Towards the development of guidelines for the surgical treatment of carotid artery disease: A tissue characterisation approach

Author
John J. Mulvihill

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Supervisor
Dr. Michael T. Walsh

DEPARTMENT OF MECHANICAL, AERONAUTICAL AND BIOMEDICAL ENGINEERING,
UNIVERSITY OF LIMERICK, IRELAND

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I hereby declare that this thesis is entirely my own work, and has not been submitted for any other awards at this or any other academic establishment. Where use has been made of the work of other people it has been fully acknowledged and referenced.

__________________________  _______________________
John Mulvihill                Dr. Michael Walsh
(Candidate)                   (Supervisor)
Abstract

Despite the proven efficacy of carotid endarterectomy (CEA), great interest has been generated in carotid angioplasty and stenting (CAS) as an alternative to CEA. The stretch caused by balloon deployment during CAS can damage the baroreceptor function and therefore alter the normal cardiac output and induce severe damage to the carotid artery layers an d plaque leading to an increased chance of restenosis. However, there is the potential to limit these damaging effects by improving the balloon device design and varying the balloon inflation parameters.

Initially, this study focused on investigating the feasibility of developing a material that can be used in an experimental model of the carotid bifurcation complete with baroreceptor response to strain, so that it may be used to identify and quantify the effects that subjecting the baroreceptor nerves to sustained circumferential stretch has to the blood flow rate as is the case during angioplasty. An electrically conductive silicone was developed and investigated using a novel test system to highlight the qualitatively similar electrical response of the material to the baroreceptor nerves undergoing strain. The mechanical behaviour of 23 human carotid plaques was characterised in order to correlate plaque behaviour to pre-operative classification data. Plaques underwent uniaxial stretch in the circumferential direction at a physiological strain rate in order to replicate the instantaneous systolic pulse experienced by the plaque in vivo. This study also evaluates the limitations of mechanically testing plaque specimens in the circumferential direction due to their random geometry, as well as in investigating the effects that changing the geometrical ratios of the specimens for uniaxial testing has on the curve-fitted strain energy function models used for finite element analysis (FEA).

The current gold standard of pre-operatively classifying plaque behaviour, duplex ultrasound, was analysed to determine whether it is an accurate predictor of the mechanical behaviour of plaques. Results demonstrate that the mechanical behaviour does not correlate with pre-operative classification as estimated by duplex ultrasound. Testing the atherosclerotic plaques until failure identified the stresses and strains that plaques can withstand prior to rupture and indicated that the majority of strains applied during a typical CAS procedure could cause plaque to rupture.

As ultrasound classification was shown to be an unsuitable diagnostic predictor of plaque material properties, an improved method is developed in this study through the use of Fourier-transform infrared (FTIR). FTIR was carried out on each plaque sample prior to mechanical testing in order to globally characterise the biological composition of the specimen. FTIR revealed that the plaques with a higher concentration of calcification contained a stiffer mechanical response compared to those with higher lipid content. Testing the atherosclerotic plaques until failure identified the stresses and strains that plaques can withstand prior to rupture and indicated that the majority of strains applied during a typical CAS procedure could cause plaque to rupture.

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Dedication

I dedicate this body of work to my family, Karrina and her family, as without you none of this would have been possible.

Our knowledge has made us cynical, our cleverness, hard and unkind. We think too much and feel too little. More than machinery we need humanity. More than cleverness we need kindness and gentleness. Let us fight for a world of reason, a world where science and progress will lead to all men’s happiness.

- Charlie Chaplin, The Great Dictator, 1940
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<th>Symbol</th>
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<td>C₀</td>
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<td>F</td>
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<tr>
<td>Iᵢ</td>
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<td>Number of families</td>
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<tr>
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<tr>
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</tr>
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<td>Level of fibre dispersion</td>
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<td>Cauchy stress</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>Engineering</td>
<td></td>
</tr>
<tr>
<td>el</td>
<td>Element</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>Deformed length</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>Number (direction)</td>
<td></td>
</tr>
<tr>
<td>o</td>
<td>Original length/area</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>True stress/strain</td>
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### Abbreviations

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<th>Definition</th>
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<td>ATR</td>
<td>Attenuate Total Reflectance</td>
</tr>
<tr>
<td>BRS</td>
<td>Baroreceptor Reflex Sensitivity</td>
</tr>
<tr>
<td>Cal:Col</td>
<td>Calcification to Collagen ratio</td>
</tr>
<tr>
<td>Cal:Li</td>
<td>Calcification to Lipid ratio</td>
</tr>
<tr>
<td>CAP</td>
<td>Carotid Artery Plaques</td>
</tr>
<tr>
<td>CAS</td>
<td>Carotid Artery Stenting</td>
</tr>
<tr>
<td>CB</td>
<td>Carotid Bifurcation</td>
</tr>
<tr>
<td>CEA</td>
<td>Carotid Endarterectomy</td>
</tr>
<tr>
<td>CPD</td>
<td>Critical Point Dryer</td>
</tr>
<tr>
<td>CREST</td>
<td>Carotid Revascularisation Endarterectomy vs. Stenting Trial</td>
</tr>
<tr>
<td>CVA</td>
<td>Cerebrovascular Accident</td>
</tr>
<tr>
<td>ECS</td>
<td>Electrically Conductive Silicone</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy Dispersive X-Ray</td>
</tr>
<tr>
<td>ERD</td>
<td>Elastic Recoil Detection</td>
</tr>
<tr>
<td>EVA-3S</td>
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</tr>
<tr>
<td>FEA</td>
<td>Finite Element Analysis</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-Transform InfraRed</td>
</tr>
<tr>
<td>GSM</td>
<td>Gray-Scale Medium</td>
</tr>
<tr>
<td>HGO</td>
<td>Holzpfel-Gasser-Ogden</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>ICSS</td>
<td>International Carotid Stenting Study</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoproteins</td>
</tr>
<tr>
<td>Li:Col</td>
<td>Lipid to Collagen ratio</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus of the Tractus Solitarius</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>SAPPHIRE</td>
<td>Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy</td>
</tr>
<tr>
<td>SEF</td>
<td>Strain Energy Function</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<tr>
<td>SPACE</td>
<td>Stent-Protected Angioplasty versus Carotid Endarterectomy</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient Ischemic Attack</td>
</tr>
<tr>
<td>WL</td>
<td>Width to Length</td>
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CHAPTER 1

INTRODUCTION
1 Introduction

Carotid Artery Stenting (CAS) is a minimally invasive procedure that deploys a stent at the site of atherosclerotic disease with the aid of balloon inflation that compresses the disease and widens the lumen to increase blood flow. The current gold standard for treating symptomatic and asymptomatic carotid stenosis is surgical endarterectomy (CEA). Initially, it was believed that CAS would replace CEA as the main procedure to treat atherosclerosis stenosis within 5 years; however, the results from various trials suggest no advantage in using CAS (Mas et al., 2008). Despite this, an uptake in the coming years is predicted due to the results of the CREST trial which show that CAS is non-inferior to CEA (Medtech Insight Report, 2011).

CAS remains the second choice for clinicians and accounts for only 26% of stroke prevention procedures performed (Paraskevas et al., 2009). This is attributed to the unfavourable effects that CAS has on the treated vasculature such as altered baroreceptor behaviour and plaque rupture peri-operatively (Gupta et al. 2005). The sustained stretch caused by balloon deployment can damage the normal baroreceptor function and induce plaque rupture which can instigate embolisation. However, there is potential to limit these damaging effects by pre-operatively de-selecting rupture prone plaques, improving the balloon angioplasty design and varying the balloon inflation parameters.

An investigation into the response of the baroreceptor function to the deployment of current balloon devices used in CAS and an improved understanding of the mechanical behaviour of carotid plaques in physiological conditions is necessary to improve the balloon design and inflation parameters. As it is not viable to measure baroreceptor function during CAS procedures, it is therefore necessary to experimentally replicate CAS conditions using a bench-top test system that employs a simulated carotid bifurcation model. However, no biomimetic material of carotid tissue and baroreceptor function exists. Also, there is a distinct lack of mechanical properties of human atherosclerotic carotid tissue and further mechanical characterisation is necessary. However, a method of identifying the composition of the plaque is important in order to improve the understanding of the key features and components that contribute to the mechanical behaviour and risk of plaque rupture.

Fourier Transform Infra-Red (FTIR) is one of the most widely used vibrational spectroscopic techniques for the identification of biological specimens. Previous work
has shown the ability of FTIR to identify and quantify the presence of lipid, collagen and calcification within the plaque in comparison to the gold standard histology (Ebenstein et al., 2009). However, it is necessary to relate the FTIR analysis results to the mechanical properties of the plaques on a global level as when plaques are treated with angioplasty balloons, the force that the balloon generates in the circumferential direction will trigger a whole plaque mechanical response. Scanning electron microscopy (SEM) has, in recent years, been used in the biological sciences specifically to image the structure of tissue in great detail. Guasti et al. (2010) demonstrates that SEM is capable of imaging the delamination and build-up of calcification in human carotid plaques. However, this study is limited to one specific plaque specimen. In comparison, this present study will examine a larger sample size of human carotid plaques in order to establish the key features present in all specimens in order to provide a better understanding of atherosclerosis development as well as plaque rupture. Energy-dispersive X-ray spectroscopy (EDX) is an elemental analysis tool which can be used to characterise the composition of biological tissue on a micro-scale. EDX spectroscopy can be carried out on samples during SEM imaging to determine the main constituents of the rupture sites of each plaque (Schembri et al., 2008) as well as to validate the vibrational spectrums produced by FTIR.

This study will examine the biological composition of arterial plaque material and how the composition relates to the mechanical behaviour and rupture potential of these plaques through the use of FTIR globally and SEM and EDX at the rupture sites. This study will also analyse the ability of FTIR to predict plaque mechanical behaviour in order to assess whether FTIR is a viable technique for use as an in vivo pre-operative imaging tool in the treatment of carotid atherosclerotic tissue. This study aims to investigate the feasibility of developing a material that could be used in an experimental model of the carotid bifurcation complete with baroreceptor response to strain, so that it may be employed in future studies to identify and quantify the deleterious effects of subjecting the baroreceptor nerves to sustained circumferential stretch such as in the case of balloon angioplasty deployment. The final objective of this study is to examine a novel balloon angioplasty device developed, using finite element analysis (FEA), and how improving balloon design and inflation parameters can reduce the potential of plaque rupture and the deleterious effect on baroreceptor function during angioplasty deployment in comparison to current balloon designs.
1.1 Objectives

1. Develop a biomimetic material of the carotid artery mechanical behaviour which incorporates the baroreceptor function.
2. Mechanically and biologically characterise human carotid plaque tissue samples.
3. Use FEA to highlight the advantages of a novel angioplasty balloon to current technology which reduces the risk of plaque rupture and the reduced over-stretching of the baroreceptor nerves which cause peri-operative complications.

1.2 Thesis Outline

These objectives are investigated in the following chapters:

Chapter 2 presents a literature review of atherosclerosis and the current methods used to treat this disease. Previous work regarding the mechanical properties of atherosclerotic diseased carotid tissue is investigated and the potential methods of mechanically and biologically characterising the tissue are also examined. The baroreceptor will be reviewed as well as the effect that current treatments for treating plaque have on the function and how to, potentially, experimentally replicate this function.

Chapter 3 investigates the feasibility of developing a material that can replicate the mechanical behaviour of tissue in a carotid bifurcation with the incorporation of the baroreceptor. The future aim of this biomimetic material development is to investigate the feasibility of experimentally examining the effect of angioplasty devices on the baroreceptor function and the change in flow rate caused by this change in function.

The second section of this study is to develop methods of mechanically and biologically characterising human plaque tissue and chapter 4 describes the equipment and processes used in this study to mechanically and biologically characterise the tissue.

Chapter 5 investigates the limitations of uniaxial testing of plaque tissue due to the random nature of the geometry using FEA and highlights the potential to define the geometrical ratios suitable for either tensile or planar shear testing.

Using the processes from chapter 4 and taking into account the limitations from chapter 5, human carotid plaque tissue will be mechanically and biologically characterised using a uniaxial test system as well as spectroscopy and microscopy methods in chapter 6. Chapter 7 discusses the effect that the level of calcium has on the mechanism of plaque rupture and highlights the ineffectiveness of current diagnostic methods regarding predicting plaque behaviour.
Chapter 1

Introduction

The final section of the work presented in chapter 8 investigates and compares the deployment of a novel two-stage perfusion balloon angioplasty device to current technology using FEA in an idealised diseased carotid artery. The purpose of this chapter is to highlight the potential of this device regarding reducing the damaging effect angioplasty has on the plaque structure and baroreceptor function by changing the inflation parameters and geometrical design.

Chapter 9 discusses the findings of the work carried out and concludes on the main objectives achieved. The final chapter describes a number of recommendations for future work that would lead on from this study.

The thesis has been prepared in the style ‘Thesis by Publication’. The referencing styles vary throughout the thesis depending on the specific journal of publication, excluding chapters 4, 8, 9 and 10 which are not published and are all Harvard style referencing.

Table 1.1 outlines the chapter and the journals in which each chapter has been published or submitted to. The status of the paper and the corresponding journal impact factor is also shown (as of April 2013).

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References


CHAPTER 2

LITERATURE REVIEW
2 Literature Review

2.1 Introduction

In this chapter the development, treatment and biological structure of atherosclerotic plaque within the carotid artery is discussed. This review chapter will describe and also discuss the major work carried out on the formation and treatment of atherosclerosis. Previous work on the mechanical characterisation methods carried out on human plaque tissue will be investigated as well as studies on the baroreceptor function and the effect surgical treatments have on the baroreceptor and atherosclerotic plaque. The objectives of this study will be refined at the end of this chapter taking into account the current literature and processes described in this chapter.

2.2 Atherosclerosis

Atherosclerosis is defined as the thickening and loss of elasticity of the walls of the arteries caused by lesions developing in the innermost layer of the artery (National Library of Medicine, MeSH C14 D050197). This thickening is an accumulation of macrophages which contain lipids (molecules such as fat, wax, cholesterol and vitamins), collagenous tissue and calcium phosphate based nodes between the endothelium lining and smooth muscle cells. Macrophages are white blood cells that are based in the tissue rather than in the bloodstream and are attracted to areas of arterial injury. Macrophages form when a leukocyte (a white blood cell which defends the body from infectious disease) reaches a damaged area of the artery wall at the endothelium (Lucas and Greaves, 2001). Atherosclerosis forms in most humans and can begin as early as ten years of age (Lusis, 2000, McGill et al., 2000). However, at the early stages of atherosclerosis the atheromatous plaques are just a build-up of excess macrophages at the artery wall that have absorbed oxidised low-density lipoproteins (LDL), in a process called endocytosis. LDLs, also known as ‘bad cholesterol’, are a carrier of lipids like cholesterol and triglyceride throughout the blood system. However, when built-up, LDLs tend to attach themselves to the endothelial layer of the artery and as previously mentioned are attracted to the macrophages in damaged areas of the artery wall.

2.2.1 Development of Atherosclerosis

Atherosclerotic plaque at this early stage is commonly described as a fatty streak type plaque. This is due to the foam cells created by the LDLs that have oxidised with the
reactive oxygen species that is produced by damaged endothelial cells. Foam cells create these fatty like streaks that can form in the plaque and eventually become the centre of the plaque at a later stage (National Library of Medicine, MeSH A11 D004587). This centre occurs when foam cells disintegrate and attract more macrophages to the plaque area therefore creating an extracellular lipid core, or ‘lipid pool’, in the more advanced atherosclerotic plaque (Lusis, 2000). The outer layer of the plaque, where the initial build-up of macrophages is located, becomes more calcified and stiff over time forming what is called a fibrous cap.

The atherosclerotic plaque can progress in size and thickness over decades and can have no symptomatic effects on the person. This increase in plaque size causes the medial layer of the artery to stretch outwards radially in order to compensate for the decreasing lumen area (cross-sectional area of the blood flow). Eventually the muscular medial layer becomes incapable of compensating for the growing plaque size and encroaches into the lumen area. At this stage of the atherosclerosis the plaque can rupture thereby releasing the lipid core and inducing thrombosis in order to heal the damaged tissue. This can occur multiple times in quick succession. Over-time the plaques can become calcified which stiffens the diseased carotid arterial tissue and greatly increases the risk of plaque rupture (Allison et al., 2004). The occurrence of thrombosis coupled with the increased size of the plaque can narrow and fully occlude the lumen which causes abnormal and restricted blood flow. This feature is known as stenosis and it is one of the main causes of stroke.

2.2.2 Atherosclerotic Plaque Composition

Advanced plaques often have a heterogeneous composition, containing extensive regions of lipid, fibrous tissue, and calcium. The occurrence of these components in varying proportions in different plaque specimens gives rise to a spectrum of multifaceted lesions. Due to this heterogeneous nature it is difficult to assign global properties to all variations of plaque.

There are two distinct plaque component types that can form in advanced lesions of atherosclerosis; the atheromatous plaque that is lipid rich and soft and the sclerotic plaque that is collagen rich and hard. The sclerotic component is the dominating component of the plaque and is relatively stable as a result of the collagen it contains. Shah et al. (2003), Cheng et al. (1993) and Huang et al. (2001) reported that the lipid component of plaque specimens is a main contributor toward the rupture of plaque and a key factor in plaque vulnerability and that calcification may stabilise the plaque.
Conversely, Wenk et al. (2011) demonstrated that the circumferential stress in the fibrous tissue increases as the volume of calcifications increases and also that the presence of calcifications can significantly alter the distribution of stress. Maldonado et al. (2012) and Vengrenyuk et al. (2006) have shown that micro-calcifications have an effect on the Young’s modulus of the surrounding tissue (inducing a five-fold increase in the stress threshold at rupture) which can increase plaque vulnerability due to the increase in voids created by the growth of micro-calcification clusters. It is deficient in the supporting collagen and so has a high potential risk of rupture (Pasterkamp and Falk, 2000, Shah, 2003). Remodelling of the vascular wall structure occurs as a result of the formation of the atherosclerotic lesions. The interaction between the cell types and the tissue determines the development of the plaque. This process becomes reactive and actively participates in the development of vascular accidents.

Growing evidence suggests that the decisive risk factor determining plaque vulnerability is plaque composition rather than the degree of luminal narrowing (Glagov et al., 1987). Most strokes and myocardial infarctions are caused by rupture or erosion of the fibrous cap of an atherosclerotic plaque, which exposes thrombogenic material of the plaque to flowing blood. This results in thrombus formation that may occlude the artery or induce embolization into the circulatory system. From a mechanical point of view, rupture of a cap will occur when the stresses in the cap exceed the strength of the cap. Determining stresses in the cap of atherosclerotic plaques using patient-specific models may therefore be a more suitable approach to assess risk of cap rupture (Huang et al., 2001, Ohayon et al., 2005). A study by Gao et al. (2009) demonstrated that the thickness of the fibrous cap is more critically important than the lipid core volume. The study’s results showed a slight decrease in the thickness of the fibrous cap caused a significant increase in the level of stress experienced by the cap. The size of the lipid core is also important in plaque rupture as studies have shown that a large lipid core that is separated from the lumen by a thin cap is at a higher risk of rupture. Collagen is the primary structural component in the fibrous cap of the plaque and provides the cap’s biomechanical strength. Reduced collagen can result in a weakened structure that can be prone to ruptures. An unfavourable plaque morphology consisting of a combination of a thin fibrous cap and low collagen content overlying a large lipid core makes a plaque more prone to rupture and thus stroke due to embolization (Schaar et al., 2004).

Due to the fact that carotid endarterectomy is regarded as the gold standard procedure for plaque alleviation, the opportunity from an engineering standpoint is to isolate
plaque types based on their mechanical properties in order to assist in the development of carotid angioplasty devices. With a detailed knowledge of how different plaque types react to mechanical loading, angioplasty balloons can therefore be tailored to interact favourably with each type as well as minimise the effect on the baroreceptor function.

2.3 Surgical Treatment of Plaque

Surgical methods are employed to eliminate the risk of stroke due to the presence of stenosis in the carotid vessels in 50% of cases for symptomatic patients and 60% of cases for asymptomatic patients (Krajcer, 2005). The two surgical procedures used are carotid endarterectomy (CEA) and carotid artery stenting (CAS). The current gold standard for treating symptomatic and asymptomatic carotid stenosis is surgical endarterectomy. Despite the proven efficacy of CEA, great interest has been generated in CAS as an alternative to open surgical therapy. CAS is less invasive compared with CEA, and has the potential to successfully treat lesions close to the aortic arch or distal internal carotid artery (Sajid et al., 2007, Jeyabalan et al., 2009). It can be performed on poor surgical candidates, used to treat stenotic lesions that are inaccessible to surgeons due to morphology or location and revision surgeries can be performed on patients relatively easily (Medtech Insight Report, 2011). However, trials conducted to establish efficacy and equivalency of CAS to CEA have often reported conflicting results and have therefore divided opinions regarding the employment of CAS in revascularisation of the carotid artery.

2.3.1 Carotid Artery Stenting (CAS)

CAS is a minimally invasive procedure that deploys a stent at the site of stenosis, compressing the plaque and widening the lumen to increase blood flow, figure 2.1. Despite the numerous advantages of CAS over traditional carotid surgery such as increased accessibility, decreased trauma and absence of general anaesthetic (Roffi et al., 2009), it remains the second choice for clinicians and accounts for only 26% of stroke prevention procedures performed (Paraskevas et al., 2009). This is attributed to unfavourable clinical studies on first generation carotid stents that cited problems related to embolic release and hemodynamic instabilities such as hypotension and bradycardia which occurs in 21% to 51% of CAS cases due to altered baroreceptor behaviour (Gupta et al., 2005).
2.3.2 Embolic Protection Devices

Distal embolization of atherosclerotic debris and thrombus is the most common complication of CAS. This high risk of embolization has been the foremost reason for slow acceptance of this procedure in the past (Kasirajan et al., 2003). To address this issue, many types of embolic protection devices have been developed. Currently, there are three main types of protection devices: occlusion balloons, filters, and flow reversal devices (Londero and Paoletti, 2004). The filter type is the most common type used and will be further discussed.

Filter type embolic protection devices incorporate a porous filter membrane in a basket or umbrella configuration that is opened and supported on a wire scaffolding structure (Tan et al., 2001). In the majority of cases, the porous membrane is made of polyurethane and the microporous holes are cut using a fine laser. Filter type devices, unlike occlusion balloons or flow reversal devices, allow blood flow to the cerebrum to be maintained throughout the carotid artery stenting procedure, while filtering any dislodged plaque from the blood. To achieve filtration of the blood, the device is located distal of the lesion during the stenting process and upon completion is collapsed and removed carrying with it all collected emboli.

The porous size of these devices is a source of great debate as it is a trade-off between the trapping capability of the filter and the disruption caused to blood flow to the cerebrum (Londero and Paoletti, 2004). It has been shown through animal experiments that emboli with a diameter of 100μm or less can cause severe damage if allowed to reach the cerebrum (Sievert and Rabe, 2002). However, it is not acceptable for filter porous size to be made less than 100μm as the disturbance to blood flow is excessive. Modern filter designs incorporate pore sizes of between 100μm and 150μm (Tan et al., 2001) although particles of diameter <100μm are unlikely to cause major cerebral damage, they may result in vision loss or cognitive changes (Henry et al., 2004a).
As the embolic protection device collects plaque debris, more and more of the filter porous become blocked. This reduces the overall porosity of the filter, increasing the resistance to blood flow and thus increasing the pressure drop across the filter (Henry et al., 2004a). Quantifying the degree of embolization that occurs during a carotid artery stenting procedure and consequently the degree to which the filter porous are blocked has currently not been determined. Therefore no research is currently available to accurately predict the actual pressure drop experienced across the filter device. The following section will highlight the efficacy and increased safety of CAS when using a protection device.

2.3.3 CEA vs. CAS Trials

The current gold standard for treating symptomatic and asymptomatic carotid stenosis is surgical endarterectomy and initially CAS was thought to offer many advantages over CEA. From the concept stage of CAS it was believed that within 5 years CAS would replace CEA as the main procedure to treat atherosclerosis stenosis however, this is currently that is not the case. Despite this, an uptake in the coming years is predicted due to the results of the Carotid Revascularisation Endarterectomy vs. Stenting Trial (CREST) showing CAS is non-inferior to CEA (Brott et al., 2010, Medtech Insight Report, 2011).

Two CAS studies; Stent-Protected Angioplasty versus Carotid Endarterectomy (SPACE), and Endarterectomy versus Angioplasty in Patients with Symptomatic Severe Carotid Stenosis (EVA-3S) trial (Carotid et al., 2010), both showed inferiority of CAS to CEA. The SPACE trial enrolled 1214 patients (601 for CEA and 613 for CAS) and failed to demonstrate equivalence between interventional approaches with CEA outperforming CAS in the intention-to-treat group at 30 days (6.6% vs. 7.4%) and 2 years (8.8% vs. 9.5%), and in the per-protocol group at 30 days (5.7% vs. 7.3%) and 2 years (7.8% vs. 9.4%) respectively (Eckstein et al., 2008). The study also found that the rate of recurrent stenosis (>70%) is significantly more frequent in the carotid artery stenting group compared with the carotid endarterectomy group. The EVA-3S trial enrolled 527 patients (262 for CEA and 265 for CAS) and reported similar trends whereby CEA outperformed CAS at 30 days (3.9% vs. 9.6%) and 6 months (6.1% vs. 11.7%) respectively (Mas et al., 2008).

It should be noted that the outcome of the CAS procedures in these trials were at an immediate disadvantage as the use of embolic protection was left to discretion of the attending physician in the SPACE trial and was only started 3 years into the EVA-3S
The results from these trials conflict with the observations from the Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy (SAPPHIRE) trial which employed distal embolic protection in its CAS procedures and treated both symptomatic and asymptomatic patients. The SAPPHIRE trial reported 30 day (20.1% vs. 12.2%, Yadav (2004)) and 3 year (26.9% vs. 24.6%, Gurm et al. (2008a)) results in favour of CAS for the primary endpoint of the incidence of death, stroke or myocardial infarction. The investigators concluded that among the patients exhibiting severe carotid-artery stenosis and coexisting conditions, carotid stenting with the use of an emboli-protection device is not inferior to carotid endarterectomy.

Two trials set up to address equivalence and efficacy of CAS over CEA, CREST and International carotid stenting study (ICSS), have recently published their early findings (Brott et al., 2010, Ederle et al., 2010b). Both trials were large, randomised, multicentre, controlled trials. CREST studied 2502 patients and ICSS studied 1713 patients and reported similar net outcomes with CAS and CEA. For the composite primary endpoint of any stroke, myocardial infarction or death during the peri-procedural period, or ipsilateral stroke on follow-up, stenting was associated with 7.2% rate of these events vs. 6.8% with surgery, a non-significant difference. However, individual risks varied within the CREST trial. When considering death and stroke alone, surgery is safer than stenting (2.6% vs. 4.8%). For the ICSS trial, the 120 day risk of stroke, death or myocardial infarction in those patients allocated to CAS was significantly higher than CEA (8.5% vs. 5.1%; HR 1.69, 1.16–2.45, p=0.006) (Forbes, 2010, Ederle et al., 2010a, Hopkins et al., 2010).

Critical differences exist between these trials making direct comparison of results difficult. ICSS studied only patients in whom carotid stenosis was recently symptomatic, whereas 47% of patients in CREST were asymptomatic. Also, within ICSS, there was an inherent disparity with respect to the level of experience of participating surgeons and interventionalists (Roffi et al., 2010). Finally, ICSS did not mandate the use of distal embolic protection devices. This is an important factor to be considered as the use of embolic protection devices can reduce the rate of stroke or death (at 30 days) by approximately 60% (Aronow and Yadav, 2004).

A particular concern highlighted by the CREST trial (Brott et al., 2010) is the propensity for complications in elderly patients, the very patients who might benefit most from a minimally invasive procedure. CREST found that at approximately age 69 and younger, stenting results were slightly better, with a larger benefit for stenting, the
younger the age of the patient. Conversely, for patients > 70 years, CEA results were slightly superior to stenting, with larger benefits for CEA, the older the age of the patient (Mantese et al., 2010). The increasing complication rate with age for CAS procedures may be a marker for some other underlying issues such as increasingly adverse anatomy or differences in pathology, so more analysis of age and its attributed variables is required before concluding outright that CEA is the best option for patients older than 70 years.

Despite the potential importance of anatomical and physiological factors for reduced CAS mortality and morbidity rates, current pre-endovascular anatomical assessment is limited. Duplex ultrasound provides information only about the region of the carotid bifurcation and conventional angiography tends to underrepresent the atherosclerotic burden of the aortic arch and exposes the patient to a measurable risk of stroke (Wyers et al., 2009). A more thorough, yet noninvasive pre-CAS anatomic evaluation may help identify patients that are at high-risk or should not undergo CAS based upon lesion assessment and tortuosity of the vasculature; including the carotid artery, and of the distal internal carotid artery. Once such method would be through the use of a non-destructive method, Fourier Transform Infrared (Li et al. 2003).

### 2.4 Mechanical Behaviour of Biological Tissue

The vasculature consists of a range of different vessels including large arteries, arterioles, capillaries, veins and venules. This complex network is used to provide the tissues with oxygen and nutrients in addition to transport unwanted carbon dioxide and waste products away from the tissue resulting in their ultimate elimination from the body. The artery wall is a complex multi-layered porous structure. Figure 2.2 portrays the different layers of the structure; the intima, the media and the adventitia.

![Artery structure illustrating the main layers: intima, media and adventitia](image)
The intima is the innermost layer and is in direct contact with the blood. It is comprised of a layer of endothelial cells and a sub-endothelial layer which mainly consists of connective tissue and collagen fibres. The outer boundary of the intima is surrounded by the internal elastic lamina an elastic tissue with fenestral pores.

The media layer consists mainly of concentric sheets of smooth muscle cells and elastic connective tissue. This layer is responsible for the artery wall’s ability to contract and relax. Arteries are classified as being an elastic or muscular type according to the relative proportions of these cellular and fibrous components found in this layer. Matrix fibres in the form of well-defined elastic lamellae and collagen bundles are abundant and prominent in elastic arteries whereas smooth muscle cells dominate in muscular arteries and there is subsequently less elastin (Lévy and Tedgui, 1999).

The adventitia is the outermost layer. The external elastic lamina is the inner layer of the adventitia. The outer layer of the adventitia is often difficult to define. It consists of fibroblasts and diagonally-to-axially orientated type I collagen. It also includes elastin fibres and nerves (Taylor and Humphrey, 2009).

2.4.1 Mechanical Variability

Arteries exhibit an anisotropic mechanical behaviour when subjected to uniform stress. This is a result of the heterogeneous and multi-layered nature of arteries. Arterial tissue consists of approximately 70% water (Holzapfel et al., 2000, Richardson, 2002, Taylor and Humphrey, 2009) and it is therefore assumed that arteries are completely incompressible. This implies that stretching in one direction will cause a proportional shrinkage in other direction so as to provide conservation of volume. This simplifies the analysis of stress and strain behaviour during arterial characterisation (Kalita and Schaefer, 2008).

Healthy arteries are highly deformable and exhibit non-linear stress-strain responses coupled with exponential stiffening at high levels of pressure due to the de-crimping of collagen fibrils. The shape of this stress-strain curve is systematically dependent on the location of the artery in the vasculature bed (Maher et al., 2012). Mechanical behaviour is perfectly elastic for proximal elastic arteries and viscoelastic for distal muscular arteries. However, the general mechanical characteristics exhibited by arteries remain constant (Holzapfel et al., 2000).

Experimental studies of multiple specimens from the same vascular location demonstrate that there is a high degree of variability regarding stress-strain behaviour.
between patients. Sommer et al. (2010) conducted an inflation-extension test on the arterial tissue of people aged 67 to 83 years in order to observe the biomechanical response. The tissue specimens exhibit strong nonlinear, pseudoelastic mechanical behaviour with small hysteresis. The resulting pressure-stretch curves from Sommer et al. (2010) study are displayed in figure 2.3.

![Figure 2.3: Inflation-extension data of 10 human intact common carotid artery sections (Sommer et al., 2010).](image)

There is a significant degree of variation between each sample in this data which highlights the variability of biological tissue. However, the three main regions of interest are still visible in each section. There is an initial linear region shown at (A), a region where the slope of the curve alters dramatically at (B) and a final linear region at (C). This corresponds to the stretch of elastin, the recruitment of crimped collagen fibres and the stretch of these collagen fibres (Sommer et al., 2010). Figure 2.3 demonstrates that while arterial tissue varies significantly between samples, the general mechanical characteristics remain the same. The inter-patient variability observed in this study provides further support for patient specific stenting (Pericovic et al., 2009). However, this arterial tissue response can significantly alter with the introduction of atherosclerotic plaque.

### 2.4.2 Diseased Arterial Tissue

At present, there is a limited number of studies that have mechanically tested the behaviour of atherosclerotic carotid artery plaques but not limited to (Stemper et al., 2005, Maher et al., 2009, Teng et al., 2009, Lawlor et al., 2011, Ebenstein et al., 2009a, Kural et al., 2012, Barrett et al., 2009). The main cause for this is the limited number of studies...
studies is the difficulty in sourcing a large number of human specimens of diseased carotid arteries which decreases the ability for these studies to fully characterise the tissue. The findings of these studies cannot distinguish the properties of carotid plaque required to fully characterise the mechanical properties due to the varied compositions of each carotid plaque specimen. It has been acknowledged that the morphology of diseased carotid arteries potentially results in a different mechanical response to balloon angioplasty deployment compared to any other vessel of the body (Herisson et al., 2011, Maher et al., 2012). Therefore, any material properties assigned to carotid plaque based on plaque mechanical data from other vasculature during computational analysis of balloon deployment limits the reliability of the subsequent results. Kural et al. (2012) characterised diseased coronary and carotid arteries for the purpose of computational modelling and demonstrated that carotid specimens were significantly less stiff than coronary specimens in the low and high-modulus regions in the longitudinal (0.91 kPa versus 4.64 kPa) and circumferential direction (1.32 kPa versus 6.38 kPa). Carotid specimens also exhibited significantly greater extensibility than coronaries in both the longitudinal (< 25%) and circumferential (< 20%) directions. The results indicate the variability of the plaque mechanical behaviour based on vasculature location and the need to develop site-specific material models for the coronary, femoral and carotid arteries from experimental studies. Table 2.1 summarises the most relevant mechanical studies on carotid plaques and their respective sample sizes.

Table 2.1: Mechanical studies of atherosclerotic plaques and their respective sample sizes and methods.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Patient No. (Sample No.)</th>
<th>Test type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learoyd and Taylor (1966)</td>
<td>7</td>
<td>Inflation Test</td>
</tr>
<tr>
<td>Ebenstein et al. (2009)</td>
<td>10</td>
<td>Indentation</td>
</tr>
<tr>
<td>Teng et al. (2009)</td>
<td>3</td>
<td>Tensile</td>
</tr>
<tr>
<td>Maher et al. (2009)</td>
<td>14</td>
<td>Compression &amp; Tensile</td>
</tr>
<tr>
<td>Maher et al. (2011)</td>
<td>8 (21)</td>
<td>Compression</td>
</tr>
<tr>
<td>Lawlor et al. (2011)</td>
<td>14 (18)</td>
<td>Tensile</td>
</tr>
<tr>
<td>Kural et al. (2012)</td>
<td>3 (5)</td>
<td>Biaxial</td>
</tr>
</tbody>
</table>

Table 2.1 highlights that the majority of sample sizes remain low, especially seen by Kural et al. (2012), Teng et al. (2009) and Learoyd and Taylor (1966) with sample sizes of 3, 3 and 7 tested respectively. Maher et al. (2009) and Lawlor et al. (2011) are the most applicable studies to the test methods set out in this study as they both utilize uniaxial testing to develop averaged constitutive models of the carotid plaques studied. These studies also contain the highest sample sizes among relevant literature conferring a greater level of reliability to their respective constitutive models.
2.4.3 Geometrical Ratios

Maher et al. (2009) prepared plaque specimens for testing immediately following removal during endarterectomy surgery and mechanically tested the resulting specimens within 2 hours. Specimens were classified before surgery into different plaque types independently by a clinician using routine duplex ultrasound with grayscale medium imaging (GSM). These classifications were then used to group and define each plaque. Each plaque specimen was grouped by carotid artery location as determined by high resolution Magnetic Resonance Imaging (MRI) and dissected at the bifurcation, separating them into common, internal and external carotid segments. Upon dissection the samples were extracted from each location and reduced to width to length ratio (WL) of 1x4mm. This WL ratio is, as according to standard protocol for engineering materials (ASTM-International, 2009), deemed suitable for tensile testing. However, the sample must be homogenous within the gauge length. In the limitations section of the study it was acknowledged by Maher et al. (2009) that such a small distance between gauge lengths can affect the stress distribution within the plaque during testing as well as the inhomogeneity of the sample for a homogenous based test protocol.

Lawlor et al. (2011) set out to mechanically classify fresh carotid arterial plaque through uniaxial tensile testing in the circumferential direction. The study was performed on 18 excised whole carotid plaques from patients undergoing endarterectomy by tensile testing the samples within 2 minutes of excision in the circumferential direction until failure. The results showed large variation across the samples investigated, similar to results from Maher et al. (2009). Lawlor et al. (2011) acknowledged limitations in the tensile testing of their particular study, as the large WL ratio of some of the tested specimens may have induced hearing deformations that are undesirable during pure tensile procedures. From this reasoning, four of the eighteen samples were excluded from the study due to the unsuitable WL ratios whereby the minor strain component deviates towards zero, away from the analytical solution for tensile testing (Lawlor et al., 2011).

Lawlor et al. (2011) hypothesises that whole plaque sampling is a key prerequisite for testing plaque following the reasoning that unlike engineering materials, biological tissues cannot be altered (dissected) to appease geometrical parameters needed for the boundary conditions to analytically develop constitutive material models. Plaque, as a biological tissue, contains interconnected smooth muscle fibres, and cutting these fibres alters the global mechanical properties of the plaque. This whole plaque requirement
will be complied with in this study similar to Lawlor et al. (2011). The preparations procedure of dissecting the tissue carried out by Maher et al. (2009) contradicts this point and demonstrates the difficulty in comparing the mechanical data between the authors mentioned above in Table 2.1 due to the different procedures undertaken between the studies.

2.4.4 **Strain Rate, Pre-Conditioning and Pre-Load**

During a zero-load configuration, straight sectioned arteries are not in a stress-free state. This means that there are residual stresses present within the walls of arterial tissue. This phenomenon can be observed when an artery is excised and cut along its length. The artery walls spring open, indicating that the tissue was subject to an internal force. This is relevant when attempting to predict the state of stress in an artery wall (Vaishnav and Vossoughi, 1987). In a number of experimental studies on biological tissue a pre-load, typically 0.01 – 0.05 N, is applied to straighten the tissue and to take into consideration the residual strain in the circumferential direction in herent in *in vivo* conditions and to ensure that the true gauge length is measured initially. A pre-load of 0.01 N will be incorporated in this study (Maher et al., 2012, Hollenstein et al., 2011).

A stress softening effect is visible in figure 2.4 as the initial loading-unloading curves shift to the right, indicating an increase in stress without an increase in strain (Holzapfel et al., 2000).

![Figure 2.4: Uniaxial stress-strain curve of arterial tissue showing the viscoelastic behaviour and strain softening effect (Holzapfel et al., 2000).](image)

This phenomenon diminishes with each load cycle until the artery exhibits repeatable behaviour. The artery is said to be pre-conditioned once this has occurred and can now be described as perfectly elastic (Point I) or viscoelastic depending on its location. Point
II displays the mechanical response of loading arterial tissue beyond its elastic/viscoelastic domain, which causes inelastic deformation. There is now a large amount of energy dissipated in order to cope with the excessive stressing. This magnitude of loading is far outside the physiological range but may occur during endovascular procedures such as balloon angioplasty. Further display of stress softening is then visible as the material is cyclically loaded and unloaded until the strain reaches Point III at which point a second instance of material preconditioning is complete (Holzapfel et al., 2000). The tissue also displays pseudoelastic behaviour whereby the stress-strain plot is different in the loading and unloading plots (Fung et al., 1979). The purpose of the preconditioning cycles is to demonstrate the necessity to apply appropriate forces or deformations several times in order to recover their in vivo state (Holzapfel et al., 2000, Maher et al., 2011). Within the tensile testing studies relevant to this current body of work, preconditioning was achieved by performing between 2 - 10 loading and unloading cycles to 5 - 10% strain at a constant strain rate. Most studies of biological tissue state that 5 cycles of loading and unloading to 10% stretch is sufficient to fully precondition a sample prior to fracture testing which will be utilised in this study for each plaque tested (Holzapfel et al., 2004, Maher et al., 2009). However, a limitation of current uniaxial tests on diseased tissue is the unrealistic strain rates applied which do not correspond to the in vivo conditions (Maher et al., 2009, Lawlor et al., 2011, Teng et al., 2009).

The majority of experimental studies carry out tensile testing on biological tissue using slow strain rates typically between 0.01 – 0.5 mm/s based on standard protocols for engineering materials (ASTM-International, 2009). The purpose of the slow strain rate is to capture the full characteristics of the mechanical behaviour as well as to quasi-statically analyse the tissue (Maher et al., 2011, Holzapfel et al., 2004, Stemper et al., 2005). However, in order to analyse the tissue in a physiologically realistic environment, the strain rate must simulate the strain rate induced by the cardiac cycle rather than a rate suited for engineering materials. Lawlor et al. (2011) stretched the samples at a constant strain rate of 0.5 mm/s with no pre-conditioning in an attempt to replicate the physiological conditions of angioplasty balloon deployment which is an almost instantaneous increase in diameter. The aim of this current body of work is to use a strain rate which is more comparable to the stretch induced by the systolic peak during the cardiac cycle i.e. 30% of the gauge length per second.
Figure 2.5 (a) shows that the peak systolic pressure of a healthy carotid artery normally reaches 120 mmHg or 16kPa (Greenfield et al., 2003). At this pressure the artery will experience the greatest circumferential stretch or deformation of the cardiac cycle. This occurs at approximately 0.2 s into the pulse cycle, figure 2.5 (b) (Vignon-Clementel et al., 2006).

![Figure 2.5. Pressure waveforms of the carotid artery (Vignon-Clementel et al., 2006) and (Greenfield et al., 2003).](image)

Figure 2.3 from section 2.4.1 demonstrates the circumferential stretch ratio ranges between 6 – 22% in a number of intact human common carotid specimens due to the applied 16 kPa a pressure from Sommer et al. (2010). This indicates that any particular artery has the potential to reach a maximum of 22% of this circumferential stretch under normal physiological conditions. This maximum level of circumferential stretch would not be possible in a diseased artery due to the reduction in lumen and stiffening effects which would indicate a maximum stretch in the lower end of the published range. Therefore, a value of 6% was used as the circumferential stretch at the peak systolic pressure of 16 kPa over 0.2s or 30% over 1 second which will be the strain rate used for fracture testing in this study.

### 2.4.5 Effect of Freezing

Venkatasubramanian et al. (2006) carried out extensive uniaxial testing on frozen porcine femoral arteries. This testing highlighted the change in the stress/strain behaviour at the lower strain ranges due to the alteration of the water content. However, in the Venkatasubramanian et al. (2006) study the tissues were tested over a small strain range that does not take into account the global mechanical behaviour of the tissue necessary for this body of work. Krag and Andreassen (1998) demonstrated that the
ultimate stress values were not significantly different between fresh and frozen biological samples undergoing tensile tests. Similarly, Ebenstein et al. (2009a) presented, using nano-indentation, that the change in Young’s Modulus was relatively small between fresh and frozen carotid plaque tissue in comparison to the four-fold increase in the modulus for the formalin fixed samples. The method of freezing is a clear limitation associated with this study as it has a stiffening effect on the specimens and a deleteriously effect on the collagen fibres of the tissue (Venkatasubramanian et al., 2006). However, the decision was made early in the study to freeze the tissue to allow time to incorporate a process for biologically characterising tissue. Stemper et al. (2007) also suggest that the freezing of tissue only has a slight stiffening effect on the tissue as long as the samples are tested within a couple of hours after tissue is equilibrated. This study also suggests that refrigerating the tissue can reduce to stiffness of the tissue by a significant amount which is in contrast to the conclusion of Loree et al. (1994b).

The freezing process, physiological strain rate and whole specimen testing hypotheses utilised in this study therefore limit the ability to compare the results from this study to similar mechanical test data on atherosclerotic plaques e.g. Maher et al. (2009), Teng et al. (2009) and Lawlor et al. (2011). However, the freezing process was undertaken due to the necessity of ascertaining the maximum amount of data from these inherently elusive samples using multiple testing techniques.

2.4.6 Rupture Definition

Plaque rupture represents the failure of the mechanics in the vessel due to morphological and geometrical changes in areas of high stress within the plaque. It is a crucial aspect of understanding the artery’s potential to failure. Loree et al. (1994) and Vito and Dixon (2003) demonstrated that the distribution of stress in plaque is a function of both the geometry and mechanical properties of the tissue. The main conclusion that can be drawn from Maher et al. (2009) is that there is a high variability, both inter-specimen and intra-specimen, in the mechanical behaviour of these plaques similar to the variability present in healthy arterial tissue. However, there is a scarcity of experimental publications regarding the mechanical and rupture properties of atherosclerotic arterial tissue. Sadat et al. (2010) highlights this point by listing the studies available of the ultimate strength of plaques from the vasculature bed, table 2.2.
Chapter 2

Table 2.2 Summary of ultimate material strength of atherosclerotic tissues of human arteries in the circumferential direction (Sadat et al. 2010).

<table>
<thead>
<tr>
<th>Artery</th>
<th>Author (year)</th>
<th>Ultimate Stress (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Lendon et al. (1993)</td>
<td>Intact fibrous cap 580</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ruptured fibrous cap 190</td>
</tr>
<tr>
<td>Iliac</td>
<td>Holzapfel et al. (2004)</td>
<td>Fibrous cap circumferential 254.8 +/- 79.8</td>
</tr>
<tr>
<td>Coronary</td>
<td>Holzapfel et al. (2005)</td>
<td>Intimal circumferential 394 +/- 223</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adventitial circumferential 1430 +/- 604</td>
</tr>
<tr>
<td>Carotid</td>
<td>Teng et al. (2009)</td>
<td>Media Circumferential 446 +/- 194</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adventitial Circumferential 1802 +/- 703</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Media Circumferential 1230 +/- 533</td>
</tr>
</tbody>
</table>

Teng et al. (2009) performed uniaxial tests on human carotid artery plaque samples in the longitudinal and circumferential direction. The samples were type II and type III atherosclerotic lesions. This study determined the ultimate strength in uniaxial tension for the various layers of the diseased artery specimen as well as intact sections. This study states that diseased carotid arteries are considerably varied in their mechanical properties similar to healthy tissue and that the stress/strain behaviour is unchanged, figure 2.6. Figure 2.6 also demonstrates two failure points caused by the varying strength of the arterial layers which highlights the difficulty to define plaque rupture from uniaxial testing.

![Figure 2.6: Stress-Stretch plot of intact carotid artery plaques from Teng et al (2009) demonstrating the two rupture points due to the layers in the artery.](image)

Stemper et al. (2005) developed a novel method to determine the mechanical properties of the human carotid artery in tension by observing the stretch properties under failure loading. In doing so there was particular emphasis put on the intimal/medial failure similar to Teng et al. (2009). The results of the test showed that the intimal layer was the first to fail under tension, figure 2.7. This layer experienced multiple failures before complete failure. Again, rendering it difficult to define at which stress/strain point initial failure occurs.

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Stemper et al. (2005) specifically assesses the failure at the fibrous cap of the plaque to improve the accuracy of predicting plaque vulnerability by analysing the fracture of the diseased intimal layer using digital videography and stress/strain data, figure 2.7. However, the Teng et al. (2009) study tests large sections of the artery intact and in layers. Teng et al. (2009) suggests that separating the specimen by layer over simplifies the complex nature of the plaque and potentially underestimates the mechanical properties (Holzapfel et al., 2000). As the general behaviour of these specimens is non-linear the use of Young’s modulus as a comparative value is not possible. Therefore, this current body of work will aim to use two rupture points, the ultimate strength and stretch to complete failure and initial rupture caused by the failure of the intima/medial layers, as values to assess plaque rupture. This study will also aim to test the plaque as a whole to avoid any underestimation of the global mechanical properties as suggested by Teng et al. (2009) and Lawlor et al. (2011).

Therefore, this study will define the stretch and stress (MPa) value at initial rupture as the first point of definite rupture where the change in slope (dy/dx) becomes negative, figure 2.8. Complete failure is defined as the point where the stress and stretch values continue to decrease for the remainder of the test procedure. Figure 2.8 illustrates a typical example of plaque behaviour of two extreme plaque types (calcified: black and lipid: grey).
In this example, the softer lipid plaque has a higher stretch ratio before rupture but at a lower stress value. However, the stress-stretch point of complete failure is more evident in the lipid plaque compared to the calcified plaque which has a series of different plaque tears most likely due to the dispersion of calcification throughout the structure of the specimen.

2.4.7 Clamping techniques

Sadate et al. (2010) describes the vascular wall as a complex material which is considered heterogeneous, anisotropic and has a random dispersion of calcification as well as varying thickness, width, shape and curvature. Therefore, it is difficult to ever truly ascertain the true material properties of plaque using a simple tensile test especially due to the varied types of clamping (Ng et al., 2005).

The uniaxial testing method is an ideal test system for testing in only one direction (circumferential). This is useful for this study as there is little need to understand the mechanical properties in the longitudinal direction when in investigating the effect that CAS has on the artery (Sommer et al., 2010). The main contentious limitation of these uniaxial systems is the method of clamping the biological tissues and the negative effects that each technique has on measuring the true mechanical properties of the specimen. In order to design an effective tensile grip for the plaque, an understanding of the various techniques available is needed i.e. fish-hooks, glue and flat clamps.

Lally et al. (2004) utilised a fish-hook method of grasping the tissue specifically in the biaxial testing of the tissue. The advantage of this method is the freedom of movement...
in the minor direction from the sutures and the hold on the collagenous fibres within the tissue. However, the main limitation of this method is the localised stress distributions produced during the high strain ranges of uniaxial testing especially for planar shear geometries.

The concept behind the glue clamping method is that the glue allows for a solid hold on the specimen without deforming the specimen when clamped and when a tensile force is being applied. Teng et al. (2009) also used superglue to attach pieces of sandpaper to the ends of the specimen. This method of clamping is not ideal for wet tissue and there is the possible risk of slippage if the glue is not coated evenly and securely.

Flat-surface clamps exert an even force over two areas at either end of a test specimen. Maher et al. (2009) and Karimi et al. (2013) used flat-surfaced clamps for their respective studies. A study undertaken by Lawlor et al. (2011) also used the flat-surface clamps to hold fresh carotid plaque during tensile load. These clamps had a spiked clamping surface to increase the grip on the atherosclerotic plaque due to the fragile nature of the material and a uniform torque force of 30 cNm was applied to each clamp during each test to failure using a torque screwdriver (BMS Inc., Limerick, Ireland).

The flat-surface method of sample gripping affects the constraint on the extracellular fibres at the bounds of the sample. The applied clamping technique not only affects how the load is transferred to the sample, but also how the load is transmitted throughout the rest of the material—thereby influencing resulting mechanical behaviour of the tissue.

Ng et al. (2005) tested various types of clamping techniques on biological tissue and determined that flat-clamping method with sandpaper and cardboard resulted in the most reliable data. This study also noted that no difference in ultimate strength between the specimens which fracture at the centre and fracture at clamps for all clamp methods.

### 2.4.8 Plaque Imaging and Mechanical Behaviour

The current method of obtaining clinical plaque data is based on morphological data that is obtained from MRI and ultrasound imaging techniques. Li et al. (2003a) determined that the current techniques have insufficient capabilities and functions in terms of predicting the composition and morphology of the plaque specimens globally. However, plaque composition characterisation is a crucial aspect of understanding the arteries but there is little published literature regarding the mechanical properties of atherosclerotic arterial tissue and correlation to biological composition.
Maher et al. (2009) characterised the radial compressive and circumferential tensile behaviour of fresh human carotid atherosclerotic plaque, figure 2.9. The plaque samples were examined using duplex ultrasound which classified the samples broadly into three categories: calcified, mixed and echolucent. The results of these tests alluded to the variation in mechanical behaviour of the samples where by the calcified samples had the stiffest response but no significant difference was noticed from varying the location of the plaque in the carotid bifurcation i.e. common, internal or external. These results suggest that the ultrasound classification method that uses Gray Scale Medium (GSM) does correspond to the mechanical behaviour. However, this current study will investigate these findings on a larger patient number.

Conversely, Lawlor et al. (2011) outlined that there was no significant relationship between post-operative mechanical stiffness and the Yeoh material coefficients developed. Three stiffness groups were identified for the carotid plaques tested by Lawlor et al. (2011), correlating to the titles of hard, mixed and soft. Also, a non-significant relationship was noticed between pre-operative disease type and ultimate tensile strength or ultimate tensile strain. This holds significant weight, as pre-operative classifications are used when considering treatments options.

Holzapfel et al. (2004) used MRI and histology to evaluate the plaque prior to mechanical testing. The results obtained from mechanical tests of the artery sample in the circumferential direction were plotted using the Cauchy stress and stretch curves which illustrated the nonlinear behaviour of the vessel as illustrated in figure 2.10. The results for the plaques were computed using a more accurate anisotropic material model. The variation in responses suggests that there is a distinct difference in the stiffness of healthy and diseased tissue. The calcified samples were found to have the stiffest response and exhibited a linear elastic response. However, Salunke et al. (2001) and
Maher et al. 2009 suggest that the mechanical behaviour of calcified plaques is non-linear which highlights the difficulty in comparing plaque samples between studies as well as patients.

Figure 2.10: Uniaxial tensile stress-stretch response of healthy and diseased media tissue (Holzapfel et al., 2004)

The results obtained from these mechanical tests are an excellent advancement in the understanding the behaviour of the atherosclerotic plaque arteries, however there is no definitive conclusion that can be drawn from these studies with regard to the specific plaque type. Further analytical techniques need to be applied to obtain more data on the heterogeneous atherosclerotic lesions so as to characterise these mechanical responses structurally with respect to the exact composition of the plaque.

Ebenstein et al. (2009) used nano-indentation to measure the mechanical properties of atherosclerotic tissue and from this observed that varying degrees of stiffness exist. Nano-indentation is a technique that permits the determination of mechanical properties for thin films, small volumes and microstructural features. By applying a number of modifications to the original method, it has been shown to be an accurate method to apply to the characterisation of vascular tissue. A novelty of this study is of the Infrared based spectroscopy to identify the plaque components to the Young’s modulus of the tissue.

The issue of pre-operative classification and identification of plaque type and vulnerability is still a non-going debate as is the efficacy and safety of balloon angioplasty. Furthermore, the issue of baroreceptor response to balloon angioplasty deployment has received little attention. The development of a biomimetic material
model of the baroreceptor could be used in a bench top test rig to assess the effect of different carotid angioplasty systems.

### 2.5 Baroreceptor

Baroreceptors are stretch sensitive, spray-like, nerve endings located within the adventitia layer of arterial tissues (Guyton, 1991, Yates and Chen, 1980). They act as blood pressure regulation mechanisms and are extremely abundant in the carotid sinus, the proximal portion of the internal carotid artery, where blood pressure regulation is critical (Sandblom and Axelsson, 2005). During an increase in blood pressure, the carotid sinus distends and the carotid baroreceptors activate. This activation causes an increase in the firing rate of action potentials which signals the nervous system to trigger a decrease in cardiac output and an increase in peripheral vascular resistance which lowers overall blood pressure (Ottesen and Danielsen, 2003). They are extremely abundant in the walls of the carotid sinus and the aortic arch. These nerves relay a constant action potential to the Nucleus of the Tractus Solitarius (NTS) through the glossopharyngeal nerve. When stretched baroreceptors activate an increase in peripheral action potentials to the NTS at an increased frequency (Ottesen and Danielsen, 2003, Sherwood et al., 1995). The NTS interprets the frequency of these action potentials as a measure of blood pressure and adjusts blood pressure accordingly.

Even a small change in baroreceptor reflex sensitivity (BRS) can significantly impair the ability of the cardiovascular system to respond to blood pressure challenges (Jordan et al., 2002, Reza Nouraei et al., 2005). Through this mechanism, baroreceptors act as a short-term regulator of heart rate and blood pressure. The nerves do not respond directly to pressure because they are not activated by a change in the absence of deformation of the artery wall (James, 1971). Pressure is sensed by the baroreceptors in a multi-step process that includes pressure-mechanical deformation in the vessel wall followed by mechanical-electrical transduction in the receptors themselves. The relationship between wall deformation and intravascular pressure is not direct (Brown, 1980). An instantaneous drop in arterial pressure is sensed by the baroreceptors when the wall of the carotid artery is stretched inward, starting a chain of events leading to an increase in heart rate, cardiac contractility, and stimulating contraction of the vessels. The responses activated by the baroreceptor nerves tend to return the arterial blood pressure to its previous value. The baroreceptor mechanism has no long-term regulatory functions. An instantaneous step increase in the carotid sinus pressure is followed by enhanced firing activity in the baroreceptor nerves themselves. This firing activity...
declines significantly the first few seconds and then decays more slowly. The decay continues and the time it takes the firing rate of the baroreceptor signals to reach the pre-stimulation value can take 1–3 days (Guyton, 1991, Ottesen, 2003, Taher et al., 1988).

2.5.1 **Atherosclerosis effect on the Baroreceptor**

Atherosclerosis damages the baroreceptor sensitivity and therefore causes a significant variability in the blood pressure of the patient due to an increase in sympathetic signals to the heart and increasing heart rate variability (Robinson et al., 1997). A study undertaken by Yun et al. (2005) proposes that atherosclerotic disease at the carotid bifurcation and sinus can interfere with baroreceptor function by buffering against accurate detection of physical parameters, such as blood pressure and vessel wall all deformation. Misperception of hypotension (low blood pressure) from the central nervous system can cause an unnecessary decrease in heart rate, thereby compromising blood flow rate and blood pressure. Studies by Akinola et al. (1999), ANGELL-JAMES (1974) and Tyden et al. (1980) have reported that the presence of rigid atheroma at the carotid sinus region is associated with a resetting of the baroreceptors to operate at a higher threshold pressure but also with a decreased baroreceptor sensitivity involving an impairment of the baroreceptor detection activity.

2.5.2 **Surgical Effects on Baroreceptors**

Baroreceptor function is deleteriously affected during CE by the incisions made through the arterial layers. These incisions strip baroreceptor nerve endings from the lumen causing increased arterial pressure instability and in extreme cases, hypertension due to decreased baroreceptor sensitivity (Sigaudo-Roussel et al., 2002, Timmers et al., 2004). CAS has a similar deleterious effect on baroreceptor function due to the sustained stretch caused by balloon deployment (McKevitt et al., 2003, Mangin et al., 2003). A study by Yun et al. (2005) has also shown that angioplasty, stenting, and carotid endarterectomy may inadvertently cause acute and chronic carotid sensor dysfunction through manipulation, atherial interposition, and balloon-induced baroreceptor injury. The over-stretching of the artery may also damage the baroreceptor and lead to decreased sensitivity of the baroreflex (Yun et al., 2005). CAS involving the bifurcation and the carotid sinuses causes marked changes in the cardiac autonomic and respiratory control systems, such as the blood pressure and heart rate, according to Mangin et al. (2003). However, there is the potential to limit this
damaging effect by varying the balloon inflation parameters which will be analysed in this study.

As it is not viable to measure baroreceptor function during CAS procedures, it is therefore necessary to experimentally replicate CAS conditions using a bench-top test system that employs a simulated carotid bifurcation (CB) model. This study aims to investigate the feasibility of developing a material that could be used in an experimental model of the CB complete with baroreceptor response to strain, so that it may be employed in future studies to identify and quantify the deleterious effects of subjecting the baroreceptor nerves to sustained circumferential stretch such as in the case of CAS.

To assess this suitability parameter, this study will perform uniaxial tensile testing on electrically conductive silicone (ECS) and develop a suitable strain energy function (SEF) for use in finite element analysis (FEA). This will allow the ECS to be fully compared to published data on carotid arterial tissue. This study will also examine the electrical behaviour of the ECS and determine if it exhibits a measurable electrical response to strain with an inherent ability to emulate the two key characteristics of baroreceptor function relating to CAS, adaption to sustained strain and recovery from strain (Ottesen and Danielsen, 2003).

2.6 Refined Objectives

From the literature reviewed in this section the objectives from section 1.1 have been refined to the following list:

1. Develop a mimetic material of the carotid artery mechanical behaviour which incorporates the baroreceptor function.
   a. Develop an electrically conductive silicone material which can be injection moulded.
   b. Develop a test method for examining the mechanical and electrical characteristics of the resultant material.
   c. Investigate the feasibility of the material regarding replicating the mechanical behaviour of carotid tissue and baroreceptor response as well as its ability to be used in the Lost Wax Method.

2. Mechanically and biologically characterise human carotid plaque tissue.
   a. Examine the limitations of the current protocol and geometrical standards of uniaxial testing biological tissue using Finite Element
Analysis in an attempt to define the width-to-length ratios suitable for mechanical testing.

b. Mechanically test human plaque tissue using physiologically realistic strain rates.

c. Develop and validate a process of biologically characterising the plaque tissue without damaging the tissue prior to mechanical testing.

d. Investigate the feasibility of predicting plaque behaviour using the developed biological characterisation method.

e. Examine the key features at the sites of rupture using electron microscopy.

3. Use FEA to highlight the advantages of a novel angioplasty balloon to current technology which reduces the risk of plaque rupture and the reduced over-stretching of the baroreceptor nerves which cause peri-operative complications.

a. Use curve-fitting models to develop material coefficients for a suitable strain energy function and apply to FEA models.

b. Compare balloon designs and highlight improved characteristics of balloon on the plaque rupture potential and baroreceptor function.

References


Chapter 2

Literature Review


Chapter 2

Literature Review


CHAPTER 3

DEVELOPMENT OF AN EXPERIMENTAL MODEL OF THE CAROTID BIFURCATION USING ELECTRICALLY CONDUCTIVE SILICONE:

AN INTRODUCTION TO THE INCORPORATION OF BARORECEPTOR FUNCTION WITHIN A MIMETIC MODEL OF THE CAROTID ARTERY

John J. Mulvihill, Eoghan M. Cunnane, Barry M. O’Connell and Michael T. Walsh*.
Centre for Applied Biomedical Engineering Research, Department of Mechanical, Aeronautical, and Biomedical Engineering, Material and Surface Science Institute, University of Limerick, Limerick, Ireland, University of Limerick, Limerick, Ireland.

The following chapter presents a paper on the development of a biomimetic material of the carotid bifurcation with baroreceptor function to be used in an experimental rig for the deployment of current balloon angioplasty devices. This chapter is published in the International Journal of Nano and Biomaterials and presented verbatim, however in a more reader friendly format.

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3 Development of an Experimental Model of the Carotid Bifurcation Using Electrically Conductive Silicone

Abstract

This study assesses the suitability of developing a material for use in an experimental model of the carotid baroreceptors. Such a model could then be used in future studies to assess the impact of carotid artery stenting on hemodynamic stability. The material must exhibit a significant measurable electrical response to strain in a fashion analogous to baroreceptor behaviour. A modified electrically conductive silicone (ECS) was examined for use as the material, which was generated from a combination of Wacker LR 3162 and silicone thinner. Samples of the ECS were subjected to uniaxial tensile testing and electrical stimulation in order to mechanically and electrically characterise the material. Testing revealed that the ECS exhibits mechanical behaviour comparable to published data on carotid arterial tissue up to 20% strain and a measurable electrical response to strain in a fashion qualitatively comparable to baroreceptor behaviour. These findings highlight the potential of this material for employment as an experimental model of the carotid baroreceptors.

Keywords: baroreceptor, carotid bifurcation, electrically conductive silicone, material characterisation, analogue material

3.1 Introduction

Stroke is a catastrophic cerebral event that can result in the necrosis of affected brain cells. Such events cause irreversible damage to an array of neurological functions in 22% to 25% of victims and death within one year for 25% of victims (Medtech Insight, 2011). The likelihood of stroke is directly related to the presence of stenosis in the carotid arteries, which are a paired set of vessels that supply oxygenated blood to the cranium (Guyton, 1991). The common carotid arteries bifurcate into the internal and external carotids which lead to the brain and face respectively. Stenosis is particularly prevalent at the site of bifurcations and this has been attributed to considerable increases in transverse average velocity gradients and viscous shear stresses that cause preferential damage to the lining of the artery and initiate or perpetuate arterial injury in
Experimental model of the carotid bifurcation

a manner that induces development of arterio-atherosclerosis (Scharfstein et al., 1963).

Carotid Artery Stenting (CAS) is a minimally invasive procedure that deploys a stent at the site of stenosis, compressing the plaque and widening the lumen to increase blood flow. Despite the numerous advantages of CAS over traditional carotid surgery such as increased accessibility, decreased trauma and absence of general anesthetic (Roffi et al., 2009), it remains the second choice for clinicians and accounts for only 26% of stroke prevention procedures performed (Paraskevas et al., 2009). This low surgical uptake is due to unfavourable clinical trial results that cited embol release and hemodynamic instability caused by altered baroreceptor behaviour as a complication in 21% to 51% of cases (Gupta et al., 2005).

Baroreceptors are stretch sensitive, spray-like, nerve endings located within the adventitia layer of arterial tissues (Guyton, 1991; Yates and Chen, 1980). They act as blood pressure regulation mechanisms and are extremely abundant in the carotid sinus, the proximal portion of the internal carotid artery, where blood pressure regulation is critical (Sandblom and Axelsson, 2005). During an increase in blood pressure, the carotid sinus distends and the carotid baroreceptors activate. This activation causes an increase in the firing rate of action potentials which signals the nervous system to trigger a decrease in cardiac output and an increase in peripheral vascular resistance which lowers overall blood pressure (Ottesen and Danielsen, 2003). When attempting to model baroreceptor behaviour, there are four non-linear phenomena that must be considered (Ottesen and Danielsen, 2003). These features are as follows:

1. A threshold and saturation pressure under and above which the baroreceptor firing rate is unresponsive to strain (Figure 3.1 (a)).
2. Asymmetric responsive behaviour indicated by hysteresis present between firing rate during baroreceptor stretching and un-stretching (Figure 3.1 (a)).
3. Adaption to a new firing rate base value when subjected to prolonged stretch as depicted by section B in figure 3.1 (b).
4. Recovery to base value following a step response to carotid sinus stimulus as depicted by section C in figure 3.1 (b).
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Figure 3.1: Characteristic behaviour of baroreceptors; (a) Asymmetric firing rate response of baroreceptors to pressure increase based on experimental data; (b) Firing rate response to a step increase in pressure from 170 to 178 mmHg at time 2.5 s. Pressure forced back to 170 mmHg at time 12.5 s (Ottesen & Danielsen, 2003).

CAS has a deleterious effect on baroreceptor function due to the sustained stretch caused by balloon deployment. However, there is potential to limit this damaging effect by varying the balloon inflation parameters. An investigation into the response of the baroreceptor function to the deployment of current balloon devices used in CAS is necessary to improve their design. As it is not viable to measure baroreceptor function during CAS procedures, it is therefore necessary to experimentally replicate CAS conditions using a bench-top test system that employs a simulated carotid bifurcation (CB) model. This paper aims to investigate the feasibility of developing a material that could be used in an experimental model of the CB complete with baroreceptor response to train, so that it may be employed in future studies to identify and quantify the deleterious effects of subjecting the baroreceptor nerves to sustained circumferential stretch such as in the case of CAS.

Current materials used in the development of simulated arteries, such as Elastosil RT 601 (Wacker-Chemie GMBH, Munich, Germany) and Sylgard 184 (Dow Corning Corp., USA), have mechanical properties similar to arterial tissue but do not possess the appropriate electrical properties \( (10^{15} \Omega \cdot \text{cm}) \) to imitate the firing rate response of the baroreceptors (Corbett et al., 2010; O’Brien et al., 2005; Wacker Chemicals Ltd., 2010). It is possible to synthesise these silicones with conductive carbon filler particles to induce electrical conductivity which occurs due to conductive carbon pathways that arise throughout the materials insulative silicone matrix (Saleem et al., 2010). There is however, a large variability in conductivity across the resulting material samples (Sau et al., 1998). A suitable alternative is to acquire a commercially available refill electrically conductive silicone (ECS). Wacker Elastosil LR3162 (Wacker-Chemie,
Munich, Germany) is one such material. However, despite its favourable electrical properties, the suitability of the ECS as an arterial mimetic material is unestablished. To assess this suitability parameter, this study will perform uniaxial tensile testing on the chosen ECS and develop a suitable strain energy function (SEF) for use in finite element analysis (FEA). This will allow the ECS to be fully compared to published data on carotid arterial tissue. This study will also examine the electrical behaviour of the ECS and determine if it exhibits a measurable electrical response to strain with an inherent ability to emulate the two key characteristics of baroreceptor function relating to CAS, adaption to sustained strain and recovery from strain (Ottesen and Danielsen, 2003).

3.2 Material and Methods

3.2.1 Material Preparation

LR3162 is a two-part, prefilled silicone-carbon composite. It has a volume resistivity of 11 Ω.cm and a viscosity of 6600 Pa.s, compared to values of 10^{15} Ω.cm and 3.5 Pa.s for Elastosil RT 601 (Wacker Chemicals Ltd., 2011; Wacker Chemicals Ltd., 2010). As future studies may wish to develop physical CB silicone models that require a wax core to create the mimic lumen, it is essential that the material be injectable at room temperature using a syringe so as not to damage the wax core. The viscosity of this ECS is relatively high compared to established mimetic silicones, which makes injecting the LR 3162 at room temperature using a syringe difficult and therefore inappropriate for this process. For this reason the LR 3162 was mixed with a silicone thinner (Dow Corning 200/5CS, Midland, MI, USA) which has a viscosity of 4.55x10^{-3} Pa.s. The addition of this silicone thinner has both a lubricating and thinning effect on the final mixture which aids in the mixing and injection processes.

The methodology employed in the mixing of an ECS has a significant effect on the electrical properties of the final material. Excessive shear during mechanical mixing degrades the carbon filler and makes the formation of conductive pathways less likely, while insufficient shear leads to poor filler distribution. The level of shear experienced during mixing is inversely proportional to the viscosity of the ECS (Zhang et al., 2007). For this reason it is desirable to develop an ECS with a viscosity just below the threshold of formability and to expose this silicone to low levels of shear for prolonged periods of time to maximise filler distribution and minimise carbon filler degradation.
The two silicone components were mixed in five different ratios using an in-house developed mechanical mixing apparatus that applies uniform rotational force to minimise excessive shearing. The ratios ranged from 50:50 to 90:10 (LR 3162:Thinner, by weight) in increments of ten. Ratios containing less than 50% LR 3162 were disregarded in an attempt to limit the reduction of viscosity and preserve carbon particle integrity during mixing. LR 3162 parts A and B were mixed in equal measures and the silicone thinner was added in the desired ratio. The resulting mixture was mixed using the aforementioned mechanical mixer.

Each ratio of the ECS was analysed in terms of curability, injectability and conductivity. Curability was defined as the ability of the material to be removed from the mould with minimal sticking and lack of colour transfer from the material after curing (Mehnert et al., 2001). The material was deemed injectable if it could be injected into a mould initially at atmospheric pressure through a 2 mm diameter syringe. Conductivity was tested by passing a current through cured, dog-bone shaped samples of the material at two points.

3.2.2 Experimental Apparatus and Procedure

The ECS was mechanically and electrically characterised using an in-house developed tensile tester and a video extensometry device, figure 3.2. The stress-strain information obtained from the tensile tester and the conductivity-strain information obtained from the video extensometer were recorded. As the strain values throughout the experiment were being measured separately using the tensile tester and the video extensometer, the two sets of values were calibrated to match during testing to minimise discrepancies and keep stress, strain and conductivity parameters in-phase.

Five samples of the ECS were subjected to a known voltage and current and exposed to four cycles of strain using the tensile tester. The first three cycles, 50% strain at 0.001 m/s, were performed to precondition the material and ensure that the results obtained were uniform over a number of training cycles by eliminating the Mullin’s effect which is responsible for initial excessive material stiffness (Sau et al., 1998). The fourth cycle stretched the samples to 150% strain at a constant rate of 0.001 m/s. This cycle was used to characterise the material and develop the SEF. A relatively large strain value was used to ensure that a fully stabilised SEF was developed from the resulting stress-strain curves. Stability of SEFs is drawn into question when attempting to predict strain ranges above those examined during experimental testing (Holzapfel et al., 2004),
therefore s training up to a near rupture value was conducted in the event that future studies wish to utilise the SEF developed in this study for larger strain applications.

Figure 3.2: Diagrammatical representation of the experimental apparatus: tensile tester, power supply, video extensometer, crocodile clips and computer used to test the cured dog-bone shaped samples of the ECS.

The baroreceptor characteristics of adaption to strain and recovery from strain were compared to the behaviour of the ECS by subjecting three samples of the ECS to a known voltage and current and straining each sample to 150% strain. This strain was maintained for ten minutes and then each sample was returned to its original length. The voltage drop across each sample was monitored during this process and also monitored for an additional ten minutes after returning the sample to its original length. The relative voltage drop across the material sample during strain $V^*$, was identified as the conductivity parameter. This was used to eliminate the effect caused by variances in crocodile clip location and was achieved by presenting each voltage drop measurement value relative to the first value.

3.2.3 Computational

An idealised CB geometric model was created in Pro/Engineer Wildfire 5.0 (Parametric Technology Corp., Needham, MA, USA) and imported into and analysed in ABAQUS/Standard 6.10-1 (Dassault Systems, SIMULA, Providence, RI, USA) to fully compare the mechanical properties of the modified LR 3162 to a SEF developed to represent carotid artery mechanical behaviour (Gasser et al., 2006). The geometric model of the CB used in this computational study is based on an idealised CB geometry proposed by Smith et al. (1996) and defined by Ding et al. (2001), table 3.1. The common carotid internal diameter of 6.1 mm is based on a study conducted by Krejza et al. (2006). The straight artery sections that represent the common, internal and external carotids were created using boundary blends and the bifurcation section was generated using a six sided patch, figure 3.3.
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Figure 3.3: (a) Cross-sectional view of the idealised CB geometric model; (b) dimensioned diagram of the apex geometry developed in order to generate the idealised CB geometric model.

Table 3.1: Dimensions used to develop the idealised CB geometric model.

<table>
<thead>
<tr>
<th>Dimension (mm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.1</td>
<td>4.21</td>
<td>4.21</td>
<td>6.34</td>
<td>6.771</td>
<td>4.39</td>
<td>11.81</td>
</tr>
<tr>
<td>H</td>
<td>5.93</td>
<td>0.5</td>
<td>11.7</td>
<td>5.98</td>
<td>6.75</td>
<td>14.64</td>
<td>60.5°</td>
</tr>
</tbody>
</table>

This idealised CB geometric model modifies the geometry defined by Ding et al. (2001) by generating a previously undefined curved apex. This apex was defined as tangent to both the inner internal and external artery walls which gave the appearance of a natural curvature similar to that found in the carotid bifurcations of young adults (Thomas et al., 2005). This geometric model also accounts for the varying artery wall thickness that features throughout the carotid bifurcation. It achieves this by integrating the work of Delfino et al. (1997) which denotes the thicknesses at ten locations across the bifurcation area. The dimensions used to create the idealised CB geometric model are listed in Table 3.1.

3.2.4 FEA Study

The idealised CB geometric model was imported into ABAQUS/Standard 6.10-1 (Dassault Systems, SIMULA, Providence, RI, USA) as a SAT file. A free 10-node quadratic tetrahedral mesh was applied with the hybrid formulation activated due to the hyperelasticity of the ECS and carotid tissue material models. Symmetry, along the axis, was applied to all three artery branches and an axisymmetric boundary condition was applied along the thickness of the CB geometric model. An internal pressure of 16 kPa was applied to mimic the peak systolic pressure within a typical carotid arterial system.
Two material models, one for carotid tissue and one for the ECS, were applied to the idealised CB geometric model and their behaviour compared under the applied load. The material model for arterial tissue was a SEF developed by Gasser et al. (2006), known as the Holzapfel-Gasser-Ogden (HGO) SEF, which is a histological and phenomenological SEF of carotid artery tissue, equation 3.1.

\[
W = \frac{\mu}{2}(\bar{I}_1 - 3) + \frac{1}{D}\left(\frac{(J_{el})^2 - 1}{2} - \ln J_{el}\right) + \frac{k_1}{2k_2}N \sum_{\alpha=1}^{N} \left\{ \exp[k_2(\bar{E}_\alpha)^2] - 1 \right\} \tag{3.1}
\]

The strain energy per unit of reference volume is represented as \(W\). \(\mu\), \(D\), \(k_1\), \(k_2\) and \(\kappa\) are temperature-dependent material parameters, \(\bar{I}_1\) and \(\bar{I}_4\) are invariants of the Cauchy Green strain tensor and \(J_{el}\) is the elastic volume ratio. \(N\) is the number of families of fibres (\(N \leq 3\)). The HGO model assumes that collagen fibre direction within each family are dispersed (with rotational symmetry) about a mean preferred direction. The parameter \(\kappa\) (\(0 \leq \kappa \leq 1/3\)) describes the level of dispersion in the fibre directions and is defined within the parameter \(\bar{E}_\alpha\), equation 3.2.

\[
\bar{E}_\alpha \equiv \kappa(\bar{I}_1 - 3) + (1 - 3\kappa)(\bar{I}_{4(\alpha \alpha)} - 1) \tag{3.2}
\]

The parameters were based on the behaviour of ten intact carotid arteries examined during a study undertaken by Sommer and Holzapfel (2012). A number of assumptions were made to simplify the material due to the complex nature of the geometry; the model was assumed incompressible (\(D = 0\)) and isotropic (\(\kappa = 1/3\)). The second step of the computational study was to create a material model based on the ECS material using a hyperelastic SEF, equation 3.3.

\[
W(I_1) = \sum_{i=1}^{3} C_{i0}(I_1 - 3)^i \tag{3.3}
\]

The material model used to characterise the ECS was a third-order polynomial SEF known as the Yeoh form, equation 3.3. The Yeoh SEF is suitable for characterising hyperelastic materials using uniaxial mechanical data as the function does not depend on the second strain invariant of the Cauchy–Green deformation tensor, \(\bar{I}_2\). \(C_{i0}\) are the material coefficients and \(I_1\) is the first-strain invariant which is based on the principal stretch ratios, equation 3.4.

\[
I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \tag{3.4}
\]
Chapter 3: Experimental model of the carotid bifurcation

The material coefficients are derived by acquiring uniaxial test data and curve-fitting to the stress-strain data. An optimisation technique was applied in this study to minimise the difference in stress values between the Yeoh SEF and the experimental data.

3.3 Results

3.3.1 ECS Preliminary Test Results

The ECS ratios between 50:50 and 70:30 were deemed to be curable, injectable and conductive while the remaining two ratios failed to inject, table 3.2.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Injectable</th>
<th>Curable</th>
<th>Conductive</th>
</tr>
</thead>
<tbody>
<tr>
<td>50:50</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>60:40</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>70:30</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>80:20</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>90:10</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The carbon filler distribution of each suitable ratio was investigated using a combination of Scanning Electron Microscopy (SEM) and Elastic Recoil Detection (ERD). Figure 3.4 (a) displays an image of the microstructure of the ECS cross-section of the 60:40 ratio obtained using SEM. Figure 3.4 (b) displays the distribution of carbon content across the black line visible in figure 3.4 (a) which represents a 15 µm “line of interest”. This figure displays an arbitrary carbon content which represents values relative to the first carbon content value obtained. The values displayed along the y-axis are arbitrary and the purpose of the chart is to portray the carbon content distribution rather than the carbon content values.

Figure 3.4: (a) SEM image of the 60:40 ECS ratio microstructure with a 15 µm “line of interest” denoted by a diagonal black line; (b) Arbitrary carbon content distribution along this “line of interest” obtained using ERD.
3.3.2 Mechanical Characterisation

The diameter of a diseased lumen can range from 10-30% of the original diameter (6.1 mm) and be increased to a lumen diameter of 70-90% of the original diameter (Krejza et al., 2006). Assuming the artery and plaque to be concentric and incompressible, to facilitate calculations in the realistic diseased artery, the following strain values (table 3.3) induced in the external diameter of the carotid artery due to circumferential stretch were hypothesised. The strain in the external diameter was examined as baroreceptors are predominantly located within the adventitia layer of arterial tissues (Yates and Chen, 1980).

Table 3.3: Strain values due to circumferential stretch during CAS when increasing the stenosed lumen (10 to 30%) to restored healthy lumen (70 to 90%) (Krejza et al., 2006).

<table>
<thead>
<tr>
<th>% of healthy lumen</th>
<th>70</th>
<th>80</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13.4</td>
<td>17.3</td>
<td>21.5</td>
</tr>
<tr>
<td>20</td>
<td>12.6</td>
<td>16.5</td>
<td>20.8</td>
</tr>
<tr>
<td>30</td>
<td>11.3</td>
<td>15.2</td>
<td>19.5</td>
</tr>
</tbody>
</table>

Therefore, the strain range within which the ECS must replicate the carotid artery mechanical behaviour is between 11.3 - 21.5% (30% to 70% - 10% to 90%), which will be taken as the approximate maximum value of 20%.

The Yeoh SEF analytical curve and the average uniaxial tensile test data of five samples of the ECS are displayed in figure 3.5, denoted as ECS – Yeoh and ECS – Expt respectively. Also included are published longitudinal uniaxial data for human carotid tissue obtained from Stemper et al. (2005) and circumferential and longitudinal uniaxial data of porcine carotid tissue obtained from Silver et al. (2003).

![Figure 3.5: Experimental and Analytical uniaxial data for ECS and carotid tissue adapted from Stemper et al. (2005) and Silver et al. (2003) (a) entire strain range; (b) up to 20% strain.](image)
Figure 3.5 (b) highlights the replicative response of the ECS to uniaxial carotid tissue behaviour up to 20% strain for human tissue and porcine tissue. The coefficients used to define the analytical curve were derived from the averaged curve of the five ECS samples, Table 3.4. These coefficients were optimised and are stable over all experimental strains.

Table 3.4: Material coefficients developed for ECS material based on the Yeoh function

<table>
<thead>
<tr>
<th>C_{10} (MPa)</th>
<th>C_{20} (MPa)</th>
<th>C_{30} (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.58e-02</td>
<td>3.68e-03</td>
<td>6.59e-04</td>
</tr>
</tbody>
</table>

### 3.3.3 FEA Study

The material coefficients were applied to the Yeoh material model that represents the ECS in the FE analysis and a comparison was carried out against the HGO material model of carotid tissue, denoted as ECS – Yeoh SEF and Carotid – HGO SEF, respectively. The data is averaged from a plane of nodes, perpendicular to the external artery wall, in the idealised CB geometric model at the base of the carotid sinus, where the baroreceptors are most abundant.

As the mechanical properties of carotid tissue vary significantly from specimen to specimen, it is more accurate to consider a range of specimens rather than just one sample. For this reason, the HGO data generated in this study is representative of the behaviour of ten intact carotid specimens from Sommer and Holzapfel (2012) applied to the HGO SEF, shaded region denoted as Carotid – HGO SEF. Figure 3.6 (a) and (b) illustrate the increase in circumferential stretch ratio (external diameter) of the CB with a steady increase in pressure, from 0-16 kPa, for both the Yeoh and HGO SEF models.

Figure 3.6: Change in circumferential stretch of the CB at the base of the carotid sinus with an increase in pressure for the carotid (HGO) and ECS (Yeoh) material models (a) entire stretch range; (b) up to 20% stretch. Shaded region, Carotid – HGO SEF, represents...
3.3.4 Electrical Characterisation

Figure 3.7 shows the averaged resulting electrical response of five ECS samples to strain. The error bars depict the spread of experimental data at each point.

![Figure 3.7: Averaged data of five samples depicting the electrical response of the ECS to strain including error bars depicting standard deviation (a) the whole strain range; (b) up to 20% strain.](image)

3.3.5 Baroreceptor Comparison

Baroreceptors located in the carotid sinus exhibit non-linear physiological phenomena. Due to this, the ECS was examined for the two most relevant parameters regarding CAS, adaption to sustained strain and recovery from strain. The averaged data for the three samples tested for these phenomena is displayed in Figure 3.8 with the baroreceptor response over a 25 s period developed by Ottesen and Danielsen (2003) inset.

![Figure 3.8: Electrical behaviour of the ECS regarding adaption to sustained strain and recovery from sustained strain which is qualitatively similar to the baroreceptor behaviour seen in Figure 3.2 (b), inset.](image)
3.4 Discussion

3.4.1 ECS Preliminary Test

Preliminary testing of the ECS material ratios was carried out to inspect for electrical conductivity through the material and assess each ratio’s suitability for injection moulding. Three ratios, 50:50, 60:40 and 70:30, were deemed suitable under both criteria and further testing using SEM and ERD highlighted that the 60:40 ratio had the most uniform distribution of carbon filler of the three ratios tested. This ratio was therefore chosen as the conductive material to be tested for use in the proposed experimental baroreceptor model. The favourable behaviour displayed by this ratio was attributed to a suitable viscosity value that resulted in ideal shearing conditions during mixing which allowed for sufficient carbon filler distribution, figure 3.4. This can be surmised as there are no obvious areas of carbon coagulation or areas of carbon deficiency within the silicone matrix.

3.4.2 Mechanical Characterisation

The uniaxial tensile testing of the optimal ECS ratio was carried out to establish if this specific type of silicone exhibits suitable mechanical behaviour to act as a mimic material of carotid arterial tissue. The testing shows that over a larger strain range the ECS behaves differently to human carotid arterial tissue as its stiffening response occurs at higher strains, figure 3.5. However, the ECS material compares favourably to circumferential mechanical properties of porcine carotid tissue, which mechanically behaves similarly to human arterial tissue in the longitudinal direction, figure 3.5 (a). Comparing the results up to the proposed averaged strain that occurs during stenting shows that all four sample sets behave similarly up to 20% strain. The deviation between the ECS and human arterial tissue may occur due to the fact that the ECS is a relatively homogenous material in comparison to arterial tissue which contains collagen fibres that stiffen at higher strains (Gasser et al., 2006) and the arterial tissue was tested in the longitudinal direction (Stemper et al., 2005).

Baroreceptors activate subject to di stension which is a function of radial strain and circumferential stretch in the artery wall. This study is limited to measuring uniaxial stretch of dog-bone shaped samples in pure tension with a uniform stress field in the length of the specimen which is then assumed to equate to circumferential stretch within the artery wall. This study does not take into account the residual stress in the circumferential and axial direction of the carotid artery as well as the non-uniform stress.
field within the artery wall. To overcome this limitation a significant sample set of dog-bone samples excised from cylindrical shaped tubes of ECS should be created and tested using a similar procedure to that which is employed in this study to take into account the circumferential residual stress. This will allow for improved mechanical and electrical characterisation of the ECS for both computational and baroreceptor feedback models.

3.4.3 FEA Study

Figure 3.6 illustrates the significant difference in mechanical behaviour over the examined strain range between the two material models. The idealised CB geometric model, commonly known as the tuning fork model, has been shown to correlate closer to the actual geometry of the CB during \textit{in vivo} and \textit{in vitro} studies than alternate idealised geometries (Ding et al., 2001, Thomas et al., 2005). The data plot, Carotid - HGO SEF, represents a range of intact specimens based on parameters from literature (Sommer and Holzapfel, 2012). Figure 3.6 (b) shows that the ECS mechanical behaviour correlates with the initial 20% stretch. However, the mechanical behaviour of both material models over the larger strain range does not correlate. The carotid arteries began to stiffen considerably after a 5 – 15% stretch unlike the ECS which behaves linearly elastically after 15% stretch. The discrepancies between the models are large as the HGO SEF is based on test data requiring $I_1$ and $I_4$ strain invariants, whereas the Yeoh SEF is based on uniaxial tensile tests, $I_1$ strain invariant only. The Yeoh SEF is not suitable to fully characterise the material for a complex multiaxial model such as the idealised CB geometric model. Further mechanical characterisation of the ECS material is necessary such as inflation-extension and planar biaxial tests. The HGO SEF, used to represent the carotid arterial tissue, was over-simplified as a constant incompressible model while the Yeoh SEF, used to represent the ECS, did not contain circumferential residual stresses or the 10% axial residual stress inherent in a realistic arterial geometry (Hariton et al., 2007).

3.4.4 Electrical Characterisation

Figure 3.7 displays a distinct change in electrical behaviour in response to strain. This occurs due to the breakdown and formation of conductive carbon pathways within the silicone matrix (Zhang et al., 2007). In this case, the breakdown of pathways is more predominant than the formation and therefore the material becomes less conductive and $V^*$ increases. Due to this distinct measurable change in electrical behaviour in response to strain coupled with the relatively limited variability present within the first 20%
strain, visible in figure 3.7 (b), this material has the potential to act as an analogue to baroreceptor firing behaviour.

This study examines electrical behaviour based on voltage drop across a set of samples during tensile testing. This method of measuring electrical behaviour does not account for the relatively minute change in current across the material. To overcome this limitation, future studies should develop a method of logging real-time values for resistance across the material that are in-phase with the corresponding values for stress and strain. However, it should be noted that in the scope of this study, \( V^* \) is an adequate electrical parameter as it is a quantifiable electrical response to strain.

### 3.4.5 Baroreceptor Comparison

In sector A of figure 3.8, the ECS exhibits a ramped response to strain that causes the resistance of the material to rise sharply. This can be attributed to the instantaneous destruction of conductive carbon pathways. In section B the ECS is maintained at a sustained strain and decay in the resistance can be observed. This decay is due to the formation of new conductive carbon pathways. Once the maximum number of available carbon pathways have aligned, the resistance of the material plateaus at a new equilibrium point. This behaviour is qualitatively similar to the reset mechanism of the baroreceptors, section B of inset. In section C the applied load is removed from the ECS and the sample is returned to 0 % elongation. This causes a further spontaneous rise in resistance, illustrated by the peak, as the newly formed carbon chains break, followed by a drop in the material resistance as the conductive carbon chains that were broken upon stretching quickly realign. This peak is not exhibited by the baroreceptor firing rate behaviour which, instead, displays a sharp drop below the original firing rate once the distension has ceased and then a gradual increase to the initial firing rate. This drop occurs as the firing rate briefly goes to zero before returning to a base value after the removal of the applied strain. Applying an initial stretch to each ECS sample during testing may help in emulating this baroreceptor occurrence by setting the electrical base value at a number higher than zero.

The rate of decay of resistance of the ECS then slows once all of the carbon particles in close proximity form conductive pathways. More distant particles continue to form pathways at a slower rate resulting in a more gradual recovery to the original equilibrium value. This behaviour is qualitatively similar to the recovery mechanism of the carotid baroreceptors, section C of inset.
By comparing the trends presented in this chart to those presented by Ottesen and Danielsen (2003) inset, it can be observed that both the ECS and the baroreceptors share a similar response to sustained strain and a similar recovery response following straining. This highlights the materials potential for use as a baroreceptor model as it allows for the examination of the short term effects on baroreceptor functionality when deploying a stent. To conduct such an examination, the material should be exposed to strain conditions similar to those experienced during CAS. As this material exhibits an electrical adaptation response qualitatively similar to the carotid baroreceptors, this examination would allow for long term feedback regarding the changes in blood flow and pressure that occur with an increase in firing activity of the baroreceptors similar to conditions experienced during CAS.

Further studies are required to fully assess the materials suitability and should address the isues of time scale and initial firing rate differences between the two feedback phenomena visible in figure 3.8 and the inset. These issues should be addressed by increasing the strain rate applied to the ECS samples in order to match strain times and also by applying residual strain to the ECS samples in order to match the initial firing rate responses. Difficulties will arise in future studies when measuring the voltage drop across the thickness of a replicative silicone model, similar to the computational model, due to relatively small wall thickness of 1 mm.

### 3.5 Summary

Preliminary uniaxial tensile test data and electrical stimulation of the ECS, examined during this study, has demonstrated the feasibility of utilising the ECS as a mimetic material of carotid tissue due to its suitable mechanical behaviour up to the physiological strain range of 20%. However, further mechanical characterisation, in the form of inflation-extension testing, of the ECS is required to develop a more accurate material model for FE analysis. This study has also demonstrated that the ECS elicits the quantifiable electrical response to sustained strain necessary for the material to act as a baroreceptor model and that it relays these responses in a fashion that is qualitatively similar to the two key characteristics of baroreceptor behaviour that relate to CAS surgery, a adaptation to sustained strain and recovery from strain. For these reasons the ECS has been deemed suitable for application as a mimetic model of the carotid artery with incorporated baroreceptor function. However, the electrical responses of the ECS to strain must be related to baroreceptor firing rate during multiaxial testing of
cylindrical specimens, under the same time and strain conditions examined by Ottesen and Danielsen (2003) to fully compare the ECS’s electrical behaviour to baroreceptor function.

CAS has been found to show equivalency to the highly invasive open artery repair which is a more commonly used surgical technique. The results from the CREST trial can potentially increase the need and use of CAS as a surgical treatment worldwide (Brott et al., 2010). Further development into the design of balloon angioplasty devices is necessary for the continuous improvement of minimally invasive treatments of carotid artery disease. However, a major concern with regard to this treatment is the rupture of the plaque due to the almost instantaneous inflation of the balloon device. To further improve the design of these devices a better understanding of the mechanical behaviour, failure of the plaque and the baroreceptor response in the circumferential direction is required. However, further mechanical characterisation of carotid plaques is necessary.

References


Chapter 3

Experimental model of the carotid bifurcation

Thomas JB, MSc; Antiga L, PhD; Che SL, BSc; Milner JS, BESc. (2005). Variation in the Carotid Bifurcation Geometry of Young versus Older Adults: Implications for Geometric Risk of Atherosclerosis. Journal of the American Heart Association. 36, 2450-2456.


CHAPTER 4

MECHANICAL AND BIOLOGICAL CHARACTERISATION METHODS
Chapter 4  

Mechanical and Biological Characterisation Methods

4.1 Introduction

The mechanical and biological characterisation background and the test setups used in this study to predict plaque mechanical response and rupture behaviour will be detailed in this chapter. The objective of this section is to highlight the processes used to develop a representative method of mechanically characterising the tissue to improve material models in FEA and to develop a biological characterisation method which can be clinically applied to improve pre-operative diagnostic methods.

4.2 Theory

4.2.1 Strain

Strain is a measure of relative deformation. It relates change in length to original length and is typically expressed as a percentage of original length assigned the dimensionless parameters, mm/mm. There are three commonly employed strain measures when working with materials undergoing large elastic deformations, engineering strain ($\varepsilon_e$), stretch ratio ($\lambda$) and the logarithmic strain ($\varepsilon_t$).

\[
\varepsilon_e = \frac{L_f - L_0}{L_0}, \quad (4.1)
\]
\[
\lambda = (\varepsilon_e + 1), \quad (4.2)
\]
\[
\varepsilon_t = \ln(\lambda) \quad (4.3)
\]

From these equations, $\varepsilon$ is the engineering strain, $L_f$ is the deformed length, $L_0$ is the original length, $\varepsilon_t$ is the logarithmic strain, and $\lambda$ is the stretch ratio and can also be represented as $\lambda = \frac{L_f}{L_0}$.

In instances of large deformation such as atherosclerosis displayed by hyperelastic materials, true strain is widely used in place of the engineering strain as this parameter accounts for the relatively large change undergone by the cross section of the material. However, the assumption that the atherosclerotic plaque material stretches in a logarithmic fashion can not be assumed and therefore the stretch ratio parameter is used in this study. The logarithmic assumption does not suit heterogeneous materials such as arterial tissue due to over-estimation of the stress at the high strain.

53
values. Engineering stress levels off at the ultimate strength but true stress continues to increase at the same strain value because it is being divided by a smaller and smaller number as the object is stretched to the point of failure.

4.2.2 Stress

Stress is a measure of force applied to a body relative to the cross-sectional area of that body. The equation for engineering stress ($\sigma_e$) is as follows:

$$\sigma_e = \frac{F}{A_0} \quad (4.4)$$

Where $F$ is the applied force and $A_0$ is the original cross-sectional area. The engineering stress measure fails to account for situations where the body being deformed undergoes a large reduction in cross-sectional area such as in the case of strain hardening. To account for instances such as this it is more appropriate to examine the true stress of a body which accounts for changes in cross-sectional area. The equation for true stress is as follows:

$$\sigma_t = \sigma_e (1 + \varepsilon_e) \quad (4.5)$$

For biological tissue, the work conjugate pair commonly used is Cauchy stress and stretch ratio which demonstrate a realistic stress-strain relationship for such heterogeneous material (Holzapfel et al., 2004). The strain measures typically have work conjugates i.e. engineering strain and engineering stress. The Cauchy stress ($\sigma_c$) is a differentiation of the strain energy function ($W$) with respect to the stretch ratio multiplied by the stretch ratio.

$$\sigma_c = \lambda \left( \frac{\partial W}{\partial \lambda} \right) \quad (4.6)$$

4.2.3 Principal Stretches

The mechanical tests necessary to characterise a material are constrained to boundary conditions which allow the following deformation assumptions. For uniaxial tensile testing, the following principal stretches hold true:

$$\lambda_1 = \lambda, \quad \lambda_2 = \lambda_3 = \frac{1}{\sqrt[3]{\lambda}} \quad (4.7)$$

For planar shear (or pure shear) testing the following principal stretches hold true:

$$\lambda_1 = \lambda, \quad \lambda_2 = 1, \quad \lambda_3 = \frac{1}{\lambda} \quad (4.8)$$
Once the boundary conditions are met, the preceding equations can be applied to the strain invariants necessary for strain energy functions. For isotropic uniaxial testing the following strain invariants are commonly used:

\[ I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \]  
\[ I_2 = \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_3^2 \lambda_1^2 \]  
\[ I_3 = \lambda_1^2 \lambda_2^2 \lambda_3^2 \]  

For biological tissue, incompressibility is assumed due to the large water content within the material, therefore \( I_3 = 1 \) (Carew et al., 1968). The first and second invariants, \( I_1 \) and \( I_2 \), are commonly used in isotropic strain energy functions using uniaxial testing only i.e. Mooney-Rivlin, Neo-Hookean and Yeoh. The strain invariants can be calculated using the principal stretch equations for both tensile and planar shear. For tensile testing they are as follows:

\[ I_1 = \lambda^2 + \frac{1}{\lambda}, \quad I_2 = 2\lambda + \frac{1}{\lambda^2} \]  

For planar shear testing the strain invariants are as follows:

\[ I_1 = I_2 = 1 + \lambda^2 + \frac{1}{\lambda^2} \]  

The strain invariants can range from \( I_1 \) to \( I_9 \) however, for anisotropic strain energy functions suitable for biological tissue the most important strain invariants used are \( I_4 \) and \( I_6 \) as they both take into account the mean fibre direction of the two collagen fibre families within biological tissue, \( \gamma \). For these invariants the principal stretches are commonly in a cylindrical coordinate system where \( r \) (radial) and \( \theta \) (circumferential) are denoted as 1 and 2 respectively in the following equations.

\[ I_4 = I_6 = \lambda_1^2 \cos^2 \gamma + \lambda_2^2 \sin^2 \gamma \]  

### 4.3 Mechanical Characterisation Methods

#### 4.3.1 Sample Acquisition and Preparation

Twenty-three carotid plaques were obtained from the Limerick University Hospital, Ireland in a manner that conformed to the Declaration of Helsinki and was approved by the hospital Ethical Research Committee. The carotid plaques were collected from consenting patients who underwent standard carotid endarterectomy surgery to treat high-grade internal carotid artery stenosis. Within this population, 58% (12/23) of the patients were male, with a median age of 65.6 years (range, 52 – 79) and the median age of the female population is 72.2 years, (range, 52 – 85). Plaques were surgically
removed from the carotid artery with preservation of plaque structural integrity emphasised to minimise possible disruption of the plaque luminal surface. The plaques were frozen in phosphate buffer solution (PBS) immediately after removal at -20°C. On the day of tissue testing the plaques were equilibrated to 37°C in PBS prior to mechanical testing. Each plaque underwent the process illustrated in figure 4.5 in section 4.3.4 and the patient details are listed in table 4.1.

![Figure 4.1: Carotid plaques post-endarterectomy and equilibrated to 37°C in PBS prior to mechanical testing.](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
<th>Gender</th>
<th>Side</th>
<th>% Stenosis</th>
<th>Indication</th>
<th>Pre-Op Disease Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>F</td>
<td>Left</td>
<td>70-79%</td>
<td>CVA</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>F</td>
<td>Left</td>
<td>90-99%</td>
<td>TIA</td>
<td>3-4</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>Left</td>
<td>70-79%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>F</td>
<td>Right</td>
<td>80-89%</td>
<td>TIA</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>Right</td>
<td>80-99%</td>
<td>TIA</td>
<td>3-4</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>F</td>
<td>Right</td>
<td>70-79%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>M</td>
<td>Left</td>
<td>50-69%</td>
<td>TIA</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>85</td>
<td>F</td>
<td>Right</td>
<td>50-69%</td>
<td>TIA</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>79</td>
<td>M</td>
<td>Left</td>
<td>90-99%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>M</td>
<td>Left</td>
<td>70-79%</td>
<td>Asym</td>
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</tr>
<tr>
<td>11</td>
<td>63</td>
<td>F</td>
<td>Right</td>
<td>90-99%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>52</td>
<td>M</td>
<td>Right</td>
<td>80-89%</td>
<td>CVA</td>
<td>1</td>
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<tr>
<td>13</td>
<td>74</td>
<td>M</td>
<td>Left</td>
<td>50-69%</td>
<td>CVA</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>52</td>
<td>F</td>
<td>Right</td>
<td>NA</td>
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</tr>
<tr>
<td>15</td>
<td>80</td>
<td>F</td>
<td>Left</td>
<td>80-89%</td>
<td>CVA</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>M</td>
<td>Left</td>
<td>50-69%</td>
<td>CVA</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>68</td>
<td>M</td>
<td>Right</td>
<td>50-69%</td>
<td>TIA</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>85</td>
<td>F</td>
<td>Right</td>
<td>70-79%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>53</td>
<td>M</td>
<td>Right</td>
<td>70-79%</td>
<td>CVA</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>74</td>
<td>M</td>
<td>Right</td>
<td>70-79%</td>
<td>Asym</td>
<td>NA</td>
</tr>
<tr>
<td>21</td>
<td>64</td>
<td>M</td>
<td>Right</td>
<td>50-69%</td>
<td>TIA</td>
<td>4</td>
</tr>
<tr>
<td>22</td>
<td>72</td>
<td>M</td>
<td>Left</td>
<td>70-79%</td>
<td>TIA</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>70</td>
<td>F</td>
<td>Right</td>
<td>70-79%</td>
<td>CVA</td>
<td>4</td>
</tr>
</tbody>
</table>

4.3.2 Mechanical Clamp Design

The clamp design used in this study is based on a combination of the fish-hook and flat clamping methods (Lawlor et al., 2011). The variability of the tissue composition from lipid to calcified component reduced the suitability of any individual clamping
technique such as the fish-hook (Lally et al., 2004), glue (Teng et al., 2009) or flat-clamp method (Maher et al., 2009). A flat-clamp based method was chosen which incorporated 1 mm diameter pins at fixed distance apart horizontally and staggered vertically to allow for varying pin formations, figure 4.2 (a). The typical formation utilised when testing plaques was to have four pins along the centre of the middle row with two pins placed in the furthest row, figure 4.2 (b). The hypothesis of this design is that the force applied by the torque screwdriver (BMS Inc., Limerick, Ireland) on the clamps would hold the soft tissue and the pins would hold the fibres embedded in the tissue at the higher stretch range.

![Figure 4.2: Images of clamp used in the study (a) showing the staggered pin holes which allow multiple formations (b) showing plaque tissue post-test which failed at the centre with no tearing at the clamp.](image)

Initial tests were carried out on porcine aortic tissue to determine the pin formation and clamp force that developed the least amount of slippage during tensile and planar shear tests. A formation with less than four pins in row 2 and 3 (figure 4 a) led to slippage during testing. The close proximity of the pins in the centre pins in rows 2 and 3 leads to a high concentration of stress which potentially could increase the failure at the clamps or premature rupture. Placing a third pin between the two pins in row 3 typically initiated tearing and was removed when testing the plaque. The clamps were lined with sandpaper and a thin piece of felt to minimise the stress distribution effect that the edges of the clamp would have on the gauge length of the plaque specimen, figure 4.2 (b). Figure 4.3 is a scanning electron microscopy (SEM) image of the surface of porcine tissue at the centre pin, two in row 2 and one in row 3, this image confirms the tearing due to the high stress concentration in the centre, this pin was subsequently removed for plaque testing.
Mechanical and Biological Characterisation Methods

Figure 4.3: SEM image of porcine tissue which demonstrates the effect of a pin at the centre of the lower row which initiated tearing in the clamp.

An experimental study on biological tissue compared different methods of clamping and showed that a clamping grip with an applied force used with a cardboard/felt type surface was the best method and also demonstrated that the results from samples that fractured close to grip did not differ in results to the mid-fracture (Ng et al. 2005). During the initial tests on porcine tissue and silicone a clamping torque force of 30 cNm was applied to the plaque specimens to ensure a full grip without causing damage to the tissue (Lawlor et al. 2011).

The whole plaque sampling is a key prerequisite for testing plaque and will be complied with in this study similar to Lawlor et al. (2011). Due to the limited sample size inherent when testing human tissue, the process of rejecting tissue from the Lawlor et al. (2011) study due to geometry is undesirable and chapter 5 analyses the potential of carrying out planar shear testing when characterising the plaque tissue, similar to Hollenstein et al. (2011). This following chapter also examines the potential to redefine the geometrical ratios suitable for either tensile or planar shear testing in order to improve the number of samples eligible for mechanical testing.

4.3.3 Mechanical Test System

Uniaxial mechanical testing was carried out on 25 specimens as a whole (from 23 patients) with a physiological strain rate of 30%/s. The uniaxial tester employed consisted of a stepper motor controlling the lead screw, a Mecmesin force gauge with a maximum force of 50 N and is accurate to within $\pm 0.25\%$ of full scale load, a Mitutoyo digital vernier callipers and an external video extensometer to verify the vernier readings, figure 4.4.
4.3.4 Mechanical Test Process

Prior to clamping, the plaque specimen is placed in a heated water bath where it is equilibrated to 37°C to ensure that the stiffness of the sample is not increased due to the lower temperature as in standard mechanical testing of biological tissue (Hollenstein et al. 2011, Holzapfel et al., 2004, and Maher et al., 2009). Once the specimen is clamped in place the tissue is re-hydrated prior to preconditioning and again prior to testing to failure, similar to Hollenstein et al. (2011). Ideally the plaque specimens should have been tested within heated water bath. However, unlike other studies on human tissue this particular test only concentrated on test to failure rather than incorporating damage models and quasi-static tests which required lengthy test times (Holzapfel et al., 2004 and Maher et al., 2011). The time taken including preconditioning for the mechanical test would never be greater than 5 minutes, negating the need to incorporate a heated water bath during testing, as long as the sample was kept hydrated during the test procedure similar to Hollenstein et al. (2011).

Geometrical parameters, width, thickness, and gauge length, were measured using vernier calipers and non-contact photography prior to clamping and again when the specimen was in the grips directly after the pre-load of 0.01 N was applied. The plaque property which this study establishes is the circumferential stretch that the plaque can withstand before rupture under a strain rate that mimics the cardiac rate to an averaged peak systolic pressure. Studies typically use very slow strain rates such as 0.01–1%/s, which is based on standard protocol for testing metals rather than biological soft tissue, rather than a more physiologically accurate strain rate of 30%/s. This assumption is...
described in greater detail in section 2.4.4. Ideally, a strain rate similar to the rate of balloon inflation would be more suitable for a realistic material model to be used in FEA, however this rate is almost instantaneous and would be difficult to simulate and attain valuable stress/stretch data.

The plaques were tested as a whole specimen rather than sectioning the structure which could potentially underestimate the global mechanical behaviour of the plaque specimen (Teng et al., 2009 and Lawlor et al., 2011). The process outlined in figure 4.5 was used to determine the stretch to complete failure of each plaque specimen. The plaques are inherently tubular and once cut in the longitudinal direction the residual stress in the circumferential direction creates an opening angle, (figure 4.5, top middle and right). For uniaxial testing the plaque is tested in a flattened shape and extended vertically, this is to simulate the force of the balloon in the circumferential direction. For planar shear testing the plaque specimen is assumed that the plaque extension is isovolumetric in nature and therefore an increase in vertical displacement will theoretically result in a limited corresponding lateral contraction (Lawlor et al., 2011). The carotid plaque is placed longitudinally in the jaws of the clamping device of the tensile tester and is extended in the circumferential direction (figure 4.5, bottom right). A pre-load of 0.01 N is applied prior to testing in order to ensure true gauge length was measured. The plaque is pre-conditioned using five loading-unloading cycles at a low strain rate of 0.1 mm/s to allow for the strain softening phenomenon of biological soft tissue (Holzapfel et al., 2000). The plaque specimen is then stretched until complete failure of the sample (figure 4.5, bottom middle). The force applied to the tissue and displacement of the clamps are measured and recorded throughout the test using a force gauge and video extensometer, respectively. This data is then converted to stress-stretch ratio values that can be used to compare each specimen. The Cauchy stress value is used as it is a better representative stress type for hyperelastic materials such as atherosclerotic plaque and the stretch ratio is the most commonly used strain measurement with Cauchy stress, detailed in section 4.2.2.
Chapter 4  Mechanical and Biological Characterisation Methods

However, this study also suggests that using a biological characterisation method to relate the mechanical properties to the content of the plaque specimen is possible. Infrared spectroscopy was employed successfully to relate the Young’s modulus of the samples to the spectroscopy results from the same location of indentation (Ebenstein et al., 2009). The aim of this current body of work will be to utilise this technique and correlate it with the mechanical test data from uniaxial testing.

4.4 Biological Characterisation of Diseased Arterial Tissue

4.4.1 FTIR Function

Fourier-Transform Infra-Red (FTIR) spectroscopy has been shown to determine the structure and relative concentration of biological molecules in tissues in a non-destructive manner (Ebenstein, 2001). The main use of FTIR is to measure how well a sample absorbs light at each wavelength. FTIR spectrometry uses a method of obtaining a broad range of infrared spectra from a sample using an interferometer, and then performing a Fourier Transform on the output signal to obtain a spectrum, figure 4.6. This infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms that the material is comprised. As each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of
material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis of tissue composition comparable to histology as shown by Li et al. (2003).

Figure 4.6: FTIR spectrometer in total reflectance mode (Spectrum 100) with microscope attachment (Spotlight 200).

Previous work undertaken by Lawlor et al. (2011) mechanically characterised fourteen fresh carotid plaques where a disassociation between the pre-operative classification and post-operative mechanical characterisations of the plaques were established. Therefore a method of biologically characterising the plaques using FTIR was developed for this work to quantify the biological content of the human plaques prior to mechanical testing. The advantages of employing FTIR spectroscopy to characterise biological materials in parallel with other characterisation procedures such as mechanical are as follows:

1. Non-destructive – it is important to characterise a biological material prior to testing without damaging the integrity of the mechanical structure of the specimen.
2. Speed – the results and spectrum are gathered quickly, which for biological tissue that degrades in air at room temperature is imperative, especially if other tests need to be carried out immediately afterwards.
3. Experimental Simplicity – the FTIR is a simple tool to use with relatively no sample preparation and no sample modification to carry out pre or post-test.
4. Accuracy – the use of a background scan, subtraction scan of water and multiple scans of the sample reduces the random noise levels. The laser systems utilised in the FTIR are self-calibrating which improves the accuracy.

A method of quantifying the tissue composition is to measure the area under the peaks ascertained from the specimens at each point (Ebenstein et al., 2009). Further to understanding the plaque type, these ratios can be related to the mechanical behaviour.
type as well as the stress value at initial or complete failure of the tissue. This information can aid in understanding what amounts of calcification or lipid correlate to plaque behaviour similar to histology (Li et al., 2003).

4.4.2 FTIR Biomedical Application

Infrared spectroscopy is a convenient tool for determining the molecular conformations of proteins, lipids, and nucleic acids, as well as identifying unknown chemical compounds. The use of FTIR as a tool for biomedical applications was first hypothesised by Hermann (1965) and Parker and Ans (1967). The use of FTIR spectrometry as a tool to characterise biological tissue increased in the ensuing years and the versatility of the instrument was highlighted when used to characterise arterial tissue (Rava et al., 1991) as well as bone (Rey et al., 1991).

4.4.2.1 Effects of Moisture

Kodali et al. (1991) examined the potential of FTIR to characterise atherosclerosis disease in arteries and use in a better understanding of the development of atherosclerotic lesions in artery walls. The study carried out FTIR analysis along the radius of a diseased artery to identify the various layers of a rabbit aorta, figure 4.7 (a). This study demonstrated that FTIR is a powerful tool on the micro scale of biological tissue characterisation especially for diseased tissue where smooth muscle proliferation has occurred. Dousseau et al. (1989) highlighted the issue of high water absorbance when testing biological tissue and suggested a method of eliminating the water spectra by subtracting the signal of water from a tissue signal for a more specific spectrum which is now a common feature in all FTIR spectrometers, figure 4.7 (b).

![Figure 4.7](image)

Figure 4.7: Kodali et al. (1991) in (a) highlights the ability of FTIR to differentiate the arterial layers, infrared spectra for a) medial layer b) intimal layer c) 20μm layer closest to lumen. (b) FTIR Spectra highlighting the advantage of water subtraction (Dosseau et al., 1989) a) spectra of water, b) spectra of a bovine serum protein with 10% water c) the water spectra subtracted revealing the true protein spectra.
Manoharan et al. (1993) was the first study to associate a wave number (cm\(^{-1}\)) of the resultant FTIR spectrum to a constituent of atherosclerotic plaque, table 4.2, which was imperative for future FTIR studies of atherosclerotic plaques. Manoharan et al. (1993) also highlighted the effect of drying the samples had on the resultant spectra (figure 4.8 (a)), further highlighting the need for water subtraction as well as to keep the tissue moist when carrying out FTIR analysis, figure 4.8 (b).

![FTIR spectra of a) human aorta intima highlighting alteration when moist and dry; b) highlighting the need for water subtraction for a moist sample: top: aorta moist before water subtraction, middle: moist aorta after water subtraction and bottom; dry aorta after subtraction (Manoharan et al., 1993).](image.png)

4.4.2.2 **Frequencies of plaque constituents**

Li et al. (2003) highlighted the ability of FTIR spectroscopy as a standalone tool for characterising biological tissue by comparing the results to histology of the same specimen of plaque. FTIR was shown in this study to ascertain similar results of the global properties of the tissue in comparison to histology. A clear advantage of histology over FTIR is the snapshot of the tissue structure through the thickness of the tissue but for global characterisation FTIR is a very powerful and easy-to-use tool. The limitation of using FTIR is the arbitrary value of absorbance used to measure the intensity of constituent within the plaque specimen and the distance infrared penetrates which can be as low as 200\(\mu\)m. However, the time and financial cost of histology emphasises the practicality of FTIR as a cheaper and quicker alternative for the same level of results (Li et al., 2003 and Ebenstien et al., 2009). In the case of Li et al. (2003) a control specimen was used to compare the plaque constituents to for a more quantitative analysis within the study. Figure 4.9 shows the typical FTIR spectra associated to four major types of atherosclerotic plaque from Li et al. (2003). The purely lipid plaque has very weak bands of calcium and phosphate associated with
calcification and the opposite can be said for the fibrous and calcified tissues where the lipid bands are weak. The necrotic tissue contains strong bands of all constituents of plaque. This figure highlights the power of FTIR to distinguish and therefore characterise the plaque type, a useful knowledge prior to mechanical testing.

![Figure 4.9: FTIR absorption spectra after water subtraction for the major types of plaque tissue from Li et al. (2003) i.e. calcified tissue (bottom), fibrous tissue and lipid based tissue (top).](image)

Studies using FTIR spectroscopy of calcified tissue and bone have highlighted the similarities of the peak locations on the spectrum for bone and calcified tissue (Boskey and Mendelsohn, 2005, Chen et al., 2005). Table 4.2 lists the main studies which carried out extensive FTIR spectroscopy on atherosclerotic plaques in the carotid artery (excluding Chen et al. (2005) and Colley et al. (2004) which were based on calcified human lens and diseased rabbit aorta, respectively) and highlights the overall agreement between the studies on the frequency values associated with the plaque constituent.

<table>
<thead>
<tr>
<th>Study</th>
<th>Plaque Constituent - Frequency (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manoharan</td>
<td>Lipid 2955, 2852, 1735, 1650, 1555, 1094 – 962</td>
</tr>
<tr>
<td>Li</td>
<td>Lipid Ester 2930, 2850, 1730, 1650, 1540, 1100 – 1000</td>
</tr>
<tr>
<td>Colley</td>
<td>Fibrous (Amide I) 2924, 1851, 1733, 1641, 1535, 1170, 1057</td>
</tr>
<tr>
<td>Chen</td>
<td>Fibrous (Amide II) - 1651, 1542, 1130 – 1000</td>
</tr>
<tr>
<td>Ebenstien</td>
<td>Calcification 2972 – 2845, 1730, 1650, - 1180 – 900</td>
</tr>
</tbody>
</table>

Ebenstein et al. (2009) used nano indentation to analyse the mechanical behaviour of a human atherosclerotic plaque specimens at certain locations which were then biochemically characterised using FTIR. This study was the first to correlate the
mechanical behaviour with the composition of the plaque characterised by FTIR. The results showed a relation between the lipid and calcification ratio with an increase in the elastic modulus. Nano indentation is a suitable mechanical test that compliments the use of FTIR as the test location can be the exact same for both procedures. Uniaxial testing is a global mechanical test needing FTIR data from more than one location of a heterogeneous specimen such as atherosclerotic plaque. A mapping technique is needed for the whole plaque specimen to quantitatively assign a plaque type to the specimen. Li et al. (2003) carried out mapping technique using FTIR with limited success, however, with the use of the mapping function on newer versions of the software the mapping technique can be carried out with more accuracy throughout the whole specimen.

4.4.2.3 Quantitative Analysis

A method of quantifying the tissue composition is to measure the area under the peaks ascertained from the specimens at each point used by Ebenstein et al. (2009) and an example applied to a plaque from this current study is illustrated in figure 4.10. The lipid and calcification constituents of the plaque were quantified in this study as a ratio to the collagen content for each region tested, i.e. Lipid:Collagen (Li:Col) and Calcification:Collagen (Cal:Col) respectively, figure 4.10. Further to understanding the plaque type (Li et al. 2003) these ratios can be related to the stress/stretch values at initial or complete failure of the tissue rather than a Young’s modulus in Ebenstein et al. (2009) due to the hyperelastic response of the specimens. This information can aid in understanding what amounts of calcification or lipid lead to plaque rupture.

Figure 4.10: Illustration of the method of quantifying the peaks from a FTIR spectrum using altered baselines suggested by Ebenstein et al. (2009). Blue: lipid, Green: Matrix and Yellow: Mineral.

Ebenstein et al. (2009) provides important data to aid in vibrational spectroscopy in clinical applications. However, it is necessary to relate the FTIR...
analysis results to the mechanical properties of the plaques on a global level rather than local level such as Ebenstein et al. (2009) as when plaques are treated with angioplasty balloons an overall response of the plaque to the balloon forces is what is important.

Tosi et al. (2012) suggests that vibrational spectroscopy is ready to be used as an in vivo method for predicting plaque type. The Tosi et al. (2012) study examines the feasibility of applying current technology and knowledge to be used in clinical applications. However, a clear limitation from this study is the lack of mechanical properties of human tissue and how FTIR can relate to this behaviour directly. This current body of work aims to utilise FTIR spectroscopy and relate the analysis to the mechanical properties using the process outlined in the following section.

4.4.3 FTIR Process

FTIR analysis (Spectrum 100, Perkin Elmer Inc., MA, USA, diamond crystal) was carried out to determine sample biological composition prior to mechanical testing. FTIR analysis was carried out at eight locations throughout the plaque using ing the attenuate total reflectance (ATR), figure 4.11. A background spectrum was run to ascertain a relative scale for the absorption intensity to remove any readings from the surrounding environment, this background spectrum was subtracted from all subsequent spectrums analysed. The ATR crystal was placed in direct contact with the specimen with an aim to limit the damage to the specimen surface or structure. Even though all plaque samples are geometrically different, an effort was made to carry out FTIR analysis in similar locations. Four locations along the centre of the plaque were taken as this is the main area of stress and will determine the mechanical behaviour. The final four locations were typically taken in the centre at either side of the initial four locations. All spectrums were ascertained using absorbance mode with a resolution of 2 cm\(^{-1}\) for 32 scans over the range of 4000 - 700 cm\(^{-1}\). The water spectrum from the PBS was subtracted from each spectrum prior to peak area calculation using inbuilt tool in the Spectrum 100 software (Dousseau et al., 1989). The C\(\text{H}_2\) stretch peaks found between 2972 - 2845 cm\(^{-1}\) correspond to the absorbance of lipid within the specimen. Also, lipid ester peaks can be found in a number of plaques at 1730 cm\(^{-1}\) which are included in the lipid peak area calculation. The collagen absorbance peak is represented by the amide I peak found between 1720 - 1585 cm\(^{-1}\). The calcification peak is defined as the phosphate absorbance peak in the 1180 - 900 cm\(^{-1}\) range. The areas under these peaks were measured using inbuilt software from Spectrum 100 (Perkin Elmer Inc., MA, USA) and from these ratios of lipid to collagen (Li:Col), calcification to...
collagen (Cal:Col) and calcification to lipid (Cal:Li) were calculated and averaged for each specimen, figure 4.11. The averages of the three respective ratios for each plaque, based on the eight locations, were used to determine the global FTIR classification to be related to the mechanical test results.

Figure 4.11: Process used to ascertain quantitative data from the FTIR results of each plaque specimen in this study.

4.4.4 Effect of FTIR on Structure

SEM imaging was carried out on a visually calcified plaque to analyse the effect of the ATR crystal had on the plaque structure, specifically a brittle node of calcification. SEM imaging was carried out on a tested area to analyse any potential damage. Figure 4.12 shows the circular mark indentation left by the ATR crystal approximately 1mm in diameter and 10µm deep.

Figure 4.12: SEM image of a calcified section of plaque highlighting the minimal indentation caused by the ATR crystal of the FTIR.
A physical alteration is present due to the ATR crystal on the node of calcification due to the brittle nature of that section of plaque. However, due to the elastic recovery behaviour of the plaque tissue no other marks were found on the lipid/fibrous sections of plaque during SEM imaging. Care and attention was used during testing so as not to crack or damage the calcified sections of the plaque tissue which could alter mechanical properties.

### 4.4.5 FTIR Imaging

Colley et al. (2004) used FTIR in conjunction with macro and micro imaging and reiterated the ability of FTIR as a standalone tool especially when used with an imaging system. Rabbit aortas were tested in situ of an aqueous solution and results from the macro test showed the location and contents of the small but distinct atherosclerotic plaques within the tissue, figure 4.13. When used in conjunction with imaging the FTIR has the potential to locate areas of plaque within arterial tissue or areas of calcification within a lipid rich plaque specimen. The aim of the Colley et al. (2004) study was to highlight the potential of FTIR imaging on the macro and micro scale for in situ testing, a closer step towards in vivo testing. However, for *in vitro* testing the macro imaging is suitable for a global characterisation of the plaque and the components within which may contribute to plaque rupture.

![Figure 4.13](image-url)

*Figure 4.13: (a) Transmission image of rabbit aorta cross-section containing atherosclerotic plaque showing mainly lipid constituents and (b) a photomicrograph (15x) of the same cross-section where the plaque is visible. (Colley et al., 2004).*

FTIR and micro-scale imaging were carried out separately in this study due to the lack of capability of the FTIR spectrometer to carry out micro-imaging in conjunction with the ATR crystal. However, SEM imaging and energy dispersive X-ray spectroscopy was used on chemically treated sections of the mechanically tested plaques to confirm the trends of composition measured by FTIR analysis.
4.5 Scanning Electron Microscopy

4.5.1 Function and Concept

Scanning electron microscopy (SEM) has many and varied applications in science and engineering and in recent years has been used specifically in the biological sciences i.e. plant and animal tissue studies. SEM imaging is a surface only analysis tool, where sample size and thickness is not a concern which is advantageous when imaging an inherently random geometrical human plaque specimen. However, as the SEM available during this study was not a low-vacuum environmental SEM the vacuum system in conventional SEMs necessitates removal of any water from the specimen which requires a process of fixation, dehydration and drying of the sample, table 4.3 in section 4.5.1.4. The sample must also be electrically conductive for the SEM to produce clear and qualitative images which are carried out by coating the sample in thin layer of a metal such as gold.

There is a scarce amount of literature on the structure of human atherosclerotic plaques using SEM imaging. SEM is an expedient technique to use for this research due to its capability to explore a large area range, varying magnification and its unrestricted access to bulk specimens (Dell’Orbo et al., 2010, Congiu et al., 2010, Schembri et al., 2008). This technique works on the basis that it scans the samples surface using the emission of electrons onto the surface where by an image is derived from the detection of these excited electrons.

4.5.1.1 Endothelial dysfunction and delamination

SEM has been used to investigate the morphological features of the endothelium in human carotid plaques (Guasti et al., 2010). The plaque specimens were analysed to investigate the characteristic effects that atherosclerosis had on the endothelial layer. The results of this analysis showed that the atherosclerosis lesion caused significant damage to the wall structure. Focusing on the endothelium, there was a significant amount of detachment between the endothelial layers and the basal lamina surface (Congiu et al., 2010). This research visually identified the consequences of the presence of the atherosclerosis in the carotid artery, figure 4.14. Congiu et al. (2010) carried out SEM imaging on the largest number of carotid plaques to date (n = 6) and the aim of this current study is to improve on the number of plaques imaged. This current work will also aim to identify the common features, such as endothelial dysfunction, that
might aid in the understanding of the main constituents that lead to the development of plaque and potential elements that instigate plaque rupture.

Figure 4.14: SEM images from Congiu et al. (2010) study which highlights (a) the endothelial dysfunction on the surface of the carotid plaque and (b) the endothelium delamination.

4.5.1.2 Calcification

Guasti et al. (2010) investigated the calcification patterns that can be visualised using SEM. Sheet-like crystals within the media layer of the carotid artery were detected, figure 4.15. There was also evidence of calcification in the tissue which progressed in a laminar pattern. This analysis was coupled with the energy dispersive x-ray (EDX) analysis which allowed an accurate characterisation of the elemental composition of the samples. Further studies have proposed that these calcifications are as a result of highly controlled processes of mineralisation (Herisson et al., 2011, Schembri et al., 2008).

Another study has shown that plaques contain hydroxyapatite mineral nano-crystals that are embedded in the collagen organic matrix (Demer et al., 2008). At a nanoscale level, apatite crystals tend to interact with cholesterol crystallites (Abela et al., 2009). With this information it is thought that mineralisation may result from a basic template pattern generated by the organic matrix at a molecular level (Demer et al., 2008).

Figure 4.15: SEM images from Guasti et al. (2010) (a) macro image of carotid artery cross-section with node of calcification (b) magnified area of the white box from (a) and (c) is a higher magnification of the white box in (b) highlighting the sheet-like formation of the calcification.
Herisson et al. (2011) performed SEM analysis on both carotid and femoral plaque specimens to characterise its composition. Four different types of calcifications were found to be present in the samples. The four types being; sheet-like calcification, nodular calcification, clear centre calcification and osteoid metaplasia. The study showed that femoral and carotid plaques have very dissimilar morphologies. The carotid arteries contained more lipid and inflammatory contents whereas the femoral arteries were more likely to calcify and to develop osteoid metaplasia. This study aids in the understanding of the main calcification types and in the identification of calcification during SEM imaging.

4.5.1.3 Energy Dispersive X-ray (EDX)

EDX is an elemental analysis tool which can be used to characterise the composition of biological tissue. EDX spectroscopy can be carried out on samples during SEM imaging to determine the main constituents of the rupture sites of each plaque as well as to validate the vibrational spectrums produced by FTIR. Schembri et al. (2008) and Guasti et al. (2010) both highlighted the potential of EDX as a useful analytical tool for examining the morphology of a plaque specimen when coupled with SEM imaging. EDX measures the elemental ratio throughout the whole thickness of the sample and for this reason, EDX is used in this study as a method of confirming the biological components identified during FTIR analysis. This additional method is necessary as FTIR only measures the biological composition of samples on the surface level and aid in confirming the level of composition identified by FTIR analysis.

4.5.1.4 Preparation of biological tissue

Biological tissue has been previously prepared for SEM using a methodology proposed by (Karcz, 1996, Karcz, 2009), table 4.3. The vacuum system in the SEM used in this study requires the sample under investigation to be completely free from water or volatile components and must be electrically conductive. To visualise cellular structures in the sample, several rigorous processing steps must be performed. These include fixation, dehydration and drying. The consequence of this preparation technique is that the tissue is in a state that it prevents it from changing and protects itself against the harmful subsequent exposure to the electron beams emitted by the SEM. For all methods of fixing, dehydrating and drying the following process is used:
Table 4.3: Process used to chemically prepare biological tissue for use in a conventional SEM in this study.

<table>
<thead>
<tr>
<th>Method</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Fixation</td>
<td>Glutaraldehyde or Methanol</td>
</tr>
<tr>
<td>2 Wash</td>
<td>Phosphate Buffer Solution (PBS) (Glutaraldehyde only)</td>
</tr>
<tr>
<td>3 Dehydration</td>
<td>Ethanol (varying grades: using distilled water)</td>
</tr>
<tr>
<td>4 Drying</td>
<td>Hexamethyldisilazane (HMDS) or Critical Point Drying (CPD)</td>
</tr>
</tbody>
</table>

4.5.2 **Fixation**

Biological specimens prepared for SEM need to be chemically fixed to limit the amount of shrinkage and structural damage during the dehydration and drying processes. The fixation technique is developed to preserve the cellular structure, so as to preserve the specimen in a form as close as possible to that of a living plant or arterial structure. Various chemical processes are used to fix the specimens (Bozzola and Russell, 1999, Schwab and Hulskamp, 2010). However, most of the protocols are based on plant tissue and insects. There has been a focus on the development of a fixative that can preserve constituents of the biological sample in the closest representative manner to in vivo as possible. There are significant differences in the molecular responses of biological tissue with different fixation methods. As a result choosing the correct fixative to optimise the observation of the tissue is a major consideration.

4.5.2.1 **Gluataraldehyde**

Primary fixation in a buffered glutaraldehyde solution followed by a post-fixation in osmium tetroxide solution is the most commonly employed means of fixing biological tissue for SEM where a number of studies have utilised this protocol as it produces the highest quality images on a nano and micro scale (Herisson et al., 2011 and Sylvian et al., 2011). The additive crosslinking method using glutaraldehyde-osmium tetroxide fixed biological tissues have been extensively used. This method works on the basis of creating chemical bonds between proteins. It preserves the natural structure of proteins (secondary & tertiary structures). It is often the case that the osmium tetroxide post fixation is omitted from the fixing step as it may destroy the tissue (Muscariello et al., 2005). The slow penetration of the glutaraldehyde and the need for periodic purification to maintain the functional aldehyde levels have greatly limited its use as a biological fixative. It has been considered whether the chemical fixation may destroy the structural integrity of some structures of interest. Glutaraldehyde cross-links the free amino acid groups of polypeptide and aminos acids and inactivates enzymes and as a result it is insufficient for enzyme localisation (Schatten, 2011).
Even though glutaraldehyde is the gold standard chemical for fixing the biological material, in recent years the use of methanol has shown to reduce the fixation time for the same qualitative results and reduced risk to health (Bomblies et al., 2008). This decrease in time is an important attribute when imaging human plaque tissue under SEM in order to truly evaluate the damage to the tissue caused by the mechanical testing.

### 4.5.2.2 Methanol

An alternative fixation method that involves using methanol has been proposed by Bomblies et al. (2008) and Schwab and Hulskamp (2010). The denaturing precipitating methanol works on the basis of reducing the solubility and disrupts the hydrophobic interactions of the proteins. It modifies the tertiary structure of the proteins as well as inactivates the enzymes. Research has shown that the use of non-cross linking alcoholic reagents yield superior results (Srinivasan et al., 2002). The methanol preserves the nucleic acids better than the aldehyde due to its minimal chemical alterations. The methanol provides instant fixation within the components of the cell walls (Srinivasan et al., 2002). However, alcohols have been found to induce some degree of cell shrinkage (Chao and Zhang, 2011). The advantages of using methanol is the significant reduction in time, potentially 48 hours to 10 minutes, the reduction in toxicity of the chemical used and the simplicity of the experiment (Bomblies et al., 2008 and Schwab and Hulskamp, 2010).

The sample is placed in 100% pure methanol for 10 minutes, at room temperature, and washed in pure molecular grade ethanol before carrying out the dehydration method. A short SEM study was carried out to evaluate the difference that the methanol and glutaraldehyde methods had on the arterial structure of porcine tissue. Figure 4.16 shows the SEM images of the cross-section of two sections of a porcine aortic artery from the same area. Figure 4.16 (a) shows the tissue prepared with glutaraldehyde where the intimal layer is on the far right and figure 4.16 (b) is the methanol prepared sample at room temperature (intimal layer at the bottom). Both images show no significant difference or advantage to either method. However, as no real disadvantage was seen between the methods, the time efficient methanol process was implemented in the study as per the protocol by Schwab and Hulskamp (2010).
4.5.3 Dehydration

Dehydrating biological tissue involves physically exchanging water for an organic solvent, such as acetone or ethanol. It has been found that ethanol is marginally superior to acetone in dehydrating the biological medium as acetone appears to cause some surface changes possibly by dissolving structural lipids (Karcz, 1996). This dehydration step needs to be performed very carefully so as to reduce the shrinkage effects as unsolicited effects on biological tissue samples have been found to be caused by the substitution of water (Pathan et al., 2008). The shape of macromolecules or membranes that exist is maintained by its interactions with water which may be destroyed by the removal of water during this process. It has been revealed that approximately 30-60% shrinkage of the sample has been reported after dehydrating the soft tissue sample this way (Schatten, 2011).

Hydrated biological samples must first be dehydrated before placing the specimen in the SEM vacuum sample chamber. This is typically achieved by passing the specimens through a graded series of ethanol-distilled water mixtures to 100% ethanol, and then drying the samples. The different grades should reduce the effect the evaporation could potentially have on the structure of the sample. The process begins with 30% ethanol and 70% distilled water up until 100% ethanol. Typically standard ethanol is used for the lower grades and for the 100% step use the molecular grade (Karcz, 2009). Ethanol dehydration has been shown to damage the cholesterol crystals within coronary arteries which are pivotal to development of plaque rupture (Abela et al., 2009). However, in a letter of reply to (Abela et al., 2012) it was suggested that the alternative of ethanol dehydration, vacuum dehydration, can affect the structure of the collagen fibrin within the tissue which are of more interest in this study due to the effect they have on the mechanical behaviour.
**Preparation**

The varying grade of ethanol solution is relatively simple to produce and the time for each step can vary from 5 to 15 minutes but no more than 30 minutes due to potential structural damage to the tissue. Once the dehydration is complete the specimen is immediately placed into a Hexamethyldisilazane (HMDS) and ethanol mixture to begin the drying process. The following process was carried out on each plaque specimen in table 4.4:

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Time (min)</th>
<th>Repetition</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% ethanol : 70% distilled water</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>50:50</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>70:30</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>80:20</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>90:10</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>95:5</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>100% molecular grade</td>
<td>10-20</td>
<td>2</td>
</tr>
</tbody>
</table>

**4.5.4 Drying**

The gold standard of drying is to use an instrument called a Critical Point Dryer (CPD). CPD is a technique that involves passing liquid CO₂ across the test specimen at a certain temperature and pressure. This method reduces the chance of unintended cell morphology, distortion and minimizes artifact production due to the minimal surface tension created during specimen dehydration. However, CPD has proven to be a time-consuming process and having a tendency to cause cell shrinkage due to the vigorous solvent exchange with the cells resulting from the temperature and pressure changes (Muscariello, et al. 2005).

Hazrin-Chong and Manefield (2012) investigated using HMDS as a drying agent as an alternative to the original CPD technique. In comparison, Jung et al. (2010) found that the hexamethyldisilazane (HMDS) can reduce the collapse and distortion of the delicate specimens during drying. The reagent cross-links proteins, reduces the surface tension and gives strength to the sample during air-drying. HMDS has been used in the tissue preparation procedures for biological specimens by Dekker et al. (1991), Botes et al. (2002) and Jung et al. (2010). Multiple samples can be dried using HMDS and the process takes only 30 minutes before it needs to air-dry for 8-12 hours (overnight). The process of HMDS drying occurs immediately after ethanol dehydration for 10 minutes. The sample is submerged in 50:50 ethanol and HMDS and another 10 minutes at 100% HMDS to ease the structural effect of removing the ethanol. The specimens are allowed to air dry over night to complete the drying process prior to SEM use.
The final process developed will incorporate molecular grade ethanol for dehydration and HMDS for drying to prepare the plaque tissue. This is a new method of preparing tissue which reduces the time (2 days to 2 hours and overnight air drying), reduces the cost when using osmium tetroxide and reduces the damage caused by CPD to the structure through shrinkage.

### 4.5.5 Gold Coating

The gold sputter coater is an apparatus which coats the mounted specimens to induce electrical conductivity on the surface of the specimen necessary for SEM. A thin layer of gold increases the image quality without any significant effect on the structure of the tissue under inspection. There are two detectors in the SEM chamber which create a signal from electrons bouncing off the gold-coated specimen. If the specimen is not finely covered with an electron-opaque substance like gold, the electron beam would travel through the specimen, creating no image and damaging the specimen. The prepared cross sections of biological tissue were mounted on an aluminium stub and using a sputter coater Emitech K.550 (Emitech Ltd., Kent U.K.) were coated in gold. The conditions applied were a gold sputtering deposition current of 30mA at a deposition rate of 10nm/min for 120 seconds, resulting in a 20nm coating.

### 4.5.6 SEM and EDX Process

A SEM Hitachi SU-70 (Hitachi High-Technologies Europe GmbH, Krefeld, Germany) was used to investigate the morphological ultrastructure of the carotid arterial samples. The microscope was set up with an accelerating voltage of 10kV and a working distance of 15mm. The SEM images the surface of the sample by scanning it with a high energy beam of electrons. The electrons interacted with the atoms that make up the sample and produce signals that contain information about the samples surface features. The SEM can ascertain images on macro-scale (2mm square image at 30x magnification) to the micro-level (5µm square image at 7000x magnification) depending on the quality of specimen preparation and gold coating.

The EDX microanalysis process was performed using INCA Energy software platform (Oxford Instruments plc., Oxon, U.K.). This program allowed the analysis of the quantitative material chemical composition. The standard imaging procedure that was required for the SEM was applied on the location desired for EDX which for this study was the complete specimen. The accelerating voltage current was increased to 20kV and a dead time of between 20-40% was desired during elemental analysis. Microscope measurements were taken at the lowest magnification of 30x to incorporate the entire
sample. The results were quantified in terms of the elemental atomic weight % in each sample. Typical results for a calcified type plaque are shown in table 4.5 confirming the presence of calcium and phosphorous similar to the calcium/phosphate peak in FTIR analysis. The ratio of Calcium and phosphorus to Carbon (Ca+P:C) was used to compare to the FTIR ratios of Calcification to Lipid (Cal:Li) and Calcification to Collagen (Cal:Col). These set of ratios are not directly or quantitatively comparable. However, they can confirm the trend under investigation which is the level of calcification to the most abundant composition feature present i.e. carbon and collagen for EDX and FTIR, respectively.

Table 4.5: EDX elemental analysis results for a calcified carotid plaque showing the different data types, percentage weight and atomic weight. The results show a large amount of carbon and oxygen with a relatively large amount of calcium and phosphate while a trace of sodium is present.

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight%</th>
<th>Atomic%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>59.09</td>
<td>68.18</td>
</tr>
<tr>
<td>Oxygen</td>
<td>33.30</td>
<td>28.84</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.67</td>
<td>0.40</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.76</td>
<td>0.79</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.18</td>
<td>1.79</td>
</tr>
</tbody>
</table>

### 4.6 Summary

To further improve in vivo imaging of carotid plaques for an improved understanding of device implantation the relationship of the mechanical behaviour and biological composition of carotid plaques is required. A limited amount of data on the mechanical behaviour of atherosclerotic plaques under physiological conditions exists. Studies undertaken by Maher et al. (2009) and Teng et al. (2009) have tested the tensile properties of the plaque in the circumferential direction at unphysiological strain rates which limits the true representation of the global properties of the plaque. This current study aims to biologically and mechanically characterise the whole plaque tissue. This study will determine if a correlation between the mechanical behaviour and biological content of the plaque exists. However, due to the lack of experimental data on the mechanical properties no standard procedure for mechanically testing biological tissue is available. A number of limitations still exist which are unavoidable when attempting to mechanically test such elusive tissue i.e. geometrical ratios. The following chapter will investigate the effect of geometrical ratios have on the test procedure and attempt to refine the ratios suitable for uniaxial mechanical tests i.e. tensile and planar shear testing.
Chapter 4

Mechanical and Biological Characterisation Methods

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CHAPTER 5

ON THE MECHANICAL BEHAVIOUR OF CAROTID ARTERY PLAQUES:

THE INFLUENCE OF CURVE-FITTING EXPERIMENTAL DATA ON NUMERICAL

John J. Mulvihill and Michael T. Walsh.

Centre for Applied Biomedical Engineering Research, Department of Mechanical, Aeronautical, and Biomedical Engineering, Material and Surface Science Institute, University of Limerick, Limerick, Ireland, University of Limerick, Limerick, Ireland.

The following chapter presents a paper on the assessment of geometrical ratios appropriate for uniaxial experimental tests of biological tissue with an aim to define the ratios that are suitable and highlight the limitations. This chapter is published in Biomechanics and Modeling in Mechanobiology and presented verbatim, however in a more reader friendly format.

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DOI: 10.1007/s10237-012-0457-9
5 On the Mechanical behaviour of carotid artery plaques: the influence of curve-fitting experimental data on numerical results

Abstract

Computational models of diseased arteries are advancing rapidly, and a need exists to develop more accurate material models of human atherosclerotic plaques. However, intact samples for in vitro mechanical testing are not readily available. Most plaque samples are harvested from carotid endarterectomies where the geometries are not suitable for the boundary parameters necessary for classical uniaxial tensile testing. Experimental studies of biological tissue, particularly human plaque tissue, have not specified the minimum width-to-length (WL) ratio necessary for appropriate tensile testing. This study proposes either tensile or planar shear testing on whole specimen samples depending on the WL ratio. However, a “grey-area” of WL ratios exists which are unsuitable for either test, between 0.5:1 and 4:1 WL ratio. Eighteen plaque samples are investigated in this study, and according to classical approaches, two of the plaque samples have WL ratios suitable for tensile testing and four are suitable for planar shear testing. The remaining twelve samples fall in the grey-area of WL ratio. The study analyses which test method is suitable for the samples in this grey-area and what effect using the incorrect test method has on results from a computational model. The study highlights that tissues above a WL ratio of 2:1 are suitable for planar shear testing, and samples below 1:1 are more suited for tensile testing. Therefore, the “grey-area” can be reduced with certain limitations applied by the minor strain assumption which need to be taken into account during experimental testing. This study also demonstrates the influence of curve-fitting experimental results using tensile- and planar shear-based boundary parameters from eighteen plaque samples.

5.1 Introduction

Stroke is a sudden necrosis of brain cells caused by an interruption of normal perfusion to the brain. Stroke has the potential to cause irreversible damage to an array of neurological functions in 22–25% of victims and death within one year for 25% of victims (Medtech-Insight 2011). One of the main instigators of a stroke event is the presence of atherosclerotic plaque in the carotid arteries (Guyton 1991). Atherosclerosis...
refers to a condition in which the walls of an artery thicken due to an accumulation of fatty deposits consisting mainly of lipid and connective tissue matrix. Over time, these deposits harden and form a fibrous cap which can rupture and cause an occlusive thrombosis reaction that can prevent normal blood flow to the brain and lead to a stroke event. Carotid artery stenting (CAS) is a minimally invasive technique of mechanically widening a diseased carotid artery, by deploying a stent at the site ofstenosis, compressing the atherosclerotic plaque and widening the lumen to increase blood perfusion. Despite the numerous advantages of CAS over carotid endarterectomy such as increased accessibility, decreased trauma and absence of general anaesthetic (Roffi et al. 2009), it accounts for only 26% of stroke prevention procedures performed due to the lack of knowledge on the mechanical effect of stents on s structur e (Paraskevas et al. 2009).

Computational studies of atherosclerotic arteries under physiological conditions have evaluated the stress distribution within the plaques to analyse the biomechanical response to certain geometrical, structural and material variations (Li et al. 2008; Kiousis et al. 2009; Creane et al. 2010; Franquet et al. 2011). A limited number of studies have also examined the biomechanical structural alterations of the plaque to the deployment of stents to improve the design of the stent (Takashima et al. 2007; Cui et al. 2010; Auricchio et al. 2011; Scherer et al. 2011). Computational studies of diseased coronary arteries are more common than the carotid (Cilla et al. 2012; Huang et al. 2001; Versluis et al. 2006) and a main limitation in these studies is the material properties representing the plaque model which are developed from experimental data based on a small sample size of human plaque from the aorta (Beattie et al. 1998; Lendon et al. 1993; Loree et al. 1994a), limiting the reliability of the stress values from these studies. The computational simulations of diseased carotid arteries also base the mechanical properties of the carotid plaque on experimental data of atherosclerosis from other parts of the vascular system which have different morphologies and potentially different mechanical responses to stent deployment (Herisson et al. 2011), excluding Creane et al. (2010) which is based on uniaxial tensile data of fresh human carotid plaques undertaken by Maher et al. (2009).

Ideally for improved material properties of diseased tissue, in vivo mechanical characterisation of carotid atherosclerotic plaques (CAP) would be carried out on a patient to develop a specific constitutive material model and apply it to their diseased artery geometry. However, there is no fully validated method of carrying out this in
vivo mechanical characterisation procedure. Current studies that mechanically characterise CAPs use in vitro mechanical test procedures developed for engineering materials with specific geometry requirements to satisfy the minor strain assumptions such as pure tension and equibiaxial testing. Limitations when mechanically characterising an excised intact plaque tissue are the random geometry and variance in mechanical response, making it difficult to test the CAP specimen as a whole in pure tension (a common test method for mechanically characterising biological tissue when using a strain energy function (SEF)). Figure 5.1 highlights the geometrical difference between CAPs excised after a carotid endarterectomy, Fig. 5.1a is from Lawlor et al. (2011) which is a fresh human CAP tested immediately after excision from the common carotid. Figure 5.1b is a defrosted human CAP from the carotid bifurcation (left-side) through the common carotid. There are many limitations to in vitro mechanical characterisation of biological tissue such as the deleterious effect on the structure and residual stresses within the plaque during excision, damage to tissues from clamping and inhomogeneity of the CAP tissue material for an ideal failure at the centre of the specimen.

In vitro studies, testing human and animal biological tissue in tension, range the width-to-length (WL) ratio from 0.5:1 to 0.167:1 in both the circumferential and longitudinal directions, Table 5.1 (excluding ASTM-International (2009) which is a standard based on engineering materials). Specifically examining the studies of human plaque, the WL ratio varies from 0.3:1 to 0.21:1. The shortest gauge lengths used in these tensile test studies were 3 and 4 mm, Richardson (2002) and Maher et al. (2009), respectively, where it was stated that such a small distance between the clamps can affect the stress distribution within the material during testing. Excluding the gauge length, the experimental plaque studies range from 7 to 15.9 mm in gauge length where in theory this length should be much longer to allow for a larger uniform stress distribution for the governing equations of major and minor strains to hold true when applying the experimental data to a SEF. However, as the tissue contains interconnected fibres, this
cutting can deleteriously affect the structure of the fibres as well as alter the global mechanical response of the plaque. For an immediate improvement in the constitutive material models of CAPs, it is necessary to mechanically characterise the whole plaque specimen as separating the specimen into smaller sections can potentially underestimate the global mechanical response (Lawlor et al. 2011; Borschel et al. 2003).

Table 5.1: List of experimental studies using tensile tests on biological tissue which also detail the gauge lengths and widths of the samples tested.

<table>
<thead>
<tr>
<th>Author</th>
<th>Length (mm)</th>
<th>Width to Length ratio</th>
<th>Tissue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stemper et al. (2005)</td>
<td>15.9</td>
<td>0.5:1</td>
<td>Human carotid artery</td>
</tr>
<tr>
<td>Lally et al. (2004)</td>
<td>9.38</td>
<td>0.37:1</td>
<td>Porcine coronary artery</td>
</tr>
<tr>
<td>Hanuza et al. (2010)</td>
<td>30</td>
<td>0.33:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Holzapfel et al. (2004)</td>
<td>7–17</td>
<td>0.31–0.13:1</td>
<td>Human iliac plaque</td>
</tr>
<tr>
<td>Loree et al. (1994a)</td>
<td>15.9</td>
<td>0.3:1</td>
<td>Human aortic plaque</td>
</tr>
<tr>
<td>Raghavan et al. (1996)</td>
<td>40</td>
<td>0.25:1</td>
<td>Human aortic aneurysm</td>
</tr>
<tr>
<td>Wang et al. (2001)</td>
<td>40</td>
<td>0.25:1</td>
<td>Human aortic thrombus</td>
</tr>
<tr>
<td>ASTM-International (2009)</td>
<td>N/A</td>
<td>0.25:1</td>
<td>Tensile testing standard</td>
</tr>
<tr>
<td>Maher et al. (2009)</td>
<td>4</td>
<td>0.25:1</td>
<td>Human carotid plaque</td>
</tr>
<tr>
<td>Xiong et al. (2008)</td>
<td>20–25</td>
<td>0.25–0.2:1</td>
<td>Human aortic aneurysm</td>
</tr>
<tr>
<td>Teng et al. (2009)</td>
<td>9</td>
<td>0.22:1</td>
<td>Human carotid plaque</td>
</tr>
<tr>
<td>Lendon et al. (1993)</td>
<td>7</td>
<td>0.21:1</td>
<td>Human aortic plaque</td>
</tr>
<tr>
<td>Silver et al. (2003)</td>
<td>10</td>
<td>0.2:1</td>
<td>Porcine carotid artery</td>
</tr>
<tr>
<td>Guinea et al. (2010)</td>
<td>10</td>
<td>0.2:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Di Martino et al. (1998)</td>
<td>22</td>
<td>0.2:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Veronda et al. (1970)</td>
<td>45.7</td>
<td>0.167:1</td>
<td>Cat skin</td>
</tr>
<tr>
<td>Richardson (2002)</td>
<td>3</td>
<td>0.167:1</td>
<td>Human coronary plaque</td>
</tr>
</tbody>
</table>

Due to the nature of the CAP geometry, when excised from the artery, this study investigates the limitations that geometric ratios associated with excised plaque specimens (WL ratios) have when mechanically tested in either pure tension or planar shear (also known as pure shear). A “grey-area” of unsuitable WL ratios is created between the correct ratios for tensile and planar shear testing. Currently this “grey-area” is between WL 0.5:1 and 4:1 according to Davis (2004). Unfortunately for whole specimen testing of CAP tissue excised from aortot endarterectomies, the sample geometry can fall into this area. Therefore, there is a need to investigate the feasibility of reducing this area and investigate the errors involved when testing inside this “grey-area”. These findings will aid in understanding the effect of changing the governing equations of principal stretches, to suit either tensile or planar shear tests for 18 aortot plaques, on a computational model of an idealised stenosed carotid artery. An understanding of the limitations and percentage error from
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On the mechanical behaviour of carotid artery plaques

theoretical minor strains for CAP samples in the grey-area will aid in improved experimental methods when testing whole CAP specimens and more accurate constitutive material models (Table 5.2).

Table 5.2: List of WL ratios used in a number of experimental studies using mechanical tests on biological tissue in planar shear.

<table>
<thead>
<tr>
<th>Author</th>
<th>Length (mm)</th>
<th>WL</th>
<th>Tissue type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis (2004)</td>
<td>N/A</td>
<td>4:1</td>
<td>Shear standard</td>
</tr>
<tr>
<td>Gao et al. (2009)</td>
<td>11</td>
<td>4:6:1</td>
<td>Porcine liver</td>
</tr>
<tr>
<td>Hollenstein et al. (2011)</td>
<td>10</td>
<td>6:1</td>
<td>Porcine skin</td>
</tr>
</tbody>
</table>

5.2 Materials and Methods

5.2.1 Ratio Analysis

A computational analysis of the stresses in a test specimen with varying geometric ratio (WL 0.1:1–10:1) was carried out with a consistent hyperelastic material property. By examining the effect of the WL ratio on the resolved stresses and minor strain of the test specimens, the errors involved when the WL ratio is within the “grey-area” for suitable mechanical testing, 0.5:1 to 4:1, were identified. Figure 5.2 illustrates two of the twelve computational models employed in this study. Figure 5.2a shows an ideal geometry for tensile testing with a WL ratio of 0.25:1, (b) illustrates a model with a WL ratio of 4:1 suitable for planar shear testing. Comparison of the minor strain at the centre of gauge length of each geometric ratio to the theoretical minor strain will highlight in an idealised computational uniaxial setup the errors involved for geometric ratios within the “grey-area” of 0.5:1–4:1 WL ratios. The “grey-area” is the range of WL ratios which are theoretically unsuitable for tensile or planar shear testing for engineering materials. However, for whole specimen biological tissue testing, this grey-area is too large and needs to be redefined to a smaller range as the current area significantly reduces the number of samples suitable for testing.

Figure 5.2: Idealised computational models of tensile and planar shear uniaxial tests. (a) WL 0.25:1 suitable for tensile testing and (b) WL 4:1 suitable for planar shear testing.
5.2.2 Idealised Diseased Artery

A two-dimensional cross-section of an idealised arterial artery was modelled using geometries analogous to work carried out by Li et al. (2008) and Franquet et al. (2011). The geometric model contains a large lipid core which is surrounded by a diseased intimal layer with a fibrous cap of 0.6 mm, Fig. 5.3. The internal diameter of the artery is 6 mm which is based on the typical minimum stenosis of a diseased artery which undergoes carotid endarterectomy. The 0.9 mm thickness of the carotid wall was based on Delfino et al. (1997), but can vary up to 1.5 mm (Li et al. 2008). The artery wall was divided into the medial and adventitial layers according to Holzapfel et al. (2000), 0.4 and 0.5 mm, respectively. The lipid core size and shape were based on Franquet et al. (2011). A peak physiological systolic pressure value of 18 kPa was applied uniformly to the wall of the lumen for each model (Cilla et al. 2012).

Figure 5.3: Dimensioned diagram of the idealised 2D planar model of a diseased carotid artery.

Grid independence was established using a 6-noded free triangular mesh applied throughout the whole finite element (FE) model. Hybrid formulation, used for incompressible and large deformation problems, and plane strain were applied to this model. Elements were concentrated around the lumen and fibrous cap region, but with at least 6 elements through the thickness of the medial and adventitial layer.

5.2.3 Material Properties

The CAP properties were based on the experimental data of fourteen CAP samples from Lawlor et al. (2011) as well as data of four CAP samples from that study which were not included due to the unsuitability of the WL ratios for tensile testing. The stress-
strain data of each plaque were fitted to a third-order reduced polynomial SEF, the Yeoh form, using an optimisation technique developed with Matlab (r2009a, Natick, MA; The Mathworks Inc., 2009) to minimise the difference in stress values between the Yeoh SEF and the experimental data, more detail in appendix B. The optimisation technique was carried out for each AP sample using both tensile and planar shear principal stretches leaving 36 material models in the numerical study.

\[ W(I_1) = \sum_{i=1}^{3} C_{i0}(I_1 - 3)^i \]  

(5.1)

The Yeoh SEF is suitable for characterising hyperelastic materials using uniaxial mechanical data, tension or planar shear, as the function does not depend on the second strain invariant of the left or right Cauchy Green tensor, \(I_2\). \(C_{i0}\) are the material coefficients, and \(I_1\) is the first-strain invariant which is based on the principal stretch ratios, equation 5.2.

\[ I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \]  

(5.2)

The principal stretches (\(\lambda_1, \lambda_2\) and \(\lambda_3\)) change depending on the uniaxial test, tensile or planar shear, where in both cases \(\lambda_1\) is the stretch in the loading direction. For tensile testing, the principal stretches are:

\[ \lambda_1 = \lambda, \quad \lambda_2 = \lambda_3 = \frac{1}{\sqrt{\lambda}} \]  

(5.3)

For planar shear testing:

\[ \lambda_1 = \lambda, \quad \lambda_2 = 1, \quad \lambda_3 = \frac{1}{\lambda} \]  

(5.4)

The WL ratio governs the degree of deformation that the minor stretch ratio component (\(\lambda_2\)) undergoes during a tensile or planar shear test. During a planar shear test, the minor principal stretch ratio (\(\lambda_2\)) is considered to be zero which equates to zero strain in the transverse direction. According to Davis (2004), the minor strain is 0.05 times the major strain when the WL ratio is 4:1 which is close to the plane stress condition necessary for the test (Palmieri et al 2009). However, any WL ratio lower than this can lead to a larger deviation from the zero strain assumption in the minor direction. For tensile testing, the minor axis experiences compressive, negative strains, as the principal strain is positive. This study developed two constitutive material models for each of the eighteen plaques based on the Yeoh form, using the tensile and planar shear boundary parameters, and analysing the effect that the WL ratio can have on the stress distribution and mechanical response in an idealised FE model. The lipid core was based on Akyildiz et al. (2011),
where the lipid core is treated as a very soft and nearly incompressible tissue, with a Young’s Modulus of 1 kPa and Poisson’s ratio of 0.45 rather than an incompressible liquid, which is based on experimental work carried out by Loree et al. (1994b). Other approaches (Loree et al. 1994b) have treated the lipid core as a nearly incompressible fluid which is not able to sustain shear stress. The effects of the 1 kPa assumption on the trends presented in this study were evaluated and discussed in the limitations section. The arterial media and adventitia layers were modelled using an anisotropic SEF developed by Gasser et al. (2006). The strain energy per unit reference volume is represented as:

\[ W = \frac{\mu}{2} (I_1 - 3) + \frac{k_1}{k_2} (\exp(k_2(\kappa(I_1 - 3) + (1 - 3\kappa)(I_4 - 1))^2) - 1) \]  

From Eq. 5, \( \mu, k_1, k_2 \) and \( \kappa \) are material parameters, \( I_1 \) is the first strain invariant and \( I_4 \) is a pseudo-invariant of the right Cauchy Green tensor that takes into account the mean fibre direction of the two collagen fibre families within biological tissue, \( \gamma \). The parameter kappa \( (0 \leq \kappa \leq 1/3) \) describes the level of dispersion in the fibre directions. The parameters for adventitial and medial layers were based on readily available data of arterial tissue from Cilla et al. (2012), listed in Table 5.3. The model was assumed incompressible, by setting the compressibility value \( (D) \) to zero for each material property, to simplify the material model (Holzapfel et al. 2000).

<table>
<thead>
<tr>
<th>Layer</th>
<th>( \mu ) (kPa)</th>
<th>( k_1 ) (kPa)</th>
<th>( k_2 ) (kPa)</th>
<th>( \kappa )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitia</td>
<td>8.44</td>
<td>547.67</td>
<td>568.01</td>
<td>0.26</td>
</tr>
<tr>
<td>Media</td>
<td>1.4</td>
<td>206.16</td>
<td>58.55</td>
<td>0.29</td>
</tr>
</tbody>
</table>

### 5.3 Results

#### 5.3.1 Ratio Analysis

Table 5.4 lists the different gauge lengths, WL ratios and plaque type of the CAP samples tested, highlighting the size and random nature of the CAP specimens. Figure 5.4 illustrates the percentage error of the minor strains of each WL ratio, from 0.1:1 to 10:1, from the theoretical minor strain suitable for either tensile (red area) or planar shear (blue area) testing. For example, any WL ratio less than 0.5:1 has minor strain values 100% in error for planar shear testing and WL ratios greater than 4:1 has minor
strain values 100\% in error for tensile testing making any of these ratios unsuitable for those test methods.

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Length (mm)</th>
<th>WL</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.19:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>2.25:1</td>
<td>Soft</td>
</tr>
<tr>
<td>3</td>
<td>4.72</td>
<td>3.51:1</td>
<td>Soft</td>
</tr>
<tr>
<td>4</td>
<td>5.8</td>
<td>0.45:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>5.55:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>6</td>
<td>7.98</td>
<td>1.04:1</td>
<td>Hard</td>
</tr>
<tr>
<td>7</td>
<td>10.05</td>
<td>1.32:1</td>
<td>Soft</td>
</tr>
<tr>
<td>8</td>
<td>3.96</td>
<td>5.02:1</td>
<td>Soft</td>
</tr>
<tr>
<td>9</td>
<td>6.1</td>
<td>1.93:1</td>
<td>Hard</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>2.55:1</td>
<td>Soft</td>
</tr>
<tr>
<td>11</td>
<td>6.42</td>
<td>2.02:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>12</td>
<td>6.11</td>
<td>2.05:1</td>
<td>Soft</td>
</tr>
<tr>
<td>13</td>
<td>5.18</td>
<td>2.53:1</td>
<td>Soft</td>
</tr>
<tr>
<td>14</td>
<td>4.57</td>
<td>0.44:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>15</td>
<td>6.89</td>
<td>4.50:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>16</td>
<td>7.08</td>
<td>1.90:1</td>
<td>Mixed</td>
</tr>
<tr>
<td>17</td>
<td>2.88</td>
<td>4.01:1</td>
<td>Soft</td>
</tr>
<tr>
<td>18</td>
<td>9.89</td>
<td>1.95:1</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

Using current standard approaches and the WL ratios in Table 5.4, only two CAP samples are suitable for tensile testing (4 and 14) and four CAP samples are suitable for planar shear testing (5, 8, 15 and 17) leaving twelve CAP samples unsuitable for either test. Excluding twelve CAP samples from this experimental study is not ideal due to the difficulty of acquiring these human CAP samples and the desire not to deleteriously cut the samples into smaller sections which could significantly alter the global mechanical properties. Therefore, it was decided to assess the current “grey-area” WL ratios to determine the theoretical minor strain errors involved with test samples in this area.
Figure 5.4: The percentage error of each CAP sample (black X) from the theoretical minor strain suitable for tensile (dark red line) and planar shear (dark blue line) for each geometric ratio that is suitable for tension (0.1:1–0.5:1), for planar shear (4:1–10:1).

FE analysis of WL ratios from 0.1:1 to 10:1 shows that ratios less than 0.5:1 and greater than 4:1 are suitable for tensile and planar shear testing, respectively, as the minor strain is correct within 5% for more than 80% of the gauge length, Fig. 5.5c, d. However, for the ratios that fall between 0.5:1 and 4:1, the minor strains are less than 80% of the gauge length, Fig. 5.5a, b (1:1 and 2:1, respectively). From Fig. 5.4, the percentage error from the theoretical minor strain of 2:1 WL is 65 and 32% from tension and planar shear, respectively, indicating that planar shear testing is more suitable. However, this is only true for 55% of the gauge length area, Fig. 5.5b. Again looking at Fig. 5.4 for the WL ratio 1:1, the percentage error is 12 and 85% from tension and planar shear, respectively, indicating that tensile testing is more suitable. However, again this is only true for 70% of the gauge length, Fig. 5.5a.
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5.3.2 Idealised Diseased Artery

An example of the von Mises stress distribution of a 2D plane strain–idealised diseased artery model is shown in Fig. 5.6, CAP sample 7, using material model developed from planar shear principal stretches (Eq. 4). The peak von Mises stress typically occurs at the point on the lumen closest to the lipid pool. The peak von Mises, maximum principal stresses and radial displacement were recorded. A constitutive model was developed for each of the CAP samples in both tension and planar shear, based on Eqs. 3 and 4, respectively. The results demonstrated that the peak stress and displacement values were typically larger in the FE models using the planar shear based SEF.

Figure 5.5: Minor Strain plots for tension and planar shear of samples with different WL ratios: a 1:1, b 2:1, c 1:1 and d 4:1, where red denotes minor strain suitable for tension (a, c) and blue is suitable for planar shear (b, d)

Figure 5.6: Von Mises stress (MPa) distribution in an idealised stenosed artery. Peak stresses occur on the lumen closest to the lipid pool and the shoulder regions of the lipid pool.
Figure 5.7 quantitatively shows the difference in radial displacement from the lumen centre along a horizontal centreline through the stenosed artery model. This graph highlights the similar behaviour of both models, but the larger radial displacement of the planar shear SEF through each section of the model of CAP sample 8.

Figure 5.7: Radial displacement from lumen centre along dashed line (inset) of stenosed artery for both the tensile (red) and planar shear (blue) models for CAP sample 8. Shaded regions on plot match the material sections outlined in Fig. 3 (inset).

Figure 5.8 illustrates the difference in peak radial displacement between the tensile-based SEF model (a) and the planar shear model (b) of CAP sample 8 where the planar shear model has larger displacements for this particular sample and for all samples, excluding sample 6. CAP sample 8 was chosen as the geometry of the specimen suggested that it should be characterised in planar shear rather than tension, and the sample best illustrates the underestimation in radial displacement for a tensile-based FE model shown in Fig. 5.8a.
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Figure 5.8: Contour of radial displacement (mm) from lumen centre in an idealised stenosed model of plaque sample 8 with a material model based on tensile (a) and planar shear (b).

Figures 5.9, 5.10 and 5.11 are data plots of the radial displacements along the circumference of the lumen, where the start point (0°) is at the right side of the lumen closest to the lipid core and continues counter-clockwise. These figures compare the radial displacement of the undeformed original lumen to the deformed lumen of both the tensile and planar shear models of CAP samples 8, 14 and 13, respectively. CAP samples 8 and 14 were chosen as the WL ratios of each are suitable for planar shear and tension, respectively. CAP sample 13 has a WL ratio of 2.53:1 which places the sample in the grey-area and creates a reduced percentage difference in lumen area between the planar shear and tension.

Figure 5.9: Radial displacement (mm) along lumen circumference of the original lumen diameter and the deformed lumen for the tensile and planar shear models for CAP sample 8, where the WL ratio (5.02:1) is suitable for planar shear testing, red box.
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Figure 5.10: Radial displacement (mm) along lumen circumference of the original lumen diameter and the deformed lumen for the tensile and planar shear models for CAP sample 14, where the WL ratio (0.44:1) is suitable for tensile testing, red box.

Figure 5.11: Radial displacement (mm) along lumen circumference of the original lumen diameter and the deformed lumen for the tensile and planar shear models for CAP sample 13, where the WL ratio (2.53:1) falls in the “grey-area” of testing.

Each of the CAP samples were assigned to averaged WL ratios, which ranges from 0.5:1 (suitable for tensile testing) to 5:1 (suitable for planar shear) in increments of 1, and the percentage difference of the radial displacements of the lumen area between the tensile and planar shear models were analysed in Fig. 5.12.
Figure 5.12: Percentage difference between lumen areas of material models based on tensile or planar shear principal stretches for each plaque sample (grouped by WL ratio). The dashed line indicates the lowest percentage error and cut-off point between using tensile and planar shear tests.

Figure 5.12 highlights that the percentage difference between the tensile and planar shear models of each CAP sample is higher at the WL ratios suitable for tensile, 0.5:1, and planar shear, 4:1 and 5:1. This suggests that reassessing the “grey-area” can alter the WL ratios limits to a maximum of WL 1:1 for tensile testing and a minimum WL of 2:1 for planar shear where only 6 of the 18 (33%) of the CAP samples are unsuitable for testing, Fig. 5.13.

Figure 5.13: The percentage error of each CAP sample (X) from the minor strain suitable for tensile (red line) and planar shear (blue line) where the limits have been reassessed to reduce the grey-area from WL 0.5:1–4:1 to 1:1–2:1.
5.4 Discussion

Recent computational studies aim to simulate the deployment of stents in diseased carotid arteries and to evaluate the stress distribution in vivo throughout the plaque. These studies make it imperative to understand the global mechanical properties of carotid plaques. Idealised FE analysis of different WL ratios highlighted the significant errors in the minor strain associated when testing a specimen outside of the suitable ratios for tensile and planar shear testing. Unlike engineering materials, biological tissue cannot be altered to suit the required geometrical parameters needed for the boundary conditions to analytically develop constitutive material models, without changing the global mechanical behaviour of the sample. The acquisition of whole human CAP specimens is difficult and limited which makes it imperative to mechanically test every available specimen when the WL ratio does not fall within current standards. Twelve of the eighteen plaques were theoretically unsuitable for either tensile or planar shear testing, Table 5.4. However, a precise knowledge of the errors involved and a reduction of this “grey-area” can aid in improved experimental methods and more accurate material models for FE analysis of stent deployment without wastage of CAP samples.

5.4.1 Ratio Analysis

The geometric ratio analysis carried out in this study illustrates the large error in minor strain between WL ratios 0.5:1 and 4:1 for both tensile and planar shear testing, Fig. 5.4. This analysis highlights the limitation when testing specimens in this “grey-area”. However, as twelve of the eighteen plaque specimens are in this area, it is a common and unavoidable issue. The use of contactless measurement of the strain in an area of the sample where the minor strain is known to be suitable for pure tension or planar shear throughout the whole test could improve the accuracy of the results. For example, WL 2:1 ratio the minor strain at 50% strain is in planar shear for only 55% of the gauge length from the vertical centre, Fig. 5.5b, whereas for WL 1:1 ratio the minor strain is correct for tension in only 70% of the gauge length at 50% strain, Fig. 5.5a. The contactless strain measurement would have to take into account this limited area for each ratio in the area throughout the test. This is necessary to ascertain accurate global mechanical behaviour of CAP samples for improved SEF material models to be used in future FE studies.
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5.4.2  Idealised Diseased Artery

The computational study on the idealised stenosed artery evaluated the effect of changing the principal stretches, to tensile or planar shear, had on the numerical model through the altered SEF. The peak von Mises stress values typically occurred at the point on the lumen closest to the lipid core due to the soft and deformable nature of the core material. The peak maximum principal stress was located on the lumen closest to the lipid core for twelve of eighteen (66 %) samples and at the shoulder cap region of the lipid core for six of the eighteen (33 %) samples. The difference between the peak stress at each location was no greater than 12 % for these CAP models for either the tensile or planar shear approaches. The peak stress values were typically larger for the planar shear-based models. The largest deformation of the lumen occurred in CAP samples 2 and 17 due to the soft nature of these samples allowing for a large displacement.

In theory, the planar shear-based SEF is expected to be stiffer than the tensile-based version when using the same experimental data. The results from the numerical model reiterate this theoretical expectation as there is decreased stress and radial displacement in CAP sample models that should be based on planar shear test rather than tensile, Fig. 5.8. This emphasises the incorrect assumption of characterising samples in the grey-area that have greater width than length to a tensile-based SEF. This assumption can lead to numerical models underestimating the realistic global mechanical behaviour of the CAP samples with these geometric ratios, Figs. 5.9, 5.10 and 5.11. Figure 5.10 shows sample 14 which is suited for tensile testing only and that the FE model using the planar shear-based SEF overestimates the lumen diameter. Assigning the CAP sample models to WL ratios in the “grey-area”, which ranges from 0.5:1 (suitable for tensile testing) to 4:1 (suitable for planar shear) in increments of 1, the greatest percentage error between lumen area occurred at the extremities, 0.5:1 and 5:1, as these ratios are farthest from the specified geometrical ratio needed for the respective test method, Fig. 5.12. For certain samples between, 1:1 and 2:1 the percentage difference between the lumen areas is reduced; as expected from Fig. 5.4 the percentage errors are similar from tension and planar shear for these WL ratios. Results show that characterising experimental data of a CAP sample with a geometric ratio unsuitable for either uniaxial test to a SEF has a significant effect on the numerical analysis. However, as acquisition of human CAP specimens suitable for in vitro mechanical testing is limited, it is necessary to characterise these particular CAP samples. Prior to this study, the “grey-area” was not
clearly defined for unsuitable geometric ratios of biological soft tissues for tensile testing as some studies used WL ratios as low as 0.4:1, but according to engineering standards, the grey-area can be between 0.25:1 and 4:1. Placement and vice versa for Fig. 5.9.

This study demonstrates that planar shear testing can be carried out on samples with WL ratios as low as 2:1 with certain errors in the minor strain involved and for samples below 1:1 tensile testing is suitable, but again with certain limitations involved for ratios that fall within the “grey-area”. Therefore, the “grey-area” is reduced from \(0.5:1 \leq WL \leq 4:1\) to \(1:1 \leq WL \leq 2:1\) (Fig. 5.13). It is also proposed that if a CAP sample has a WL ratio that falls within this new “grey-area” the gauge length should be altered when mounting the specimen in the clamps to achieve a WL ratio outside this “grey-area” avoiding the larger errors involved and improving the accuracy of the material model developed to characterise the CAP sample.

### 5.4.3 Limitations

The simplified computational analysis of the idealised ratios with uniform thickness was carried out to highlight the errors these geometric ratios contain when compared to theoretical minor strain. The idealised 2D plane strain models were used in the numerical study is an over-simplification of a diseased artery. However, as this was a comparative study, this setup was deemed suitable as the exclusion of residual strains and geometrical variations of the CAP samples. The effects of the 1 kPa assumption on the trends of this study were evaluated by varying the Young’s Modulus of the lipid core down to zero and this affected the peak von Mises stress, peak displacement, and deformed lumen area results by 3.33, 6.08 and 1.57 %, respectively. The CAP samples were characterised using the Yeoh SEF which assumes the material is isotropic and homogenous. However, as the main priority of this study is to analyse the plaque behaviour in the circumferential direction, the authors believe that an isotropic and homogenous assumption for the material properties is appropriate. This methodology of intact plaque testing is not without its own limitations, and the authors ultimately wanted to assess the strength of these tissues as a whole. Biaxial testing of plaque samples is necessary in future studies that aim to understand the anisotropic behaviour of carotid plaques (Holzapfel et al. 2004). The sample size of CAPs used in this study was small, but this limitation further highlights the need to redefine the ratios grey-area that at a certain b e u sed f or u niaxial testing a s w ell a s ta king in to a ccount th e experimental errors to improve the data needed for curve fitting of SEFs.
5.4.4 Conclusions

This study highlights that using the incorrect boundary parameters in a SEF can affect the peak stress and radial displacement values in a numerical model. The percentage error radial displacement is largest for CAP samples with WL ratios of 0.5:1 and 5:1 between the constitutive models developed for tensile and planar shear. It is important to characterise the sample using the correct boundary conditions, tensile or planar shear, for samples with certain geometrical ratios. However, as many whole CAP specimens typically do not have the required geometric ratio to suit these parameters, this study suggests to characterise samples to a SEF that have a WL ratio of 1:1 or less using tensile parameters and for samples greater than WL 2:1 to a planar shear setup. There are significant limitations when following these assumptions and the use of contactless strain measurement in a sample where the minor strain is known to be correct is imperative for more accurate experimentation of these CAP samples in the grey-area.

References


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CHAPTER 6

MECHANICAL AND BIOLOGICAL CHARACTERISATION OF CAROTID PLAQUE TISSUE

J.J. Mulvihill\textsuperscript{a}, E.M. Cunnane\textsuperscript{a}, S. McHugh\textsuperscript{b}, E. G. Kavanagh\textsuperscript{b}, S.R. Walsh\textsuperscript{b}, and M. T. Walsh\textsuperscript{a}

\textsuperscript{a}: Centre for Applied Biomedical Engineering Research, Department of Mechanical, Aeronautical, and Biomedical Engineering, Material and Surface Science Institute, University of Limerick, Limerick, Ireland.

\textsuperscript{b}: Department of Vascular Surgery, Limerick University Hospital, Limerick, Ireland.

The following chapter presents a paper on a novel approach of mechanically and biologically characterising human plaque tissue using uniaxial testing as well as spectroscopy and microscopy to identify the composition of the plaques. This chapter is under review in Acta Biomaterialia and presented verbatim, however in a more reader friendly format.

Acta Biomaterialia (\textit{In review})
6 Mechanical, biological and structural characterisation of in vitro ruptured human carotid plaque tissue

Abstract

Recent experimental studies performed on human carotid plaques have focused on mechanical characterisation for the purpose of developing material models for finite element analysis without quantifying the tissue composition or relating mechanical behaviour to pre-operative classification. This study characterises the mechanical and biological properties of 25 human carotid plaques as well as investigates the common features that lead to plaque rupture during mechanical testing by performing circumferential uniaxial tests, Fourier Transform Infra-Red (FTIR) and scanning electron microscopy (SEM) on each specimen to relate plaque composition to mechanical behaviour. Mechanical results revealed large variations between plaque specimen behaviour with no correlation to pre-operative ultrasound prediction. However, FTIR classification demonstrated a statistically significant relationship between stress and stretch values at rupture and the level of calcification (p = 0.002 and p = 0.009). Energy dispersive X-ray spectroscopy was carried out to confirm that the calcium levels observed using FTIR analysis were accurate. This work demonstrates the potential of FTIR as an alternative method to ultrasound of predicting plaque mechanical behaviour. SEM imaging at the rupture sites of each specimen highlighted voids created by the nodes of calcifications in the tissue structure which could lead to increased vulnerability of the plaque.

Keywords: tissue characterisation, atherosclerosis, carotid, FTIR, microscopy, biomechanics.

6.1 Introduction

Plaque rupture remains one of the most difficult clinical events to predict accurately and one of the leading causes of stroke worldwide [1, 2]. Despite significant advancements in in vivo imaging of atherosclerotic plaque, the standard for predicting the likelihood of plaque rupture remains the luminal stenosis level and plaque type, both of which are determined through the use of duplex ultrasound [3-5]. However, it has been reported that duplex ultrasound measurement cannot underestimate the severity of the
atherosclerotic plaque or under predict the risk of plaque rupture [6]. Tosi, Giorgini [7] suggest that the clinical use of vibrational spectroscopy is ready to be developed as an in vivo analysis technique for characterising atherosclerotic plaques. However, there are limited studies that relate the mechanical behaviour and rupture data of arterial plaques to vibrational spectroscopic data [8].

Fourier Transform Infra-Red (FTIR) is one of the most widely used vibrational spectroscopic techniques for the identification of biological components within tissue specimens [9]. FTIR functions by obtaining a broad range of infrared spectra from a sample based on the vibration of the molecules present and therefore can be used to generate a positive identification of the composition of a biological tissue [8]. Previous work has shown the ability of FTIR to identify and quantify the presence of lipid, collagen and calcification within plaque tissue [9]. Evenstein, Coughlin [8] use FTIR analysis to correlate the mechanical data from nano-indentation to the biological content ratios calculated from FTIR. This study provides important data to aid in the use of vibrational spectroscopy in clinical applications. However, it is necessary to relate the FTIR analysis results to the mechanical properties of the plaques on a global level rather than localised areas such as Evenstein, Coughlin [8] as carotid artery stenting (CAS) applies forces in the circumferential direction that will trigger a whole plaque mechanical response. Also, such a study would demonstrate whether FTIR is truly predicting the biological composition of the plaque as a whole and the features that may increase plaque vulnerability.

The morphology and composition features that contribute to plaque vulnerability have been extensively studied [10, 11]. However, the role of calcifications in plaque vulnerability is still under debate. Previous studies have suggested that calcifications stabilise the plaque [12-14] and that fibrous cap thickness [12, 15] and peak circumferential stress [16, 17] are the main contributors toward plaque vulnerability. Conversely, Wenk [18] demonstrated that the circumferential stress in the fibrous tissue increases as the volume of calcifications increases and that the presence of calcifications can significantly alter the distribution of stress and shift the peak stress from the main location of ruptures, such as the cap shoulder noted by Cheng, Loree [12] and Versluis, Bank [17]. Maldonado, Kelly-Arnold [19] and Vengrenyuk, Carlier [20] have shown that micro-calcifications have an effect on the Young’s modulus of the surrounding tissue (inducing a five-fold increase in the stress threshold at rupture).
which can increase plaque vulnerability due to the increase in voids created by the growth of micro-calcification clusters.

In order to examine the effect that calcifications have on the surrounding tissue structure, this study will use scanning electron microscopy (SEM) to observe the areas of rupture in the plaque in vitro induced by mechanical testing. SEM has, in recent years, been used in the biological sciences specifically to image the structure of tissue in great detail. Guasti, Marino [21] and Dell’Orbo, Quacci [22] demonstrate that SEM is capable of imaging the delamination and build-up of calcification in human carotid plaques. However, these studies are limited to one specific plaque specimen. Congiu, Schembri [23] examined a greater sample size (n=6) and identified the delamination and dysfunction of the endothelium which led to the build-up of disease in the specimens. In comparison, this present study examines an even larger sample size of human carotid plaques in order to establish the key features present in all specimens in order to provide a better understanding of atherosclerosis development and in vitro plaque rupture. In parallel to SEM imaging, energy-dispersive X-ray spectroscopy (EDX), an elemental analysis tool, can be used to characterise the composition of biological tissue in the areas of interest observed during imaging. EDX spectroscopy can determine the main constituents at the rupture sites of each plaque [24] as well as validate the vibrational spectrums and trends produced by FTIR.

This paper examines the biological composition of arterial plaque material and how the composition relates to the mechanical behaviour and rupture potential of these plaques through the use of FTIR globally and SEM and EDX. In order to achieve this, twenty-five carotid plaques are examined using each of these techniques as well as mechanical testing. This paper also analyses the ability of FTIR to predict plaque mechanical behaviour in order to assess whether FTIR is a viable technique for use as an in vivo pre-operative imaging tool in the treatment of carotid atherosclerotic tissue as well as assessing the contribution of calcifications toward plaque vulnerability. Finally, SEM imaging is carried out to investigate the morphology and structure of the in vitro rupture sites of the plaque specimens.

6.2 Materials and Methods

6.2.1 Sample Acquisition

Twenty-three carotid plaques were obtained from the Limerick University Hospital, Limerick, Ireland in a manner that conformed to the Declaration of Helsinki and was...
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approved by the hospital Ethical Research Committee. The carotid plaques were collected from consenting patients who underwent standard carotid endarterectomy surgery to treat high-grade carotid artery stenosis. Within this population 58% (12/23) of the patients were male, with a median age of 65.6 years (range, 52 – 79) and the median age of the female population was 72.2 years, (range, 52 – 85). Plaques were surgically removed from the carotid artery with preservation of plaque structural integrity emphasised to minimise possible disruption of the plaque luminal surface, figure 6.1. The ultrasound pre-operative identification was based on the type classification by AbuRahma and Bergan [25] where plaques are classified from Type I (soft echolucent plaque) to Type IV (hard echogenic plaque).

![Figure 6.1: Carotid plaques post-endarterectomy and equilibrated to 37°C in PBS prior to mechanical testing.](image)

The plaques were frozen in phosphate buffer solution (PBS) immediately after removal at -20°C. On the day of tissue testing, the plaques were equilibrated to room temperature in PBS and, after FTIR analysis, were further heated to 37°C prior to mechanical testing. Each plaque underwent the process illustrated in figure 6.2.

![Figure 6.2: Mechanical and biological characterisation process for each plaque tested.](image)

6.2.2 Mechanical Testing

Uniaxial mechanical testing was carried out on twenty-five whole specimens obtained from the twenty-three patients. Plaques 3 and 16 were divided into two separate pieces during the surgical removal and were sufficiently large to be tested individually. This increased the sample size to n = 25 (i.e. plaques 3a, 3b, 16a and 16b). Prior to mechanical testing, the specimens were placed into clamps designed for soft biological tissue, a uniform force was applied to the clamps using a torque screwdriver [26]. Measurements of the gauge length, thickness and width were taken using a vernier calipers and also with a non-contact photography system to validate the values. The plaques typically had width-to-length ratios greater than 4:1 which was suited for planar shear (also known as pure shear) testing. Figure 6.3 illustrates the mechanical testing process for each whole plaque specimen.
When the specimens were placed in the clamps, the geometrical ratio was taken into account to reduce the errors associated with unsuitable width-to-length ratios based on previous work by Mulvihill and Walsh [27]. As the samples were tested as a whole, the width and gauge lengths between each sample varied, 22.89 ± 5.94 mm and 4.157 ± 1.75 mm respectively. However, there was a larger variation in the sample thickness which is inherent in these samples due to difference in fibrous cap thickness, level of stenosis and overall artery size between each specimen (1.521 ±1.278 mm). This current study evaluates the circumferential stretch and stress value that the plaque can withstand prior to rupture, figure 6.3, at a physiological strain rate that replicates the instantaneous systolic pulse experienced by the plaque in vivo i.e. 30% of the gauge length per second (%/s) which is based on a typical circumferential stretch at 16 kPa [28]. Mechanical characterisation studies typically use relatively slow strain rates such as 0.1–1%/s which are ideal for complex constitutive models [29-32]. However, these strain rates may greatly underestimate the stress values which these plaques undergo in vivo or under stent and angioplasty deployment [26, 31]. In this study the plaque samples were preconditioned using five loading-unloading cycles to account for strain softening [33]. Plaque specimens were then stretched to complete failure, figure 6.3. The stretch and stress values at the point of plaque rupture are defined as the final point of definitive rupture where the plaque tissue does not exhibit an increase in stress with an increase in stretch, figure 6.3 [31].
6.2.3 FTIR analysis

FTIR analysis (Spectrum 100, Perkin Elmer Inc., MA, USA, Diamond Crystal) was carried out before and after chemical preparation for SEM in order to determine sample composition prior to mechanical testing and afterward to confirm specimen dehydration prior to SEM analysis. The analysis was performed at eight locations over the plaque luminal surface using the attenuate total reflectance (ATR) prior to dehydration, figure 6.4. After a background spectrum was removed, the ATR crystal was placed in direct contact with the specimen. All spectrums were ascertained using absorbance mode with a resolution of 2 cm⁻¹ for 32 scans over the range of 4000 - 700 cm⁻¹. The water spectrum was subtracted from each spectrum prior to peak area calculation [34]. The CH₂ stretch peaks found between 2972 - 2845 cm⁻¹ correspond to the absorbance of lipid within the specimen. Also, lipid ester peaks can be found in a number of plaques at 1730 cm⁻¹ which are included in the lipid peak area calculation. The collagen absorbance peak is represented by the amide I peak found between 1720 - 1585 cm⁻¹. The calcification peak is defined as the phosphate absorbance peak in the 1180 - 900 cm⁻¹ range. The areas under these peaks were measured using inbuilt software from Spectrum 100 (Perkin Elmer Inc., MA, USA) and from this, the ratios of lipid to collagen (Li:Col), calcification to collagen (Cal:Col) and calcification to lipid (Cal:Li) were calculated and averaged for each specimen [8] following the process outlined in figure 6.4.

Figure 6.4: Process used to ascertain quantitative data from the FTIR results of each plaque specimen

6.3 Scanning Electron Microscopy (SEM)

SEM imaging was carried out on the cross-section of the rupture site of all twenty-five samples after mechanical testing. Each rupture site was identified during post-
processing of the video footage from the mechanical test. However, as a conventional SEM was used in this study, it was necessary to chemically prepare each specimen to be used in the vacuum chamber. This preparation procedure requires a process of fixation, chemical dehydration and drying of the sample to minimise damage and shrinkage to the tissue structure. The sample must also be electrically conductive for the SEM to produce clear and quantitative images. This conductivity was attained by coating the sample in a thin layer of gold. To the author’s knowledge, there are only four studies which have used SEM to image the macro and micro structure of atherosclerotic carotid arteries [22-24, 35]. Therefore a consensus on a procedure for preparing human atherosclerotic tissue for SEM analysis that does not damage the structure, induce sample shrinkage or reduce image quality does not exist. The procedure employed in this study involved the samples being firstly cleaned in fresh PBS and then fixed in methanol for 10 minutes (as methanol is a more rapid and safer method of fixation than using glutaraldehyde or formaldehyde [36]). The fixed samples are then washed in 100% ethanol prior to the dehydration process whereby the specimens are passed through seven increments of ascending grades of molecular grade ethanol and distilled water mixture (30% ethanol up to 100% ethanol). The samples are then chemically dried in hexamethyldisilazane (HMDS) which has been shown to be a cheaper and more practical method of drying than critical point drying [37]. The samples are then gold plated at 30mA for 120 seconds to ensure a complete and even deposition of gold. EDX is used in this study to help identify the calcified and collagenous regions as well as validating the FTIR classification results. EDX measurements were taken at the lowest magnification of 30x to incorporate the entire sample during SEM imaging. The accelerating voltage current was increased to 20 keV and a dead time of between 20-40% was desired during elemental analysis. The EDX microanalysis process was performed using INCA Energy software platform (Oxford Instruments plc., Oxon, UK). The results were quantified in terms of the elemental atomic weight % in each sample and the ratio of Calcium and Phosphorus to Carbon (Ca+P:C) was compared to the FTIR ratios of Calcification to Lipid (Cal:Li) and Calcification to Collagen (Cal:Col). The results from the global FTIR analysis, carried out prior to chemical dehydration, are compared to the EDX data, carried out during SEM imaging, in order to determine if the same plaque components and trends were identified using both methods. All statistical data was generated using a one-way ANOVA test performed using SPSS statistical software (SPSS 20 Inc., Chicago Illinois, USA). This test was carried out to compare the statistical relevance between the means of the FTIR ratios of each plaque to
their respective stress and stretch data at rupture. A value of \( p < 0.05 \) was defined as the value of significance in this study.

### 6.4 Results

Table 6.1 lists the demographic, pre-operative, FTIR and mechanical rupture data of each specimen that was mechanically and biologically characterised in this study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pre-Op Data Type</th>
<th>FTIR Data</th>
<th>Mechanical Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Li:Col</td>
<td>Cal:Col</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0.199</td>
<td>0.309</td>
</tr>
<tr>
<td>2</td>
<td>3-4</td>
<td>0.234</td>
<td>0.289</td>
</tr>
<tr>
<td>3a</td>
<td>2</td>
<td>0.754</td>
<td>0.398</td>
</tr>
<tr>
<td>3b</td>
<td>2</td>
<td>0.720</td>
<td>0.576</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.670</td>
<td>0.238</td>
</tr>
<tr>
<td>5</td>
<td>3-4</td>
<td>0.381</td>
<td>0.729</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.971</td>
<td>0.664</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1.547</td>
<td>0.626</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0.311</td>
<td>0.473</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0.336</td>
<td>0.275</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0.401</td>
<td>0.324</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0.616</td>
<td>0.300</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>1.020</td>
<td>0.357</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>0.301</td>
<td>0.355</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>0.219</td>
<td>0.307</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>0.403</td>
<td>0.391</td>
</tr>
<tr>
<td>16a</td>
<td>2</td>
<td>0.516</td>
<td>0.335</td>
</tr>
<tr>
<td>16b</td>
<td>2</td>
<td>0.896</td>
<td>0.427</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>0.836</td>
<td>0.286</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>0.942</td>
<td>0.590</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>0.355</td>
<td>0.279</td>
</tr>
<tr>
<td>20</td>
<td>NA</td>
<td>0.477</td>
<td>0.291</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>0.324</td>
<td>0.364</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>0.406</td>
<td>0.679</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>0.200</td>
<td>0.188</td>
</tr>
</tbody>
</table>
Figure 6.5 presents the Cauchy Stress vs. Stretch Ratio data to ultimate failure of each plaque specimen that was mechanically tested. The colour of the lines in figure 6.5 (Type I: orange, Type II: blue, Type III: purple, Type III/IV: red and Type IV: green) indicates the pre-operative classification of that plaque based on the definitions from AbuRahma and Bergan [25]. The statistical values obtained for pre-operative type to stress and stretch were $p = 0.353$ and $p = 0.328$, respectively. The non-conformity of each line colour highlights that there is no particular correlation between mechanical behaviour and ultrasound classification. Figure 6.5 also demonstrates the wide range of plaque mechanical behaviour. This indicates that certain plaques may be more susceptible to plaque rupture in vivo or during stenting and angioplasty.

The plots shown in figure 6.6 are of the Cauchy Stress vs. Stretch Ratio data grouped by Cal:Li type whereby a black line corresponds to a Cal:Li ratio greater than 1 and conversely for the grey dashed lines. Figure 6.6 demonstrates that the higher calcification to lipid content (Cal:Li $>1$), classified by FTIR analysis, relates to a stiffer mechanical response whereas a lower calification to lipid less than one (Cal:Li $<1$) indicates softer plaque behaviour. A significant statistical relation was found between the rupture stress and strain values and the FTIR classification i.e. Cal:Li $> 1$ ($p = 0.002$).
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and $p = 0.009$, respectively).

Figure 6.6: Cauchy Stress vs. Stretch Ratio plot of the plaques classified by FTIR biological content ratio of Cal:Li > 1 (black lines) and Cal:Li < 1 (grey dashed lines). The graph highlights that the plaques with the higher calcification content have a stiffer mechanical behaviour compared to the more lipid plaques which are softer.

SEM imaging was carried out on the mechanically tested specimens in order to determine common features at the site of rupture. FTIR was carried out at the sites of rupture to confirm full dehydration of the plaque specimens for use in the SEM vacuum chamber. SEM analysis revealed typical features such as endothelial separation from the basal lamina and collagen delamination in the cross-section, figure 6.7, as well as endothelial dysfunction exposing the basal lamina layer, figure 6.8, from surface analysis.

Figure 6.7: SEM images of (a) endothelium separation and (b) stratum delamination through the cross section of the plaque.
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Figure 6.8: SEM images of endothelial dysfunction on the surface (a) the basal lamina layer (arrow) protruding through the stretched endothelium (b) absence of endothelium where leukocyte adhesion (arrows) is prevalent.

Figure 6.9 is a macro image taken from the cross-section of two human carotid plaque specimens at the site of rupture, figure 6.9 (a) had a Cal:Li greater than 1 and figure 6.9 (b) was less than 1. Both images show node s of calcification dispersed throughout fibrous tissue which contributed to the failure of these plaques. A higher level of calcification in these sites of rupture was observed in the Cal:Li > 1 based plaques. Figure 6.9 (a) demonstrates the dominance of calcified nodes in the cross-section and the effect it has on the surrounding tissue by creating voids which according to Maldonado, Kelly-Arnold [19] can lead to specimen rupture.

Figure 6.9: SEM image of the cross-section of a plaque specimen at the site of plaque rupture where nodes of calcification (arrows) and fibrous tissue are present in a Cal:Li > 1 type (a) and a Cal:Li < 1 type (b).

The most common formations of calcification within these observed plaques were nodular and sheet-like, figure 6.10 (a) and (b), respectively. Various levels of these calcification formations were observed in all plaque specimens, including micro-calcifications, which were dispersed through the stratum (figure 6.11 b), and large nodes of calcifications, which dominated the whole plaque structure (figure 6.9 a) and according to Maldonado, Kelly-Arnold [19] can create voids due to clustering of the calcifications. The key variation observed between both plaque types (Cal:Li > 1 and
Cal:Li < 1) was the development of these calcification nodes and their domination of the surrounding tissue structure that led to the higher Cal:Li ratio, figure 6.9 (a). Figure 6.11 (a) and (b) show the interaction of the calcification with the surrounding fibrous tissue and how it separates the collagen layers creating voids evidencing work reported by Maldonado, Kelly-Arnold [19] and highlighting a feature which could increase the vulnerability of the plaque tissue structure.

Figure 6.10: SEM images of two calcification types commonly found in the carotid plaques analysed in the study: (a) nodular and (b) sheet-like.

Figure 6.11: SEM images of a node of calcification within a carotid plaque and its effect on the surrounding tissue (a) separating the stratified tissue and (b) tissue stretched and wrapped around the node.

EDX was carried out on the site of rupture to analyse the level of calcification in each specimen. The plaques analysed by EDX were grouped by the FTIR classification and table 6.2 lists the average percentages of each element as well as the calcium and phosphorous to carbon ratio. Table 6.2 demonstrates that EDX indicated higher amounts of calcium and phosphorous in the plaques which FTIR had classified as calcified.
Table 6.2: EDX element data and FTIR biological ratios which highlights a large presence of calcification in both the EDX (phosphate and calcium) and FTIR results for the Cal:Li>1 plaques and a lower ratio for Cal:Li<1 plaques.

<table>
<thead>
<tr>
<th>Element</th>
<th>Col:Li &gt;1</th>
<th>Col:Li &lt;1</th>
<th>Percentage Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>51.8</td>
<td>60.85</td>
<td>14.87</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>37.97</td>
<td>32.45</td>
<td>14.54</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0.52</td>
<td>0.45</td>
<td>13.46</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>2.84</td>
<td>1.79</td>
<td>36.97</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>7.06</td>
<td>3.62</td>
<td>48.73</td>
</tr>
<tr>
<td>Ca+P:C (%)</td>
<td>19.56</td>
<td>8.8</td>
<td>54.55</td>
</tr>
</tbody>
</table>

FTIR – Cal:Li 1.486 0.625 57.95
FTIR – Cal:Col 1.88 0.58 69.14

6.5 Discussion

In this study mechanical testing of whole carotid plaque specimens acquired from carotid endarterectomies was carried out to ascertain the mechanical behaviour and rupture data of each plaque at a physiological strain rate. These results were compared to the pre-operative ultrasound classification and post-operative FTIR and EDX analyses to determine if any trend and/or statistical relation are present. SEM imaging was qualitatively analysed to determine if any common features were present in the sections of the plaques which failed during mechanical testing.

Despite duplex ultrasound being a widely used pre-operative classification method to predict plaque type prior to undertaking carotid endarterectomy [38], the large spread of data in figure 6.5 and table 6.1 demonstrates that it is not a good predictor of plaque mechanical behaviour or rupture potential which is essential information required prior to CAS due to the risk of embolization during surgery. The pre-operative classification typing based on AbuRahma and Bergan [25] did not show any significant relation to rupture data based on a one-way ANOVA test.

Tosi, Giorgini [7] suggest that vibrational spectroscopy is ready to be used as an in vivo method for predicting plaque type. This finding allows for this study to relate the global mechanical properties of plaque to FTIR analysis. Averaged FTIR analysis at eight locations of each plaque specimen indicated that eight (32%) of the twenty-five samples tested had a high calcification content as the Cal:Li ratio was greater than one. The remaining seventeen (68%) specimens had a Cal:Li ratio less than one indicating a low level of calcification. Figure 6.6 presents the mechanical data grouped by FTIR classification and suggests a clear distinction between the stiffer group of plaques due to calcification (Cal:Li > 1) and the softer more lipid plaques (Cal:Li < 1) suggesting that
FTIR has the potential to predict the global mechanical behaviour of carotid plaques albeit in an in vitro environment. These findings correspond with the results of Ebenstein, Coughlin [8] where nano-indentation was used to show that the stiffness of plaque tissue increases with higher calcification content at a specific location through the use of FTIR analysis. Stress values at rupture in this study found that the average rupture stress value for the lipid dominant plaques (Cal:Li < 1) is 0.342 ± 0.16 MPa. This value is comparable to the rupture threshold stress value of 0.3 MPa based on experimental data [15] but overestimates in vivo rupture stresses predicted by Maldonado, Kelly-Arnold [19] which suggest that non-calcified tissue ruptures at 0.107 MPa. Shah [39], Cheng, Loree [12] and Huang, Virmani [13] reported that the lipid component of plaque specimens is a main contributor toward the rupture of plaque and a key factor in plaque vulnerability and that calcifications may stabilise the plaque. However, this averaged rupture value for lipid based plaques is low in comparison to the stiffer plaques (Cal:Li > 1) with an average rupture stress of 0.618 ± 0.23 MPa indicating that these lipid plaques will experience rupture at a lower applied force. The Cal:Li > 1 averaged stress value is double the threshold stress value of 0.3 MPa from Lendon, Davies [15] and concurs with the in vivo rupture work by Maldonado, Kelly-Arnold [19] and Wenk [18] and in vitro experimental work by Ebenstein, Coughlin [8] which all demonstrate that calcifications significantly increase the stiffness of plaque tissue. The results presented in this study show that the lipid based plaques have a higher level of stretch at rupture (1.927 ± 0.26 stretch) compared to the more calcified plaques (1.631 ± 0.17 stretch). This would suggest that plaques with a higher calcium composition are more likely to rupture when exposed to the stretching caused by the circumferential forces of an expanding carotid stent and potentially more vulnerable to CAS in contrast to previous studies that have reported that rupture of carotid plaques during CAS is more likely in plaques with large lipid composition [39, 40]. This study also suggests that calcifications have a significant role in the vulnerability of the plaque with regard to a lower stretch ratio at rupture when exposed to the stretching caused by the circumferential forces of an expanding carotid stent and potentially more vulnerable to CAS in contrast to previous studies that have reported that rupture of carotid plaques during CAS is more likely in plaques with large lipid composition [39, 40]. This study also suggests that calcifications have a significant role in the vulnerability of the plaque with regard to a lower stretch ratio at rupture when exposed to the stretching caused by the circumferential forces of an expanding carotid stent and potentially more vulnerable to CAS in contrast to previous studies that have reported that rupture of carotid plaques during CAS is more likely in plaques with large lipid composition [39, 40].
and $p = 0.009$ demonstrates the potential of F TIR in predicting the vulnerability of plaque based on ultimate stretch and stress values in vitro.

SEM imaging was carried out on the sections of the plaque samples that experienced rupture. This was performed in order to qualitatively analyse the common rupture site components evident between all of the specimens examined in this study. On a macro-level, the key features within the rupture sites of the plaque samples were delamination of the collagen layers, separation of the endothelial layer and presence of calcification nodes through the cross-section of the plaques and its effect on the fibrous stratum, figures 6.7-6.11. The SEM preparation procedure employed in this study involved the use of chemicals to fix, dehydrate and dry the samples using methanol, ethanol and HMDS, respectively. Ethanol dehydration has been shown to damage the cholesterol crystals within coronary arteries which are pivotal to development of plaque rupture [41]. However, in a letter of reply to Abela, Shamoun [42] it was suggested that the alternative to ethanol dehydration, vacuum dehydration, can affect the structure of the collagen fibrin within the tissue which are of more interest in this study due to the effect they have on the mechanical behaviour.

Endothelial dysfunction was present in all plaques observed in the form of complete separation of the endothelium from the basal lamina layer, stretching and tearing of the endothelium on the surface and the delamination of the stratum through the cross-section similar to images shown in Congiu, Schembri [23]. Endothelial dysfunction on the surface of the plaques was apparent in all plaque samples whereby the endothelium was either stretched, figure 6.8 (a), or entirely absent, figure 6.8 (b), thereby exposing a reticulum of fibrin from the basal lamina layer. This endothelial deterioration promotes the adhesion of leukocytes to the damaged endothelium and leads to an increase in the permeability of the arterial structure to low-density lipoprotein which leads to the growth of atherosclerosis [43]. These leukocytes are prevalent in all imaged plaque samples and an example of the leukocytes are shown in figure 6.8 (b) in which they have adhered to and penetrated through the exposed basal lamina layer. This emphasises that over stretching of the plaque during CAS aids in the promotion of further adhesion of leukocytes post-operatively allowing for the development of restenosis [44].

The presence of calcification throughout the cross section of the diseased tissue has a profound effect on the global structure of the artery as illustrated by the mechanically stiff and soft plaques shown in figure 6.9 (a) and (b), respectively. However, in both cases the presence of calcification nodes was shown to be a main contributor to the
rupture of these plaques. Figure 6.9 (a) highlights the damage that the large nodes of calcification caused to the tissue structure as well as the multiple formations of these calcified nodes within one plaque sample which created a void in the cross section that may have contributed to failure in the plaque [19, 20]. The most prevalent calcification types observed during imaging were nodular calcifications, figure 6.10 (a), and sheet-like calcifications in figure 6.10 (b). There was no clear evidence of osteoid-metaplasia or clear-centre type calcifications as defined by Herisson, Heymann [35] in the specimens imaged during this study. However, further examination into the micro- and nano-level of these specimens may show indications of these calcification types. Figure 6.11 (a) and (b) demonstrate the effect that the formation of calcification nodes has on the tissue. It illustrates the effects of separating the stratum and subsequent voids which can weaken the mechanical properties of the plaque.

Schembri, Congiu [24] and Guasti, Marino [21] both highlighted the potential of EDX as a useful analytical tool for examining the morphology of a plaque specimen when coupled with SEM imaging. EDX measures the elemental ratio and is used in this study as a method of confirming the biological components identified during FTIR analysis. EDX revealed that all of the samples, soft and stiff types, contained at least a trace of phosphorous and calcium. However, EDX results also demonstrated that the plaques which had a Cal:Li ratio greater than one had higher levels of calcium and phosphorous than those with a ratio less than one, Table 6.2. The carbon content is comparatively smaller in the plaque samples with a Cal:Li ratio greater than one due to the higher presence of calcification nodes which do not contain carbon as highlighted by EDX results in Schembri, Congiu [24]. The calcium and phosphorus to carbon ratio of the averaged results in Table 6.2 for both plaque types evidences the large presence of calcification in the mechanically stiffer plaques compared to the softer specimens. This confirms that a similar trend is identifiable in both the FTIR and EDX methods which suggest that the stiffer mechanical behaviour is due to the higher level of calcification within the carotid plaque.

A limitation of this study is the preparation of the tissue for SEM which can potentially distort the images and alter key features such as cholesterol crystals [41]. Ideally, the use of a low-vacuum SEM, which requires no preparation, rather than a conventional SEM would eradicate this limitation. Also, plaque rupture in vivo is a multi-axial and multi-factorial process that in vitro uniaxial testing in the circumferential direction does not fully represent. Uniaxial testing in the eccentric circumferential direction was chosen as...
circumferential stress is a key determinant in understanding the forces that the plaque specimens can withstand in vivo [20] which, in combination with FTIR analysis and SEM imaging, can aid in further understanding the mechanics and structural features responsible for plaque rupture and vulnerability. However, a caveat for this study is that further comparison to in vivo rupture studies is necessary to demonstrate the potential of these characterisation techniques and the accuracy of stress values ascertained from in vitro mechanical testing to in vivo conditions.

6.6 Conclusion

Duplex ultrasound measurement classification has been shown to be an unsuitable diagnostic predictor of plaque material properties. An improved method of classification is developed in this study through the use of vibrational spectroscopy, specifically using FTIR. FTIR has demonstrated that the plaques with a higher concentration of calcification than lipid content produced a stiffer mechanical response than those with higher lipid content which conversely displayed a softer response. To validate the FTIR predictions, EDX spectroscopy was carried out on plaque samples imaged using SEM to analyse the elemental content of the plaques at the area of rupture within each specimen after mechanical testing to failure. EDX reiterated the main outcome of the FTIR results i.e. that there is a higher content of calcification in the mechanically stiffer plaques in comparison to the softer plaques. This study also concludes that SEM, coupled with EDX spectroscopy, can be a useful tool in analysing the key features that lead to plaque rupture. An important conclusion of this study is the identification of the voids created by the calcification clusters in the tissue structure at the rupture sites of both lipid and calcified dominant plaques. This suggests that calcification does not stabilise the plaque tissue but rather increases the vulnerability and difficulty in predicting the location of the rupture. These large nodes of calcification not only created voids in the stratified structure of the tissue but de laminated the collagen layers that could further lead to plaque failure. This conclusion is an important and novel factor in the debate on the role of calcification in the determination of plaque vulnerability as this paper experimentally demonstrates that calcification can potentially destabilise the plaque tissue structure.

Acknowledgements

The authors would like to acknowledge the Irish Research Council, Dublin 4, Ireland for the funding from the EMBARK Initiative. The authors would also like to thank Ms. Hilary Barrett for her work on the study specifically with the SEM and EDX work.
References


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CHAPTER 7

CAROTID PLAQUE COMPOSITION AND MECHANISM OF BEHAVIOUR:

EFFECT OF PLAQUE CALCIFICATION ON RISK OF RUPTURE

J.J. Mulvihill¹, S. M. CHugh², E. M. Cunnane¹, E. K.avanagh², S. R. Walsh², and M. T. Walsh¹

¹: Centre for Applied Biomedical Engineering Research, Department of Mechanical, Aeronautical, and Biomedical Engineering, Material and Surface Science Institute, University of Limerick, Limerick, Ireland.

²: Department of Vascular Surgery, Limerick University Hospital, Limerick, Ireland.

The following chapter presents a paper on the effect of calcification on the risk of rupture and the potential of vibrational spectroscopy as a new diagnostic method of predicting plaque material type pre-operatively. This chapter is submitted in the European Journal of Vascular and Endovascular Surgery and presented verbatim, however in a more reader friendly format.

European Journal of Vascular and Endovascular Surgery (In Review)
7 Carotid plaque composition and mechanism of behaviour – Effect of plaque calcification on risk of rupture

Abstract

Objective: The main objective of this work is to determine whether biological plaque composition had any correlation with plaque mechanical behaviour, with particular focus on risk of rupture when undergoing stresses comparable with balloon inflation during carotid artery stenting (CAS).

Approach: Carotid bifurcation plaques were collected from consecutive patients undergoing standard endarterectomy to treat high-grade internal carotid artery stenosis. Fourier Transform Infra-Red (FITR) was carried out on eight locations throughout each of the plaques. Uniaxial mechanical testing was carried out on 23 specimens as a whole to ascertain the global mechanical properties. The plaque property that this study establishes is the stress at rupture due to a circumferential stretch that the plaque can withstand before rupture under a strain rate that mimics the cardiac rate of an averaged healthy peak systolic pressure.

Results: Carotid bifurcation plaques were collected from 23 consecutive patients. FTIR analysis of each plaque specimen indicated that eight (32%) of the plaques had a higher calcification as the Calcification to Lipid ratio (Ca:Li) ratio was greater than one. These plaques were noted to have a significantly increased initial rupture stress value ($p = 0.003$). In plaques with a Cal:Li ratio less than one there was rupture at a lower stress and higher stretch value.

Conclusions: Carotid plaques with a higher calcium composition are more stable and less likely to rupture in physiological conditions. However, in patients undergoing CAS, FTIR can identify calcified plaques that may be more likely to rupture from the circumferential force of an expanding balloon.
7.1 Introduction

Carotid endarterectomy (CEA) has long been the standard treatment for symptomatic carotid artery stenosis. The introduction of carotid artery stenting (CAS) in recent years has evolved into an alternative modality. Carotid artery stenting is less invasive compared with CEA, and has the potential to successfully treat lesions close to the aortic arch or distal internal carotid artery.\(^1\,^2\) Randomised controlled trials conducted to establish efficacy and equivalency of CAS to CEA have often reported conflicting results and therefore divided opinions regarding the employment of CAS in revascularisation of the carotid artery.\(^3\,^4\,^5\)

A particular concern highlighted by several trials is the increasing complication rate with age for CAS procedures compared with CEA in a comparable population.\(^3\,^4\,^6\,^7\) This may be a marker for underlying issues such as increasingly adverse anatomy or differences in pathology. Despite the potential importance of anatomical and physiological factors for reduced CAS mortality and morbidity rates, current pre-endovascular assessment is limited. Duplex ultrasound is most commonly used for pre-operative evaluation of degree of carotid stenosis and plaque structural morphology.

We sought to determine carotid plaque structural characteristics using Fourier Transform Infra-Red (FTIR). Fourier Transform Infra-Red is one of the most widely used spectroscopic techniques in the identification of biological specimens. Previous work has shown the ability of FTIR to identify and quantify the presence of lipid, collagen and calcification within vascular plaques as an equivalent tool to histology to measure and identify biological composition.\(^8\)

Carotid plaque structure and composition also has a significant bearing on the risk of cerebral infarct. A recent study has reported that carotid plaques with a higher amount of lipid composition were associated with higher rates of cerebrovascular events than those with higher levels of calcification.\(^9\,^10\) Plaque rupture initiates the release of embolic debris, and ruptured plaques have also been reported as having an increased incidence of neurological symptoms.\(^12\,^13\)

Carotid artery stenting is associated with high rates of cerebrovascular events in some trials but is an attractive intervention in selected candidates. Plaque rupture during CAS may trigger adverse events therefore there is a need to identify which plaques are at high risk of rupture during CAS. The key moment with regard to plaque rupture during
CAS is balloon inflation. Therefore we sought to determine whether biological plaque composition determined using F ITR had any correlation with plaque mechanical behaviour, with particular focus on risk of rupture when undergoing stretches comparable with balloon inflation during CAS.

7.2 Materials and Methods

Following hospital ethical research committee approval, carotid bifurcation plaques were collected from patients undergoing standard CEA to treat high-grade internal carotid artery stenosis and who underwent duplex ultrasound imaging. Plaques were endarterectomised in toto with preservation of plaque structural integrity and minimisation of disruption to the plaque luminal surface. The plaques were taken from the hospital and frozen in phosphate buffer solution (PBS) at -20°C. The plaques were equilibrated to 37°C in fresh PBS and tested as a whole specimen rather than sectioning the structure which could potentially underestimate the global mechanical behaviour of the plaque specimen.

Uniaxial mechanical testing was carried out on 23 specimens as a whole at a physiological strain rate of 30%/s. The plaque property that this study establishes is the circumferential stretch that the plaque can withstand before rupture under a strain rate that mimics the cardiac rate of an averaged healthy peak systolic pressure. The carotid plaques are placed longitudinally in the clamping jaws of a uniaxial tester and are extended in a circumferential direction. The plaques were pre-conditioned using five loading-unloading cycles to facilitate for the strain softening phenomenon exhibited by biological soft tissue. The plaque specimen is then stretched until sample failure. The force and displacement values are recorded throughout the test and are then converted to stress-stretch values that can be used to compare each specimen. The Cauchy stress value is used as it is a better representative stress type for hyperelastic materials such as arterial plaque and the stretch ratio is the most commonly used strain measurement with Cauchy stress. The stretch and stress (MPa) value at rupture is defined as the first point of definitive rupture where the change in slope of the curve (dy/dx) becomes negative, figure 7.1.
Fourier Transform Infra-Red was carried out (Spectrum 100, Perkin Elmer Inc., MA, USA, diamond crystal) on eight locations throughout the plaque using the attenuate total reflectance method. All spectra were ascertained using absorbance with a resolution of 2cm\(^{-1}\) and 32 scans over the range of 4000 - 700cm\(^{-1}\). After the water subtraction was applied to each of the spectra the areas under the peaks of interest (Lipid: 2980-2810cm\(^{-1}\), Lipid Ester: 1750 -1715cm\(^{-1}\), Collagen: 1715 -1585cm\(^{-1}\) and Calcification: 1180 -900cm\(^{-1}\)) were measured and the ratios of lipid to collagen (Li:Col), calcification to collagen (Cal:Col) and calcification to lipid (Cal:Li) were calculated and averaged for each specimen, where a Cal:Li ratio greater than one indicates a highly calcified plaque and conversely for Cal:Li less than one.\(^{17}\)

All statistical data was generated using a one-way ANOVA test and performed using SPSS statistical software (SPSS 20 Inc., Chicago Illinois, USA). A value of \(p < 0.05\) was defined as value of significance in this study.

### 7.3 Results

Carotid bifurcation plaques were collected from 23 consecutive patients who underwent standard CEA to treat internal carotid artery stenosis. Within this population 58% (n=12/23) of the patients were male with a median age of 65.6 years (range 52 – 79), and 42% (n=11/23) were female with a median age of 72.2 years (range 52 – 85).
Indications for CEA were transient ischemic attack (TIA) (n = 13, 57.5%), cerebrovascular accident (n = 7, 30.5%), high-grade asymptomatic carotid stenosis (n = 2, 8.5%) and one with no data (4.5%).

From pre-operative ultrasound classification, 4.5% (n=1) were type I, 39% (n=9) type II, 30.5% (n=7) type III, 8.5% (n=2) as type III/IV and 13% (n=3) type IV, one could not be classified (4.5%). Average Stress at rupture for types I, II, III, III/IV and IV were 0.17, 0.298, 0.316, 0.622 and 0.371 MPa, respectively. The Cauchy Stress versus Stretch Ratio data of each plaque obtained from the population analysed is represented in figure 7.2. The shading and style of the lines in figure 7.2 (Type I: grey dots, Type II: grey line, Type III: black dashes, Type III/IV: grey dashes, N/A: black dots and Type IV: black line) indicates the pre-operative classification of that plaque which highlights a large spread of the data with no particular correlation with mechanical behaviour and ultrasound classification. Figure 7.2 and table 7.1 also demonstrate the spread of mechanical behaviour of plaques emphasising how certain plaques can potentially be more susceptible to plaque rupture during stenting and angioplasty.

Table 7.1: Pre-operative patient data, ultrasound classification and post-operative mechanical properties of each plaque. Li:Col: Lipid to Collagen ratio; Cal:Col: Calcification to Collagen ratio, Cal:Li: Calcification to Lipid Ratio.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
<th>Gender</th>
<th>% Stenosis</th>
<th>Pre-Op Disease Classification</th>
<th>Li:Col</th>
<th>Cal:Col</th>
<th>Cal:Li</th>
<th>Initial Stretch</th>
<th>Initial Stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>F</td>
<td>70-79%</td>
<td>4</td>
<td>0.199</td>
<td>0.309</td>
<td>1.794</td>
<td>1.325</td>
<td>0.367</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>F</td>
<td>90-99%</td>
<td>3-4</td>
<td>0.234</td>
<td>0.289</td>
<td>1.230</td>
<td>1.396</td>
<td>0.592</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>70-79%</td>
<td>2</td>
<td>0.754</td>
<td>0.398</td>
<td>0.528</td>
<td>1.425</td>
<td>0.132</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>F</td>
<td>80-89%</td>
<td>3</td>
<td>0.670</td>
<td>0.238</td>
<td>0.324</td>
<td>1.422</td>
<td>0.242</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>80-99%</td>
<td>3-4</td>
<td>0.381</td>
<td>0.729</td>
<td>1.910</td>
<td>1.903</td>
<td>0.653</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>F</td>
<td>70-79%</td>
<td>2</td>
<td>0.971</td>
<td>0.664</td>
<td>0.663</td>
<td>1.314</td>
<td>0.308</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>M</td>
<td>50-69%</td>
<td>3</td>
<td>1.547</td>
<td>0.626</td>
<td>0.436</td>
<td>1.252</td>
<td>0.179</td>
</tr>
<tr>
<td>8</td>
<td>85</td>
<td>F</td>
<td>50-69%</td>
<td>3</td>
<td>0.311</td>
<td>0.473</td>
<td>1.399</td>
<td>1.221</td>
<td>0.317</td>
</tr>
<tr>
<td>9</td>
<td>79</td>
<td>M</td>
<td>90-99%</td>
<td>2</td>
<td>0.336</td>
<td>0.275</td>
<td>0.782</td>
<td>1.837</td>
<td>0.461</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>M</td>
<td>70-79%</td>
<td>2</td>
<td>0.401</td>
<td>0.324</td>
<td>0.772</td>
<td>1.287</td>
<td>0.063</td>
</tr>
<tr>
<td>11</td>
<td>63</td>
<td>F</td>
<td>90-99%</td>
<td>2</td>
<td>0.616</td>
<td>0.300</td>
<td>0.581</td>
<td>1.689</td>
<td>0.609</td>
</tr>
<tr>
<td>12</td>
<td>52</td>
<td>M</td>
<td>80-89%</td>
<td>1</td>
<td>1.020</td>
<td>0.357</td>
<td>0.350</td>
<td>1.871</td>
<td>0.170</td>
</tr>
<tr>
<td>13</td>
<td>74</td>
<td>M</td>
<td>50-69%</td>
<td>3</td>
<td>0.301</td>
<td>0.355</td>
<td>1.170</td>
<td>1.389</td>
<td>0.699</td>
</tr>
<tr>
<td>14</td>
<td>52</td>
<td>F</td>
<td>NA</td>
<td>2</td>
<td>0.219</td>
<td>0.307</td>
<td>1.620</td>
<td>1.164</td>
<td>0.206</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>F</td>
<td>80-89%</td>
<td>3</td>
<td>0.403</td>
<td>0.391</td>
<td>0.777</td>
<td>1.481</td>
<td>0.234</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>M</td>
<td>50-69%</td>
<td>2</td>
<td>0.896</td>
<td>0.427</td>
<td>0.324</td>
<td>1.329</td>
<td>0.064</td>
</tr>
<tr>
<td>17</td>
<td>68</td>
<td>M</td>
<td>50-69%</td>
<td>3</td>
<td>0.836</td>
<td>0.286</td>
<td>0.463</td>
<td>1.534</td>
<td>0.294</td>
</tr>
<tr>
<td>18</td>
<td>85</td>
<td>F</td>
<td>70-79%</td>
<td>2</td>
<td>0.942</td>
<td>0.590</td>
<td>0.786</td>
<td>1.513</td>
<td>0.131</td>
</tr>
<tr>
<td>19</td>
<td>53</td>
<td>M</td>
<td>70-79%</td>
<td>3</td>
<td>0.355</td>
<td>0.279</td>
<td>0.776</td>
<td>1.358</td>
<td>0.244</td>
</tr>
<tr>
<td>20</td>
<td>74</td>
<td>M</td>
<td>70-79%</td>
<td>NA</td>
<td>0.477</td>
<td>0.291</td>
<td>0.643</td>
<td>1.432</td>
<td>0.301</td>
</tr>
<tr>
<td>21</td>
<td>64</td>
<td>M</td>
<td>50-69%</td>
<td>4</td>
<td>0.324</td>
<td>0.364</td>
<td>1.238</td>
<td>1.596</td>
<td>0.507</td>
</tr>
<tr>
<td>22</td>
<td>72</td>
<td>M</td>
<td>70-79%</td>
<td>2</td>
<td>0.406</td>
<td>0.679</td>
<td>1.526</td>
<td>1.337</td>
<td>0.432</td>
</tr>
<tr>
<td>23</td>
<td>70</td>
<td>F</td>
<td>70-79%</td>
<td>4</td>
<td>0.200</td>
<td>0.188</td>
<td>0.896</td>
<td>1.741</td>
<td>0.239</td>
</tr>
</tbody>
</table>
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Figure 7.2: Cauchy Stress vs. Stretch ratio plots of each plaque, demonstrating the distribution of plaque behaviour following uniaxial testing. Disparity between pre-operative plaque classification demonstrated within the plots; Type I = grey dots, Type II = grey line, Type III = black dashes, Type III/IV = grey dashes, Type IV = black line and N/A = black dots.

Figure 7.3 presents the stretch and stress values at fracture for each of the plaques and also indicates the stretch limits of 53% and 200% required of each carotid plaque when restoring a 30% stenosed lumen to a 70% lumen and also when a 10% lumen is restored to a post-operative 90% lumen, respectively. This second scenario is of particular interest in this study as the average level of pre-operative stenosis for this group of patients is 90%. It can be seen that only six of the 23 plaques (26%) would have a stretch threshold high enough to successfully undergo the CAS surgical procedure to achieve the 70% lumen. Furthermore, none of the 23 (0%) plaques would successfully undertake the 90% lumen procedure. This high failure rate in both cases does raise serious concerns regarding the use of CAS as a treatment option for diseased carotid arteries.
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Figure 7.3: Cauchy Stress (black squares) and Stretch Ratios (bars) of each plaque divided into pre-operative types. Dashed black lines represent the lowest and highest idealised stretch the plaque undergoes during CAS.

Figures 7.4 and 7.6 display the averaged Cauchy Stress and Stretch Ratio values at initial rupture of the plaque in the groupings of pre-operative classification type and FTIR type based on the Cal:Li ratio, respectively. Figure 7.4 shows no distinct correlation between the pre-operative ultrasound classification types in either the stretch or stress values. Conversely, figure 7.6 illustrates a trend with FTIR type and the stretch and stress values at initial rupture. Where Cal:Li greater than one there is a higher stress and lower stretch at rupture. Where Cal:Li was less than one there was rupture at a lower stress and higher stretch value.
Pre-operative classification did not show any significance in relation to the stress and stretch values at initial rupture of the plaque ($p = 0.154$ and $p = 0.134$). There was no significant correlation between pre-operative classification and patient demographics ($p = 0.428$, $p = 0.854$ and $p = 0.191$ for age, gender and indication respectively) or level of stenosis ($p = 0.165$). Stenosis level on pre-operative duplex also showed a non-significant relation to stress and stretch at plaque rupture ($p = 0.102$ and $p = 0.059$).

FTIR analysis of each plaque specimen indicated that eight (35%) of the plaques had a higher calcification as the Cal:Li ratio was greater than one. The remaining 15 (65%) specimens had a Cal:Li ratio less than one indicating a low level of calcification. The eight plaques with Cal:Li greater than one (Plaques 1, 2, 5, 8, 13, 14, 21 and 22) had a stiffer behaviour with a higher stress value at rupture as well as lower stretch values than that of the 15 specimens (Plaques 3, 4, 6, 7, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20 and 23) which had a Cal:Li ratio less than one which stretched further before rupture but at a lower stress value.
Figure 7.5: Cauchy Stress vs. Stretch Ratio plot of the plaques grouped by FTIR biological content ratio of Cal:Li greater than one (black lines) and Cal:Li less than one (grey dashed lines). The graph highlights that the plaques with the higher calcification content have a stiffer mechanical behaviour compared to the more lipid plaques which are softer.

Figure 7.5 shows an apparent trend between the mechanical behaviour and the FTIR type which is based on the Cal:Li ratio being greater than or less than one. However, as there is no means of quantifying the mechanical behaviour in terms of a Young’s’ Modulus due to the non-linear elastic behaviour the FTIR type was compared to the initial rupture data.

Figure 7.6: Averaged Stress and Stretch values at initial rupture grouped by FTIR biological content, Cal:Col > 1 (black square) and Cal:Col > 1 (grey circle) (error bars indicate standard deviation of the groupings).
There was no significant correlation between patient age and the ratios of Li:Col, Cal:Col and Cal:Li ($p = 0.549$, $p = 0.815$ and $p = 0.976$) and gender compared to the ratios ($p = 0.479$, $p = 0.558$ and $p = 0.638$). Stenosis level on pre-operative ultrasound also showed a non-significant relation to the ratios of the ratios of Li:Col, Cal:Col and Cal:Li ($p = 0.624$, $p = 0.637$ and $p = 0.982$). A significant relation was found between the initial rupture stress value and the FTIR type based on whether the ratio of Cal:Li was greater or less than one ($p = 0.003$). However, the relationship between the FTIR type and the stretch value at initial rupture shows no significance ($p = 0.383$).

### 7.4 Discussion

Our results indicate that plaque rupture occurred at higher levels of stress in plaques with a higher level of calcification. Furthermore plaques with higher lipid content had a greater stretch potential before rupturing, although at lower overall stress values.

Evidence has suggested that atherosclerotic plaque composition and anatomy are important predictors of plaque stability and clinical outcome as well as the degree of vessel stenosis. A recent Swiss study assessed 62 patients with high grade asymptomatic carotid stenosis with a median follow up of 18.9 months. They noted a significantly increased risk of stroke in those patients whose carotid plaques were classified as lipid-rich based upon magnetic resonance imaging (MRI) (HR 7.21; 95% CI 1.12-46.28; $p = 0.037$). A study in 2012 of 126 symptomatic carotid stenosis patients undergoing multisequence MRI noted a significantly increased risk of recurrent ipsilateral clinical ischemic events in patients with a lipid rich necrotic core (Hazard ratio (HR) = 3.2001; 95% CI, 1.078 to 9.504; $p = 0.036$). In this study the presence of a thin or ruptured fibrous plaque cap was also noted to be a risk factor for cerebral ischemia (HR = 5.756; 95% CI, 1.913 to 17.324; $p = 0.002$).

Previous studies investigating plaque rupture as a significant clinical predictor included a large study correlating histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms. The study authors noted a significantly decreased time to cerebral event in patients with plaque rupture ($p = 0.02$). Similarly a more recent study assessed 161 patients with carotid plaques and assessed plaque characteristics between symptomatic and asymptomatic patients and time since stroke. Researchers noted a significantly increased incidence of fibrous plaque rupture in the symptomatic patient group compared with the asymptomatic group (30 vs. 9%; $p = 0.001$). These findings are in keeping with previous studies noting a strong...
association between plaque rupture on histology and surface ulceration on angiography, and between angiographic ulceration and risk of subsequent stroke.\textsuperscript{20,21}

Our results correlate with previously reported studies. Our analysis of plaque composition indicates that those with a higher lipid component (i.e. Cal:Li ratio $< 1$) underwent rupture at a lower stress value than those with a higher proportion of calcification. Calcification-rich carotid plaque (i.e. Cal:Li ratio $> 1$) are less likely to rupture at a comparable stress value to those with a predominant amount of lipid component from the previously noted publications that untreated patients whose plaques have a higher Ca:Li and Cal:Col ratios will have a lower risk of plaque rupture and subsequent decreased stroke rate. However, our analysis of plaque stress values suggest that high calcium compositions considered stable in those undergoing surveillance may not correlate with stability during carotid intervention, in particular CAS.

Peri-procedural stroke for CAS has been proposed to be related to plaque manipulation resulting in plaque rupture, subsequent superimposed thrombus formation, and embolisation of plaque debris.\textsuperscript{22,23} The effect of the CAS process on a plaque involves the increase in carotid lumen cross sectional area by the circumferential strain placed upon the plaque by the expanding stent. The theoretical increase in luminal cross sectional area from 30\% (i.e. a carotid stenosis of 70\%) to 70\% requires a stretching of the carotid plaque surface of 53\%, as the expected post-procedural luminal circumference length would be 1.53 times the pre-procedural length.

Previous studies have reported that rupture of carotid plaques during manipulation in stenting procedures is more likely in plaques with a large lipid composition.\textsuperscript{11,24} Interestingly our results show that in fact plaques with higher lipid composition have a significantly greater capacity to stretch without rupture. This would suggest that plaques with a higher calcium composition are more likely to rupture when exposed to the stretching caused by the circumferential forces of an expanding carotid stent.

Multiple previous subgroup analysis of several randomized trials not ed that elderly patients undergoing CAS have an increased peri-procedural risk of stroke.\textsuperscript{3,4,6,7} Furthermore it has been reported that increasing patient age is associated with a higher proportion of more unstable plaques with higher amount of large lipid as compared with younger patients.\textsuperscript{25} However, a recent in vivo study of emboli produced during CAS reports on the increased potential for stroke in more calcified plaques, with researchers
noting that “calcium-rich” plaques produced significantly greater numbers of emboli captured on the embolic protection device \((p = 0.02)\).\(^{26}\)

Even though there is a small sample size due to the difficulty in sourcing a large number of samples, our results add to debate in this regard. We suggest that increased calcification may lead to an increased rate of plaque rupture during CAS, therefore patient cohorts who have a theoretically higher rate of plaque calcification may be at increased risk of stroke during CAS. It is essential that patient and plaque characteristics be considered in patients potentially undergoing CAS in order to pre-select those who may be at a higher peri-procedural risk.

### 7.5 Conclusions

Carotid plaques under surveillance with a higher calcium composition are more stable and less likely to rupture than those with a larger lipid composition. However, those with higher lipid content have a greater capacity to increase in length without rupture. In patients undergoing CAS, circumferential force from an expanding stent may be more likely to cause rupture in patients with a higher plaque calcium composition.

#### 7.5.1 Acknowledgements

None

#### 7.5.2 Sources of Funding


#### 7.5.3 Disclosures

None.

### 7.6 Significance

The results presented in this study add to debate around the use of minimally invasive techniques to treat carotid artery disease and, in particular the rupture potential due to plaque composition. Our analysis of plaque stretch and stress values in the circumferential direction suggest that high calcium compositions considered stable in those undergoing surveillance may not correlate with stability during carotid intervention, in particular, CAS. We suggest that increased calcification may lead to an increased rate of plaque rupture during CAS, therefore patient cohorts who have a theoretically higher rate of plaque calcification may be at increased risk of stroke during CAS. It is suggested that for patients potentially undergoing CAS, plaque biological
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composition and the corresponding relationship to mechanical properties, such as rupture potential, be considered in order to screen out those who may be at a higher peri-procedural risk.

References

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CHAPTER 8

FINITE ELEMENT ANALYSIS OF A NOVEL BALLOON ANGIOPLASTY DEVICE
Chapter 8

Finite element analysis of balloon angioplasty

8 Finite Element Analysis of a Novel Balloon Angioplasty Device

8.1 Introduction

The following section is based on the computational studies of balloon angioplasty devices deployed in a 90% occluded atherosclerotic carotid plaque with a reduced inflation parameter to decrease the potential of the balloon activating the baroreceptor nerves and therefore reducing the blood pressure peri-operatively. This section reviews the current finite element analysis (FEA) based studies of the stress distribution of carotid artery plaques undergoing balloon deployment as well as the strain energy functions that replicate the arterial tissue and atherosclerotic plaques. The objective of this section is to compare the effect that a novel double helical angioplasty balloon design has on the rupture potential of the plaques compared to current technology using calcified and lipid material models of plaque from this study which were classified from the FTIR analysis.

The following section reports and discusses the stress distribution and varied compression capabilities of a drug eluting balloon device which was designed and patented by Dr. Michael Walsh, Adrian Lynch, and I at the Centre for Applied Biomedical Engineering at the University of Limerick. The initial concept and development conducted on the device thus far was done so through a separate project which was financed by a separate funding body. The following section merely applies the numerical modelling methods and techniques developed in this study to demonstrate the improved capabilities of the ‘Transporter’ drug eluting balloon over current drug delivery devices.

8.2 Transporter Balloon

The transporter balloon involves a two-stage double helical balloon preferably incorporating an internal lumen to allow blood perfusion across the balloon during inflation as well as stepped coils which can be inflated separately, figure 8.1.
The ability of this design to maintain blood perfusion during inflation allows for an increase in inflation time of the balloon and thus greater time for drug transport to occur into the artery wall. Also the invention draws on knowledge obtained from drug eluting stents in the area of artery wall compression (O’Connell and Walsh, 2010). The design involves a two-stage non-uniform outer surface along its length. The smaller coils allow for an initial inflation that opens up the lumen of the occluded artery without damaging the disease and initiating embolisation. A paclitaxel based drug or lipid solidifying agent can be coated on these coils depending on the body lumen.

In the second stage a larger coil is inflated to completely open the vessel and again diffuse drug into the vessel. The larger helical coil provides the structural stability required to open the blockage and compress the artery wall, while the smaller coils in the helical balloon will be used to reduce artery wall compression. The primary purpose of these smaller coils is to optimise the distribution of therapeutic levels of drugs into the artery wall by reducing the compression and increasing the diffusion of drug in these areas of the disease.

Reducing arterial compression has clear advantages from both a mechanical and a mass transport point of view. Due to the variable approach a reduction in mechanical injury would occur. This variable compression is achieved by incorporating a two stage process where smaller coils from the first stage will not penetrate as deeply as a traditional balloon with a uniform diameter longitudinally. This also reduces the
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damage caused to the artery which could in turn reduce the amount of drugs required to suppress the smooth muscle cell proliferation responsible for restenosis. The objective of this study is to examine the viability of this technology in reducing damage to the plaque during deployment. Appendix C contains more illustrations and detail on the patented transporter balloon design.

8.3 Strain Energy Functions

A hyperelastic material can be characterised by a nonlinear constitutive equation known as a strain energy function (SEF) (Holzapfel et al., 2000 and Humphrey, 1995). A SEF relates the strain energy of a material to the deformation gradients from mechanical testing. The strain in variants manipulated to relate to the principal loading stretch are applied to these SEFs to ascertain a plot of stress-strain similar to the experimental data. There are a variety of SEFs some limited to isotropic behaviour and some can take into account a anisotropic behaviour. However, for a anisotropic biological tissue there are a limited number of SEFs to utilise. Table 8.1 lists the common isotropic SEFs which can be used to characterise the hyperelastic behaviour of arterial tissue. The SEFs can have simplistic forms such as the Neo-Hookean which is based on the first strain invariant and one variable. However, this limits the suitability of the function to the stress-strain response of a material. The Mooney-Rivlin and Signorini form are based on the first and second invariants whereas Yeoh and Delfino are based on the first invariant but with more definable coefficients for an accurate model in comparison to the Neo-Hookean.

Table 8.1: List of common SEFs used to characterise biological tissue for use in FEA.

<table>
<thead>
<tr>
<th>SEF</th>
<th>Equation</th>
<th>Equation No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neo-Hookean</td>
<td>$C_{10}(I_1 - 3)$</td>
<td>8.4</td>
</tr>
<tr>
<td>Mooney-Rivlin</td>
<td>$C_{10}(I_1 - 3) + C_{01}(I_2 - 3)$</td>
<td>8.5</td>
</tr>
<tr>
<td>Signorini form</td>
<td>$C_{10}(I_1 - 3) + C_{01}(I_2 - 3) + C_{20}(I_1 - 3)^3$</td>
<td>8.6</td>
</tr>
<tr>
<td>Yeoh Form</td>
<td>$C_{10}(I_1 - 3) + C_{20}(I_1 - 3)^2 + C_{30}(I_1 - 3)^3$</td>
<td>8.7</td>
</tr>
<tr>
<td>Delfino type</td>
<td>$\frac{a}{b} \left{ exp \left[ \frac{b}{2}(I_1 - 3) \right] - 1 \right}$</td>
<td>8.8</td>
</tr>
</tbody>
</table>

8.3.1 Yeoh Function

A number of SEFs exist to characterise a hyperelastic materials such as arterial tissue and polymers. The Yeoh Function is tailored specifically to simulate the mechanical behaviour of carbon filled rubbers (Yeoh, 1990). The Yeoh model is a three termed SEF
that only features the first strain invariant. The second strain invariant is neglected because \( \frac{\partial W}{\partial I_2} \) is assumed to equal zero as it is reported to be numerically close to zero (Kawabata and Kawai, 1977).

\[
W = C_{10}(I_1 - 3) + C_{20}(I_1 - 3)^2 + C_{30}(I_1 - 3)^3 \tag{8.9}
\]

Using principal stretches in equation 4.7 and the first strain invariant in equation 4.9 from section 4.2.3, equation 8.9 can now be rewritten as:

\[
W(\lambda) = C_{10}(\lambda^2 + \frac{2}{\lambda} - 3) + C_{20}(\lambda^2 + \frac{2}{\lambda} - 3)^2 + C_{30}(\lambda^2 + \frac{2}{\lambda} - 3)^3 \tag{8.10}
\]

Engineering Stress, \( \sigma_e \), is defined as the derivative of \( W \) with respect to the stretch ratio. \( \sigma_e \) is therefore be represented as:

\[
\sigma_e = \frac{\partial W}{\partial \lambda} \tag{8.11}
\]

By applying equation 8.11 to equation 8.10, \( \sigma_e \) can therefore be defined by equation 8.12.

\[
\sigma_e = C_{10}(2\lambda - \frac{2}{\lambda^2}) + 2C_{20}\left(\lambda^2 + \frac{2}{\lambda} - 3\right)(2\lambda - \frac{2}{\lambda^2}) + 3C_{30}\left(\lambda^2 + \frac{2}{\lambda} - 3\right)^2(2\lambda - \frac{2}{\lambda^2}) \tag{8.12}
\]

Cauchy Stress, \( \sigma_c \), is defined as the derivative of \( W \) with respect to the relevant stretch ratio multiplied by the same stretch ratio. \( \sigma_c \) is therefore be represented as:

\[
\sigma_c = \lambda \frac{\delta W}{\delta \lambda} \tag{8.13}
\]

### 8.3.2 Holzapfel-Gasser-Ogden (HGO) form

The material property assigned to the carotid bifurcation model is based on an anisotropic hyperelastic strain energy function proposed by Holzapfel et al. (2000) and Gasser et al. (2006) for modelling arterial layers with distributed collagen fibre orientations:

\[
W = \frac{\mu}{2}(I_1 - 3) + \frac{1}{D}\left(\frac{(f^{ett})^2}{2} - 1 - \ln f^{ett}\right)
+ \frac{k_1}{2k_2} \sum_{\alpha=1}^{N} \left\{ \exp[k_2(E_{\alpha})^2] - 1 \right\} \tag{8.14}
\]

With
\[
\bar{E}_\alpha \equiv \kappa (I_1 - 3) + (1 - 3\kappa)(I_{4(aa)} - 1), \quad (8.15)
\]

The strain energy per unit of reference volume is represented as \(W\). \(\mu, D, k_1, k_2\) and \(\kappa\) are material parameters, \(I_1\) is the first strain invariant and \(I_4\) is a pseudo-invariant of the Cauchy Green tensor and \(f^{st}\) is the elastic volume ratio. \(N\) is the number of families of fibres which is typically 2 for biological tissue. The HGO model assumes that collagen fibre direction within each family are dispersed (with rotational symmetry) about a mean preferred direction (Simulia, 2011). The parameter \(\kappa\) \((0 \leq \kappa \leq 1/3)\) describes the level of dispersion in the fibre directions and is defined within the strain-like elastic based parameter \(\bar{E}_\alpha\), equation 8.15, the parameter \(\kappa\) is defined as (Simulia, 2011):

\[
\kappa = \frac{1}{4} \int_0^\pi \rho(\Theta) \sin^3 \Theta d\Theta \quad (8.16)
\]

It is also assumed that all families of fibres have the same mechanical properties and the same dispersion. When \(\kappa = 0\) the fibres are perfectly aligned (no dispersion). When \(\kappa = \frac{1}{3}\) the fibres are randomly distributed and the material becomes isotropic; this corresponds to a spherical or orientation density function. The strain-like quantity \(\bar{E}_\alpha\) characterizes the deformation of the family of fibres with mean direction \(A_\alpha\). \(\bar{E}_\alpha = I_{4(aa)} - 1\) for perfectly aligned fibres \((\kappa = 0)\), and \(\bar{E}_\alpha = I_1 - 3\) for randomly distributed fibres \((\kappa = \frac{1}{3})\) (Simulia, 2011).

### 8.4 Computational Studies

A main limitation in computational studies of diseased arteries is the material properties representing the plaque model which are developed from experimental data based on a small sample size of human plaque from the aorta (Beattie et al., 1998; Lendon et al., 1993 and Loree et al., 1994) which limits the reliability of the stress values from these studies (Cilla et al., 2012; Huang et al., 2001 and Versluis et al., 2006). The computational simulations of diseased carotid arteries also base the material properties of the carotid plaque on experimental data of atherosclerosis from other vasculature which have a different morphology and potentially different mechanical response to stent deployment (Herisson et al., 2011 and Maher et al., 2012), excluding Creane et al. (2010) which is based on uniaxial tensile data of fresh human carotid plaques undertaken by Maher et al. (2009).
Many studies have been carried out on the effects of stent design on plaque stress. Balossino et al., (2008) examined different stent designs in one artery type and it was concluded that the greater the distance between stent links, the greater the circumferential stress concentrations between the stent links. Lally et al., (2005) investigated and compared two stent designs each with varying radial pattern. Toner et al., (2006) analysed the stent strut thickness, and concluded that tents with thicker struts had much higher stress concentrations when expanded. Despite this very few studies have investigated balloon deployment solely. Some articles include balloon deployment as part of the FEA, however in such studies the balloon is not in direct contact with the plaque and artery (Chua et al, 2004). One such study was carried out by Chua et al. (2004) where a finite element model was created which included an artery, plaque, stent and balloon. Using this model a simulation of the stent implantation procedure was performed, figure 8.3 (Chua et al., 2004). The balloon was placed inside the stent. The outside diameter of the balloon was set to the inner diameter of the stenosed artery.

![Figure 8.2: FEA assembly showing the interaction between the balloon, stent, plaque and artery (Chua et al. 2004).](image)

From expanding the balloon and stent against the plaque and artery it was found that the locations of highest stress corresponded with regions, which most plaque ruptures occur such as the fibrous cap shoulder. The stress concentrations occurred at the plaque, artery interface and the plaque cap. This was due to stiffness variances between the plaque and the artery, and the plaque and the stent (Chua et al., 2004). This current study will simulate the balloon deployment similar to this work excluding the stent deployment.
Other studies by Early et al. (2009), Cui et al. (2010) and Gasser and Holzapfel (2007) used the same half-length and quarter model geometry of the artery.

8.5 Methods

8.5.1 Mechanical Testing
Uniaxial mechanical testing was carried out on twenty-five specimens obtained from the twenty-three patients, section 4.3.1. The plaque specimens were uniformly stretched in the circumferential direction rather than the longitudinal as the majority of cardiac cycle induced strain occurs in this direction. This study establishes the circumferential stretch that the plaque can withstand before rupture at a physiological strain rate that replicates the instantaneous systolic pulse experienced by the plaque in vivo, 30% of the gauge length per second, section 2.4.4. Material coefficients for the Yeoh SEF were derived by fitting the stress-stretch experimental plots to an analytical stress-stretch solution using a non-linear direct method optimisation procedure developed with MATLAB (The MathWorks, Inc., Natick, Massachusetts), Appendix B.

8.5.2 Geometry

Artery Model
A quarter artery model was developed to simulate a 6 mm length centre section of an idealised concentric diseased common carotid to compare the inflation of a standard angioplasty balloon and a two-stage double helical perfusion balloon; at full length and half length, figures 8.4 and 8.5. The arterial geometry mimics the same idealised 3D setup where there are two separate layers of healthy tissue and plaque according to Early et al. (2009), Early and Kelly (2010), Cui et al. (2010), Chau et al. (2004) and Gasser and Holzapfel, (2007). The internal diameter of the artery is 6 mm (Krejza et al., 2006) with a thickness of 1 mm (Delfino et al., 1997). The stenosis lumen is 2 mm in diameter which corresponds to a 33% stenosis lumen based on diameter and approximately 90% stenosis by area, a typical stenosis for symptomatic patients. The aim of this setup is to increase this significantly diseased lumen to 4.5 mm which equates to 75% lumen of the original 6 mm or 60% of the original cross sectional area. The aim of angioplasty is typically to open the diseased lumen to the original lumen, approximately 6 mm. However, the aim of this study was to examine the feasibility of changing this inflation parameter by taking into account the conclusion of chapter 3, which is to reduce the over-stretching of the balloon to...
minimise the damaging effect of angioplasty on baroreceptor function post-operatively. The plaque section, 2mm in thickness, does not take into account a lipid core or nodes of calcification, as the material model this diseased layer is based on is of plaque tested as a whole, chapter 6, which does not take into account the local properties of calcification and lipid.

**Balloon Models**

The standard balloon section is modelled at the centre of the balloon with a 0.1 mm material thickness and a cramped outer diameter of 1 mm, figure 8.4 (a). This full length model does not take into account the curvature at the balloon ends or the dog-boning effect during inflation. The novel two-stage double helical perfusion balloon is based on a patented design from this group. The hypothesis of this design is to deploy a double helical balloon in two stages where blood can perfuse through the lumen centre upon deployment. The purpose of the first stage of the transporter balloon is to open the lumen to a diameter of 3.5 mm which will allow healthy perfusion to the brain. There are two separate balloons which are longitudinally coiled in parallel to one another, figure 8.4 (b). Section 8.2 summarises and illustrates the main details of the transporter balloon design. The seven large balloons have a diameter of 0.5 mm and the eight small balloons are 0.25 mm and the undeformed central lumen diameters of the large and small balloons are 1 mm and 1.25 mm, respectively. Figure 8.4 illustrates the two models setups in the arterial geometry, red: artery, blue: plaque and green/yellow: balloon model.

![Figure 8.3: Assembled geometries of the full length (a) standard balloon and the (b) transporter balloon prior to deformation, red: artery, blue: plaque and green/yellow: balloon.](image)

A half-length model of the balloon was also designed using the same arterial model but with half the length of the balloons, figure 8.5. The geometrical design of the curvature of the standard balloon is based on a typical carotid angioplasty balloon, figure 8.5 (a),
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(ABT Medical, 2013). For the transporter balloon the number of balloons was correspondingly decreased with no change to diameters or lumen size, figure 8.5 (b).

Figure 8.4: Assembled geometries of the half-length (a) standard balloon and the (b) transporter balloon prior to deformation.

8.5.3 Mesh and Grid Independence
The two models required two separate grid independence studies to demonstrate that the results from the models were not dependent on or affected by the density of the mesh. For the standard and transporter balloon models the seeding density was increased incrementally for arterial and plaque section four times, both mesh densities of the respective balloons were kept as standard size as results were taken from the artery and plaque not the balloons. A criterion of 5% difference between the von Mises stresses at a time step of one was used to confirm grid independency in both cases (black lines in figure 8.7 and 8.8). The stresses were taken from the contact point along the plaque lumen wall for both models (inset of figure 8.6 and 8.8).

Standard Balloon
The seeding density for the arterial in the standard balloon model was incrementally decreased from 0.2 to 0.05 by 0.05, where 0.2, referred to as Version 1, gave an element size of 69,983 and 0.05 (Version 4) gave an element size of 221,519. Figure 8.6 shows the von Mises stresses along the artery wall (red line, inset) for each of the mesh densities. Version 1 underestimated the stresses along the wall due to the poor mesh quality and figure 8.7 highlights the large percentage difference between version 1 and 2. Figure 8.7 shows that the percentage difference between versions 3 and 4 is small (0.54 ± 1.09%), highlighting that version 3 mesh density is suitable for further testing. Incremental points along the artery wall were used to highlight percentage difference in figure 8.7.
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Figure 8.5: von Mises stress values along the plaque wall for four different mesh density versions applied to the standard balloon model. Inset: red line indicates location of nodes used to acquire the stress values.

Figure 8.6: Percentage difference in von Mises stress at each node along the plaque lumen wall between the different versions for the standard balloon model. Black lines indicate the threshold value of ±5% used to indicate grid independence.

Transporter Model

The mesh density was increased in the artery and plaque by similarly decreasing the seed value incrementally in the transporter model from 0.3 to 0.15 in 0.05 increments. However, due to the circular nature of the balloons in contact and the large deformation of the elements in contact a section 0.2 mm thick into the plaque was partitioned to greatly increase the mesh density to improve the element size along contact section, figure 8.10 (right). For this section, the number of elements along the artery wall was increased from 125 in versions 1 (100,876 elements) and 2 (203,224 elements) to 200 in
versions 3 (255,325 elements) and 4 (303,378 elements). In each version the elements along the first 0.2 mm through the plaque was set to 6. Figure 8.8 shows the von Mises stress in the transporter model for the first stage, the inset shows the red line along the plaque lumen wall where the stress values were measured.

Figure 8.7: von Mises stress values along the plaque wall for four different mesh density versions applied to the transporter balloon model. Inset: red line indicates location of nodes used to acquire the stress values.

Figure 8.9 shows the percentage difference between each incremental version of mesh density used in this grid independence study. The percentage difference between version 3 and 4 is below the criterion of 5% throughout the artery wall (0.81 ± 2.19%), therefore version 3 (255,327 elements) will be used for this model throughout the study. Due to the high level of strain undertaken by the elements at the wall of the plaque in this complex geometry there was a degree of variation in stress illustrated in Figure 8.9. However, due to large mesh necessary and time taken for an idealised model the mesh was not further improved at the wall to reduce and smooth this inherent variation in stress along the wall.
Figure 8.8: Percentage difference in von Mises stress at each node along the plaque wall between the different versions for the transporter balloon model. Black lines indicate the threshold value of ±5% used to indicate grid independence.

Figure 8.10 shows the final meshes used for each model, standard and transporter, left and right respectively. In both cases the mesh type used was a 4 node linear tetrahedral and hybrid mesh where the Ngleom function was on for each step used. For the transporter model a 0.2 mm thick section along the artery wall had an increased mesh density due to large deformation present.

8.5.4 Material Properties

Three different sections were defined in the models; balloon, artery and plaque. The material definitions for the balloon and artery sections were kept the same throughout the different models. However, the plaque material parameters were changed to analyse the difference in stress distribution within a diseased carotid artery between the two FTIR classifications of behaviour based on Cal:Li ratio from chapter 6.

Balloon
The balloon material definition was based on a linear elastic model from the study by Takashima et al. (2007) where the balloon was almost incompressible due to the use of water as the inflation method and the almost incompressible nature of the material used. A balloon with a Young’s modulus of 200 GPa and a Poisson’s ratio of 0.495 was used in order to avoid compression in the balloon thickness.

**Artery**

The material definition of the carotid artery is based on a phenomenological, anisotropic and hyperelastic SEF developed by Holzapfel et al. (2000) and the material parameters are based on the averaged data of an intact human common carotid from the experimental study by Sommer and Holzapfel (2012). These parameters were chosen as the Sommer and Holzapfel (2012) study is the only experimental data available that characterises the human carotid artery as a whole as well as the individual layers as shown in table 8.2.

| Table 8.2: Material Coefficients for the HGO strain energy function used for the carotid artery material definition. |
|-----------------|-----------|-----------|-----------|-----------|-----------|
| Type            | $\mu$ (kPa) | $k_1$ (kPa) | $k_2$ | $\Phi$ (°) | $\rho$ |
| Intact Wall     | 44.5      | 28.4      | 68.9 | 20.4       | 0.8      |

**Plaque**

The material definition of the plaque section uses the Yeoh SEF and the material properties are based on the averaged mechanical data of the stiff and soft plaques based on the FTIR classification from figure 8.11 and section 6.4. The Yeoh SEF is suitable for characterising hyperelastic materials using uniaxial mechanical data only, in tension or planar shear (also known as pure shear), as the function does not depend on the second strain invariant which would require biaxial data. Material coefficients ($C_{i0}$) for the Yeoh SEF were derived by fitting the averaged stress-stretch experimental plots based on FTIR classification displayed in figure 8.11 (red and yellow dashed lines for the stiff and soft plaques, respectively) to an analytical stress-stretch solution using a non-linear direct method optimisation procedure developed with Matlab to minimise the difference between the stress values, appendix B.
The Matlab program developed is based on an optimised “fsolve” function which is used to solve nonlinear equations based on a tolerance until the question converges. This function is used to apply values to the material coefficients of the Yeoh SEF which it solves and compares to the experimental data of each specimen until a tolerance is met and the system converges. A tolerance of $10^{-6}$ was used when fitting the equation and a total difference between the stress values was calculated using a least squares method. Certain constraints were in place on the solver to ensure the material parameters were stable upon completion i.e. $C_{10}$ and $C_{30} > 0$ and the stability was confirmed using the evaluation function in ABAQUS. Table 8.3 lists the resulting coefficients for the average plots based on the FTIR classifications as well as the $R^2$ difference found at each stress value once the system was converted.

Table 8.3: Material coefficients for the averaged FTIR classifications based on calcification to lipid content for the Yeoh SEF.

<table>
<thead>
<tr>
<th>Type</th>
<th>$C_{10}$</th>
<th>$C_{20}$</th>
<th>$C_{30}$</th>
<th>$R^2$ Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal:Li &gt; 1</td>
<td>2.505 E-01</td>
<td>-1.913 E-01</td>
<td>1.114 E-01</td>
<td>1.031 E-03</td>
</tr>
<tr>
<td>Cal:Li &lt; 1</td>
<td>5.656 E-02</td>
<td>7.893 E-04</td>
<td>8.57 E-03</td>
<td>5.382 E-04</td>
</tr>
</tbody>
</table>

8.5.5 Boundary Conditions

The undeformed geometric model of the artery is designed to imitate a concentric diseased carotid artery with a lumen stenosis 2 mm. The lumen gain for both balloon models was achieving a 4.5 mm open lumen diameter using a radial displacement boundary condition applied to the whole of the balloon. For the transporter model two steps were used to carry out this lumen gain. The first stage simulated the small
balloons being inflated to open up the lumen to a diameter of 3.5 mm which allows for healthy perfusion to the brain without applying a level of stress to the plaque to initiate rupture and allow plaque softening drug to transport into the plaque, figure 8.12 (a). The second stage was to inflate the larger balloons to increase the lumen gain by using a radial displacement to 4.5 mm and apply a varied compression to promote drug transport, figure 8.12 (b). Figures 8.12 illustrates the radial displacement boundary conditions placed separately to the large and the small balloons for both stages.

Symmetry boundary conditions constrain the movement of the plaque, artery and balloon models along symmetric lines. A symmetry boundary condition was applied to the distal and proximal ends of the arterial geometry in the axial direction to simulate an extension of the artery longitudinally, figure 8.13. A limitation of this boundary condition is that it presumes that the plaque section continues longitudinally either side of the whole artery. Therefore, the half-length model is necessary to examine the effect of the stress at the balloon ends and negates the limitation caused by this symmetry boundary condition (Chau et al. 2004 and Gasser and Holzapfel, 2007). The plaque model is not longitudinally symmetric. However, as it is long enough, it is assumed that the effect of this difference is negligible.
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Figure 8.12: Axial symmetry applied to the proximal and distal ends of the arterial geometry in the z-direction. An axi-symmetric boundary condition was applied to the faces of the balloon and artery along the longitudinal direction to simulate a full geometry model due to the symmetrical nature of a concentric diseased artery, figure 8.14 (Cui et al., 2010, Early and Kelly, 2010).

Figure 8.13: Axi-symmetric boundary condition applied the longitudinal faces of the arterial and balloon geometries of the quarter model.

8.5.6 Contact Parameters

Two sets of contact were applied to the models, a surface-to-surface between the balloon and plaque lumen wall with a frictionless tangential behaviour and surface-to-surface self-contact between the small and large balloons in the transporter model. Between the balloons and the artery friction was not considered and the augmented Lagrangian method was used as the contact algorithm (Takashima et al., 2007). For the contact pairing between the plaque and balloon a "finite sliding" formulation was used as there is substantial tangential movement between the balloon and plaque. A "small sliding" formulation will result in better convergence behaviour than a "finite sliding" formulation. However, when there is substantial tangential movement between the surfaces the "finite sliding" formulation will be more accurate. Over-closure can be an issue with hyperelastic models undergoing large amounts of stretch and the separate
surfaces can penetrate especially when contacting surfaces that are represented by multi-faceted meshed surfaces that might not align exactly. This over-closure is a main source of convergence difficulties and varied stress results along the contact wall. However, by selecting "Adjust only to remove over-closure", the slave nodes will be adjusted without any additional strain into the slave surface so that they are not initially over-closed. This will greatly reduce the number of iterations, reduce convergence difficulties and stress variation in the analysis but still varied stress values will occur to a smaller degree as shown in figure 8.9. Figure 8.15 illustrates the two sets of contact pairs used in this study for balloon models.

![Figure 8.14: Surface-to-surface contact parameters applied to the model. (a) tangential contact between balloon and artery, (b) self-contact between small and large balloons of the transporter model.](image)

### 8.5.7 Method to generate results

The stress values of interest for this study are along the longitudinal length of the plaque in direct contact with the balloon models (red line in figure 8.16) and 0.2 mm and 1 mm through the plaque parallel to the red line to show the reduction in stress through the thickness which aids in the varied compression. The nodes along the artery wall were selected and the average values and peak values were measured. The von Mises stress was used to compare the various models in the study; standard balloon vs. transporter, Lipid vs. Calcified material and full length models vs. half-length models. This stress type is used similar to studies by Chau et al. (2004), Early et al. (2009), Early and Kelly (2010) and Auricchio et al. (2011) to capture the stress for a hyperelastic material.
The purpose of the half-length models is to analyse the edge effects of the balloons on the plaque and how the stress peaks at the edge (figure 8.17) compare to those in the centre of the respective balloons and to be a closer simulation of the Chua et al. 2004, Cui et al. 2010 and Early et al. 2009 studies.

The key stress values used to compare each model will be based on a threshold stress value of plaque rupture. A value of 300 kPa is commonly used in computational studies without damage models (Cilla et al., 2012, Ohayon et al., 2005 and Cheng et al., 1993). However, this threshold value is based of uniaxial tensile testing of atherosclerotic plaques from the aorta (Lendon et al. 1991) which does not truly represent the potential rupture threshold of plaques from other vasculature as Herisson et al. (2011) and Maher et al. (2012) has shown to the different morphology, tissue composition and mechanical properties. The threshold stress values of rupture used in this study are based on the experimental results presented earlier in chapter 7. From the experimental study the whole plaque specimen typically had two fracture points, the initial rupture which is potentially due to the intima/media layer fracture and the complete rupture which was a combination of the media and remaining adventitial layer dependent on surgical removal (Teng et al., 2009). These two rupture points were investigated based on the FTIR classification of calcification and lipid content, figure 8.18.
8.6 Results

8.6.1 Lipid versus Calcified material
Two different material definitions based on the FTIR classification from Table 8.3 were applied to the plaque section of the arterial model and the standard and transporter balloons were deployed to analyse the difference in material behaviour. Figures 8.19-8.21 illustrate von Mises stress along the artery wall at 0 mm for the standard balloon, stage 1 transporter and stage 2 transporter balloon, respectively. These figures demonstrate that the calcified plaque models were considerably stiffer (14-36 times stiffer) in all balloon models and led to a higher level of stress directly along the artery wall in comparison to the lipid material model.
Figure 8.18: Comparison of the von Mises stress directly along the artery wall (0 mm) of the calcified and lipid plaque models for the standard balloon.

Figure 8.19: Comparison of the von Mises stress directly along the artery wall (0 mm) of the calcified and lipid plaque models for the first stage of the transporter balloon.
Figure 8.20: Comparison of the von Mises stress directly along the artery wall (0 mm) of the calcified and lipid plaque models for the second stage of the transporter balloon.

Table 8.4 highlights that none of the calcified plaques would withstand the level of stress applied by the standard or transporter balloons without rupturing. This table confirms the need to identify these plaques pre-operatively as they would not be able to undergo the CAS procedure. As a result of this outcome the following sections of this study will only analyse the results from the lipid material models.

Table 8.4: List of the initial and complete rupture stresses for each calcified based plaques tested and whether the balloon models would rupture the plaques based on the average von Mises Stress values of each model (X – rupture, O – no rupture)

<table>
<thead>
<tr>
<th>Plaque No.</th>
<th>Standard Balloon</th>
<th>Transporter Stage 1</th>
<th>Transporter Stage 2</th>
<th>Initial Rupture Stress</th>
<th>Complete Rupture Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>19.650</td>
<td>5.491</td>
<td>17.494</td>
<td>0.505</td>
<td>0.651</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.348</td>
<td>1.011</td>
<td>4.684</td>
<td>0.125</td>
<td>0.244</td>
</tr>
<tr>
<td>1</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.367</td>
<td>0.459</td>
</tr>
<tr>
<td>2</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.591</td>
<td>1.14</td>
</tr>
<tr>
<td>5</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.644</td>
<td>0.767</td>
</tr>
<tr>
<td>8</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.317</td>
<td>0.405</td>
</tr>
<tr>
<td>13</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.699</td>
<td>0.865</td>
</tr>
<tr>
<td>14</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.483</td>
<td>0.483</td>
</tr>
<tr>
<td>21</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.507</td>
<td>0.666</td>
</tr>
<tr>
<td>22</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.432</td>
<td>0.426</td>
</tr>
<tr>
<td>No. Survived</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% Survived</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
8.6.2 Standard versus Transporter

This next section compares the average stress values along the artery wall between the standard balloon and the two stages of the transporter balloon to analyse whether the transporter can improve on the current gold standard device in relation to stress distribution. Figure 8.22 shows the stress values directly along the artery wall (0 mm) for each balloon model. This plot illustrates the consistent stress distribution for the standard balloon and conversely the cyclical nature of the transporter balloon in both stages. The second stage of the transporter balloon on average along the wall is less than that of the standard balloon, table 8.5. However, the large balloons of the transporter induce a large level of stress due to the decreased contact area which is a disadvantage caused by the design of the balloon. The main goal of the transporter is the application of a drug during the first stage of deployment which would aim in softening the plaque while allowing healthy blood perfusion. The average and peak stress values applied from the first stage are lower than the standard balloon. Analysing the average stress values applied by the balloons, the standard and second stage of the transporter would rupture 95% of the tested plaques, table 8.5. However, 42% of the plaques would have survived the first stage of the transporter balloon without complete rupture.

Figure 8.21: Comparison of the von Mises stress directly along the artery wall (0 mm) between the standard and the two stages of the transporter balloon.
Table 8.5 shows which plaques the balloons would have ruptured at initial rupture and complete (X) and on es t hat c ould pot entially ha ve s urvived (O) based on t heir experimental plots. Table 8.5 highlights that stage 1 of the transporter reduce the risk of rupture with a 42% survival rate at complete rupture and with the addition of plaque softening that it could lead to an improved stage 2 survival rate which currently only has a 5% survival rate for this level of stenosis.

Table 8.5: List of the initial and complete rupture stresses for each lipid based plaques tested and whether the balloon models would rupture the plaques based on the average von Mises Stress values of each model (X – rupture, O – no rupture)

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Standard Balloon</th>
<th>Transporter Stage 1</th>
<th>Transporter Stage 2</th>
<th>Initial Rupture Stress</th>
<th>Complete Rupture Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.543</td>
<td>0.386</td>
<td>0.352</td>
<td>0.234</td>
<td>0.342</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.007</td>
<td>0.052</td>
<td>0.108</td>
<td>0.136</td>
<td>0.155</td>
</tr>
<tr>
<td>3a</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.132</td>
<td>0.281</td>
</tr>
<tr>
<td>3b</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.248</td>
<td>0.248</td>
</tr>
<tr>
<td>4</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.242</td>
<td>0.240</td>
</tr>
<tr>
<td>6</td>
<td>X / X</td>
<td>X / O</td>
<td>X / X</td>
<td>0.308</td>
<td>0.414</td>
</tr>
<tr>
<td>7</td>
<td>X / O</td>
<td>X / O</td>
<td>X / X</td>
<td>0.179</td>
<td>0.527</td>
</tr>
<tr>
<td>9</td>
<td>X / O</td>
<td>O / O</td>
<td>X / O</td>
<td>0.461</td>
<td>0.667</td>
</tr>
<tr>
<td>10</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.063</td>
<td>0.115</td>
</tr>
<tr>
<td>11</td>
<td>O / X</td>
<td>O / O</td>
<td>O / X</td>
<td>0.609</td>
<td>0.537</td>
</tr>
<tr>
<td>12</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.170</td>
<td>0.324</td>
</tr>
<tr>
<td>15</td>
<td>X / X</td>
<td>X / O</td>
<td>X / X</td>
<td>0.234</td>
<td>0.428</td>
</tr>
<tr>
<td>16a</td>
<td>X / X</td>
<td>O / O</td>
<td>X / X</td>
<td>0.404</td>
<td>0.520</td>
</tr>
<tr>
<td>16b</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.064</td>
<td>0.087</td>
</tr>
<tr>
<td>17a</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.294</td>
<td>0.311</td>
</tr>
<tr>
<td>18</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.131</td>
<td>0.168</td>
</tr>
<tr>
<td>19</td>
<td>X / X</td>
<td>X / O</td>
<td>X / X</td>
<td>0.244</td>
<td>0.423</td>
</tr>
<tr>
<td>20</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.301</td>
<td>0.266</td>
</tr>
<tr>
<td>23</td>
<td>X / X</td>
<td>X / X</td>
<td>X / X</td>
<td>0.239</td>
<td>0.283</td>
</tr>
<tr>
<td># Survived</td>
<td>1 / 1</td>
<td>3 / 7</td>
<td>1 / 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% Survived</td>
<td>5 / 5</td>
<td>17 / 42</td>
<td>5 / 5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The compression through the thickness is an important feature to analyse and compare for both balloons as the lipid pools and nodes of calcification which contribute to plaque rupture are embedded in the tissue structure as well as drug transport being dependent on the level of compression value due to the stratified nature of the diseased artery layers (O’Connell and Walsh, 2010). The stress values were measured along the artery wall at 0, 0.2 and 1 mm. Figure 8.23 highlights the lower stress values through the wall for the first stage of the transporter balloon (black plots) in comparison to the standard balloon (grey plots) showing improved stress distribution through the thickness. Also
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the cyclical nature of the stress values caused by the geometry diminishes at 1 mm line for the transporter balloon.

Figure 8.22: von Mises stress values of the standard balloon and first stage of the transporter balloon at different levels through the thickness of the plaque.

Table 8.6 shows how the two stages of the transporter compare to the standard balloon in terms of average stress at each line (0, 0.2 & 1 mm) through the thickness along the artery wall. This highlights that stage 1 has lower stress along the artery wall which lowers through the thickness of the plaque. Also, the table highlights the decrease in stress through the thickness and that the transporter has better reduction in stress especially between 0 and 0.2 mm (30.8 & 33.4% compared to 25.2%).

Table 8.6: Average stress values of the balloons along the plaque wall and through the thickness at 0.2 mm and 1 mm highlighting the difference in stress between the balloons and the reduction through the thickness.

<table>
<thead>
<tr>
<th>Average</th>
<th>Standard Balloon</th>
<th>Transporter Stage 1</th>
<th>Transporter Stage 2</th>
<th>Std. vs. Stage 1</th>
<th>Std. vs. Stage 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mm</td>
<td>0.543</td>
<td>0.386</td>
<td>0.552</td>
<td>28.8</td>
<td>3.2</td>
</tr>
<tr>
<td>0.2 mm</td>
<td>0.406</td>
<td>0.267</td>
<td>0.368</td>
<td>34.1</td>
<td>9.5</td>
</tr>
<tr>
<td>1 mm</td>
<td>0.157</td>
<td>0.101</td>
<td>0.132</td>
<td>35.3</td>
<td>18.4</td>
</tr>
<tr>
<td>0 vs. 0.2 %</td>
<td>25.2</td>
<td>30.8</td>
<td>33.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.2 vs. 1 %</td>
<td>61.3</td>
<td>62.1</td>
<td>64.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

8.6.3 Half Model versus Full Model
A limitation of this study is the idealised nature of the geometry of the balloons at the centre of the plaques. The following section aims to analyse the effect the ends of the
balloons had on the peak stress values. Another limitation of this following section is the geometry of the plaque which normally would not be present at the ends of the balloon and they would be ideally placed in direct contact with healthy arterial tissue. However, it was the aim of this study to examine the increase in the peak and average stress values for each balloon using the lipid material model. Figure 8.24 illustrates that there is a large peak of stress at the edge of the balloon as expected for all balloons at the 0 mm line. The increase in peak stress at the edge of the balloons is 38, 38 and 40% higher than the average stress value along the artery wall for the full-length models of the standard balloon, first stage and second stage transporter balloons, respectively.

Figure 8.23: von Mises stress values along the artery wall of the half-length balloon models highlighting the peak stresses caused by the edge of the balloons.

Figure 8.25 compares the stress values along the 0 mm line for the stage 1 of the transporter balloon which highlights the average increase in the stress values caused by the change in model.
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Figure 8.24: Comparison of the half-length and full length models of the first stage of the transporter balloon highlighting an increase in the peak stresses along the artery wall.

Table 8.7 shows the percentage increase of the peak stresses along the plaque wall due to the change in boundary conditions from the half model. The large peaks at the edge increased the peak stress values which indicate that the full length model may underestimate the stress values. However, these edge effects are seen for both standard and transporter balloon, it is assumed that the full length models can be employed for a comparative analysis to investigate any possible benefits that the transporter may have over the standard balloons.

Table 8.7: Comparison of the percentage increase in average stress between the half and full length models.

<table>
<thead>
<tr>
<th></th>
<th>Half Model</th>
<th>Full Model</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mm</td>
<td>0.2 mm</td>
<td>1 mm</td>
</tr>
<tr>
<td>Standard Balloon</td>
<td>0.595</td>
<td>0.406</td>
<td>0.152</td>
</tr>
<tr>
<td>Transporter – Stage 1</td>
<td>0.426</td>
<td>0.289</td>
<td>0.107</td>
</tr>
<tr>
<td>Transporter – Stage 2</td>
<td>0.665</td>
<td>0.439</td>
<td>0.152</td>
</tr>
</tbody>
</table>

Figure 8.26 shows again that the effect of the peak stresses diminishes at the 1mm line and the peak stress value at the balloon end of the transporter is relatively the same as the peaks at the centre.
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8.7 Discussion and Conclusion

The purpose of this study was to compare a novel two-stage perfusion balloon to current balloon devices to highlight how the reduction in inflation parameters, a conclusion from chapter 3 in order to decrease the damaging effect on the baroreceptor, may improve the stress distribution on the plaque to reduce risk of rupture.

The geometrical model under investigation in this study is of one particular scenario where a 90% occluded artery is treated to allow for a lumen gain of 75% of original diameter or 60% of original cross sectional area. This scenario is an alternative method to the typical over-stretching of the artery required for a CAS surgery.

This particular study used material models specific to plaques from the carotid artery unlike other computational studies which have used material properties from other vasculature to simulate carotid plaques (Versilius et al., 2006 and Cilla et al., 2012). Experimental studies have shown that diseased tissue does not accurately represent the mechanical behaviour or morphology of other arteries location (Maher et al., 2012 and Herisson et al., 2011). Similar studies which analyse the peak stresses without damage models use a value of 300 kPa as the rupture point of carotid plaques (Ohayon et al., 2005). This current study uses rupture points developed from the experimental section which again are specific to the plaques from the carotid artery. Teng et al. (2009) and Stemper et al. (2005) reported different levels of plaque rupture due to the varied

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Figure 8.25: Comparison of the stress values through the thickness of the plaque for the first stage of the transporter model showing that the effect of the varied balloons diminishes through the thickness again in the half-length model.
strength of the layers in the plaque. For this study two rupture points were identified, initial rupture where the slope of line initially became negative and the complete failure where the stress did not increase with an increase in stretch for the rest of the mechanical test. Initial rupture is important to analyse as this can be associated with the intima layer rupturing which could lead to embolisation peri-operatively. Therefore, complete rupture would underestimate stress value that could lead to embolisation.

FTIR classification was used to group the mechanical behaviour of the plaques into two types which were curve-fit to the Yeoh SEF. Initial and complete rupture stress values were determined for each group which highlights that the 300 k Pa underestimates the initial and complete rupture values for the calcified plaque and overestimates the plaque rupture point for the initial rupture of the lipid plaques in figure 8.18. Analysing the FEA results for both the standard and transporter balloons it is evident that the calcified based material models exhibited significant levels of stress which exceeded the initial and complete rupture values (0.505 and 0.618 M Pa, respectively). The average stress values of the standard and transporter at stage 1 and 2 was 19.7, 5.5 and 17.6 M Pa, respectively, which was 36, 14 and 31 times higher than the average stress values experienced by the lipid classified plaques. This demonstrates that the calcified plaques would not be able to undergo any form of balloon angioplasty based on FTIR classification without rupture. This finding demonstrates the effectiveness of the FTIR classification and supports the theory that CAS should not be performed on these more calcified plaques. This analysis highlights the importance of using a pre-operative plaque diagnostic tool such as FTIR which could potentially reduce the risk of peri-operative plaque rupture by identifying these calcified plaques unsuitable for CAS.

A comparative study of the stress distribution of the standard and second stage of the transporter balloons in the lipid model shows that the standard balloon exhibits an ideal uniform stress along the wall of the artery. Even though the average stress values are higher in the standard model, the transporter model design inherently creates peak stress values along the artery wall due to the lower contact area to spread the force which is a limitation that could induce plaque rupture at the peak stress points. However, the aim of this transporter is to deploy in two stages. The purpose of the first stage is to open the lumen to 3.5 mm which allows for healthy flow through the central lumen of the balloon during deployment. The average stress values and peak stresses along the wall for this stage are lower than that of the standard balloon. Analysing the initial and complete rupture stress values for the first stage of the transporter balloon, this stage
ruptures 83% of the plaques at initial rupture and 52% at complete rupture would have failed peri-operatively. This is a considerable decrease compared to the standard balloon which would have ruptured 95% of the plaques in both cases.

The secondary purpose of the first stage is to deploy drug into the artery over a longer period of time than the standard balloon due to the normal blood flow through the central lumen during deployment. The drug would be designed to soften the plaque which could decrease the rupture potential of the plaques during the second stage of deployment in the transporter balloon. A tertiary goal of this design is to limit the damaging effect on the baroreceptor by reducing the instantaneous over-stretching and allowing the baroreceptors to adapt before the second stage of deployment. This has the potential to limit the post-operative hypotension suffered by patients of current balloon angioplasty devices (Yun et al., 2005, McKevitt et al., 2003 and Mangin et al., 2003).

Table 8.6 demonstrates the improved stress dissipation of the transporter balloon through the artery wall and figure 8.23 illustrates that the peak stresses dissipate at 1mm into the plaque model. The stress distribution through the wall of the artery is important from a drug transport view as a decrease in compression can promote increase penetration of the drug into the plaque (O’Connell and Walsh, 2010). As the arterial layers are stratified the mass transport properties are considerably anisotropic, the mass transport longitudinally is more significant than in the radial direction (Tarbell, 2003). An increase in compression can reduce the diffusion in the radial direction even further which is not ideal for large drug particles (O’Connell et al., 2010). The standard balloon applies a uniform stress along the artery wall which does not promote an increase in drug transport through the plaque. However, the stepped balloon diameter of the transporter model varies the compression which promotes an increased transport of drug at the site of the small balloons for the same lumen gain.

A limitation of this current FE study is the arterial model of the central section of a plaque with no geometrical change along the length. The plaque model is assumed continuous through the length of the model which does not truly represent the plaque geometry and certain important geometrical features which contribute to plaque rupture such as the fibrous cap and cap shoulders. However, the aim of this study is to compare the balloon models with the material models and rupture points developed from the experimental data.

The transporter balloon has numerous advantages over the current balloon design such as perfusion during deployment reducing the possibility of peri-operative complications,
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varied compression to promote drug transport and a two-stage deployment to reduce risk of rupture to the plaque and by deploying tissue softening drug in the first stage. The second stage of the transporter balloon does not offer any advantage of stress distribution into the artery in comparison to the standard balloon due to the reduced contact area. However, the purpose of this device is to improve the compression and therefore the transport of drug into the plaque during the first stage and to alter the inflation parameter by reducing the over-stretching of current balloon designs. The transporter balloon is key to the development and improvement of drug coated balloons due to the perfusion which increases the time allowed during the two stages of deployment and the improved compression into the plaque to promote drug transport and allow for baroreceptor adaptation (Ottesen and Danielsen, 2003). Experimental analysis is necessary to validate these results. Therefore, a biomimetic material model of the diseased carotid bifurcation which incorporates the baroreceptor function and plaque is necessary. This current body of work is the initial step toward developing a test system that could potentially highlight the improvements of this novel angioplasty design.

References

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CHAPTER 9

DISCUSSIONS AND CONCLUSIONS
The overall objective of this study was towards the development of guidelines for the surgical treatment of carotid artery disease using a tissue characterisation approach. However, there are two main limitations before fully analysing the carotid bifurcation which is the limited mechanical data on the atherosclerotic carotid plaques and limited experimental data of the effect of angioplasty on the baroreceptor function peri-operatively.

Due to the difficulty of measuring baroreceptor function during angioplasty in vivo a biomimetic model of the carotid bifurcation which incorporates the baroreceptor response of flow rate and blood pressure alteration to an increase in stretch was investigated. The aim of this part of the study was to incorporate a material into an idealised carotid bifurcation geometry within which angioplasty devices would be deployed. The change in diameter caused by the deployment would correspond to a change in flow rate and blood pressure and a measureable change in baroreceptor firing rate which could highlight if a threshold of safety was surpassed by the balloon device. This material model could highlight a device’s effect on the peri-operative flow rate and blood pressure and to the post-operative complications due to over-stretching of baroreceptor nerves which leads to hypotension in the patient. The results from this section demonstrated that it is possible to develop a material using electrically conductive silicone (ECS) that can replicate the mechanical behaviour and baroreceptor response to stretch up to 20% strain necessary for the stretch induced on the baroreceptor nerves in the adventitial layer. However, there are limitations with the material as it does not have the same hyperelastic response in the higher levels of strain. The electrical response of the ECS is only qualitatively similar to that of the baroreceptor function to strain over time. However, further work is possible to validate this similarity over a realistic time scale to relative resistivity rather than relative voltage.

This study did demonstrate that it is necessary to examine baroreceptor function when designing and examining balloon angioplasty due to the peri- and post-operative complications associated with this device due to over-stretching of the baroreceptor nerves at the site of the disease. A balloon which inflates to a lower level of stretch first could help the baroreceptor nerves to adapt to this stretch without any
effect on the cardiac output similar to the adaptation function which occurs during exercise where an increase in stretch does not correspond to a decrease in cardiac output after a length of time exercising (Scheuer and Tipton, 1977).

There is a lack of published data on the diseased carotid plaques for the development of a mimetic material of plaque which could be incorporated into a carotid bifurcation for realistic testing of balloon angioplasty deployment. Furthermore, the lack of mechanical data reduces the reliability of stress distribution from computational studies analysing the effect of angioplasty devices on the risk of plaque rupture. Human carotid plaque samples are challenging to source on a consistent level which makes it difficult to test large sample sizes for a full mechanical characterisation of the tissue. As the samples are heterogeneous and geometrically varied it is difficult to develop a general mechanical response for these tissues. This varying geometry also makes the specimens unsuitable to undergo standard uniaxial testing protocols i.e. tensile testing.

A number of studies that tensile tested carotid plaques dissected the tissue to suit the boundary parameters necessary for the test protocol. However, this dissection causes a limitation by deleteriously affecting the fibrous structure of the plaques. This thesis examines the feasibility in defining the geometrical ratios suitable for tensile and planar shear testing as no standard geometrical ratio exists in literature for mechanical testing of biological tissue.

This study highlighted that these ratios can be refined to allow for a larger range of width-to-length eligible for both tensile and planar shear testing to increase the number of plaques suitable for the respective test. This study was borne from the limitation in the Lawlor et al. (2011) study which rejected four plaque samples due to the geometrical ratios being unsuited for tensile. However, the ratios would have allowed for planar shear testing. These plaques could have been tested in planar shear rather than in tension and characterised accordingly with the Yeoh strain energy function (SEF).

Fourier transform infrared (FTIR) analysis successfully detected the characteristic peaks that identify the different constituents present in the plaque samples. These findings were correlated with the results of the mechanical tests to characterise the variation in mechanical response. A plaque mechanical behaviour with a high Cauchy stress and low stretch corresponded to a high level of calcification (Cal:Li > 1) whereas a low Cauchy stress and high stretch response was classified as a lipid type (Cal:Li < 1). From this it is surmised that this higher calcification content is linked to the stiffness of the plaque in the artery. The results indicate that plaque rupture occurred at higher levels of
stress in plaques with a higher level of calcification. Furthermore, plaques with higher lipid content had a greater stretch potential before rupturing, although at lower overall stress values. Calcification classified carotid plaques (i.e. Cal:Li > 1) are less likely to rupture at comparable stress values to those with a predominant amount of lipid component. However, our analysis of plaque stretch values suggest that high calcium compositions considered stable in those undergoing surveillance may not correlate with stability during carotid intervention, in particular CAS. The results of this part of the study has determined that FTIR is a non-destructive method to evaluate atherosclerotic tissue samples and is a worthy tool to characterise the composition of carotid plaques.

In coupling the morphological analysis using the SEM with the EDX, the quantities of calcification in the samples were evaluated to assess the viability for the FTIR readings to characterise the composition. EDX spectroscopy determined the chemical composition in each sample type based on elemental content. Calcium and phosphorus to carbon ratios were averaged and grouped based on FTIR classification which showed a higher level of calcification in the mechanically stiffer plaques in comparison to the softer plaques. This analysis reaffirmed that the results obtained from the FTIR analysis were accurate based on the trends observed in the calcification and lipid content. Furthermore, from investigating the capabilities of current pre-operative diagnostic techniques to evaluate atherosclerotic plaque in vivo (e.g. duplex ultrasound), it is clear there are inadequacies associated with each technique (Li et al., 2003). Tosi et al. (2012) suggests that FTIR is ready to be used as a preoperative diagnostic tool in diagnosing atherosclerosis to compete against ultrasound. This thesis confirms FTIR is capable of evaluating atherosclerotic plaque given its capability in assessing the existence and quantification of calcification, lipid and collagen.

Another objective of this thesis was to develop a method that successfully fixed, dehydrated and dried the biological tissue samples. This ensured optimum preservation was achieved so that the tissue sample would not alter in the subsequent analytical and characterisation techniques that it was to be subjected to. A preliminary investigation confirms that the use of methanol to fix biological tissue is an adequate alternative to the gold standard glutaraldehyde. SEM imaging demonstrated that calcification was present in most plaque specimens and was more prevalent in the plaques classified as calcified using FTIR. SEM imaging identified the two prominent types of calcification in the samples as sheet-like and nodular. There was also a high level of delamination in the intimal surface where the layers had become detached. In
extreme cases the layers were completely absent. This level of damage suggests that these vessels were subjected to a significant amount of disruption from turbulent blood flows when in vivo which promoted the adhesion of leukocytes for the development of atherosclerosis. Combining a chemical based method of fixation (methanol), dehydrating (molecular grade ethanol) and drying (HMDS) is a new approach which has shown expedient results under SEM that demonstrate the capability of this approach in replacing the gold standard of glutaraldehyde and CPD, a more expensive and time consuming process.

The transporter balloon has shown to have numerous advantages over the current balloon design such as perfusion during deployment, varied compression to promote drug transport and a two-stage deployment to reduce risk of rupture to the plaque and reduce the damaging effect on the baroreceptor function by deploying tissue softening drug in the first stage. The second stage of the transporter balloon does not offer any advantage of stress distribution into the artery in comparison to the standard balloon due to the reduced contact area. However, the purpose of this device is to improve the compression and therefore the transport of drug into the plaque during the first stage of deployment (O’Connell et al., 2010 and O’Connell and Walsh, 2010). The transporter balloon is key to the development and improvement of drug coated balloons due to the perfusion which increases time allowed during the two stages of deployment and the improved compression into the plaque to promote drug transport and allow for baroreceptor adaption by reducing the over-stretching based inflation parameter (Ottesen and Danielsen 2003).

The results of plaque rupture and the relation of mechanical behaviour to calcification adds to the debate of CAS vs. CEA. The results suggest that a drug coated balloon can reduce the rupture rate of carotid plaques which are lipid dominated. However, regardless of device design plaques with increased calcification may lead to an increased rupture in a fo PL plaques rupture during CAS, therefore patient cohorts who have a theoretically higher rate of plaque calcification may be at increased risk of stroke during CAS. It is essential that patient and plaque characteristics be considered in patients potentially undergoing CAS in order to pre-select those who may be at a higher peri-procedural risk. This study also hypothesises that FTIR is a viable technique for use as an in vivo pre-operative imaging tool to aid in this pre-selection.
Conclusions

1. This study showed that it is feasible to develop a biomimetic material of the carotid bifurcation with baroreceptor function which can alter flow in a bench-top system for the deployment and testing of balloon angioplasty devices.
   a. Electrically conductive silicone was manufactured to simulate the baroreceptor function using the electrical response to stretch which can be used to control the flow rate, similar to the baroreceptor response to stretch, through the test system during balloon angioplasty deployment.
   b. A uniaxial test system was developed to stretch the ECS and measure the mechanical behaviour and electrical response to stretch using relative voltage as a value of measurement.
   c. The ECS developed and tested in this study was found to have a mechanical response to pressure in an idealised carotid bifurcation geometry similar to the realistic carotid arterial behaviour up to 20% stretch. The electrical response to a uniaxial stretch resulted in a qualitatively similar behaviour to the baroreceptor when subjected to sustained stretch.

2. A method of mechanically and biologically characterising human carotid plaques was developed which led to an improved understanding of the composition of plaques that ruptured and a potential diagnostic method to improve patient outcome for CAS.
   a. There is a current lack of mechanical data and standard protocols to mechanically test the plaques. This study refines the geometrical ratios suitable according to the standard protocol to improve the number of plaques that can be tested.
   b. Current mechanical testing studies use strain rates based on engineering material protocols and this study used a strain rate which simulates the systolic peak in the cardiac cycle, similar to the \textit{in vivo} environment of the plaque.
   c. FTIR and EDX spectroscopy were used to develop a method of biologically characterising the composition of the plaque tissue to relate to the mechanical properties of plaque specimen.
   d. FTIR analysis showed that the stiffness of the mechanical response was related to the level of calcification to lipid composition. EDX was used to......
reaffirm this trend found in these plaques, that there was a higher level of calcification in the stiffer based plaques.

e. SEM imaging was used to highlight the key features at the sites of rupture within the plaques. Endothelial dysfunction was present in all plaques in the form of endothelium stretching or an absence of endothelium which promoted the adhesion of 1 eukocytes that led to or restenosis. Calcification was present in most plaques, typically nodular and sheet-like formations. The nodes of calcification significantly affected the stratified nature of the tissue cross section which could have led to the lower stretch values upon rupture for the more calcified plaques.

3. An idealised diseased arterial geometry was built in FEA and a standard and novel two-stage perfusion angioplasty balloons were deployed in the diseased model with two different material models based on the FTIR classification of Cal:Li greater than or less than one.

a. A curve-fitting tool in Matlab was created to develop material coefficients for the Yeoh SEF of the two material models based on FTIR classification of the plaque behaviours and compositions.

b. These material models were applied to the diseased section of the arterial model and the two balloon models were deployed showing that the calcified plaques classified by FTIR would all rupture during deployment of either device based on the initial and complete rupture points of each plaque in that FTIR grouping. This supports the potential of FTIR as a diagnostic tool prior to undergoing CAS surgery to improve patient outcomes by identifying these vulnerable plaques.

c. The novel two-stage transporter device has general improvements over current technology such as the constant perfusion throughout deployment, the varied compression through the diseased artery wall which would aid in drug transport and the reduction of stress distribution in the first stage of balloon deployment. This varied deployment could potentially reduce risk of rupture and deploy a plaque softening drug to improve the second deployment stage as well as reduce the effect of over-stretching on the baroreceptor nerves. This could lead to improved post-operative complication rates with the reduction in the number of patients suffering from hypotension due to damaged baroreceptor nerves by altering the level of inflation to 75% of original lumen diameter.
References


CHAPTER 10

RECOMMENDATIONS FOR FUTURE WORK
Chapter 10

10 Future Work

10.1 Introduction

This section will examine the various directions of research this current body of work can continue as well as demonstrate the level of work carried out highlighting the research potential of the processes developed in this study can achieve.

10.2 Femoral Plaque Tissue

The biological and mechanical characterisation methods developed in this study can be applied to any type of biological tissue in the body not just vascular tissue. For this study five femoral plaques were ascertained from surgery. However, comparing the mechanical and biological properties of the carotid and femoral tissue did not fit the ethos of the main body of the thesis. There is potential in the future to increase the sample number of the femoral plaques and compare the morphology of the two plaque types similar to Herrison et al. (2011) but with a comparative analysis of the mechanical behaviours of human tissue rather than porcine in a similar study by Maher et al. (2012).

The major difference evident between carotid and femoral morphology was the degree of calcification and the growth of the plaque type in the wall structure. Two main types of calcifications were found in the samples: sheet-like and nodular. In comparing the femoral artery with the carotid there was significantly higher evidence of sheet-like calcification in the femoral than here in the carotid samples, which consisted of a higher amount of nodular calcification. Figure 10.1 (a) is a scanning electron microscopy (SEM) image of the lumen surface of a femoral plaque which has a developed ‘bubble’ formation of calcification. This is an unprecedented discovery of calcification and demonstrates the significantly calcified nature of this plaque type when calcification is allowed to build up in the lumen wall in contact with blood flow.

Figure 10.1: SEM images of the site-specific arterial cracks and rupture points A) femoral B) carotid plaque.
Energy dispersive X-ray (EDX) spectroscopy determined the composition in each of the femoral plaques where the femoral samples contained the highest calcification content in comparison to the calcified carotid plaques. Table 10.1 summarises the average atomic percentage of the main elements C, O, P and Ca that were obtained for the femoral plaques. Table 10.1 shows that the femoral plaques have a high amount of calcium and a lower amount of carbon.

Table 10.1: EDX elemental results for the femoral atherosclerotic calcified plaque type.

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic %</th>
<th>Median</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>45.69</td>
<td>47.26</td>
<td>0.13</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>41.60</td>
<td>39.83</td>
<td>0.09</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>3.56</td>
<td>3.50</td>
<td>0.28</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>8.35</td>
<td>8.64</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 10.2 summarises the ratios that were determined from the main elements C, O, P and Ca that were obtained for each sample type. The three sample types that were analysed had varying amounts of calcium and phosphorus. The calcium and phosphorus to carbon ratio results indicate that the Fourier Transform Infrared (FTIR) classified lipid samples contain the lowest amount of calcification, increasing in the calcified samples and increasing again to the highest amount in the femoral samples. The overall calcium to phosphorus ratio for each sample type was calculated to show the presence of hydroxyapatite which is an indication of the amount of calcification.

Table 10.2: Comparison of the ratios developed from the EDX analysis of the carotid plaque types classified through FTIR and comparison to femoral plaques.

<table>
<thead>
<tr>
<th>Average % Atomic</th>
<th>Type</th>
<th>(Ca+P):C</th>
<th>Ca:C</th>
<th>P:C</th>
<th>Ca:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal:Li &lt; 1</td>
<td>0.09</td>
<td>0.06</td>
<td>0.03</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td>Cal:Li &gt; 1</td>
<td>0.19</td>
<td>0.14</td>
<td>0.05</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>Femoral</td>
<td>0.26</td>
<td>0.18</td>
<td>0.08</td>
<td>2.35</td>
<td></td>
</tr>
</tbody>
</table>

Figure 10.2 illustrates the mechanical results of the four femoral artery plaque samples. One femoral plaque was completely occluded and was unable to undergo mechanical testing due to high level of calcification. The results shown in this graph indicate the majority of samples were hard plaques as each was able to withstand a high Cauchy stress resistance and a low stretch ratio before complete failure.
Further SEM imaging of the carotid and femoral plaques is necessary to analyse important micro and nano level features which were not noted during the current study such as cholesterol crystals that have been shown to have a significant effect on potential plaque rupture, figure 10.3 (Abela et al., 2009, Silvain et al., 2011). This feature was not analysed as the current work concentrated on features which would have contributed to the global failure mechanics.

**Figure 10.3:** SEM image of carotid plaque lumen surface where the development of cholesterol crystals is evident.

### 10.3 Biaxial Testing

Biaxial testing is a necessary step toward full characterisation of biological tissue. It is a natural progression of correlating the biological characterisation method to the data from biaxial tests. A preliminary equibiaxial test was carried out on a section of human carotid plaque to 10% stretch at 0.1mm/s. Figure 10.4 illustrates the anisotropic nature of this specific plaque with a two-fold increase in stress at 10% strain in the longitudinal direction (inset table). Further equibiaxial and quasi-static biaxial tests could analyse...
and relate the mechanical and anisotropic behaviour to FTIR and EDX data. From this proposed work a strain energy function (SEF) using the Holzapfel-Gasser-Ogden function could be developed for improved modelling of carotid tissue in finite element analysis.

Figure 10.4: Stress-Stretch plots of the longitudinal and circumferential directions of human carotid plaque tissue.

### 10.4 Analogous Material of Plaques

A future objective of this study would be to develop an inflation-extension bench top test rig capable of assessing the mechanical impact of angioplasty on the carotid artery which incorporates the baroreceptor function and diseased plaque geometry with realistic material properties, as suggested by the work in chapter 3. Carotid artery plaque mechanical behaviour can be replicated using a polymer as these materials have a hyperelastic response to stretch. Since in vivo testing of balloon angioplasty devices is difficult, the proposed design process is to develop a synthetic biomimetic material that accurately replicate the same stress-strain profiles of the varying types of carotid artery plaques tested in this current body of work. Balloon angioplasty devices can then be deployed within this bench top test rig with the diseased carotid bifurcation model. This would also require an accurate biomimetic material that fulfilts the mechanical behaviour of the carotid artery plaque and the baroreceptor function which is validated in a multiaxial environment and can alter the flow rate in the system when undergoing a stretch from the angioplasty device. Figure 10.5 describes the result of an investigation carried out which compared the carotid plaques tested in this study to commercially
available polymers. The carotid plaques are grouped into four types by mechanical behaviour, Type 1: soft to Type 4: stiff, rather than by FTIR classification. These polymers were tested with the same pre-conditioning and strain rate parameters as the carotid plaque tissue.

Figure 10.5 Material selection method of polymers compared to results from chapter 6 (denoted as Mul et al). Displayed is Polytek 50, Slygard 184 at 9:1, and RT601 at 11:1 and 18:1.

Results from this current body of work demonstrate strong correlation with the polymers mechanical behaviour in the physiological stretch ratio up to 1.3. The sum of squares approximations, shown in Table 10.4, describes strong correlation of the polymers with the plaque types. The most successful correlations were Type 2 with the 18:1 ratio at 0.0017 least square difference and Type 2 with 11:1 at 0.003 least squares difference. Type 4 (stiff) also showed quite accurate correlation with Slygard 184 9:1 and Polytek 50 returning sum of squares differences of 0.0299 and 0.0405, respectively. These results indicate that through this mechanical response of these materials can replicate the mechanical performance of carotid plaques in an inflation-extension test rig. These strong correlations are defined using coefficient of determination and described in Table 10.4.

Table 10.3: Sum of the squares approximation study of material polynomials against the tested carotid plaque types from this study.

<table>
<thead>
<tr>
<th>Carotid Plaque</th>
<th>Polymer Material</th>
<th>Sum of Squares ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td>RT601 18:1</td>
<td>0.0017</td>
</tr>
<tr>
<td>Type 3</td>
<td>RT601 11:1</td>
<td>0.0030</td>
</tr>
<tr>
<td>Type 4 (Hard)</td>
<td>Slygard 184 9:1</td>
<td>0.0299</td>
</tr>
<tr>
<td>Type 4 (Hard)</td>
<td>Polytek 50</td>
<td>0.0405</td>
</tr>
</tbody>
</table>
This method indicates the potential successful integration of these polymers into the diseased carotid bifurcation which incorporates the baroreceptor function. However, further work needs to be carried out to design a mould which can incorporate the carotid plaque geometry similar to Pazos et al. (2010).

References

Development of an experimental model of the carotid bifurcation using electrically conductive silicone:
an introduction to the incorporation of baroreceptor function within a mimetic model of the carotid artery

John J. Mulvihill, Eoghan M. Cunnane,
Barry M. O’Connell and Michael T. Walsh*

‘CABER’ Centre for Applied Biomedical Engineering,
Department of Mechanical Aeronautical and Biomedical Engineering,
Materials and Surface Science Institute,
University of Limerick,
Castletroy, Limerick, Ireland
E-mail: john.mulvihill@ul.ie
E-mail: eoghan.cunnane@ul.ie
E-mail: barry.oconnell@ul.ie
E-mail: michael.walsh@ul.ie
*Corresponding author

Abstract: This study assesses the suitability of developing a material for use in an experimental model of the carotid baroreceptors. Such a model could then be used in future studies to assess the impact of carotid artery stenting on hemodynamic stability. The material must exhibit a significant measurable electrical response to strain in a fashion analogous to baroreceptor behaviour. A modified electrically conductive silicone (ECS) was examined for use as the material, which was generated from a combination of Wacker LR 3162 and silicone thinner. Samples of the ECS were subjected to uniaxial tensile testing and electrical stimulation in order to mechanically and electrically characterise the material. Testing revealed that the ECS exhibits mechanical behaviour comparable to published data on carotid arterial tissue up to 20% strain and a measurable electrical response to strain in a fashion qualitatively comparable to baroreceptor behaviour. These findings highlight the potential of this material for employment as an experimental model of the carotid baroreceptors.

Keywords: baroreceptor function; electrically conductive silicone; ECS; analogue biomaterial; material characterisation; carotid bifurcation.


Biographical notes: John J. Mulvihill graduated with a first class honours degree in Mechanical Engineering from the University of Limerick in 2009 and is currently a Postgraduate Researcher of Biomedical Engineering in Centre for Applied Biomedical Engineering Research and the Materials and Surface Science Institute at the University of Limerick. His research areas include simulated tissue modelling and biological tissue characterisation.
1 Introduction

Stroke is a catastrophic cerebral event that can result in the necrosis of affected brain cells. Such events cause irreversible damage to an array of neurological functions in 22% to 25% of victims and death within one year for 25% of victims (Medtech Insight, 2011). The likelihood of stroke is directly related to the presence of stenosis in the carotid arteries, which are a paired set of vessels that supply oxygenated blood to the cranium (Guyton, 1991). The common carotid arteries bifurcate into the internal and external carotids which lead to the brain and face, respectively. Stenosis is particularly prevalent at the site of bifurcations and this has been attributed to considerable increases in transverse average velocity gradients and viscous shearing stresses that cause preferential damage to the lining of the artery and initiate or perpetuate arterial injury in a manner that induces the development of arterio-atherosclerosis (Scharfstein et al., 1963).

Carotid artery stenting (CAS) is a minimally invasive procedure that deploys a stent at the site of stenosis, compressing the plaque and widening the lumen to increase blood flow. Despite the numerous advantages of CAS over traditional carotid surgery such as increased accessibility, decreased trauma and absence of general anaesthetic (Roffi et al., 2009), it remains the second choice for clinicians and accounts for only 26% of stroke prevention procedures performed (Paraskevas et al., 2009). This low surgical uptake is due to unfavourable clinical trial results that cited emboli release and hemodynamic instability caused by altered baroreceptor behaviour as a complication in 21% to 51% of cases (Gupta et al., 2005).
Baroreceptors are stretch sensitive, spray-like, nerve endings located within the adventitia layer of arterial tissues (Guyton, 1991; Yates and Chen, 1980). They act as blood pressure regulation mechanisms and are extremely abundant in the carotid sinus, the proximal portion of the internal carotid artery, where blood pressure regulation is critical (Sandblom and Axelsson, 2005). During an increase in blood pressure, the carotid sinus distends and the carotid baroreceptors activate. This activation causes an increase in the firing rate of action potentials which signals the nervous systems to trigger a decrease in cardiac output and an increase in peripheral vascular resistance which lowers overall blood pressure (Danielsen and Ottesen, 2003).

When attempting to model baroreceptor behaviour, there are four non-linear phenomena that must be considered (Danielsen and Ottesen, 2003). These features are as follows:

1. A threshold and saturation pressure under and above which the baroreceptor firing rate is unresponsive to strain [Figure 1(a)].

2. Asymmetric responsive behaviour indicated by hysteresis present between firing rate during baroreceptor stretching and un-stretching [Figure 1(a)].

3. Adaptation to a new firing rate base value when subjected to prolonged stretch as depicted by section B in Figure 1(b).

4. Recovery to base value following a step response to carotid sinus stimulus as depicted by section C in Figure 1(b).

Figure 1  Characteristic behaviour of baroreceptors, (a) asymmetric firing rate response of baroreceptors to pressure increase based on experimental data; (b) firing rate response to a step increase in pressure from 170 to 178 mmHg at time 2.5 s

Note: Pressure forced back to 170 mmHg at time 12.5 s

Source: Danielsen and Ottesen (2003)

CAS has a deleterious effect on baroreceptor function due to the sustained stretch caused by balloon deployment. However, there is potential to limit this damaging effect by varying the balloon inflation parameters. An investigation into the response of the baroreceptor function to the deployment of current balloon devices used in CAS is necessary to improve their design. As it is not viable to measure baroreceptor function
during CAS procedures, it is therefore necessary to experimentally replicate CAS conditions using a bench-top test system that employs a simulated carotid bifurcation (CB) model. This paper aims to investigate the feasibility of developing a material that could be used in an experimental model of the CB complete with baroreceptor response to strain, so that it may be employed in future studies to identify and quantify the deleterious effects of subjecting the baroreceptor nerves to sustained circumferential stretch such as in the case of CAS.

Current materials used in the development of simulated arteries, such as Elastosil RT 601 (Wacker-Chemie GMBH, Munich, Germany) and Sylgard 184 (Dow Corning Corp., USA), have mechanical properties similar to arterial tissue but do not possess the appropriate electrical properties \((10^{15} \, \Omega \cdot \text{cm})\) to imitate the firing rate response of the baroreceptors (Corbett et al., 2010; O’Brien et al., 2005; Wacker Chemicals Ltd., 2010). It is possible to synthesise these silicones with conductive carbon filler particles to induce electrical conductivity which occurs due to conductive carbon pathways that arise throughout the materials insulative silicone matrix (Saleem et al., 2010). There is however, a large variability in conductivity across the resulting material samples (Sau et al., 1998). A suitable alternative is to acquire a commercially available prefilled electrically conductive silicone (ECS). Wacker Elastosil LR3162 (Wacker-Chemie, Munich, Germany) is one such material. However, despite its favourable electrical properties, the suitability of the ECS as an arterial mimetic material is unestablished.

To assess this suitability parameter, this study will perform uniaxial tensile testing on the chosen ECS and develop a suitable strain energy function (SEF) for use in finite element analysis (FEA). This will allow the ECS to be fully compared to published data on carotid arterial tissue. This study will also examine the electrical behaviour of the ECS and determine if it exhibits a measurable electrical response to strain with an inherent ability to emulate the two key characteristics of baroreceptor function relating to CAS, adaption to sustained strain and recovery from strain (Danielsen and Ottesen, 2003).

2 Methods

2.1 Material preparation

LR3162 is a two-part, prefilled silicone-carbon composite. It has a volume resistivity of 11 \(\Omega \cdot \text{cm}\) and a viscosity of 6,600 Pa.s, compared to values of \(10^{15} \, \Omega \cdot \text{cm}\) and 3.5 Pa.s for Elastosil RT 601 (Wacker Chemicals Ltd., 2010, 2011). As future studies may wish to develop physical CB silicone models that require a wax core to create the mimetic lumen, it is essential that the material be injectable at room temperature using a syringe so as not to damage the wax core. The viscosity of this ECS is relatively high compared to established mimetic silicones, which makes injecting the LR 3162 at room temperature using a syringe difficult and therefore inappropriate for this process. For this reason, the LR 3162 was mixed with a silicone thinner (Dow Corning 200/5CS, Midland, MI, USA) which has a viscosity of 4.55 \(\times 10^{-3}\) Pa.s. The addition of this silicone thinner has both a lubricating and thinning effect on the final mixture which aids in the mixing and injection processes.

The methodology employed in the mixing of an ECS has a significant effect on the electrical properties of the final material. Excessive shear during mechanical mixing degrades the carbon filler and makes the formation of conductive pathways less likely,
while insufficient shear leads to poor filler distribution. The level of shear experienced during mixing is inversely proportional to the viscosity of the ECS (Zhang et al., 2007). For this reason, it is desirable to develop an ECS with a viscosity just below the threshold of formability and to expose this silicone to low levels of shear for prolonged periods of time to maximise filler distribution and minimise carbon filler degradation.

The two silicone components were mixed in five different ratios using an in-house developed mechanical mixing apparatus that applies uniform rotational force to minimise excessive shearing. The ratios ranged from 50:50 to 90:10 (LR 3162: Thinner, by weight) in increments of ten. Ratios containing less than 50% LR 3162 were disregarded in an attempt to limit the reduction of viscosity and preserve carbon particle integrity during mixing. LR 3162 parts A and B were mixed in equal measures and the silicone thinner was added in the desired ratio. The resulting mixture was mixed using the aforementioned mechanical mixer.

Each ratio of the ECS was analysed in terms of curability, injectability and conductivity. Curability was defined as the ability of the material to be removed from the mould with minimal sticking and by the lack of colour transfer from the material after curing (Mehnert et al., 2001). The material was deemed injectable if it could be injected into a mould initially at atmospheric pressure through a 2 mm diameter syringe. Conductivity was tested by passing a current through cured, dog-bone shaped samples of the material at two points.

2.2 Experimental apparatus and procedure

The ECS was mechanically and electrically characterised using an in-house developed tensile tester and a video extensometery device, Figure 2. The stress–strain information obtained from the tensile tester and the conductivity-strain information obtained from the video extensometer were recorded. As the strain values throughout the experiment were being measured separately using the tensile tester and the video extensometer, the two sets of strain values were calibrated to match during testing to minimise discrepancies and keep stress, strain and conductivity parameters in-phase.

Five samples of the ECS were subjected to a known voltage and current and exposed to four cycles of strain using the tensile tester. The first three cycles, 50% strain at 0.001 m/s, were performed to precondition the material and ensure that the results obtained were uniform over a number of straining cycles by eliminating the Mullin’s effect which is responsible for initial excessive material stiffness (Sau et al., 1998). The fourth cycle stretched the samples to 150% strain at a constant rate of 0.001 m/s. This cycle was used to characterise the material and develop the SEF. A relatively large strain value was used to ensure that a fully stabilised SEF was developed from the resulting stress-strain curves. Stability of SEFs is drawn into question when attempting to predict strain ranges above those examined during experimental testing (Holzapfel et al., 2004), therefore straining up to a near rupture value was conducted in the event that future studies wish to utilise the SEF developed in this study for larger strain applications.

The baroreceptor characteristics of adaption to strain and recovery from strain were compared to the behaviour of the ECS by subjecting three samples of the ECS to a known voltage and current and straining each sample to 150% strain. This strain was maintained for ten minutes and then each sample was returned to its original length. The voltage drop across each sample was monitored during this process and also monitored for an additional ten minutes after returning the sample to its original length. The relative
voltage drop across the material sample during strain V*, was identified as the conductivity parameter. This was used to eliminate the effect caused by variances in crocodile clip location and was achieved by presenting each voltage drop measurement value relative to the first value.

Figure 2 Diagrammatical representation of the experimental apparatus: tensile tester, power supply, video extensometer, crocodile clips and computer used to test the cured dog-bone shaped samples of the ECS.

2.3 Computational

An idealised CB geometric model was created in Pro/Engineer Wildfire 5.0 (Parametric Technology Corp., Needham, MA, USA) and imported into and analysed in ABAQUS/Standard 6.10-1 (Dassault Systems, SIMULA, Providence, RI, USA) to fully compare the mechanical properties of the modified LR 3162 to a SEF developed to represent carotid artery mechanical behaviour (Gasser et al., 2006). The geometric model of the CB used in this computational study is based on an idealised CB geometry proposed by Smith et al. (1996) and defined by Ding et al. (2001), Table 1. The common carotid internal diameter of 6.1 mm is based on a study conducted by Krejza et al. (2006). The straight artery sections that represent the common, internal and external carotids were created using boundary blends and the bifurcation section was generated using a six sided patch.

Table 1 Dimensions used to develop the idealised CB geometric model

<table>
<thead>
<tr>
<th>Dimension (mm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.1</td>
<td>4.21</td>
<td>4.21</td>
<td>6.34</td>
<td>6.771</td>
<td>4.39</td>
<td>11.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dimension (mm)</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.93</td>
<td>0.5</td>
<td>11.7</td>
<td>5.98</td>
<td>6.75</td>
<td>14.64</td>
<td>60.5°</td>
</tr>
</tbody>
</table>

This idealised CB geometric model modifies the geometry defined by Ding et al. (2001) by generating a previously undefined curved apex. This apex was defined as tangent to both the inner internal and external artery walls which gave the appearance of a natural
curvature similar to that which is found in the CBs of young adults (Thomas et al., 2005). This geometric model also accounts for the varying artery wall thickness that features throughout the CB. It achieves this by integrating the work of Delfino et al. (1997) which denotes the thicknesses at ten locations across the bifurcation area. The dimensions used to create the idealised CB geometric model are listed in Table 1.

**Figure 3** (a) Cross-sectional view of the idealised CB geometric model; (b) dimensioned diagram of the apex geometry developed in order to generate the idealised CB geometric model (see online version for colours)

### 2.4 FEA study

The idealised CB geometric model was imported into ABAQUS/Standard 6.10-1 (Dassault Systems, SIMULA, Providence, RI, USA) as a SAT file. A free ten-node quadratic tetrahedral mesh was applied with the hybrid formulation activated due to the hyperelasticity of the ECS and carotid tissue material models. Symmetry, along the axis, was applied to all three artery branches and an axisymmetric boundary condition was applied along the thickness of the CB geometric model. An internal pressure of 16 kPa was applied to mimic the peak systolic pressure within a typical carotid arterial system.

Two material models, one for carotid tissue and one for the ECS, were applied to the idealised CB geometric model and their behaviour compared under the applied load. The material model for arterial tissue was a SEF developed by Gasser et al. (2006), known as the Holzapfel-Gasser-Ogden (HGO) SEF, which is a histological and phenomenological SEF of carotid artery tissue, equation (1).

\[
\Psi = C_{10} \left( T_1 - 3 \right) + \frac{1}{D} \left( \frac{(J^d)^2}{2} - 1 \right) - \ln J^d + \frac{k_1}{2k_2} \sum_{a=1}^{N} \left\{ \exp \left[ k_2 \left( \bar{E}_a \right)^2 \right] - 1 \right\}
\]

The strain energy per unit of reference volume is represented as \( \Psi \). \( C_{10}, D, k_1, k_2 \) and \( \kappa \) are temperature-dependent material parameters, \( T_1 \) and \( T_4 \) are invariants of the Cauchy Green strain tensor and \( J^d \) is the elastic volume ratio. \( N \) is the number of families of fibres (\( N \leq 3 \)). The HGO model assumes that collagen fibre direction within each family
are dispersed (with rotational symmetry) about a mean preferred direction. The parameter \( \kappa \) \((0 \leq \kappa \leq 1/3)\) describes the level of dispersion in the fibre directions and is defined within the parameter \( E_\alpha \) , equation (2).

\[
E_\alpha \overset{\text{def}}{=} \kappa (\bar{T}_1 - 3) + (1 - 3\kappa)(\bar{T}_{\langle\alpha\alpha\rangle} - 1)
\] (2)

The parameters were based on the behaviour of ten intact carotid arteries examined during a study undertaken by Sommer and Holzapfel (2012). A number of assumptions were made to simplify the material due to the complex nature of the geometry; the model was assumed incompressible \((D = 0)\) and isotropic \((\kappa = 1/3)\). The second step of the computational study was to create a material model based on the ECS material using a hyperelastic SEF, equation (3).

\[
\Psi(I_1) = \sum_{i=0}^{3} C_i (I_1 - 3)^i
\] (3)

The material model used to characterise the ECS was a third-order polynomial SEF known as the Yeoh form, equation (3). The Yeoh SEF is suitable for characterising hyperelastic materials using uniaxial mechanical data as the function does not depend on the second strain invariant of the Cauchy-Green deformation tensor, \( \bar{T}_2 \). \( C_{i0} \) are the material coefficients and \( I_1 \) is the first-strain invariant which is based on the principal stretch ratios, equation (4).

\[
I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2
\] (4)

The material coefficients are derived by acquiring uniaxial test data and curve-fitting to the stress-strain data. An optimisation technique was applied in this study to minimise the difference in stress values between the Yeoh SEF and the experimental data.

3 Results

3.1 ECS preliminary test results

The ECS ratios between 50:50 and 70:30 were deemed to be curable, injectable and conductive while the remaining two ratios failed to inject.

Table 2 List of ratios (ECS:Thinner) manufactured and tested for injectability, curability and conductivity

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Injectable</th>
<th>Curable</th>
<th>Conductive</th>
</tr>
</thead>
<tbody>
<tr>
<td>50:50</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>60:40</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>70:30</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>80:20</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>90:10</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The carbon filler distribution of each suitable ratio was investigated using a combination of scanning electron microscopy (SEM) and elastic recoil detection (ERD). Figure 4(a)
displays an image of the micro structure of the ECS cross-section of the 60:40 ratio obtained using SEM. Figure 4(b) displays the distribution of carbon content across the black line visible in Figure 4(a) which represents a 15 μm ‘line of interest’. This figure displays an arbitrary carbon content which represents values relative to the first carbon content value obtained. The values displayed along the y-axis are arbitrary and the purpose of the chart is to portray the carbon content distribution rather than the carbon content values.

**Figure 4**  (a) SEM image of the 60:40 ECS ratio microstructure with a 15 μm ‘line of interest’ denoted by a diagonal black line (b) Arbitrary carbon content distribution along this ‘line of interest’ obtained using ERD
3.2 Mechanical characterisation

The diameter of a diseased lumen can range from 10% to 30% of the original diameter (6.1 mm) and be increased to a lumen diameter of 70% to 90% of the original diameter (Krejza et al., 2006). Assuming the artery and plaque to be concentric and incompressible, to facilitate calculations in the realistic diseased artery, the following strain values induced in the external diameter of the carotid artery due to circumferential stretch were hypothesised.

Table 3  Strain values due to circumferential stretch during CAS when increasing the stenosed lumen (10% to 30%) to restored healthy lumen (70% to 90%)

<table>
<thead>
<tr>
<th>% of healthy lumen</th>
<th>70</th>
<th>80</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13.4</td>
<td>17.3</td>
<td>21.5</td>
</tr>
<tr>
<td>20</td>
<td>12.6</td>
<td>16.5</td>
<td>20.8</td>
</tr>
<tr>
<td>30</td>
<td>11.3</td>
<td>15.2</td>
<td>19.5</td>
</tr>
</tbody>
</table>

Source: Krejza et al. (2006)

Therefore, the strain range within which the ECS must replicate the carotid artery mechanical behaviour is between 11.3% to 21.5% (30% to 70% – 10% to 90%), which will be taken as the approximate maximum value of 20%.

The Yeoh SEF analytical curve and the average uniaxial tensile test data of five samples of the ECS are displayed in Figure 5, denoted as ECS – Yeoh and ECS – Expt respectively. Also included are published longitudinal uniaxial data for human carotid tissue obtained from Stemper et al. (2005) and circumferential and longitudinal uniaxial data of porcine carotid tissue obtained from Silver et al. (2003).

Figure 5  Experimental and analytical uniaxial data for ECS and carotid tissue, (a) entire strain range (b) up to 20% strain

Source: Adapted from Stemper et al. (2005) and Silver et al. (2003)
Figure 5  Experimental and analytical uniaxial data for ECS and carotid tissue, (a) entire strain range (b) up to 20% strain (continued)

Figure 5(b) highlights the replicative response of the ECS to uniaxial carotid tissue behaviour up to 20% strain for human tissue and porcine tissue. The coefficients used to define the analytical curve were derived from the averaged curve of the five ECS samples, Table 4. These coefficients were optimised and are stable over all experimental strains.

Table 4  Material coefficients developed for ECS material based on the Yeoh function

<table>
<thead>
<tr>
<th>C10 (MPa)</th>
<th>C20 (MPa)</th>
<th>C30 (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.58e-02</td>
<td>3.68e-03</td>
<td>6.59e-04</td>
</tr>
</tbody>
</table>

3.3 FEA study

The material coefficients were applied to the Yeoh material model that represents the ECS in the FE analysis and a comparison was carried out against the HGO material model of carotid tissue, denoted as ECS – Yeoh SEF and carotid – HGO SEF, respectively. The data is averaged from a plane of nodes, perpendicular to the external artery wall, in the idealised CB geometric model at the base of the carotid sinus, where the baroreceptors are most abundant.

As the mechanical properties of carotid tissue vary significantly from specimen to specimen, it is more accurate to consider a range of specimens rather than just one sample. For this reason, the HGO data generated in this study is representative of the behaviour of ten intact carotid specimens from Sommer and Holzapfel (2012) applied to the HGO SEF, shaded region denoted as carotid – HGO SEF. Figures 6(a) and 6(b) illustrate the increase in stretch ratio (external diameter) of the CB with a steady increase in pressure, from 0–16 kPa, for both the Yeoh and HGO SEF models.
Development of an experimental model of the carotid bifurcation using ECS

Figure 6  Change in circumferential stretch of the CB at the base of the carotid sinus with an increase in pressure for the carotid (HGO) and ECS (Yeoh) material models (a) entire stretch range; (b) up to 20% stretch

Note: Shaded region, carotid – HGO SEF, represents ten intact human carotid arteries.

3.4 Electrical characterisation

Figure 7 shows the averaged resulting electrical response of five ECS samples to strain. The error bars depict the spread of experimental data at each point.
3.5 Baroreceptor comparison

Baroreceptors located in the carotid sinus exhibit non-linear physiological phenomena. Due to this, the ECS was examined for the two most relevant parameters regarding CAS, adaption to sustained strain and recovery from strain. The averaged data for the three samples tested for these phenomena is displayed in Figure 8 with the baroreceptor response over a 25 s period developed by Danielsen and Ottesen (2003) inset.
Figure 8  Electrical behaviour of the ECS regarding adaption to sustained strain and recovery from sustained strain which is qualitatively similar to the baroreceptor behaviour seen in Figure 1(b), inset

4 Discussion

4.1 ECS preliminary test

Preliminary testing of the ECS material ratios was carried out to inspect for electrical conductivity through the material and to assess each ratio’s suitability for injection moulding. Three ratios, 50:50, 60:40 and 70:30, were deemed suitable under both criteria and further testing using SEM and ERD highlighted that the 60:40 ratio had the most uniform distribution of carbon filler of the three ratios tested. This ratio was therefore chosen as the conductive material to be tested for use in the proposed experimental baroreceptor model. The favourable behaviour displayed by this ratio was attributed to a suitable viscosity value that resulted in ideal shearing conditions during mixing which allowed for sufficient carbon filler distribution, Figure 4. This can be surmised as there are no obvious areas of carbon coagulation or areas of carbon deficiency within the silicone matrix.

4.2 Mechanical characterisation

The uniaxial tensile testing of the optimal ECS ratio was carried out to establish if this specific type of silicone exhibits suitable mechanical behaviour to act as a mimetic material of carotid arterial tissue. The testing shows that over a larger strain range the ECS behaves differently to human carotid arterial tissue as its stiffening response occurs at higher strains, Figure 5. However, the ECS material compares favourably to circumferential mechanical properties of porcine carotid tissue, which mechanically behaves similarly to human arterial tissue in the longitudinal direction, Figure 5(a). Comparing the results up to the proposed averaged strain that occurs during stenting
shows that all four sample sets behave similarly up to 20% strain. The deviation between the ECS and human arterial tissue may occur due to the fact that the ECS is a relatively homogenous material in comparison to arterial tissue which contains collagen fibres that stiffen at higher strains (Gasser et al., 2006) and the arterial tissue was tested in the longitudinal direction (Stemper et al., 2005).

Baroreceptors activate subject to distension which is a function of radial strain and circumferential stretch in the artery wall. This study is limited to measuring uniaxial stretch of dog-bone shaped samples in pure tension with a uniform stress field in the length of the specimen which is then assumed to equate to circumferential stretch within the artery wall. This study does not take into account the residual stress in the circumferential and axial direction of the carotid artery as well as the non-uniform stress field within the artery wall. To overcome this limitation, a significant sample set of dog-bone samples excised from cylindrical shaped tubes of ECS should be created and tested using a similar procedure to that which is employed in this study to take into account the circumferential residual stress. This will allow for improved mechanical and electrical characterisation of the ECS for both the computational and baroreceptor feedback models.

4.3 FEA study

Figure 6 illustrates the significant difference in mechanical behaviour over the examined strain range between the two material models. The idealised CB geometric model, commonly known as the tuning fork model, has been shown to correlate closer to the actual geometry of the CB during \textit{in vivo} and \textit{in vitro} studies than alternate idealised geometries (Ding et al., 2001; Thomas et al., 2005). The data plot, carotid – HGO SEF, represents a range of ten intact specimens based on parameters from literature (Sommer and Holzapfel, 2012). Figure 6(b) shows that the ECS mechanical behaviour correlates with the initial 20% stretch. However, the mechanical behaviour of both material models over the larger strain range does not correlate. The carotid arteries began to stiffen considerably after a 5% to 15% stretch unlike the ECS which behaves linearly elastically after 15% stretch. The discrepancies between the models are large as the HGO SEF is based on test data requiring \( T_1 \) and \( T_4 \) strain invariants, whereas the Yeoh SEF is based on uniaxial tensile tests, \( T_1 \) strain invariant only. The Yeoh SEF is not suitable to fully characterise the material for a complex multiaxial model such as the idealised CB geometric model. Further mechanical characterisation of the ECS material is necessary such as inflation-extension and planar biaxial tests. The HGO SEF, used to represent the carotid arterial tissue, was over-simplified as an isotropic and incompressible model while the Yeoh SEF, used to represent the ECS, did not contain circumferential residual stresses or the 10% axial residual stress inherent in a realistic arterial geometry (Hariton et al., 2007).

4.4 Electrical characterisation

Figure 7 displays a distinct change in electrical behaviour in response to strain. This occurs due to the breakdown and formation of conductive carbon pathways within the silicone matrix (Zhang et al., 2007). In this case, the breakdown of pathways is more predominant than the formation and therefore the material becomes less conductive and
V* increases. Due to this distinct measurable change in electrical behaviour in response to strain coupled with the relatively limited variability present within the first 20% strain, visible in Figure 7(b), this material has the potential to act as an analogue to baroreceptor firing behaviour.

This study examines electrical behaviour based on voltage drop across a set of samples during tensile testing. This method of measuring electrical behaviour does not account for the relatively minute change in current across the material. To overcome this limitation, future studies should develop a method of logging real-time values for resistance across the material that are in-phase with the corresponding values for stress and strain. However, it should be noted that in the scope of this study, V* is an adequate electrical parameter as it is a quantifiable electrical response to strain.

4.5 Baroreceptor comparison

In sector A of Figure 8, the ECS exhibits a ramped response to strain that causes the resistance of the material to rise sharply. This can be attributed to the instantaneous destruction of conductive carbon pathways.

In section B, the ECS is maintained at a sustained strain and decay in the resistance can be observed. This decay is due to the formation of new conductive carbon pathways. Once the maximum number of available carbon pathways have aligned, the resistance of the material plateaus at a new equilibrium point. This behaviour is qualitatively similar to the reset mechanism of the baroreceptors, section B of inset.

In section C, the applied load is removed from the ECS and the sample is returned to 0% elongation. This causes a further spontaneous rise in resistance, illustrated by the peak, as the newly formed carbon chains break, followed by a drop in the materials resistance as the conductive carbon chains that were broken upon stretching quickly realign. This peak is not exhibited by the baroreceptor firing rate behaviour which, instead, displays a sharp drop below the original firing rate once the distension has ceased and then a gradual increase to the initial firing rate. This drop occurs as the firing rate briefly goes to zero before returning to a base value after the removal of the applied strain. Applying an initial stretch to each ECS sample during testing may help in emulating this baroreceptor occurrence by setting the electrical base value at a number higher than zero.

The rate of decay of resistance of the ECS then slows once all of the carbon particles in close proximity form conductive pathways. More distal particles continue to form pathways at a slower rate resulting in a more gradual recovery to the original equilibrium value. This resistance recovery is qualitatively similar to the recovery mechanism of the carotid baroreceptors, section C of inset.

By comparing the trends present in this chart to those presented by Danielsen and Ottesen (2003) inset, it can be observed that both the ECS and the baroreceptors share a similar response to sustained strain and a similar recovery response following straining. This highlights the materials potential for use as a baroreceptor model as it allows for the examination of the short-term effects on baroreceptor functionality when deploying a stent. To conduct such an examination, the material should be exposed to strain conditions similar to those experienced during CAS. As this material exhibits an electrical adaption response qualitatively similar to the carotid baroreceptors, this examination would allow for long-term feedback regarding the changes in blood flow and
pressure that occur with an increase in firing activity of the baroreceptors similar to conditions experienced during CAS.

Further studies are required to fully assess the materials suitability and should address the issues of time scale and initial firing rate differences between the two feedback phenomena visible in Figure 8 and the inset. These issues should be addressed by increasing the strain rate applied to the ECS samples in order to match strain times and also by applying residual strain to the ECS samples in order to match the initial firing rate responses. Difficulties will arise in future studies when measuring the voltage drop across the thickness of a replicative silicone model, similar to the computational model, due to relatively small wall thickness of 1 mm.

5 Conclusions

Preliminary uniaxial tensile test data and electrical stimulation of the ECS, examined during this study, has demonstrated the feasibility of utilising the ECS as a mimetic material of carotid tissue due to its suitable mechanical behaviour up to the physiological strain range of 20%. However, further mechanical characterisation, in the form of inflation-extension testing, of the ECS is required to develop a more accurate material model for FE analysis.

This study has also demonstrated that the ECS elicits the quantifiable electrical response to strain necessary for the material to act as a baroreceptor model and that it relays these responses in a fashion that is qualitatively similar to the two key characteristics of baroreceptor behaviour that relate to CAS surgery, adaption to sustained strain and recovery from strain. For these reasons, the ECS has been deemed suitable for application as a mimetic model of the carotid artery with incorporated baroreceptor function.

However, the electrical responses of the ECS to strain must be related to baroreceptor firing rate during multiaxial testing of cylindrical specimens, under the same time and strain conditions examined by Danielsen and Ottesen (2003) to fully compare the ECS’s electrical behaviour to baroreceptor function.

References


On the mechanical behaviour of carotid artery plaques: the influence of curve-fitting experimental data on numerical model results

John J. Mulvihill · Michael T. Walsh

Abstract Computational models of diseased arteries are advancing rapidly, and a need exists to develop more accurate material models of human atherosclerotic plaques. However, intact samples for in vitro mechanical testing are not readily available. Most plaque samples are harvested from carotid endarterectomies where the geometries are not suitable for the boundary parameters necessary for classical uniaxial tensile testing. Experimental studies of biological tissue, particularly human plaque tissue, have not specified the minimum width-to-length (WL) ratio necessary for appropriate tensile testing. This study proposes either tensile or planar shear testing on whole specimen samples depending on the WL ratio. However, a “grey-area” of WL ratios exists which are unsuitable for either test, between 0.5:1 and 4:1 WL ratio. Eighteen plaque samples are investigated in this study, and according to classical approaches, two of the plaque samples have WL ratios suitable for tensile testing and four are suitable for planar shear testing. The remaining twelve samples fall in the grey-area of WL ratio. The study analyses which test method is suitable for the samples in this grey-area and what effect using the incorrect test method has on results from a computational model. The study highlights that tissues above a WL ratio of 2:1 are suitable for planar shear testing, and samples below 1:1 are more suited for tensile testing. Therefore, the “grey-area” can be reduced with certain limitations applied by the minor strain assumption which need to be taken into account during experimental testing. This study also demonstrates the influence of curve-fitting experimental results using tensile- and planar shear–based boundary parameters from eighteen plaque samples.

Keywords Carotid artery disease · Plaque properties · Mechanical characterisation · Modelling approaches

1 Introduction

Stroke is a sudden necrosis of brain cells caused by an interruption of normal perfusion to the brain. Stroke has the potential to cause irreversible damage to an array of neurological functions in 22–25 % of victims and death within one year for 25 % of victims (Medtech-Insight 2011). One of the main instigators of a stroke event is the presence of atherosclerotic plaque in the carotid arteries (Guyton 1991). Atherosclerosis refers to a condition in which the walls of an artery thicken due to an accumulation of fatty deposits consisting mainly of lipid and connective tissue matrix. Over time, these deposits harden and form a fibrous cap which can rupture and cause an occlusive thrombosis reaction that can prevent normal blood flow to the brain and lead to a stroke event. Carotid artery stenting (CAS) is a minimally invasive technique of mechanically widening a diseased carotid artery, by deploying a stent at the site of stenosis, compressing the atherosclerotic plaque and widening the lumen to increase blood perfusion. Despite the numerous advantages of CAS over carotid endarterectomy such as increased accessibility, decreased trauma and absence of general anaesthetic (Roffi et al. 2009), it accounts for only 26 % of stroke prevention procedures performed due to the lack of knowledge on the mechanical effect of stents on the plaque structure (Paraskevas et al. 2009).
Computational studies of atherosclerotic arteries under physiological conditions have evaluated the stress distribution within the plaques to analyse the biomechanical response to certain geometrical, structural and material variations (Li et al. 2008; Kiousis et al. 2009; Creane et al. 2010; Franquet et al. 2011). A limited number of studies have also examined the biomechanical structural alterations of the plaque to the deployment of stents to improve the design of the stent (Takahima et al. 2007; Cui et al. 2010; Auricchio et al. 2011; Scherer et al. 2011). Computational studies of diseased coronary arteries are more common than the carotid (Cilla et al. 2012; Huang et al. 2001; Versluis et al. 2006) and a main limitation in these studies is the material properties representing the plaque model which are developed from experimental data based on a small sample size of human plaque from the aorta (Beattie et al. 1998; Lendon et al. 1993; Loree et al. 1994a), limiting the reliability of the stress values from these studies. The computational simulations of diseased coronary arteries also base the material properties of the carotid plaque on experimental data of atherosclerosis from other parts of the vasculature which have different morphologies and potentially different mechanical responses to stent deployment (Herisson et al. 2011), excluding Creane et al. (2010) which is based on uniaxial tensile data of fresh human carotid plaques undertaken by Maher et al. (2009).

Ideally for improved material properties of diseased tissue, in vivo mechanical characterisation of carotid atherosclerotic plaque (CAP) would be carried out on a patient to develop a specific constitutive material model and apply it to their diseased artery geometry. However, there is no fully validated method of carrying out this in vivo mechanical characterisation procedure. Current studies that mechanically characterise CAPs use in vitro mechanical test procedures developed for engineering materials with specific geometry requirements to satisfy the minor strain assumptions such as pure tension and equi-biaxial testing. Limitations when mechanically characterising any type of excised intact plaque tissue are the random geometry and variance in mechanical response, making it difficult to test the CAP specimen as a whole in pure tension (a common test method for mechanically characterising biological tissue when using a strain energy function (SEF)). Figure 1 highlights the geometrical difference between CAPs excised after a carotid endarterectomy, Fig. 1a is from Lawlor et al. (2011) which is a fresh human CAP tested immediately after excision from the common carotid. Figure 1b is a defrosted human CAP from the carotid bifurcation (left-side) through to the common carotid. There are many limitations to in vitro mechanical characterisation of biological tissue such as the deleterious effect on the structure and residual stresses within the plaque during excision, damage to tissues from clamping and inhomogeneity of the CAP tissue material for an ideal failure at the centre of the specimen.

In vitro studies, testing human and animal biological tissue in tension, range the width-to-length (WL) ratio from 0.5:1 to 0.167:1 in both the circumferential and longitudinal directions, Table 1 (excluding ASTM-International (2009) which is a standard based on engineering materials). Specifically examining the studies of human plaque, the WL ratio varies from 0.3:1 to 0.21:1. The shortest gauge lengths used in these tensile test studies were 3 and 4 mm, Richardson (2002) and Maher et al. (2009), respectively, where it was stated that such a small distance between the clamps can affect the stress distribution within the material during testing. Excluding this gauge length, the experimental plaque studies range from 7 to 15.9 mm in gauge length where in theory this length should be much longer to allow for a larger uniform stress distribution for the governing equations of major and minor strains to hold true when applying the experimental data to a SEF. However, as the tissue contains interconnected fibres, this cutting can deleteriously affect the structure of the fibres as well as alter the global mechanical properties of the plaque. For an immediate improvement in the constitutive material models of CAPs, it is necessary to mechanically characterise the whole plaque specimen as separating the specimen into smaller sections can potentially underestimate the global mechanical properties (Lawlor et al. 2011; Borschel et al. 2003).

Due to the nature of the CAP geometry, when excised from the artery, this study investigates the limitations that geometric ratios associated with excised plaque specimens (WL ratios) have when mechanically tested in either pure tension or planar shear. A “grey-area” of unsuitable WL ratios is created between the correct ratios for tensile and planar
Table 1 List of experimental studies using tensile tests on biological tissue which also detail the gauge lengths and widths of the samples tested

<table>
<thead>
<tr>
<th>Author</th>
<th>Length (mm)</th>
<th>WL</th>
<th>Tissue type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stemper et al. (2005)</td>
<td>15.9</td>
<td>0.5:1</td>
<td>Human carotid artery</td>
</tr>
<tr>
<td>Lally et al. (2004)</td>
<td>9.38</td>
<td>0.37:1</td>
<td>Porcine coronary artery</td>
</tr>
<tr>
<td>Hanuza et al. (2010)</td>
<td>30</td>
<td>0.33:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Holzapfel et al. (2004)</td>
<td>7–17</td>
<td>0.31–0.13:1</td>
<td>Human iliac plaque</td>
</tr>
<tr>
<td>Loree et al. (1994a)</td>
<td>15.9</td>
<td>0.3:1</td>
<td>Human aortic plaque</td>
</tr>
<tr>
<td>Raghavan et al. (1996)</td>
<td>40</td>
<td>0.25:1</td>
<td>Human aortic aneurysm</td>
</tr>
<tr>
<td>Wang et al. (2001)</td>
<td>40</td>
<td>0.25:1</td>
<td>Human aortic thrombus</td>
</tr>
<tr>
<td>ASTM-International (2009)</td>
<td>N/A</td>
<td>0.25:1</td>
<td>Tensile testing standard</td>
</tr>
<tr>
<td>Maher et al. (2009)</td>
<td>4</td>
<td>0.25:1</td>
<td>Human carotid plaque</td>
</tr>
<tr>
<td>Xiong et al. (2008)</td>
<td>20–25</td>
<td>0.25–0.2:1</td>
<td>Human aortic aneurysm</td>
</tr>
<tr>
<td>Teng et al. (2009)</td>
<td>9</td>
<td>0.22:1</td>
<td>Human carotid plaque</td>
</tr>
<tr>
<td>Lendon et al. (1993)</td>
<td>7</td>
<td>0.21:1</td>
<td>Human aortic plaque</td>
</tr>
<tr>
<td>Silver et al. (2003)</td>
<td>10</td>
<td>0.2:1</td>
<td>Porcine carotid artery</td>
</tr>
<tr>
<td>Guinea et al. (2010)</td>
<td>10</td>
<td>0.2:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Di Martino et al. (1998)</td>
<td>22</td>
<td>0.2:1</td>
<td>Human aortic artery</td>
</tr>
<tr>
<td>Veronda et al. (1970)</td>
<td>45.7</td>
<td>0.167:1</td>
<td>Cat skin</td>
</tr>
<tr>
<td>Richardson (2002)</td>
<td>3</td>
<td>0.167:1</td>
<td>Human coronary plaque</td>
</tr>
</tbody>
</table>

Table 2 List of WL ratios used in a number of experimental studies using mechanical tests on biological tissue in planar shear

<table>
<thead>
<tr>
<th>Author</th>
<th>Length (mm)</th>
<th>WL</th>
<th>Tissue type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis (2004)</td>
<td>N/A</td>
<td>4:1</td>
<td>Shear standard</td>
</tr>
<tr>
<td>Gao et al. (2009)</td>
<td>11</td>
<td>4.6:1</td>
<td>Porcine liver</td>
</tr>
<tr>
<td>Hollenstein et al. (2011)</td>
<td>10</td>
<td>6:1</td>
<td>Porcine skin</td>
</tr>
</tbody>
</table>

Fig. 2 Idealised computational models of tensile and planar shear uniaxial tests. a WL 0.25:1 suitable for tensile testing and b WL 4:1 suitable for planar shear testing.

planar shear tests for 18 carotid plaques, on a computational model of an idealised stenosed carotid artery. An understanding of the limitations and percentage error from theoretical minor strains for CAP samples in the grey-area will aid in improved experimental methods when testing whole CAP specimens and more accurate constitutive material models (Table 2).

2 Materials and methods

2.1 Ratio analysis

A computational analysis of the stresses in a test specimen with varying geometric ratio (WL 0.1:1–10:1) was carried out with a consistent hyperelastic material property. By examining the effect of the WL ratio on the resolved stresses and minor strain of the test specimens, the errors involved when the WL ratio is in the “grey-area” for suitable mechanical testing, 0.5:1 to 4:1, were identified. Figure 2 illustrates two of the twelve computational models employed in this study. Figure 2a shows an ideal geometry for tensile testing with a WL ratio of 0.25:1, (b) illustrates a model with a WL ratio of 4:1 suitable for planar shear testing. Comparison of the minor strain at the centre of gauge length...
2.2 Idealised diseased artery

A two-dimensional cross-section of an idealised atherosclerotic artery was modelled using geometries analogous to work carried out by Li et al. (2008) and Franquet et al. (2011). The geometric model contains a large lipid core which is surrounded by a diseased intimal layer with a fibrous cap of 0.6 mm, Fig. 3. The internal diameter of the artery is 6 mm which was based on Franquet et al. (2011) and reiterated in experimental work by Krejza et al. (2006). The lumen diameter was chosen to be 30% of the artery diameter, 1.8 mm, which is based on the typical minimum stenosis of a diseased artery which undergoes carotid endarterectomy. The 0.9 mm thickness of the carotid wall was based on Delfino et al. (1997), but can vary up to 1.5 mm (Li et al. 2008). The artery wall was divided into the medial and adventitial layers according to Holzapfel et al. (2000), 0.4 and 0.5 mm, respectively. The lipid core size and shape were based on Franquet et al. (2011). A peak physiological systolic pressure value of 18 kPa was applied uniformly to the wall of the lumen for each model (Cilla et al. 2012).

Grid independence was established using a 6-noded free triangular mesh applied throughout the whole finite element (FE) model. Hybrid formulation, used for incompressible and large deformation problems, and plane strain were applied to this model. Elements were concentrated around the lumen and fibrous cap region, but with at least 6 elements through the thickness of the medial and adventitial layer.

2.3 Material properties

The CAP properties were based on the experimental data of fourteen CAP samples from Lawlor et al. (2011) as well as data of four CAP samples from that study which were not included due to the unsuitability of the WL ratios for tensile testing. The stress-strain data of each plaque were fitted to a third-order reduced polynomial SEF, the Yeoh form, using an optimisation technique developed with Matlab (r2009a, Natick, MA; The Mathworks Inc., 2009) to minimise the difference in stress values between the Yeoh SEF and the experimental data. The optimisation technique was carried out for each CAP sample using both tensile and planar shear principal stretches leaving 36 material models in the numerical study.

\[
\Psi(I_1) = \sum_{i=1}^{3} C_i (I_1 - 3)^i
\]  

The Yeoh SEF is suitable for characterising hyperelastic materials using uniaxial mechanical data, in tension or planar shear, as the function does not depend on the second strain invariant of the Cauchy Green deformation tensor, \( I_2 \). \( C_i \) are the material coefficients, and \( I_1 \) is the first-strain invariant which is based on the principal stretch ratios, Eq. 2.

\[
I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2
\]  

The principal stretches (\( \lambda_1, \lambda_2 \) and \( \lambda_3 \)) change depending on the uniaxial test, tensile or planar shear, where in both cases \( \lambda_1 \) is the stretch in the loading direction. For tensile testing, the principal stretches are:

\[
\lambda_1 = \lambda, \lambda_2 = \lambda_3 = \frac{1}{\sqrt{\lambda}}
\]  

For planar shear testing:

\[
\lambda_1 = \lambda, \lambda_2 = 1, \lambda_3 = \frac{1}{\lambda}
\]  

The WL ratio governs the degree of deformation that the minor stretch ratio component (\( \lambda_2 \)) undergoes during a tensile or planar shear test. During a planar shear test, the minor principal stretch ratio (\( \lambda_2 \)) is considered to be 1 which equates to zero strain in the transverse direction. According to Davis (2004), the minor strain is 0.05 times the major strain when the WL ratio is 4:1 which is close to the plane strain condition necessary for the test (Palmieri et al 2009). However, any WL ratio lower than this can lead to a larger deviation from the zero strain assumption in the minor direction. For tensile testing, the minor axis experiences compressive, negative strains, as the principal strain is positive. This study developed two constitutive material models for each of the eighteen plaques based on the Yeoh form, using the tensile and planar shear boundary parameters, and analysing.
the effect that the WL ratio can have on the stress distribution and mechanical response in an idealised FE model. The lipid core was based on Akyildiz et al. (2011), where the lipid core is treated as a very soft and nearly incompressible tissue, with a Young’s Modulus of 1 kPa and Poisson’s ratio of 0.45 rather than an incompressible liquid, which is based on experimental work carried out by Loree et al. (1994b). Other approaches (Loree et al. 1994b) have treated the lipid core as a nearly incompressible fluid which is not able to sustain shear stress. The effects of the 1 kPa assumption on the trends presented in this study were evaluated and discussed in the limitations section. The arterial media and adventitia layers were modelled using an anisotropic SEF developed by Gasser et al. (2006). The strain energy per unit of reference volume is represented as \[ \Psi = \mu (I_1 - 3) + \frac{k_1}{k_2} \left( \exp\left( \frac{k_2}{k_2} (\kappa (I_1 - 3) + (1 - 3\kappa)(I_4 - 1))^2 \right) - 1 \right) \] (5)

From Eq. 5, \( \mu, k_1, k_2 \) and \( \kappa \) are material parameters, \( I_1 \) and \( I_4 \) are invariants of the Cauchy Green strain tensor. The parameter kappa \( (0 \leq \kappa \leq 1/3) \) describes the level of dispersion in the fibre directions. The parameters for adventitial and medial layers were based on readily available data of arterial tissue from Cilla et al. (2012), listed in Table 3. The model was assumed incompressible, by setting the compressibility value (D) to zero for each material property, to simplify the material model (Holzapfel et al. 2000).

### 3 Results

#### 3.1 Ratio analysis

Table 4 lists the different gauge lengths, WL ratios and plaque type of the CAP samples tested, highlighting the size and random nature of the CAP specimens. Figure 4 illustrates the percentage error of the minor strains of each WL ratio, from 0.1:1 to 10:1, from the theoretical minor strain suitable for either tensile (red area) or planar shear (blue area) testing. For example, any WL ratio less than 0.5:1 has minor strain values 100% in error for planar shear testing and WL ratios greater than 4:1 has minor strain values 100% in error for tensile testing making any of these ratios unsuitable for those test methods.

Using current standard approaches and the WL ratios in Table 4, only two CAP samples are suitable for tensile testing (4 and 14) and four CAP samples are suitable for planar shear testing (5, 8, 15 and 17) leaving twelve CAP samples unsuitable for either test. Excluding twelve CAP samples from this experimental study is not ideal due to the difficulty of acquiring these human CAP samples and the desire not to deleteriously cut the samples into smaller sections which could significantly alter the global mechanical properties. Therefore, it was decided to assess the current “grey-area” WL ratios to determine the theoretical minor strain errors involved with test samples in this area.

FE analysis of WL ratios from 0.1:1 to 10:1 shows that ratios less than 0.5:1 and greater than 4:1 are suitable for tensile and planar shear testing, respectively, as the minor strain is correct within 5% for more than 80% of the gauge length, Fig. 5c, d. However, for the ratios that fall between 0.5:1 and 4:1, the minor strains are less than 80% of the gauge length, Fig. 5a, b (1:1 and 2:1, respectively). From Fig. 4, the percentage error from the theoretical minor strain of 2:1 WL is 65 and 32% from tension and planar shear, respectively, indicating that planar shear testing is more suitable. However, this is only true for 55% of the gauge length area, Fig. 5b. Again looking at Fig. 4 for the WL ratio 1:1, the percentage error is 12 and 85% from tension and planar shear, respectively, indicating that tensile testing is more suitable.

<table>
<thead>
<tr>
<th>Plaque Length (mm)</th>
<th>WL</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.19:1</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>2.25:1</td>
</tr>
<tr>
<td>3</td>
<td>4.72</td>
<td>3.51:1</td>
</tr>
<tr>
<td>4</td>
<td>5.8</td>
<td>0.45:1</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>5.55:1</td>
</tr>
<tr>
<td>6</td>
<td>7.98</td>
<td>1.04:1</td>
</tr>
<tr>
<td>7</td>
<td>10.05</td>
<td>1.32:1</td>
</tr>
<tr>
<td>8</td>
<td>3.96</td>
<td>5.02:1</td>
</tr>
<tr>
<td>9</td>
<td>6.1</td>
<td>1.93:1</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>2.55:1</td>
</tr>
<tr>
<td>11</td>
<td>6.42</td>
<td>2.02:1</td>
</tr>
<tr>
<td>12</td>
<td>6.11</td>
<td>2.05:1</td>
</tr>
<tr>
<td>13</td>
<td>5.18</td>
<td>2.53:1</td>
</tr>
<tr>
<td>14</td>
<td>4.57</td>
<td>0.44:1</td>
</tr>
<tr>
<td>15</td>
<td>6.89</td>
<td>4.50:1</td>
</tr>
<tr>
<td>16</td>
<td>7.08</td>
<td>1.90:1</td>
</tr>
<tr>
<td>17</td>
<td>2.88</td>
<td>4.01:1</td>
</tr>
<tr>
<td>18</td>
<td>9.89</td>
<td>1.95:1</td>
</tr>
</tbody>
</table>
Fig. 4 The percentage error of each CAP sample (black X) from the theoretical minor strain suitable for tensile (dark red line) and planar shear (dark blue line) for each geometric ratio that is suitable for tension (0.1:1–0.5:1), for planar shear (4:1–10:1) and in the “grey-area” (0.5:1–4:1).

Fig. 5 Minor Strain plots for tension and planar shear of samples with different WL ratios: a 1:1, b 2:1, c 1:1 and d 4:1, where red denotes minor strain suitable for tension (a, c) and blue is suitable for planar shear (b, d).

However, again this is only true for 70% of the gauge length, Fig. 5a.

3.2 Idealised diseased artery

An example of the von Mises stress distribution of a 2D plane strain–idealised diseased artery model is shown in Fig. 6, CAP sample 7, using material model developed from planar shear principal stretches (Eq. 4). The peak von Mises stress typically occurs at the point on the lumen closest to the lipid pool. The peak von Mises, maximum principal stresses and radial displacement were recorded. A constitutive model was developed for each of the CAP samples in both tension and planar shear, based on Eqs. 3 and 4, respectively. The results demonstrated that the peak stress and displacement values were typically larger in the FE models using the planar shear–based SEF.

Figure 7 quantitatively shows the difference in radial displacement from the lumen centre along a horizontal centre-line through the stenosed artery model. This graph highlights the similar behaviour of both models, but the larger radial displacement of the planar shear SEF through each section of the model of CAP sample 8. Figure 8 illustrates the difference in peak radial displacement between the tensile-based SEF model (a) and the planar shear model (b) of CAP sample 8 where the planar shear model has larger displacements for this particular sample and for all samples, excluding sample 6. CAP sample 8 was chosen as the geometry of the specimen suggested that it should be characterised in planar shear rather than tension, and the sample best illustrates the underestimation in radial displacement for a tensile-based FE model shown in Fig. 8a.
Figures 9, 10 and 11 are data plots of the radial displacements along the circumference of the lumen, where the start point ($0^\circ$) is at the right side of the lumen closest to the lipid core and continues counter clockwise. These figures compare the radial displacement of the undeformed original lumen to the deformed lumen of both the tensile and planar shear models of CAP samples 8, 14 and 13, respectively. CAP samples 8 and 14 were chosen as the WL ratios of each are suitable for planar shear and tension, respectively. CAP sample 13 has a WL ratio of 2.53:1 which places the sample in the grey-area and creates a reduced percentage difference in lumen area between the planar shear and tension.

Each of the CAP samples were assigned to averaged WL ratios, which ranges from 0.5:1 (suitable for tensile testing) to 5:1 (suitable for planar shear) in increments of 1, and the percentage difference of the radial displacements of the lumen area between the tensile and planar shear models were analysed in Fig. 12. Figure 12 highlights that the percentage difference between the tensile and planar shear models of each CAP sample is higher at the WL ratios suitable for tensile, 0.5:1, and planar shear, 4:1 and 5:1. This suggests that reassessing the “grey-area” can alter the WL ratios limits to a maximum of WL 1:1 for tensile testing and a minimum WL of 2:1 for planar shear where only 6 of the 18 (33%) of the CAP samples are unsuitable for testing, Fig. 13.

4 Discussion

Recent computational studies aim to simulate the deployment of stents in diseased carotid arteries and to evaluate the stress distribution in vivo throughout the plaque. These studies make it imperative to understand the global mechanical properties of carotid plaques. Idealised FE analysis of different WL ratios highlighted the significant errors in the minor strain associated when testing a specimen outside of the suitable ratios for tensile and planar shear testing.
Unlike engineering materials, biological tissue cannot be altered to suit the required geometrical parameters needed for the boundary conditions to analytically develop constitutive material models, without changing the global mechanical behaviour of the sample. The acquisition of whole human CAP specimens is difficult and limited which makes it imperative to mechanically test every available specimen even when the WL ratio does not fall within current standards. Twelve of the eighteen plaques were theoretically unsuitable for either tensile or planar shear testing, Table 4. However, a precise knowledge of the errors involved and a reduction of this “grey-area” can aid in improved experimental methods and more accurate material models for FE analysis of stent deployment without wastage of CAP samples.

4.1 Ratio analysis

The geometric ratio analysis carried out in this study illustrates the large error in minor strain between WL ratios 0.5:1 and 4:1 for both tensile and planar shear testing, Fig. 4. This analysis highlights the limitation when testing specimens in this “grey-area”. However, as twelve of the eighteen plaque specimens are in this area, it is a common and unavoidable issue. The use of contactless measurement of the strain in an
The percentage error of each CAP sample (black X) from the theoretical minor strain suitable for tensile (dark red line) and planar shear (dark blue line) where the limits have been reassessed to reduce the "grey-area" from WL 0.5:1–4:1 to WL 1:1–2:1.

area of the sample where the minor strain is known to be suitable for pure tension or planar shear throughout the whole test could improve the accuracy of the results. For example, WL 2:1 ratio the minor strain at 50 % strain is in planar shear for only 55 % of the gauge length from the vertical centre, Fig. 5b, whereas for WL 1:1 ratio the minor strain is correct for tension in only 70 % of the gauge length at 50 % strain, Fig. 5a. The contactless strain measurement would have to take into account this limited area for each ratio in the area throughout the test. This is necessary to ascertain accurate global mechanical behaviour of CAP samples for improved SEF material models to be used in future FE studies.

4.2 Idealised diseased artery

The computational study on the idealised stenosed artery evaluated the effect of changing the principal stretches, to tensile or planar shear, had on the numerical model through the altered SEF. The peak von Mises stress values typically occurred at the point on the lumen closest to the lipid core due to the soft and deformable nature of the core material. The peak maximum principal stress was located on the lumen closest to the lipid core for twelve of eighteen (66 %) samples and at the shoulder cap region of the lipid core for six of the eighteen (33 %) samples. The difference between the peak stress at each location was no greater than 12 % for these CAP models for either the tensile or planar shear approaches. The peak stress values were typically larger for the planar shear–based models. The largest deformation of the lumen occurred in CAP samples 2 and 17 due to the soft nature of these samples allowing for a large displacement.

In theory, the planar shear-based SEF is expected to be stiffer than the tensile-based version when using the same experimental data. The results from the numerical model reiterate this theoretical expectation as there is decreased stress and radial displacement in CAP sample models that should be based on planar shear test rather than tensile, Fig. 8. This emphasises the incorrect assumption of characterising samples in the grey-area that have greater width than length to a tensile-based SEF. This assumption can lead to numerical models underestimating the realistic global mechanical behaviour of the CAP samples with these geometric ratios, Figs. 9, 10 and 11. Figure 10 shows sample 14 which is suited for tensile testing only and that the FE model using the planar shear–based SEF overestimates the lumen displacement and vice versa for Fig. 9.

Assigning the CAP sample models to WL ratios in the "grey-area", which ranges from 0.5:1 (suitable for tensile testing) to 4:1 (suitable for planar shear) in increments of 1, the greatest percentage error between lumen area occurred at the extremities, 0.5:1 and 5:1, as these ratios are farthest from the specified geometrical ratio needed for the respective test method, Fig. 12. For certain samples between, 1:1 and 2:1 the percentage difference between the lumen areas is reduced; as expected from Fig. 4 the percentage errors are similar from tension and planar shear for these WL ratios. Results show that characterising experimental data of a CAP sample with a geometric ratio unsuitable for either uniaxial test to a SEF has a significant effect on the numerical analysis. However, as acquisition of human CAP specimens suitable for in vitro mechanical testing is limited, it is necessary to characterise these particular CAP samples. Prior to this study, the "grey-area" was not clearly defined for unsuitable geometric ratios of biological soft tissues for tensile testing as some studies used WL ratios as low as 0.4:1, but according to engineering standards, the grey-area can be between 0.25:1 and 4:1.

This study demonstrates that planar shear testing can be carried out on samples with WL ratios as low as 2:1 with certain errors in the minor strain involved and for samples below 1:1 tensile testing is suitable, but again with certain
limitations involved for ratios that fall within the “grey-area”. Therefore, the “grey-area” is reduced from 0.5:1 ≤ WL ≤ 4:1 to 1:1 ≤ WL ≤ 2:1 (Fig. 13). It is also proposed that if a CAP sample has a WL ratio that falls within this new “grey-area” the gauge length should be altered when mounting the specimen in the clamps to achieve a WL ratio outside this “grey-area” avoiding the larger errors involved and improving the accuracy of the material model developed to characterise the CAP sample.

4.3 Limitations

The simplified computational analysis of the idealised ratios with uniform thickness was carried out to highlight the errors theses geometric ratios contain when compared to theoretical minor strain. The idealised 2D plane strain models were used in the numerical study is an over-simplification of a diseased artery. However, as this was a comparative study, this setup was deemed suitable as was the exclusion of residual strains and geometrical variations of the CAP samples. The effects of the 1 kPa assumption on the trends of this study were evaluated by varying the Young’s Modulus of the lipid core down to zero and this affected the peak von Mises stress, peak displacement and deformed lumen area results by 3.33, 6.08 and 1.57 %, respectively. The CAP samples were characterised using the Yeoh SEF which assumes the material is isotropic and homogenous. However, as the main priority of this study is to analyse the plaque behaviour in the circumferential direction, the authors believe that an isotropic and homogenous assumption for the material properties is appropriate. This methodology of intact plaque testing is not without its own limitations, and the authors ultimately wanted to assess the strength of these tissues as a whole. Biaxial testing of plaque samples is necessary in future studies that aim to understand the anisotropic behaviour of carotid plaques (Holzapfel et al. 2004). The sample size of CAPs used in this study was small, but this limitation further highlights the need to redefine the ratios grey-area that can be used for uniaxial testing as well as taking into account the experimental errors to improve the data needed for curve fitting of SEFs.

5 Conclusion

This study highlights that using the incorrect boundary parameters in a SEF can affect the peak stress and radial displacement values in a numerical model. The percentage error radial displacement is largest for CAP samples with WL ratios of 0.5:1 and 5:1 between the constitutive models developed for tensile and planar shear. It is important to characterise the sample using the correct boundary conditions, tensile or planar shear, for samples with certain geometrical ratios. However, as many whole CAP specimens typically do not have the required geometric ratio to suit these parameters, this study suggests to characterise samples to a SEF that have a WL ratio of 1:1 or less using tensile parameters and for samples greater than WL 2:1 to a planar shear setup. There are significant limitations when following these assumptions, and the use of contactless strain measurement in an area of the sample where the minor strain is known to be correct is imperative for more accurate experimentation of these CAP samples in the grey-area.

References


Springer
On the mechanical behaviour of carotid artery

Paraskevas KI, Mikhailidis DP, Veith FJ (2009) Carotid artery stenting may be losing the battle against carotid endarterectomy for the management of symptomatic carotid artery stenosis, but the jury is still out. Vascular 17(4):183–189
Appendix B

The code is divided into two separate Matlab files, the first is used to input the raw force-displacement data which is then converted into the experimental stress-stretch data. This data is plotted and the fsolve function is used to find suitable coefficients for the Yeoh function to fit the experimental data. The fsolve function calls on the second matlab file called “Solution 3” which is iterated numerous time, changing the coefficients incrementally each time, until coefficients are found which match the set out tolerances. Once the fsolve is converged matlab returns to first file which calls on matlab to plot the experimental against the solved analytical data as well as list the coefficients with the least square resultant value.

File 1

```matlab
clc
clear all
format long
Sample = dlmread('Plaque 3.TXT');
%***********************************
Distance = abs(Sample(:, 1));
%***********************************
Force = Sample(:, 2);
%***********************************
csa = 49.74;
length = 4.72;
%***********************************
EStress = Force./csa;
EStrain = Distance./length;
lambda = (EStrain + 1);
CStress = EStress.*lambda;
%***********************************
% Filter_Strain = [min(lambda): (max(lambda)-min(lambda))/60 :max(lambda)]';
% [p] = polyfit(lambda, CStress,3);
% p(end) = 0;
% lambda_Filter = polyval(p,Filter_Strain);
%***********************************
figure(1)
set(gca,'fontsize',20)
plot(EStrain,EStress,'bo','LineWidth',2)
hold on
plot(lambda,CStress,'r-','LineWidth',3)
xlabel('Eng. Strain','Fontsize',20,'FontName','Ariel')
ylabel('Stress(MPa)','Fontsize',20,'FontName','Ariel')
% title('Hard Plaque','Fontsize',20,'FontName','Ariel')
legend('Exp Data',2)
hold off
%*****************************************************************************
EXP_Data_3 = [lambda CStress];
save('EXP_Data_3','EXP_Data_3');
%*****************************************************************************
Init_Guess = [0.01 0.01 0.01];
```
options = optimset('Display','iter','MaxIter',5000,'MaxFunEvals',5000); %
'options','MaxIter',5000,'MaxFunEvals',5000); %
'options'); Coeffs = fsolve(@Solution_3,Init_Guess,options)
%**************************************************************************
C1 = Coeffs(1); C2 = Coeffs(2); C3=Coeffs(3);
l1 = sym('l1');
l2 = 1;
l3 = 1/(l1);
% l1 = sym('l1'); % l2 = 1/(sqrt(l1)); % l3 = 1/(sqrt(l1));
I1 = l1^2 + l2^2 + l3^2;
W = C1*(I1 -3) + C2*(I1-3)^2 +C3*(I1-3)^3;
S = (diff(W,l1));
S_New2 = lambda.*(S_New);
figure(3)
plot(EStrain,EStress,'bo','LineWidth',2)
hold on
plot(EStrain,S_New2, 'r-')
hold off
% ************************************************************************

File 2 – Solution 3

function Cost = Solution_3(Init_Guess)
C1 = Init_Guess(1); C2 = Init_Guess(2); C3 = Init_Guess(3);
%*****************************************************
load EXP_Data_3
Strain = EXP_Data_3(:,1)';
Stress = EXP_Data_3(:,2)';
%*****************************************************
l1 = sym('l1');
l2 = 1;
l3 = 1/(l1);
I1 = l1^2 + l2^2 + l3^2;
W = C1*(I1 -3) + C2*(I1-3)^2 +C3*(I1-3)^3
%*****************************************************
lambda1 = sqrt(2*Strain+1);
S = (diff(W,l1));
S_New2 = lambda1.*(S_New);
%*****************************************************
figure(4)
plot(Strain, Stress, 'b+')
hold on
plot(Strain, S_New2, 'r-')
hold off
Cost = sum((Stress - S_New2).^2)
%*****************************************************
Stable_Range = [0:0.01:2.0];
W_def = double(subs(W,'l1',Stable_Range));
if any(W_def < 0)
    Cost = 1e+20
end
if any(C3 < 0)
    Cost = 1e+20
end
%*****************************************************
end
Appendix C

The proposed design in this document is just one embodiment in a number of helically coiled balloon designs, but it demonstrates that a variable compression coiled balloon will sufficiently increase the device's ability to transport therapeutic levels of drugs throughout the diseased artery wall. This design can include larger number of smaller or larger coils with varying diameters and distances between one another as well as the incorporation of a sheath in the longitudinal direction on either side of the balloons. There is a non-exhaustive list of therapeutic agents and coating systems that could potentially be used with this device, for example a paclitaxel based drug, a lipid pool solidifying agent, a clotting/embolisation agent and a sphere based delivery system. This device can be used for any type of medical procedure in a body lumen or visceral organ for example, artery angioplasty, brachytherapy, colon angioplasty etc. Due to the increased effectiveness of the therapeutic agent, this should lower unit cost, by way of reducing the amount of agent required which is a clear disadvantage for DEBs compared to DESs.

Brief Description of the Figures

The invention will be more clearly understood from the following description of some embodiments thereof, given by way of example only, in which:

Fig. A (comparative) is a sectional view of a conventional drug eluting balloon (DEB) in an inflated state in a vessel;

Fig. 1 is an illustration of a catheter of the invention;
Fig. 2 is a sectional, partially perspective, view of a catheter according to the invention having a drug eluting balloon formed from two interspersed balloons in a double helix formation;

Fig. 3 is a sectional view of catheter of the invention having a drug eluting balloon formed from three interspersed balloons in a triple helix formation;

Fig. 4 is a sectional, partially perspective, view of a catheter of Fig. 3;
Fig. 5 is a detailed sectional, partially perspective, view of the catheter of Fig. 1; and

Fig. 6 is a sectional view of a conventional drug eluting balloon (Traditional) and two drug eluting balloons of the invention (Transporter 1 and 2) in an inflated state in a vessel.