
<td>$c_1$</td>
<td>excluded due to alfa</td>
<td>9.45 (kPa)</td>
</tr>
<tr>
<td>$c_2$</td>
<td>excluded due to alfa</td>
<td>14.14</td>
</tr>
<tr>
<td>$c_{1,\text{circ}}$</td>
<td>fitted ($c_{1,\text{circ}} \geq 0$)</td>
<td>16.13 (kPa)</td>
</tr>
<tr>
<td>$c_{2,\text{circ}}$</td>
<td>fitted ($c_{2,\text{circ}} \geq 0$)</td>
<td>15.11</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0, 90</td>
<td>-65.7, 0, 65.7, 90 ($^\circ$)</td>
</tr>
<tr>
<td>$T_m$</td>
<td>fitted ($T_m \geq 0$)</td>
<td>39.73 (kPa)</td>
</tr>
<tr>
<td>$\lambda_o$</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>$\lambda_m$</td>
<td>2.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Table 5.1: Overview of approximations and constrains to parameters in this study and the parameters found by fitting procedure of Masson [67] on human carotid*

The constitutive model includes four collagen-fiber families characterized by fiber angles $\alpha_k$. We reduce that to two fibers in circumferential and axial direction and assume that we can grasp the contribution of the four fibers in only two fibers directions. One family is assumed to be oriented axially ($\alpha_1 = 0^\circ$) and another circumferentially ($\alpha_2 = 90^\circ$). We also assumed diagonal and axial collagen fibers to have similar behaviors ($c_{1(1)} = c_1$) and ($c_{2(1)} = c_2$), whereas the circumferential fibers may differ ($c_{1(2)} = c_{1,\text{circ}}$ and $c_{2(2)} = c_{2,\text{circ}}$) because of possible interactions with circumferentially oriented smooth muscle. Noting that the residual stress-related axial stretch $\Lambda$ is typically near unity (Chuong and Fung, [72]; Humphrey, [68]), we let $\Lambda = 1$. The residual stress related values are $R_m$, $\Theta_o$ and $\lambda$. The radius in unloaded state ($\rho_m$) is found by linear interpolation from lower pressure response of the experimental data. From experimental work of Van de Broek [34], we know that the $\Theta_o$ is approximately 110$^\circ$. With the $\Theta_o$ and $\rho_m$ and the use of simple kinematics we can calculate the $R_m$ at 2.39. The axial pre-stretch, $\lambda$, in the porcine LAD is in the physiological state 1.4. The $\lambda_m$ and $\lambda_o$ found by Masson [67] for the human LAD are 1.7 and 0.96, respectively. Since we know that the stretch values of porcine LAD are a proximally twice as high as in human LAD we assume the $\lambda_m$ is 2.4. We leave $\lambda_o$ 0.96 because we have no data on differences in compression. The perivascular effect on the LAD segment is assumed to be zero since it is dissected from it’s surrounding. So after approximating: $R_m$, $\Theta_o$, $\lambda$, $\lambda_m$, $\lambda_o$ and choosing only fibers in circumferential and axial direction, we end up with the values in table 5.1. These assumptions simplify the components of the Cauchy stress tensor to:
\[ \sigma_{rr} = -p + c \lambda_1^2 \]
\[ \sigma_{\theta\theta} = -p + c \lambda_2^2 + \frac{c}{c_1\text{circ}} \left( \lambda_2^2 - 1 \right) \exp \left( \frac{c_2\text{circ}}{c_1\text{circ}} \left( \lambda_2^2 - 1 \right) \right) + T_m \lambda_2 \left[ 1 - \left( \frac{\lambda_m - \lambda_2}{\lambda_m - \lambda_1} \right)^2 \right] \] (5.17)
\[ \sigma_{zz} = \text{cons} \tan t \]

**Fitting**

From equations (5.6, 5.7, 5.11, 5.12 and 5.16) and the aforementioned assumptions, 4 parameters must be determined: passive wall properties \((c, c_{1\text{circ}}, c_{2\text{circ}})\) and muscle activation \((T_m)\). Best-fit values of these parameters were determined using a nonlinear least squares (Levenberg–Marquardt) minimization of the difference between computed and measured intraluminal pressures over a pressure loop. The following objective function was minimized.

\[ e = \sum_{j=1}^{N} \left[ \left( P_i^{\text{th}}(u) - P_i^{\text{exp}} \right)^2 \right] \] (5.18)

where \(N\) is the number of data points, \(u\) the vector of parameters to be optimized, \(P_i^{\text{th}}\) the computed intraluminal pressure from equation 5.16, and \(P_i^{\text{exp}}\) the measured inner pressure. As a measure of the goodness of fit, we computed the root mean square of the fitting error:

\[ \text{RMSE} = \sqrt{\frac{e}{N}} \] (5.19)

The behavior of the model depends critically on some parameters, and will have a weak dependence on other parameters. This dependence can be quantified by the normalized parameter sensitivity. This is defined as the differential change in the quantitative behavior of the model (measured by some metric \(X\)) for a given fractional change in each parameter of interest \((J)\). The normalized parameter sensitivity is:

\[ \text{Sensitivity} = \frac{\Delta X}{\Delta J} \times \frac{x}{X} \] (5.20)

With these assumptions we can fit the model to the data to identify the material properties of the healthy porcine LAD.

**5.3 Results**

**5.3.1 First loading loop**

We started the fitting procedure with the best-fit values of Masson [67] as the initial guess. The fitting procedure and the aforementioned assumptions resulted in a good fit to
the applied pressures for the loading ramp of the first pressure loop (figure 5.2), and set of best-fit values of the parameters (table 5.2). The best fitted parameters give an $e = 58$ and RMSE of 2.7. All parameter values are higher than zero and thus fulfill the constrain stated by Holzapfel [73, 74]. Parameters $c$, $c_{1\text{circ}}$ and $T_m$, are all in line with de parameters found in literature. However, $c_{2\text{circ}}$ is a low compared to values found in literature.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First loading loop</th>
<th>Human Carotid (Masson)</th>
<th>Porcine Basilar (Hu)</th>
<th>Mice Carotid (Gleasen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$ [kPa]</td>
<td>13.4</td>
<td>30</td>
<td>2.8</td>
<td>20</td>
</tr>
<tr>
<td>$c_{1\text{circ}}$ [kPa]</td>
<td>15.3</td>
<td>16.13</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>$c_{2\text{circ}}$ [-]</td>
<td>0.92</td>
<td>15.11</td>
<td>40</td>
<td>12.5</td>
</tr>
<tr>
<td>$T_m$ [kPa]</td>
<td>36</td>
<td>39.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 5.2: Fitted parameters of the loading curve of the first loop, and the parameters found in literature of different vessels.*

*Figure 5.2: The experimental data of the loading the first pressure loop ($o$) and the best fit curve.*

The calculated response of the fitting procedure constitutes from the contribution of three components elastin, collagen and SMC. The contribution of the different components is
separately plotted in figure 5.3. The elastin matrix contribution is visualized by the black line that has almost a linear character and is almost independent of the applied pressure. The SMC contribution is visualized with the green line that has a non-linear character that would reach a maximum at the stretch equal to $\lambda_m$. Since we do not reach $\lambda_m$ in this experiment the contribution of the SMC becomes larger at higher pressure, but the relative increase is more in the smaller deformation region. The collagen contribution is visualized with the red line that also has a non-linear character. The contribution grows exponentially as the pressure increases. Thus the elastin and SMC contribution is relative larger at the lower pressure range creating the linear elastic behavior of the vessel in this pressure range. The collagen contribution increases exponentially as pressure increases creating the well-known stiffening effect of arteries.

![Figure 5.3: The response curve of the best-fit parameters of the first loading loop (blue) and the separate contributions of elastin (black), collagen (red) and SMC (green).](image)

The separate contributions of the three components in the total response are sensitive to the fitted parameters in the equation. The parameter $c$ is only influencing the elasticin contribution, $c_{1\text{circ}}$ and $c_{2\text{circ}}$ are influencing the collagen contribution and $T_m$ influences the SMC contribution. We varied the parameters between upper and lower boundary values to evaluate the influence of the parameters on the total response. The upper boundary of the parameters $c$, $c_{1\text{circ}}$ and $T_m$ is twice the value found by Masson [67]. The upper boundary of $c_{2\text{circ}}$ is approximately three times the value of the best fit value in this study. The lower boundary for $c$, $c_{1\text{circ}}$, $c_{2\text{circ}}$ and $T_m$ is chosen at a value that results in
nearly zero contribution of the related component. The result of a change in one of the four parameters on the fitted curve and the three separate contributions are visualized in figure 5.4. Elastin contribution depends only on parameter c. Increasing c increases the stiffness of elastin, making the total response of the artery stiffer and almost linear in our pressure range. The effect of a changing c between 1-50 [kPa], is visualized by the change in elastin and total response in figure 5.4A. The collagen contribution is dependent on parameters c_{1circ} and c_{2circ}. Decreasing these values decreases the stiffness of the fibers and delays the moment of engagement. Making the total response of the artery more compliant and the maximum CSA higher. Figure 5.4B visualizes the change of the total response to a c_{1circ} change between 1-30 [kPa]. Figure5.4C visualizes the change of collagen and total response due to a change in c_{2circ} between 1-5 [-]. The SMC response depends on the SMC activation T_m. figure 5.4D visualizes the change of collagen and total response due to change in T_m between 1-100 [kPa]. The change of T_m has a maximum contribution that is dependent on the λ_m, which is 2.4. For this case it means that the change in T_m results in more SMC contribution in the high-pressure region. If one would go to even higher stretch regions this effect can reverse.
Figure 5.4: A: Elastin(black) and total response(blue) at a changing $c$ value. B: Collagen(red) and total response(blue) at a changing $c_{1\text{circ}}$ value. C: Collagen(red) and total response(blue) at a changing $c_{2\text{circ}}$ value. D: SMC(green) and total response(blue) at a changing $T_m$ value.

To see how sensitive a parameter are and whether this sensitivity is changing when the artery deforms, the sensitivity is calculated at different states of strain, at $r = 1.2$ and $r = 1.4$, as shown in table 5.3.

<table>
<thead>
<tr>
<th>Radius</th>
<th>c</th>
<th>$c_{1\text{circ}}$</th>
<th>$c_{2\text{circ}}$</th>
<th>$T_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>0.35</td>
<td>0.1</td>
<td>0</td>
<td>0.55</td>
</tr>
<tr>
<td>1.4</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 5.3: Sensitivity per parameter at different radii of the radius response
The change of $c$ has approximately the same sensitivity in the low and high stretch regions. The change of $c_{1\text{circ}}$ and $c_{2\text{circ}}$ become more sensitive in the higher stretch regions. For this case the change in $T_m$ becomes more sensitive in the higher region.

Taking a closer look at the visualization of the response change due to change in SMC activation $T_m$ (figure 5.5), we see a shift of the total response curve that looks very similar to the changes that we see in the experiments. When the activation of the SMC goes down the artery becomes more compliant having a bigger CSA at the same pressure. We see that this effect is seen in over the complete pressure range and has the most influence on average physiological pressure.

![Figure 5.5: SMC (green) and total response (blue) at a changing $T_m$ value between 1 an 100.](image)

5.3.2 Consecutive pressure loops

To evaluate whether the SMC activity can cause the change in behavior shown in the experiment, we fixed $c$, $c_{1\text{circ}}$, $c_{2\text{circ}}$ at the fitted values and lowered $T_m$ to 1 kPa. The shifted response curve, due to the change in $T_m$, is shown in figure 5.6. The radius response is higher at a lower $T_m$, relaxation of the SMC make the vessel more compliant.
We see that it is possible to shift the first loading curve closer to the data of the second loading data, by changing only $T_m$ from 36 to 1 kPa. However the change of behavior seen in the complete experiment cannot be described by changing $T_m$.

Since it is not possible to obtain the elastic change due to changing the SMC activation only, we relieved the constrains that the material properties of elastin and collagen are not changing. The one constrain that we put upon the parameters is that they must have a lower value than the values found in the previous loop or be close to the previous found value. If we fit every loading curve with four parameters, we obtain these results (figure 5.7 and table 5.4):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Loading first loop</th>
<th>Loading second loop</th>
<th>Loading third loop</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$ [kPa]</td>
<td>13.4</td>
<td>0.0938</td>
<td>0</td>
</tr>
<tr>
<td>$c_{1circ}$ [kPa]</td>
<td>15.3</td>
<td>15.2969</td>
<td>13.1992</td>
</tr>
<tr>
<td>$c_{2circ}$ [-]</td>
<td>0.92</td>
<td>1.0294</td>
<td>1.0294</td>
</tr>
<tr>
<td>$T_m$ [kPa]</td>
<td>36</td>
<td>26.1719</td>
<td>6.5391</td>
</tr>
<tr>
<td>$e$</td>
<td>58</td>
<td>158.6166</td>
<td>146.3</td>
</tr>
</tbody>
</table>

*Table 5.4: Best fit parameters of all three loading curves and the error of the fits*
If we analyze the values of the parameters of the best fits of the second and third loop, the $c$ value goes down to zero. This would imply that the elastin matrix is not contributing in the elastic behavior of the artery. Figure 5.7 shows that the pressure values in the second and third loop are always overestimated and never underestimated by the model. Consequently the error values of the best fit of the second and third loops are high. This does not happen with the fitting of the first loop data and is most likely due to the constrains that we use in the fitting procedure.

5.4 Discussion & Conclusion

We applied a mathematical model to fit the data from the MRI experiment to test the hypothesis that SMC are responsible for the change in behavior of the LAD. We selected the fiber model of Beak et al. [22], since this allows us to study the contributions of each component separately. Our main result is that we can characterize the behavior of LAD segment from in vitro MRI measurements of CSA and intraluminal pressure measurements of the first loading loop using a nonlinear, fiber-reinforced, hyperelastic, incompressible model of the wall that includes residual stresses and smooth muscle tone. The material parameters found in this fitting procedure were reasonable compared to literature, only $c_{2\text{circ}}$ was very low.
We employed a phenomenological model of active stress generation introduced by Rachev and Hayashi [71], to investigate whether change of SMC activation was the cause of the elasticity change. Changing the activation of the active part of the model shows that the change of SMC activity can cause the change in elastic behavior. But the change of elastic behavior of the LAD segment over the complete experiment was too large to be modeled by the change in SMC activity only. Thus other effects next to SMC activation must play a role in the change of elastic behavior.

The results of the fitting of the second and third loop have a value of \( c \) that goes to zero and a high error. This implies that the elastin matrix is not contributing in the elastic behavior of the artery. Since we know from literature that elastin is a very durable material [41], this does not seem to be reasonable. Thus the model is not able to describe the elastic behavior of the second and third loop, given our constrains.

Viscoelastic effects, such as creep, are typically in arteries (Holzapfel et al. [75]). Boutouyrie et al. [76] showed that the viscosity, measured in vivo in intact animals, is threefold lower than the viscosity of the arterial wall, measured in vitro. Nevertheless, in the model it is assumed that the wall exhibits a hyper elastic behavior. This could be the reason why it was impossible to fit the model to the second and third pressure loop with reasonable material parameters. Thus the model is forced to lower the material parameters to values that are physiologically not acceptable, to compensate for the viscoelastic effect that is probably contributes to the change in elastic behavior.

The model of Beak et al. [22] uses 14 parameters to fit the model to the data. Since we only have 8 data points to fit the model we simplified the model. We reduced the number of fitting parameters to four: \( c, c_{1\text{circ}}, c_{2\text{circ}}, T_m \) and reduced the number fiber families to two. The other parameters that were fitted in Masson’s study [67] and are fixed in this study. These choices are a result of known parameters from literature and reasoning from the basic wall mechanics. These assumptions simplify the model, but maintain the realistic character due to the substantiated choice of the values of the fixed parameters. One of the primary challenges in estimating the four model parameters from pressure–CSA data using nonlinear regression is identifying unique values. The success achieved herein resulted primarily from our ability to use structurally motivated functional forms of the constitutive relations and reasonable values of the initial guesses for each parameter.

In conclusion, we identified geometric and material parameters directly from MRI pressure-CSA data of the first loading loop and showed that the elastic change seen in the experiment cannot be caused by change of SMC activation only.
Chapter 6

Discussion & Conclusion

In this study, we investigated the influence of time and the loading protocol on the mechanical properties of the healthy coronary arteries. In this section we will discuss our experimental results and methods and formulate conclusions and recommendations for future research on vessel mechanics and vulnerable plaque rupture.

Experimental results
The loading response was different from the unloading response in every pressure loop. Due to this the end response at 20 mmHg was always significantly higher than the start response at 20 mmHg. The following pressure loop started with approximately the same response as the previous pressure loop ended, independent on the time in between the pressure loops. The loading curve of the following pressure loop follows the previous unloading curve and becomes larger in the higher-pressure ranges. Therefore the elastic behavior of the coronary artery was different in every repeated pressure loop. Consequently we conclude that not the time being in the setup, but the loading protocol, meaning loading the vessel with 15 static pressure steps of 10 minutes each, is inducing structural changes in the vessel wall. The IVUS and MRI experiments gave qualitatively similar results. The results of the MRI experiments were in line with results found in literature, [65, 66]. The results of the IVUS experiments gave a stiffer response than the results of the MRI experiments. But it seems plausible that the IVUS results are stiffer due to mounting effect.

Mathematical model
We used a model to fit the experimental MRI data. The applied model assumes that the arterial wall consist of a mixture of an elastin-dominated amorphous matrix, families of locally parallel collagen fibers and SMC and has 14 parameters to fit the model to the data. We reduced the number of fitting parameters to four parameters: c, c_1circ, c_2circ, T_m and reduced the number fiber families to two. Our main result is that we can characterize the elastic behavior of LAD segment from in vitro MRI measurements of CSA and intraluminal pressure measurements of the first loading loop using a nonlinear, fiber-reinforced, hyperelastic, incompressible model of the wall that includes residual stresses and smooth muscle tone. The material parameters found in this fitting procedure were compared well to literature. The model showed that SMC activation can change the elastic behavior in a way that is similar to the changes seen in the experiments. However, the change of elastic behavior of the LAD segment over the complete experiment was too large to be modeled by the change in SMC activity alone. Fitting the model to the second and third loop with four parameters was accomplished by decreasing the values of the material parameters every loop. This implies that the material becomes more compliant every loop. This is not in line with the results found in the distensibility and stress-strain
analysis, where we found that the material becomes stiffer every loop. This contradiction is caused by the fact that the data does not contain enough information to fit model adequately and because the model does not account for plastic deformation.

**Interpretation of results**
The change in SMC activation can be influenced by pressurization and can thereby change the elastic behavior of the LAD segment during the pressure loop. Data from literature on the influence of SMC activation on elastic behavior show changes in elastic behavior that are similar to the change that we see in our experiments. But the model showed that the change of elastic behavior of the LAD segment over the complete experiment was too vast to be modeled by the change in SMC activity alone. Thus other effects next to SMC activation must play a role in the change of elastic behavior. Degeneration of elastin, collagen or SMC during the experiment could cause the observed change in elastic behavior. Since such processes are time dependent and time itself does not influence the response, we can assume that degeneration of any of the individual components in the vessel is not causing the change in elastic behavior. Sorop et al. [50] showed that the remodeling of the wavy collagen network takes more than 24 hours. Thus, large-scale reorientation of collagen does therefore seem not to be the principal mechanism underlying the change in elastic behavior in our experiment. Three other possible mechanisms are (a) creep processes in the elastin matrix surrounding the collagen fibers, (b) creep within the fibers themselves, or (c) plastic effects at the interface between the collagen fibers and the elastin matrix. The observation that the most creep occurs at high pressure, when significant forces are borne by the collagen fibers, indicates that the latter two explanations are more likely than the first. Additionally, Davis et al. [41] and Shaprio et al. [42] showed that elastin is an extremely durable material, thus it is not expected that during the experiment the mechanical properties of elastin matrix change. It therefore seems likely that the creep mechanism resides within the collagen fibers or at the interface between fiber and matrix. These possibilities could be quite similar if the fibers are viewed as composite hierarchical structures of discontinuous supramolecular assemblies bound together by interfibrillar matrix, Cribb et al. [77]. Stress-relaxation within individual molecules of a fibrous protein has been demonstrated in the case of the cytoskeletal protein titin found in muscle, Tshkovrebova et al. [78].

**Setup**
The setup made it possible to visualize the in vitro elastic behavior of the coronary artery in physiological conditions, with MRI and IVUS. The adjustable luminal flow, luminal pressure, prestretch and temperature create the possibility to do a variety of experiments to investigate the influence on the behavior of the LAD. The constantly refreshed tissue bath ensures a fresh buffer in the tissue bath and creates the possibility to introduce drugs in the tissue bath to investigate the influence of drugs, e.g. vasodilatation and constriction inducing agents, on the behavior of the LAD.

In this study the rate of failure of the experiments was high, mainly due to air entrapment. Improved control of the pressure pump will reduce rate of failure rate significantly.
One main drawback is the low temperature in the tissue bath of the MRI experiment. Although we do not expect that this will change the conclusion of this study, we would like to control the temperature. To reach the 39°C in the setup Pelletier elements can be used to heat the buffer inside the MRI room just before it enters the setup.

The setup design makes it impossible to position the sample in the iso-center of the MRI scan. Due to this we have warping artifacts in the image. The setup can easily be adjusted to have the possibility to position the sample in the iso-center of the MRI-scan and thereby loose the warping artifacts.

*Future work*

Although we aimed at generating more experimental data, we only analyzed a limited number of arterial segments. The experiment could be repeated for several times, to quantify the change in elastic behavior more accurately.

To investigate the contribution of the different components of the wall several approaches can be followed. Elastin and collagen can be degraded selectively and the SMC activation / relaxation can be achieved by vasodilatation and constriction inducing agents. Selective degradation or elimination of each component may provide insight into the contribution of each component to the changing behavior.

If we want to improve the determination of the material properties by fitting the experimental data to the model, we need to increase the sampling pressure-CSA curves. Especially extending the measurements to the non-linear low pressure regime would improve the fitting procedure. Additionalli measurements, including (zero-stress) e.g. opening angle and axial force, might be essential to improve the feasibility of the model.

In future experiments that try to induce a rupture in a vulnerable plaque it must be taken into account that the loading protocol can change the elastic behavior of the vessel. In our vision the following protocol could give a more stable behavior of the LAD and has the possibility to induce a rupture of the vulnerable plaque. The protocol that could be applied is depicted in figure 6.1. In between the pulsating pressure, on could introduce repeating pressure pulses varying from 120 mmHg to a pressure that induces a rupture. The varying pressure pulses generate data for the response to pressures higher than 120 mmHg and will finally induce rupture of the vulnerable plaque. The physiological pulsatile response will give information about whether the behavior is changing and will induce a physiological state of the vessel. During the pulsatile protocol the LAD can be imaged with triggered MRI. Although it is not possible to image vulnerable plaque during the rupture it will be clear at which pressure the plaque ruptured and how it looked just before it ruptured.
Conclusions

The designed setup has proven to be feasible to test the elastic properties of a healthy porcine LAD. Time being in the setup does not seem to influence the elastic behavior, but the loading protocol induces a change of elastic behavior of the LAD segment. The found change in elastic behavior could be caused by various effects such as: change in SMC activation, creep effects within collagen fibers and plastic effects at the interface between collagen fiber and elastin matrix. The mathematical model showed that the change in SMC activation alone cannot cause the change in elastic behavior seen in our experiments. To determine the role of collagen, elastin or SMC in the changing elastic behavior of the LAD segment, further investigations are needed. Selective degradation or elimination of each component may provide insight into the contribution of each component.

Figure 6.1: Apply a pulsating pressure to the artery and image with triggered MRI.
Bibliography


61. Dijkstra, J.P.D., *Quantitative Intra Vascular ultraSound (QCU)*. Leiden University Medical Center; Radiology department.


Appendix A

In this appendix we will first discuss and describe the design of the setup. Additionally we will show the results and conclusion on the temperature control experiments.

The design of the setup

The setup is designed to fit inside an 18 mm coil to gain a resolution in MRI that can identify the different components of the vulnerable plaque. The design makes it possible to accurately apply luminal pressure, luminal flow, prestretch on the tested vessel and hold a constant buffer temperature in a tissue bath that continuously gets refreshed. Additionally it is possibility image with IVUS and to measure the temperature and pressure inside the tissue bath and lumen of the tested vessel. Thus the setup is able to visualize the in vitro elastic behavior of the coronary artery in physiological conditions, with MRI and IVUS. The adjustable luminal flow, luminal pressure, prestretch and temperature create the possibility to do a variety of experiments to investigate the influence on the elastic behavior of the tested vessel. The constantly refreshed tissue bath ensures a fresh buffer in the tissue bath and creates the possibility to introduce drugs in the tissue bath to investigate the influence of drugs on the elastic behavior of the vessel.

The cannulated LAD segment was installed into the setup. The proximal part of the LAD was connected to the piston and the distal part to the tissue bath. The position of the piston was adjusted to an axial prestretch of 1.4. The pump (Micropump, Watson-marlow, US) delivered a constant flow from the heated reservoir (MGW Lauda M3, US), through the tissue bath back to the reservoir. At a flow rate of approximately 30 [ml/min] the temperature in the setup remained stable at 39+/-0.5 °C. In the heated reservoir the buffer is heated to 46 °C and aerated with Carbogen (95% O₂ + 5% CO₂) to hold the pH at 7.4. A pressure pump (pressure myograph 110P, Danish Myo Technology) was used to apply an intraluminal pressure between 0 – 160 mmHg. The extraluminal pressure was dependent on the flow of the buffer and was during the experiments 3 mmHg. The IVUS catheter (Atlantis™ SR Pro 40Mhz Coronary Imaging Catheter), connected to the IVUS system (Galaxy 2 system), was introduced in the lumen and positioned at point where the catheter images a circular part of the lumen. The catheter was fixed at this position by the hemostasis valve. Instead of introducing the IVUS catheter to image the lumen, we used an 18 mm receiver coil in the MRI experiments. The receiver coil, was positioned in the middle of the LAD segment around the tissue bath and was connected to the MRI system, (figure D.1).
Figure D.1: Schematic drawing of the setup used in the MRI experiments.

Design
In this section we will explain the choices we made in the design of the setup. The complete setup is made of plastic to be MRI compatible and is fixated in a box that fits in the 55 cm diameter hole of the MRI scan, (figure D2).

Figure D.2: Assembly drawing of the setup.
The setup is placed in this box to prevent any water leaks from the setup to the MRI scan. In figure D2 a schematic presentation of the setup is given. The tissue bath (A) is connected to the outflow cylinder (E), which is fixated to the box. The piston (B) is introduced in to the tissue bath and the cannulated LAD is connected to the piston and the tissue bath (figure D3). The piston is connected to the piston positioner (G), the piston positioner is connected to the screw (H) and the rails (F). This screw can be rotated by the handle (I). If we rotate the handle we move the piston positioner over the rails and thereby the piston, resulting in a change of pre-stretch of the LAD. The coil holder (C) is to fixate the receiver coil. The coil holder can be moved over the rails (D) to position the coil at the desired imaging area, as shown in figure D.3.

![Figure D.3 Schematic drawing of cross-sectional view of the setup: the tissue bath (A), the piston (B), the coil holder (C), the rails (D), the outflow cylinder (E), the rails (F), the piston positioner (G), the screw (H), the handle (I).](image)

The complete tissue bath fits inside an MRI-receiver coil with an 18 mm diameter, (figure D.4). The hole where the cannule of the distal part of the LAD segment is connected can be used to introduce measurement catheters (IVUS, pressure, temperature) into the lumen of the LAD segment. The buffer outflow of the bath has a circular distribution, which gives a homogeneous distributed outflow holes. The outflow resistance is very low, so only low pressure is build up in the tissue bath. The pressure in the tissue bath is approximately 3 mmHg, thus preventing air to enter the bath through the o-ring connection of the piston. The tissue bath is 125 mm long to be sure that we have enough room to apply prestretch at any coronary artery.
The piston is designed to facilitate pre-stretch, intraluminal pressure, inflow to the tissue bath and the connection of the LAD. The proximal part of the LAD segment is connected to the piston. The position of the piston can be controlled with an accuracy of 0.1 mm, which makes it possible to accurately apply pre-stretch. Through the piston it is possible to apply a luminal pressure and luminal flow through the LAD segment, created with the pressure pump. This entrance can also be used to introduce measurement catheters (IVUS, pressure, temperature) into the lumen of the LAD segment. The buffer inflow of the tissue bath is facilitated by three holes in the top of the piston (1.1 mm diameter) and the inflow is homogenous distributed through a foam mesh before entering the bath.

**Figure D.3: View of the piston.**

**Figure D.4: View of the tissue bath.**

**Temperature control in the Setup**

The mechanical behavior of the LAD segment is temperature dependent so it is important to have a stable temperature in the tissue bath. In the following section we will show the results from the temperature control experiments.

**IVUS**

In the IVUS experiments, we were able to obtain stable 39 °C in the tissue bath with a pump setting of 1500, equal to 24 ml/min and a buffer temperature of 46 °C in the heated bath. In figure B.1 we can see that after 40 minutes the temperature in the tissue bath is approximately at stable temperature, starting from approximately room temperature.
Figure B.1: Development and stabilization of the temperature in the setup, during start up of the setup. Temperature inside the tissue bath (blue), heated bath (red) and temperature of the heater.

**MRI**

We want to investigate if we can reach 39°C in the tissue bath and quantify the influence of flow on the temperature to be sure that we do not have unnecessary flow during the measurement.

In MRI pilots we observed that at flows higher than 24 ml/min, will result in bright flow pattern in the image. With bright flow patterns in the image it is impossible to distinguish the arterial wall. Thus maximum flow we can use in MRI experiments with our imaging settings is 24 ml/min. To quantify the effect of flow on the temperature in the setup we use the following protocol and measured the temperature: inside the LAD(red), at the outflow of the tissue bath (blue) and at the heated bath(green). First we applied a flow of 24 ml/min, after 40 minutes the temperature was stable at approximately 30°C. After 140 min we stopped the flow for 15, 30 and 35 minutes, which resulted in temperature drops to approximately 28, 26 and 25 °C. When the flow was switched the flow on again it took approximately 15 minutes until the temperature reached 30 °C. After this we varied the flow at 24, 16 and 8 ml/min, which resulted in stable temperatures of 27,5 and 25 °C. We switched the flow settings after the stable temperature was reached in the current setting.
Figure B.2: Temperature measurement of at the outflow of the tissue bath (blue), in the LAD (red) and in the heated bath (Green). We stopped the flow three times for 15, 30 and 35 minutes and we varied the flow at 24, 16 and 8 ml/min.

Thus we can conclude that the temperature changes quickly if the flow is switched off. The maximum flow that can be applied during imaging can be 24 ml/min. Additionally we quantified the temperature flow relation between flows of 8 and 24 ml/min. Consequently we chose to do the measurements at a flow of 24 ml/min. This flow will not disturb the measurements and will give are stable temperature, (30°C), which is too low and needs to be improved in future experiments.
Appendix B:

Intravascular Ultrasound Imaging:

In this section we will discuss how we experimentally checked the calibration of the catheter and the results of the failed IVUS experiments. First we will discuss influences of N.U.R.D, non-concentric positioning and the actual calibration check. Secondly we will explain the findings from the failed IVUS experiments.

If we measure coronary surface area in the setup we want to know how: N.U.R.D, non-concentric positioning of the catheter, are influencing the measurement. And if the fact that we measure in a 39°C Krebs solution is influencing the measurements. To quantify the image deformation due to the aforementioned factors and to check the calibration a phantom was imaged with the Galaxy 2 system and Atlantis™ SR Pro 40Mhz Coronary Imaging Catheter. The phantom consists of eight rows of thin steel wires at an angle of 45 degrees with a distance of 0.5 mm from each other within each row. A side and top view of the phantom is shown in figure A.1.a and A.1.b, respectively. The path of the guiding wire and the temperature of the Krebs buffer is the same as it is in the IVUS experiment. The IVUS catheter is introduced in the center of all the eight rows to image the thin steel wires. An IVUS image of the phantom is shown in figure A.1.c, the measurement of the angles and distances between the wires in the image are shown in figure A.1.d.

Figure A.1: A.1.a: Side view of the phantom. A.1.b: Top view of the phantom. A.1.c: The gray-scaled image of the eight rows of thin steel wires. A.1.d: Measurement of the angel and the distance between the wires.
The image A.1.b shows that the catheter gives image quality decrease at the wires further away from the catheter. The angle between the wires found in this experiment is 45\(\pm\)2 degrees. The distance between the wires found in this experiment is 0.50\(\pm\)0.01 mm. Thus we observe no effects of N.U.R.D. in the angle measurements, no effect of geometrical deformation in the distance measurements and no effect of temperature or buffer, in this phantom study. So we can expect that the measurements from IVUS images made in the setup of the coronary artery in the 39\(^\circ\)C Krebs buffer will be the actual values.

Four LAD segments were visualized during the complete loading protocol. Results from the second and third experiment were not used in the analysis due to this unrealistic CSA response. The second and third experiment in figure A2, have a discontinuous character. It is impossible that the CSA response is constant in between two pressure steps. During these measurements the IVUS catheter probably moved relative to the vessel wall, different cross-sections of the LAD segment were measured due to this movement. For mechanical stress-strain calculations, the different loading states of one cross-section of the LAD are needed. Since the second and third experiment are useless due to the unrealistic CSA response only two experiments could be analyzed, the first and fourth experiment.

Figure A2: The second and third experiment relatively in graph A and B. In the pressure-CSA response are discontinuous, this is visible in the marked areas were the CSA response has a unexpected trend.
Appendix C:

Preparation of the LAD

In the present study, porcine hearts (age range 3-5 months) were harvested during the slaughter process in the abattoir (Westfort v.o.f) within 30 minutes from death. Until excision, the hearts were stored in a krebs buffer at 4 °C. Within 5 hours from death the LAD segments were excised, 5 to 10 mm distal to the ostium of the left coronary arteries, right after the first curve and side branch, (figure C.1). The LAD segments are 30 to 50 mm long with an inner diameter of 2 to 4 mm. This part of the LAD is quite straight and has usually 3 to 5 side branches, which were ligated with surgical suture. Both ends of the LAD were connected to cannules to install the LAD in the testing setup. The details of the excision procedure are described in this appendix.

Figure C.1: The LAD segment.

- Take the heart out of the cooled Krebs buffer.
- Inspect if the LAD shows any irregularities.
- Make a 2 cm deep incision in the left ventricle perpendicular to the LAD 3 cm under the apex of the heart (figure C.2.A).
- Make two incisions, also 2 cm deep, parallel to the LAD at 2 cm distance of the LAD from the previous incision to the left atrium and pulmonary artery, left and right from the LAD (figure C.2.A).
- Make an incision 2 cm underneath the LAD parallel to the surface, from the apex to the atria.
- Cut the piece of tissue free from the heart, perpendicular to the LAD through the left atrium and the aortic wall (figure C.2.B).
• Free the LAD and the side branches from the surrounding tissue with the blunt ends of the scissors, figure (C.2.C).
• Ligate the side branches with the surgical suture and insert the cannules.
• Clamp the cannules inside the LAD with the suture.
• Remove all the surrounding tissue from the LAD.
• Pressurize the LAD and check for leakages and repair them with suture (figure C.2.D).
• Store the cannulated LAD in Krebs buffer until installation in the setup.

Figure C.2: A: Incisions under and around the LAD. B: Incision to cut the cubic piece of tissue, containing the LAD, out of the heart. C: LAD partly free from the surrounding tissue. D: Cannulated LAD with ligated side branches.