Tailoring of the Biomechanics of Tissue-regenerative Vascular Scaffolds

by

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Department of Surgery
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Declaration

I, Hugo Krynauw, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: [Signed by candidate]
Date: 28 May 2016
Abstract

The lack of long term patency of small diameter synthetic vascular grafts currently available on the market has directed research towards improving the performance of these grafts. Improved radial compliance matching and appropriate tissue ingrowth into the graft structure are main goals for an ideal vascular graft. In addition, the use of biodegradable materials offers the promising prospect of leaving behind a near native vessel with no synthetic material remaining. Tissue ingrowth into grafts alters their mechanics. This, combined with a loss of mechanical integrity over time, in the case of biodegradable scaffolds, brings the need to investigate how these changes play out and how to tailor them for optimal graft healing.

This project set out to investigate the mechanics of electrospun Pellethane® 2363–80AE (Dow Chemicals) and DegraPol® (ab medica S.p.A) biostable DegraPol® DP0 and biodegradable DegraPol® DP30 scaffolds during in vivo animal studies. DegraPol® DP30 findings were used to investigate the scaffolds’ potential use for vascular grafts by means of a finite element graft model.

Porous, electrospun scaffolds were manufactured and implanted into two subcutaneous and one circulatory rat models. All studies consisted of four time points, namely 0, 7, 14 and 28 days. Scaffold morphology was characterised, and tissue ingrowth was quantified by histological analysis of explanted samples. Orthogonal, uni-axial tensile testing measured scaffold mechanical response of in-fibre and cross-fibre deformation.

Tissue ingrowth brought about considerable changes in biostable DegraPol® DP0 scaffold mechanics. Tensile testing of degradable DegraPol® DP30 scaffolds in their load bearing circumferential direction showed a balance between a loss in mechanical strength and an increase in strength by tissue ingrowth. This resulted in constant radial compliance of 4.47 ± 0.14%/100 mmHg between 80 and 120 mmHg for the four week period predicted with the numerical models. The finite element model based on DegraPol® DP30 scaffold mechanics for 6 mm grafts showed better, i.e. higher, radial compliance than current grafts used clinically (polyethylene terephthalate and expanded polytetrafluoroethylene grafts). This stability in compliance, coupled with good tissue ingrowth is of scientific importance as it shows that highly aligned, porous electrospun DegraPol® DP30 scaffolds are a viable option for vascular grafting to achieve long term graft patency.
I  Acknowledgements

There is much to be said about journeys, but a road is seldom walked alone. I hereby acknowledge and thank those who enabled and enriched my journey through, and to, this degree, including:

My supervisors, without whom there would have been neither start nor finish. Tom Franz for patience, motivation and an endless supply of good advice and humour; Deon Bezuidenhout for the polymer knowledge and positive energy he always carries at his fingertips; and Matthieu de Beule for my time spent at Ghent University.

All those who made contributions towards scientific endeavours of this project. Georges Limbert, for the work on the constitutive material model; Josepha Köhne and Jannik Büscher for assistance with the animal work; Helen Ilsley for all histology preparations; and Bruno Orlandi for mechanical workshop assistance.

My friends and colleagues at the Cardiovascular Research Unit, University of Cape Town. You were all a major ingredient to the enjoyment of the expedition.

My family, for walking with me, for believing in me, and for your continued support throughout. A special thanks to my wife, Carolyn, for her part in keeping the wheels rolling and the double expansion our household saw during this work.

ab medica S.p.A for donating the DegraPol® material for this project. ETH Zurich and University of Zurich are owners and ab medica S.p.A is exclusive licensee of all IP Rights of DegraPol®.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

The South African Medical Research Council Inter-University Cape Heart Research Unit (MRC IUCHRC) for further financial support.
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V.I Scaffold characterisation

- $C$: Radial vessel or vascular graft compliance (%/100 mmHg)
- $m_{\text{dry}}$: Mass of the dry scaffold (mg)
- $m_{\text{eth.fibres}}$: Mass of ethanol with volume of fibres (mg)
- $m_{\text{eth.pores}}$: Mass of ethanol to fill pores in scaffold (mg)
- $m_{\text{submerged}}$: The mass of the scaffold submerged in the beaker of ethanol (mg)
- $m_{\text{wet}}$: Mass of the scaffold after removal from the ethanol, but retaining the ethanol in the pores (mg)
- $P_1$: Lower luminal pressures for compliance calculation (mmHg)
- $P_2$: Upper luminal pressures for compliance calculation (mmHg)
- $P$: Scaffold porosity (%)
- $V_{\text{fibres}}$: Volume of fibres (mm$^3$)
- $r_{p1}$: Graft inner radius at pressure $P_1$ (mm)
- $r_{p2}$: Graft inner radius at pressure $P_2$ (mm)
- $V_{\text{pores}}$: Volume of pores
- $V_{\text{total}}$: Total volume of scaffold, including fibres and pores (mm$^3$)

V.II Constitutive material model

- $c_1, c_2, p$: Constitutive material parameters
- $\beta$: Parameter for compressibility
- $\xi_a / \xi_b$: Ratio of the dimensions of the unit cell
- $\phi_{\text{chain}}$: Volume fraction of the fibre phase
- $\phi_{\text{bulk}}$: Volume fraction of the isotropic bulk phase
- $\psi_{\text{bulk}}$: Strain energy of the bulk material
- $\psi_{\text{chain}}$: Strain energy of the fibre chain
- $I_1, I_3, I_4$: Tensor invariants 1, 3, 4
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<tr>
<td>AIH</td>
<td>Anastomotic Intimal Hyperplasia</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>ePTFE</td>
<td>Expanded polytetrafluoroethylene</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin (stain)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolactone</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene Terephthalate (Dacron®)</td>
</tr>
<tr>
<td>PEUU</td>
<td>Poly(etherurethane urea)</td>
</tr>
<tr>
<td>PGA</td>
<td>Polyglycolic Acid</td>
</tr>
<tr>
<td>PLA</td>
<td>Polylactic Acid</td>
</tr>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>SEDF</td>
<td>Strain Energy Density Function</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth Muscle Cell</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>UMAT</td>
<td>User Material (Abaqus)</td>
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## VII Definitions

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<td>Anastomosis</td>
<td>The surgical connection between two hollow organs, here referring to the surgical connection between the graft and natural vessel</td>
</tr>
<tr>
<td>Aneurysm</td>
<td>Localised swelling of the arterial wall</td>
</tr>
<tr>
<td>Anisotropy</td>
<td>Directionally dependent, specifically referring to mechanical properties</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>Thickening and hardening of arterial walls</td>
</tr>
<tr>
<td>Autologous</td>
<td>Obtained from the same person, referring to vessels from the same person used for grafts</td>
</tr>
<tr>
<td>Axial</td>
<td>Along the blood vessel or graft cylindrical axis</td>
</tr>
<tr>
<td>Biodegradable</td>
<td>Not biostable - material which naturally decomposes in its designated environment, the human body</td>
</tr>
<tr>
<td>Biostable</td>
<td>Not biodegradable - material which remains intact in its designated environment, the human body</td>
</tr>
<tr>
<td>Bypass</td>
<td>Surgery to bypass a section of diseased or blocked artery</td>
</tr>
<tr>
<td>Circumferential</td>
<td>Along the vessel or graft circumference</td>
</tr>
<tr>
<td>Collagen</td>
<td>Structural protein found in connective tissue</td>
</tr>
<tr>
<td>Compliance</td>
<td>The property of a material to change in shape with applied load, referring to vessels' change in diameter with internal pressure</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>An artery supplying blood to the heart muscle</td>
</tr>
<tr>
<td>Distal</td>
<td>Further away from the reference point - the heart, in context of vascular context</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Layer of specialised cells lining the cavity of certain organs, with reference to blood vessels.</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>An artery in the thigh</td>
</tr>
<tr>
<td>Fibrin</td>
<td>An insoluble fibrous protein formed during the clotting of blood</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>A cell found in connective tissue which produces collagen and other proteins in the extracellular space.</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>A thickening and scarring of connective tissue, normally occurring after injury or surgery</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>The loss of blood from a ruptured blood vessel</td>
</tr>
<tr>
<td>Heparin</td>
<td>A naturally occurring compound acting as anti-coagulant, medically used to treat thrombosis</td>
</tr>
<tr>
<td>Homologous</td>
<td>Similar in structure, position and origin</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>Abnormal growth caused by excessive multiplications of cells</td>
</tr>
<tr>
<td>In vitro</td>
<td>A process occurring outside a living organism, such as a test tube</td>
</tr>
<tr>
<td>In vivo</td>
<td>A process occurring inside a living organism</td>
</tr>
<tr>
<td>Lumen</td>
<td>The cavity of a hollow organ, with reference to blood vessels</td>
</tr>
<tr>
<td>Macrophage</td>
<td>A cell which removes foreign material, waste and harmful organisms</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
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<tr>
<td>-------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mandrel</td>
<td>A rod around which material formed, with reference to electrospinning fibres around the mandrel</td>
</tr>
<tr>
<td>Occluded</td>
<td>Blocked</td>
</tr>
<tr>
<td>Patency</td>
<td>Duct or tube that is naturally open, with reference to blood vessels</td>
</tr>
<tr>
<td>Patency rate</td>
<td>The percentage graft still patent (open) after a certain period of time.</td>
</tr>
<tr>
<td>Pathological</td>
<td>Relating to pathology, the cause and effect of disease</td>
</tr>
<tr>
<td>Pathology</td>
<td>The science of the cause and effect of disease</td>
</tr>
<tr>
<td>Porogen</td>
<td>Particle used in the creation of pores</td>
</tr>
<tr>
<td>Proximal</td>
<td>Closer to the reference point - the heart, in context of vascular context</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>Vein that runs from the foot to the thigh</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>A type of muscle cell found in blood vessels</td>
</tr>
<tr>
<td>Spinnerette</td>
<td>Hollow tube through which material for electrospinning flows</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>The presence or formation of blood clots in the cardiovascular system.</td>
</tr>
<tr>
<td>Tissue engineering</td>
<td>The study of the growth of new tissue and organs from cells and scaffolds.</td>
</tr>
<tr>
<td>Transanastomotic</td>
<td>Occurring across the anastomosis, with reference to graft-vessel junction</td>
</tr>
<tr>
<td>Transmural</td>
<td>Occurring across the wall of a structure, with reference to the vascular graft wall</td>
</tr>
<tr>
<td>Tunica adventitia</td>
<td>The outer layer of a blood vessel</td>
</tr>
<tr>
<td>Tunica intima</td>
<td>The inner layer of a blood vessel</td>
</tr>
<tr>
<td>Tunica media</td>
<td>The middle layer of a blood vessel</td>
</tr>
<tr>
<td>Vascular graft</td>
<td>An artificial blood vessel</td>
</tr>
</tbody>
</table>
1. Introduction and Problem Definition

This chapter opens with a brief outline of the background to the project. It then identifies the problem to be addressed and the importance of doing so. It closes with a statement of the researcher’s aim for the project and the objectives set out in order to achieve the aim.
1.1. Background to the project
Cardiovascular disease is the leading cause of death in Western countries (Chlupac et al. 2009, El-Kurdi et al. 2008, Kannan et al. 2005, Sankaran et al. 2015) with atherosclerosis and arteriosclerosis accounting for a large portion of fatalities. These refer to the narrowing and stiffening of arteries in the body and, when left untreated, can cause ischemia and infarction in distal organs. Treatment usually involves surgical intervention in the forms of reopening the vessel with balloon angioplasty; supporting the vessel with internal stenting; or bypassing the diseased section with a vascular graft. This research looks into one of the many aspects of vascular grafts.

The ideal bypass surgery of blood vessels remains autologous grafts - the patient’s own artery or vein. Autologous grafts are, however, often unavailable due to either disease or previous use for bypass grafting (Berglund and Galis 2003, Chlupac et al. 2009, Ratcliffe 2000, Teo and Ramakrishna 2009, Wang et al. 2007). Currently available vascular prostheses made from polyethylene terephthalate (PET) such as Dacron®, and expanded polytetrafluoroethylene (ePTFE) perform well as large-calibre replacements, but their long-term patency is discouraging in small to medium graft (<7 mm) applications (Chlupac et al. 2009, Ratcliffe 2000, Zilla et al. 2007). This failure is mainly a result of a poor healing process with surface thrombogenicity due to the lack of endothelial cells, as well as anastomotic intimal hyperplasia (Berglund and Galis 2003, Limbert et al. 2016). Although attempts have been made to improve these grafts by means of various coatings, the results are not conclusive (Berglund and Galis 2003, Gosselin et al. 1996, Herring et al. 1994, Ritter et al. 1998). An ideal small-diameter vascular graft has become a major focus in research, with tissue engineering providing a promising outlook (Ratcliffe 2000).

1.2. Problem identification and importance
An ideal tissue engineering graft would be described by both its mechanical and post-implant healing response. Combining these would yield appropriate physiological behaviour during the healing process, from implant to ideally full synthetic material absorption, leaving behind a tissue engineered vessel with no synthetic material.

Tissue ingrowth into prosthetic grafts alters their mechanics. This, combined with loss of mechanical integrity over time in the case of biodegradable scaffolds, brings about the need to investigate how these changes play out and how to balance them. Once this interplay has been identified by means of mechanical testing, the results can be used in a constitutive model in order to simulate graft behaviour.

Detailed knowledge of the mechanical characteristics of materials is still lacking, with many studies only reporting response to loading as linear, even though scaffold and tissue commonly show non-
linear behaviour. With the combination of the two, coupled with changes over time due to ingrowth and degradation, more in-depth knowledge about their mechanical behaviour is required. This is needed for both the direct assessment and comparison of mechanical properties between various grafts, and to enable characterisation by mathematical constitutive models for use in computational modelling of grafts.

1.3. Aim and objectives

The aim of this project was to investigate the changes in mechanics occurring in biodegradable (DegraPol® DP30) electrospun scaffolds during implantation in animal models, and to computationally evaluate the suitability of the scaffolds for use in arterial grafts. In pursuing this aim, the changes in mechanics of biostable scaffolds (Pellethane® and DegraPol® DP0) in animal models were to be investigated as foundation studies.

The objectives for the project were:

a) To manufacture biostable and biodegradable scaffolds by electrospinning Pellethane®, DegraPol® DP0 and DegraPol® DP30
b) To implant and explant scaffolds in animal models, using both subcutaneous and circulatory rat models to generate tissue ingrowth
c) To characterise the scaffolds morphologically and mechanically, including fibre size and alignment, scaffold porosity, and mechanical strength both with and without tissue ingrowth
d) To develop and verify finite element models and assess computationally DegraPol® DP30 scaffold for use in vascular grafts
2. Literature Review and Theory

This literature review commences with a brief look at vascular physiology and relevant pathology, followed by an overview of currently used vascular grafts. Next, the basics of tissue engineering, with relevance to vascular grafts are discussed. Tissue engineered vascular grafts and scaffolds are the focus of this project, and some of the materials used for them are discussed in Section 2.3. Methods of manufacturing porous synthetic grafts are then discussed, with a specific focus on electrospinning in Section 2.4. Since the natural vessel material and synthetic scaffold material exhibit non-linear mechanical properties, Section 2.5 covers constitutive material modelling of non-linear porous materials, focusing on vascular and tissue engineering use. In closing, Section 2.6 looks at some of the work which has been done toward finite element models of vascular structures and grafts.
2.1. Blood vessels physiology
The circulatory system is a transport system for oxygen, carbon dioxide, nutrients, waste and hormones. It consists of the heart - a muscular, four chamber double pump; blood; and blood vessels which form a network of conduits throughout the body. The blood flow through the body is illustrated in Figure 2.1 where oxygenated blood (red) from the lungs passes through the left atrium of the heart, into the left ventricle of the heart. When the muscular left ventricle contracts, the blood is ejected into the aorta from where it is distributed throughout the body via the arterial system. Deoxygenated blood (blue) returns to the heart via the venous system and enters the right atrium. From here it enters the right ventricle and is ejected to the lungs for oxygenation (Fox 2006).

![Figure 2.1. Illustration of the circulatory system (Fox 2006).](image)

The current project concerns the arterial system which transports oxygenated blood at high, pulsatile pressure throughout the body and more specifically, small diameter arterial grafts, less than 6 mm in diameter.

There are three main types of blood vessels: arteries, capillaries and veins. Arteries and veins have three layers: tunica intima, tunica media and tunica adventitia. The intima is a layer of endothelial cells lining the lumen of the vessel, and serves as a barrier to blood plasma. The media gives most of the mechanical strength to the vessel, with smooth muscle cells embedded in a matrix of elastin and collagen fibres. The adventitia serves to attach the vessel to its surrounding tissue (Van Vlimmeren et al. 2011).
The mechanical behaviour of vessels is governed by the specific composition of each of these layers, predominantly by that of the media. This composition is specialised and depends on the function of the vessel. Veins greatly differ from arteries, and the exact position of the artery or vein in the circulatory system requires further specialisation, expressed by a variation in the composition of the layers (Levick 2013). Large arteries have thick, elastic walls to absorb the pulsatile flow. They consist mostly of elastin (40%), with near equal volumes of collagen and smooth muscle cells (27 and 25%, respectively). Smaller to medium arteries (<7 mm) mostly consist of smooth muscle cell (60%) with 20% collagen and 10% elastin and they form the majority of the distribution network (Levick 2013). The smooth muscle cells play a large role in controlling blood flow throughout the body by constricting or relaxing the vessel through stimulation by the sympathetic nervous system. In conjunction with this is the more stable contribution by collagen and elastin. Collagen is relatively stiff, with an elastic modulus of around 1 GPa and low extensibility, whilst elastin has a low elastic modulus, between 0.3 and 0.6 MPa, and a high extensibility (Shadwick 1999, Yeoman 2004). The effect of these two materials on the arterial mechanics was shown by Roach and Burton in 1957, when they illustrated the effect of collagen and elastin by digesting the other from samples of human artery (Shadwick 1999), as depicted in Figure 2.2.

![Non-linear arterial stiffening and the contribution to this by elastin and collagen](image)

Figure 2.2. Non-linear arterial stiffening and the contribution to this by elastin and collagen, adapted from Shadwick (1999).

Considering the non-linear behaviour, it is important to be able to express vessel mechanical behaviour in simple, standardised ways. One of these is radial compliance (Nisbet et al. 2009, Seifalian et al. 1999). It is expressed as the change in a vessel diameter with a specific blood pressure increase per 100 mmHg increase, and is written as:
\[ C = \frac{r_p^2 - r_h^2}{r_h} \times \frac{10^4}{P_2 - P_1} \% / 100 \text{mmHg} \]  

(2-1)

Where \( P_1 \) and \( P_2 \) are the pressures in mmHg between which compliance must be calculated and \( r_p \) is the inner graft radius at pressure \( P \).

Another important factor in arterial mechanics is that they are in a state of axial stretch. Arteries are under a constant 10% axial pre-stretch (Holzapfel et al. 2000, Wakatsuki and Elson 2002).

### 2.2. Current grafts

Synthetic grafts have successfully been used for medium to large vascular grafts (7-9 mm) since 1957, with 5 year patency rates exceeding 90% (Kannan et al. 2005). This cannot be said of smaller grafts, where patency rates are much lower. Currently, autologous saphenous vein grafts still yield the best long term patency for small diameter arterial grafts, and is the preferred graft source. Up to 40% of patients, however, do not have saphenous veins available for grafting, either due to disease or previous graft use (Seifalian et al. 1999, Teebken and Haverich 2002). This results in the use of synthetic grafts, even though they have low patency rates. Table 2.1 summarises the 5 year patency of various grafts and also highlights the correlation between graft radial compliance and patency.

<table>
<thead>
<tr>
<th>Graft type</th>
<th>Compliance (%/100 mmHg)</th>
<th>5 year patency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host artery</td>
<td>5.9 ± 0.5</td>
<td>n/a</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>4.4 ± 0.8</td>
<td>75%</td>
</tr>
<tr>
<td>Umbilical vein</td>
<td>3.7 ± 0.5</td>
<td>60%</td>
</tr>
<tr>
<td>Bovine heterograft</td>
<td>2.6 ± 0.3</td>
<td>59%</td>
</tr>
<tr>
<td>PET</td>
<td>1.9 ± 0.3</td>
<td>50%</td>
</tr>
<tr>
<td>ePTFE</td>
<td>1.6 ± 0.2</td>
<td>40%</td>
</tr>
</tbody>
</table>

Matching compliance between graft and vessel is seen as one of the key requirements for long term graft patency (Seifalian et al. 1999, Vorp et al. 1995, Zilla et al. 2007). A compliance mismatch between graft and adjoining vessel alters the resistance to pulsatile flow at the anastomosis. Specifically, grafts with significantly lower compliance than adjacent arteries pose a resistance to the pulsatile component of the blood flow, affecting distal hemodynamic (Kannan et al. 2005). The sudden change in compliance from vessel to graft causes an attempt to smooth the transition by increased tissue growth in the peri-anastomotic regions, leading to anastomotic intimal hyperplasia, and often graft occlusion.
Several synthetic materials are currently used for vascular grafts, however the most commonly used is still extruded Polytetrafluoroethylene (ePTFE) and Polyethylene terephthalate (PET or Dacron®). Lindholt et al. (2011) showed improved patency rates of heparin bonded PTFE (Propaten®) grafts over regular PTFE ones, however this does not address the compliance mismatch between the artery and graft.

2.3. Tissue engineered vascular graft scaffolds and materials

Tissue regeneration provides a promising avenue for the development of vascular grafts. Whether aiming for composite implants combining synthetic materials with living cells and tissue or the complete replacement of initially implanted materials by regenerated tissue ingrowth permitting (i.e. porous) scaffolds with structural properties tailored for implantation in the arterial circulation and mimicking arterial mechanics is required. The complete healing of the porous vascular graft implies interconnected pores to permit transmural tissue ingrowth rather than the initially realised trans-anastomotic ingrowth (Mooney et al. 1996, Pennel et al. 2013, Zilla et al. 2007). The lack of transmural ingrowth limited endothelialisation to the region bordering the anastomosis, leaving synthetic material exposed to the lumen. When using biodegradable materials, tissue ingrowth throughout the scaffold is required in order to prevent overall failure with the onset of degradation.

Both natural and synthetic materials are being used for graft scaffolds. Section 2.3.1 and 2.3.2 will briefly outline some materials which have been used, whilst Section 2.3.3 reports on some of the previous applications of DegraPol® in tissue engineering.

2.3.1. Natural materials

Natural materials considered for tissue engineering are biological tissues with an existing extracellular matrix (ECM), such as arteries (Gui et al. 2009, Tamura et al. 2003) or ureters (Derham et al. 2008, Field 2003). This provides an inherently bio-compatible scaffold, the largest advantage of using natural material (Wang et al. 2007). In order to prevent immunoreactivity and rejection due to antigenic differences between the source and recipient, decellularising the ECM has been attempted (Conklin et al. 2002, Gui et al. 2009). Conklin et al. (2002) found arterial remodelling in a canine carotid artery bypass, using porcine carotid derived grafts, to be complete after two months. In another decellularised graft study, they found smooth muscle cell (SMC) repopulation from both host artery migration as well as infiltration from luminal and adventitial sides after three weeks. Large-scale availability is a problem with naturally derived, intact scaffolds, and thus much research has gone the manufacture of scaffolds, both of natural and synthetic material.
Several natural materials have been isolated and manufactured into scaffolds for use in tissue engineering. Collagen, as the main structural element of the ECM, has been isolated and formed into gels, which was then used to manufacture vascular scaffolds by casting (Ratcliffe 2000) as well as electrospinning (Matthews et al. 2003). Collagen has also been combined with other substances such as natural elastin (Boland et al. 2004) and synthetic poly(L-lactic acid)-co-poly(ε-caprolactone) (He et al. 2005). Silk fibroin is another natural material that has been electrospun for tissue engineered scaffolds, as well as hyaluronic acid (Liang et al. 2007).

2.3.2. Synthetic materials

With the low patency of the original PET and ePTFE grafts, the first step to improving synthetic grafts were based on these grafts by means of coating the lumen with agents known to inhibit thrombogenesis or to promote anticoagulation, as well as modifying the lumen surface and seeding it with autologous endothelial cells (Wang et al. 2007). However, these improvements could still not satisfy the requirements. Although natural materials have a non-thrombogenic advantage over synthetic materials, the ability to tailor material properties, as well as their availability and reproducibility, has favoured synthetic materials in tissue engineering research. This flexibility and availability directly lead to a large array of materials and combination of materials being used in research with regards to tissue engineered vascular grafts. Some of the materials of note, based simply on the vast number of research publications incorporating them in vascular grafts, are PET, ePTFE, polycaprolactone (PCL), poly-glycolic acid (PGA), polypropylene (PP) and the polyurethane family.

2.3.3. DegraPol® meshes

Although DegraPol® has not yet been used in scaffolds for vascular grafts, it has been used in research for numerous other tissue engineering scaffolds, such as bone (Saad et al. 2000), cartilage (Brizzola et al. 2009, Tan et al. 2009, Yang et al. 2003), skeletal muscle (Riboldi et al. 2008, Riboldi et al. 2005) and smooth muscle cell culturing (Danielsson et al. 2006). DegraPol® can be produced with a variety of mechanical and degradation properties, which, together with its hemocompatibility and non-toxicity, makes this material a promising option. DegraPol® scaffolds are often manufactured by electrospinning, and work has been undertaken to explore optimising the various spinning parameters in order to tailor the scaffold morphology (Milleret et al. 2011).
2.4. Manufacturing methods for porous graft scaffolds

2.4.1. Particulate leaching

Particulate leaching is a method of manufacturing highly porous polymeric scaffolds. The basic process includes adding particles (porogen), of known size to a liquid polymer, either molten or in solution. After casting, the solvent is allowed to evaporate from the solution, or the molten polymer allowed to cool, leaving the composite material behind. This is then placed in a solution that dissolves the particles, leaching their residue out under vacuum and leaving behind a porous structure (Murphy et al. 2002). With particulate leaching, both the size and the porosity of the scaffold can be readily controlled by changing parameters such as the particle type, size, and concentration (Jun and West 2005). Figure 2.3 shows a Scanning Electron Microscope (SEM) image of an example scaffold produced by particulate leaching.

![Figure 2.3. SEM image of a scaffold produced by particulate leaching (Murphy et al. 2002).](image)

2.4.2. Thermally induced phase separation

Thermally induced phase separation does not require solid particles for the creation of a porous scaffold. In this technique, a polymer is dissolved into a solvent, after which water is added to the solution. The two liquids are then mixed to form an emulsion and cooled to below the solvent melting point before they can separate. Vacuum drying is then used to sublime the solvent, leaving a porous scaffold behind. Pore size and distribution; and the interconnectivity of the foam depends on a delicate balance of several parameters such as polymer concentration, quenching rate, quenching depth, solvent / non-solvent composition and the presence of additives (Nam and Park 1999a). Nam and Park (1999b) explored the resultant scaffolds at different configurations of these parameters and showed how this influences foam morphology. Figure 2.4 shows an example of a scaffold produced by thermally induced phase separation.
2. Literature Review and Theory

2.4.3. Electrospinning

Electrospinning is a process by which micro- to nanometer thick polymer fibres are deposited on a target by means of electrostatic forces. This technique has received increasing attention in literature as a means of producing non-woven material consisting of fibres with diameters of a few nanometers in diameter (Greiner and Wendorff 2007). Figure 2.5 shows a SEM image of electrospun PET.

The basic principle behind electrospinning involves applying a high DC voltage between the spinneret and a conducting target. The spinneret is fed with the polymer solution, resulting in the formation of a pendent polymer droplet at the spinneret tip. The electrical field deforms the charged droplet into a cone, called the Taylor cone. When the electrostatic force overcomes the surface tension of the cone, a charged polymer jet is formed and flows away from the spinneret towards the mandrel. During travel, the solvent evaporates and the jet solidifies into a fibre, which whips in a spiral pattern towards the target (Greiner and Wendorff 2007, Reneker and Hou 2004). Hohman et al. (2001) developed an extensive mathematical model to predict the fibre dynamics from spinneret to target. It was found that the curved path is formed due an electrically driven bending instability of the
charged jet as well as the increase in viscosity as the solvent evaporates (Reneker and Hou 2004). Fibre thinning occurs due to stretching and acceleration in this unstable region. (Li and Xia 2004).

Figure 2.6 depicts the basic requirements for an electrospinning machine.

![Figure 2.6. Basic apparatus used for electrospinning.](image)

The shape and motion of the target determines the final product shape. Target motion can also be used to control fibre alignment, for instance, when rotating the target (Figure 2.6), varying the rotational speed changes fibre alignment (Ayres et al. 2006). Several parameters influence electrospinning results. Some are intrinsic to the polymer solution (type of polymer and solvent; polymer concentration; viscosity; electrical conductivity; and polarity and surface tension of solvent), whilst others are operationally controlled (electric field strength; distance between spinneret and target; polymer feeding rate; and motion of target). Lee et al. reported that the solvent choice had a greater effect on fibre morphology than the other parameters (Lee BR et al. 2002), with some solvents causing higher variations in fibre diameter than other for the same spun polymer. Solvent selection is however not open-ended as it is limited by the polymer used, often even more so with degradable materials.

Solvent selection can play a further role, as high solvent volatility can lead to dimpled surfaces (Lee BR et al. 2002, Teo and Ramakrishna 2009). Another cause for dimples on the fibre surface is high humidity when spinning, due to condensate on the fibres. Dimpling increases the overall surface area of the fibres, but can also serve to alter fibre mechanical properties, as well creating cellular attachment sites on a scale smaller than the fibre diameter (Teo and Ramakrishna 2009).

Temperature and humidity also has an effect on results. Many of these are interrelated, requiring balancing the various aspects for optimal results. Milleret et al. (2011) illustrated this well with DegraPol®, obtaining an array of scaffolds by using varying spinning parameters.
Electrospinning is one of the commonly used methods of manufacturing tissue engineering scaffolds. Besides modifying the spinning conditions as mentioned above, other techniques have been developed to enhance the spun scaffold. Grafts made with multiple layers of different material and thread properties have been made to simulate the various layers of the natural artery (Mcclure et al. 2010, Soletti et al. 2010, Thomas et al. 2007, Vaz et al. 2005). Furthermore, co-spinning two different polymers simultaneously can combine advantages of different materials into a single scaffold. Another way to achieve this advantage is by coaxial spinning, with one material as the core surrounded by another one. Coaxial spinning is also used for controlled drug release by encapsulating the drug within a degradable polymer sheath (Blackstone et al. 2014, Zhang et al. 2006).

Controlling and understanding the mechanical response is of importance for tissue regenerative scaffolds. The stress-strain behaviour of electrospun scaffolds is a function of the bulk material, as well as the scaffold characteristics, including fibre thickness, fibre orientation and the degree to which fibres merged during spinning. (Lee K et al. 2005).

Increasing fibre alignment will decrease scaffold anisotropy. This can have a twofold effect on the tissue engineered structure. Firstly, few tissues are isotropic, so by controlling the fibre alignment, closer matches to the behaviour of natural tissue can be achieved without any tissue ingrowth. Secondly, cells prefer propagating along the fibres, thus aligned fibres can create a preferred cellular direction (Greiner and Wendorff 2007).

Mechanical deformation of electrospun scaffolds causes changes on the fibre level. Fibres can slide over each other and rearrange and individual fibres can start failing without the whole scaffold failing. These changes lead changes in the mechanical response of the scaffold, often stress softening seen as lowered stress values for consecutive strain cycles. In addition to this, loading beyond the previous maximum strain returns the behaviour to near that of an uncycled sample. This effect is very similar to that reported when testing filled rubbers, and is known as the Mullins effect (Diani et al. 2009, Lee K et al. 2005). The softening is most likely caused by both the chemical and fibre merge bonds undergoing damage during the cyclic loading.

2.5. Constitutive modelling of non-linear elastic porous polymers for arterial grafts

A constitutive material model is a mathematical model of a material that can describe the material’s behaviour. The model is developed with a certain goal in mind, such as mechanical deformation under load and thus has constrains on the conditions under which it is valid.

The choice of a constitutive model depends on the behaviour to be modelled. Blood vessels are complex structures displaying intricate behaviour in situ. Certain assumptions need to be made in
order to simplify the model, however keeping it realistic enough to be relevant in the study. Aspects to consider include heterogeneity, residual stresses and smooth muscle contractility (Vito and Dixon 2003). Although tissue regeneration in grafts introduces these three factors over time, they are ignored in this study in favour of a wider scope of degradation and tissue regeneration.

The most commonly used concept used in constitutive models of biological tissues is that of a Strain Energy Density Function (SEDF). Strain energy is expressed as a function in terms of the deformation gradient tensor and various unknown, experimentally determined parameters (Vito and Dixon 2003). Two of the main criteria that must be satisfied in order to use a SEDF is material hyperelasticity, thus the stress purely depends on strain state, and path independence between strain states. When using a SEDF, constitutive models of blood vessels can be treated in four different ways, namely: pseudoelastic, randomly elastic, poroelastic, or viscoelastic. These four models are briefly summarised in Table 2.2.

<table>
<thead>
<tr>
<th>Type</th>
<th>Assumptions</th>
<th>Major limitation</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudoelastic</td>
<td>The vessel is treated as one hyperelastic material in loading and another in unloading</td>
<td>The equation separately models loading or unloading data, where both are considered.</td>
<td>The approach is simple and captures vessel deformation. Pseudoelastic models form the backbone of other model types</td>
</tr>
<tr>
<td>Randomly elastic</td>
<td>The strain response is centred around a definite value that lies on a well-defined curve</td>
<td>Data is noisier than when separately modelling loading or unloading values</td>
<td>The equation models both loading and unloading data simultaneously</td>
</tr>
<tr>
<td>Poroelastic</td>
<td>The tissue is treated as a fluid-saturated porous medium</td>
<td>The movement of fluid through the porous tissue may be irrelevant within the timescale of the experiment Nonlinear models include many unknown parameters</td>
<td>The equation models the fluid inside the porous scaffold’s contribution to tissue properties</td>
</tr>
<tr>
<td>Viscoelastic</td>
<td>The strain response is a function of the stress history</td>
<td>The response is usually modelled using a discrete number of elements behaviour Data from one viscoelastic test may be insufficient to model characteristics from other viscoelastic tests</td>
<td>The equation models observed viscoelastic behaviour (creep, hysteresis and stress relaxation)</td>
</tr>
</tbody>
</table>
2.6. Finite element modelling of arteries, vascular grafts and graft reinforcing structures

After the implementation of a constitutive material model, graft behaviour must be modelled. This can be done either theoretically (Chaudhry et al. 1997, Rachev and Hayashi 1999, Vito and Dixon 2003) or with a finite element approach (Vito and Dixon 2003, Vorp et al. 1995, Williams 2006). The choice of a finite element approach is based on much wider implementation in current literature, as well as the possibility to model complex geometry and incorporate other structures such as reinforcing around the mesh material, making the overall structure and mesh-reinforcing interaction too complex for a theoretical approach.

Vorp et al. (1995) used a two-dimensional finite element study to validate their ten coefficient constitutive material model. Although the finite element computational component of the study was simple, results agreed well with experimentally determined artery behaviour.

Zidi and Cheref (2003) constructed a fibre reinforced material and analysed and compared its behaviour to a commercial ePTFE graft, as well as porcine artery samples. They utilised a neo-Hookean SEDF with an extra constitutive parameter for the fibre reinforcement to model their material, and showed that they were better able to match natural compliance than what the ePTFE graft could.

Yeoman et al. (2009) investigated the combined use of finite element modelling and genetic algorithms to obtain optimised material parameters for a fabric graft reinforcing. The study showed how the specifically designed fabric reinforcement successfully lowered the compliance of porous PU grafts into the desired range. The fabric reinforcing was modelled by a six coefficient exponential SEDF and the polyurethane as a generic hyperelastic foam. Various mechanical tests were conducted on the fabric, after which a genetic algorithm was used to optimise the SEDF coefficients.

Van Der Merwe et al. (2008) have analysed the mechanics of a knitted Nitinol external saphenous vein support with finite element methods. The study showed how the mesh reacts under physiological loading conditions and also how various mesh geometries affected performance. Sirry et al. (2010) used the same reinforcing mesh, but furthered the work by investigating the interaction between the mesh and a porous poly-urethane scaffold, as well as considering the effect of embedding the mesh into the graft scaffold.
3. Materials and Methods

This chapter describes the materials and methods used during the research project. First off the materials used for, and the manufacturing of the scaffolds as well as the preparation of samples for testing and the testing procedures. This is followed by the details of the three \textit{in vivo} studies, followed by a description of the finite element modelling and outline of the statistical methods used.
3.1. Scaffold materials and manufacturing

3.1.1. Materials

Pellethane® is a family of thermoplastic polyurethane elastomers owned by The Lubrizol Corporation (Wickliffe, Ohio, USA). They are used in a variety of high-value elastomer applications including footwear; medical tubing and devices; automotive; film and industrial and engineering applications (The lubrizol corporation 2009). Pellethane® 2363–80AE is a biostable aromatic poly(ether urethane) with a hard segment consisting of 4,4-methylenebis(phenylisocyanate) and 1,4butanediol, and soft segments consisting of poly(tetramethylene oxide). The electrospinning solution was prepared by dissolving Pellethane® in Tetrahydrofuran to form a 15% by weight concentration at room temperature. The solution was placed in a laboratory thermal oven on rollers for agitation, at a temperature of 37 °C for 8 hours.

DegraPol® (ab medica S.p.A, Lainate, Italy) is a biodegradable polyester-urethane that consists of poly(ε-caprolactone-co-glycolide)-diol soft segments and poly(3-(R-hydroxybutyrate)-co-(ε-caprolactone))-diol hard segments. Both polymer segments are biodegradable and their degradation products are non-toxic (Milleret et al. 2009). By using different ratios of the hard and soft segments, the mechanical properties of the final product can be modulated, whereas changing the ratio of ε-caprolactone to glycolide affects the degradation characteristics. This versatility, combined with the non-toxicity and hemocompatibility makes DegraPol® a promising choice for tissue engineering scaffolds. Currently DegraPol® is mainly being used in the research field, focussing on medical application where biodegradation is required. There are currently no commercial application publically listed. DegraPol® DP0 and DP30 have ε-caprolactone-to-glycolide ratio of 100:0 and 70:30 respectively, and both have a hard-to-soft segment ratio of 40:60 (unpublished data). The electrospinning solutions were prepared by dissolving, separately, DegraPol® DP0 and DP30 in chloroform to form 20% by weight concentrations at room temperature and subsequently sonicating at 37°C for 90 min.

3.1.2. Electrospinning

Polymer solutions for spinning were prepared and used on the same day. Spinning parameters were determined in a separate study (Khatib 2013). Requirements for the resulting scaffold were moderate to high fibre alignment and consistent fibres. The spinning rig, shown in Figure 3.1, consisted of: (a) the custom made high voltage power supply; (b) syringe pump (SE400B syringe pump, Fresenius, Bad Homburg, Germany), syringe and needle; (c) translation and rotation stage with their power and control; and (d) the target mandrel (hypodermic tubing, Small Parts, Loganport, USA).
Study specific spinning parameter values are provided for each study in Sections 3.4.1-3.4.3. These include flow rate, applied voltage, spinning distance and rotational speed.

### 3.1.3. Scaffold sample preparation

After each spinning session was completed, the mandrel was removed from the spinning rig and the scaffold soaked in ethanol to aid removal. Figure 3.2 shows the entire scaffold on the mandrel prior to removal. Once removed, the scaffold was cut into sample rings as shown in Figure 3.2 and Figure 3.3.
3: Materials and Methods

Figure 3.2. Scaffold on spinning target (top) and opened circumferential ring before cutting into individual samples (lower).

Figure 3.3 illustrates how circumferential (in fibre direction) and axial (cross fibre) scaffolds were cut. Degradable scaffolds were stored under vacuum in a desiccator with silica to avoid the onset of degradation.

Figure 3.3. Sample cutting schematic, showing an axial (A) and circumferential (C) sample.

Each scaffold sample was stored in an individual, numbered tube. Sample numbers were randomised for each experiment, ensuring that samples from various scaffolds were distributed in the various experimental groups.

Prior to all in vivo studies, samples were sterilised. Pellethane® samples were sterilised by submersion in a 70% ethanol solution for a minimum of 24 hours, after which they were dried under vacuum (Townson & Mercer, Stretford, England), again for at least 24 hours. DegraPol® hydrolyses when exposed to ethanol for extended periods of time, thus samples were sterilised by means of ethylene oxide gas, followed by at least six hours under vacuum to ensure all ethylene oxide had been removed.
3.2. Morphological scaffold characterisation

Scaffold morphological characterisation included fibre diameter, fibre alignment, scaffold porosity, and molecular weight determination. These are discussed in the sections below. In addition to these quantitative measures, scaffolds were also assessed qualitatively, using SEM images (FEI Nova NanoSEM 230; FEI Hillsboro, Oregon, USA) to identify typical fibre characteristics, such as the specific surface texture and the prevalence of broken and merged fibres. Low magnification stereo microscope images were used to document aspects such as the extent of tissue adhering to explanted in vivo samples.

3.2.1. Fibre dimensions

Fibre diameter was determined by measuring 10 random fibres on x750 SEM images. In each study, each group was represented by a group of n=3, with images from both sides of the sample and from two locations on each side, one near the cutting edge and another near the centre. This resulted in fibre diameter being measured from 12 images per group. Figure 3.4 shows a typical SEM image with an example of the fibres selected for measurement highlighted.

![SEM image showing an example of fibres selected for determining fibre diameter, indicated in red.](image)

3.2.2. Fibre alignment

The fibre alignment in scaffolds was determined by measuring the fibre dispersion in x100 SEM images processed in imaging software Fiji, based on ImageJ 1.48 (Schindelin et al. 2012). The Directionality plug-in in Fiji (written by JY Tinevez) was used to quantify dispersion by means of a Fourier component analysis, as previously used by Hoogenkamp et al. (2015), and Woolley et al. (2011). The plug-in divided an image into square components, calculating the Fourier power spectra for each. Each was analysed in a polar coordinate system, measuring the power in user specified
angular increments, (2° increments were used). A Gaussian function is fitted to the highest peak from which dispersion is calculated as the standard deviation of the Gaussian. This dispersion factor was used as an indication of fibre alignment. Figure 3.5 illustrates this with completely aligned, drawn lines (left); aligned fibres (centre); and random fibres (right). The “goodness” factor indicates the similarity between the Gaussian fit and the measured data with a 0-1 range, where 1 is a perfect match. The randomness of non-aligned electrospun scaffolds yields a low “goodness” factor due to the poor Gaussian fit, as illustrated in Figure 3.5 (right). Three samples were used from each study, with images from two locations on each sample.

![Image of fibre directionality assessment](image)

**Figure 3.5.** Fibre directionality assessment, showing example images (top row) Gaussian fit (middle row) and results (bottom row) for example line (left) aligned fibres (centre) and random fibres (right).

### 3.2.3. Scaffold porosity

Scaffold porosity, $P$, formed by the fibrous network, was described by the ratio between pore volume $V_pores$ and total volume $V_total$ of scaffold samples as:

$$P = \frac{V_pores}{V_total}$$  \hspace{1cm} (3-1)

As measuring either of these quantities is not feasible with basic lab equipment, a method was derived in order to estimate porosity by means of hydrostatic weighing, using a lab balance scale (XS105S analytical balance; Mettler Toledo, Greifensee, Switzerland).

The scaffold porosity is described in Eq. 3-1 as a volumetric ratio, can also be written as a mass ratio for a single material representing said volumes, since $volume = mass / density$. In this case ethanol
is used for hydrostatic weighing, rewriting Eq. 3-1 in terms of $m_{\text{eth.pores}}$, $m_{\text{eth.fibres}}$ and $m_{\text{eth.total}}$, the masses of ethanol representing the respective volumes, gives porosity as:

$$P = \frac{V_{\text{pores}}}{V_{\text{total}}}$$

$$= \frac{V_{\text{pores}}}{V_{\text{pores}} + V_{\text{fibres}}}$$

$$= \frac{m_{\text{eth.pores}}}{m_{\text{eth.pores}} + m_{\text{eth.fibres}}} \quad (3-2)$$

The equation with measurable quantities used for determining the porosity is:

$$P = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{wet}} - m_{\text{submerged}}} \quad (3-3)$$

where $m_{\text{dry}}$ is the mass of the dry scaffold; $m_{\text{submerged}}$ is the mass of the scaffold submerged in the beaker of ethanol; and $m_{\text{wet}}$ is the mass of the scaffold after removal from the ethanol, but retaining the ethanol in the pores. These quantities and are illustrated in Figure 3.6.

Figure 3.6. The three measurements for hydrostatic weighing, used to determine scaffold porosity, with a) the dry scaffold, b) the scaffold submerged in ethanol, and c) the scaffold with ethanol trapped in the pores.

Equation 3-3 relates to Eq. 3-2 as follows:

$$P = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{wet}} - m_{\text{submerged}}}$$

$$= \frac{(m_{\text{fibres}} + m_{\text{eth.pores}}) - (m_{\text{fibres}})}{(m_{\text{fibres}} + m_{\text{eth.pores}}) - (m_{\text{fibres}} - m_{\text{eth.fibres}})}$$

$$= \frac{m_{\text{eth.pores}}}{m_{\text{eth.pores}} + m_{\text{eth.fibres}}} \quad (3-4)$$
The porosity of each spun scaffold for each study was measured as described above with n=1 per spun scaffold. Physical sample sizes for weighing were kept as large as possible so as to reduce measurement error.

### 3.2.4. Molecular weight

Changes in the weight average molar weight, Mw, during degradation were determined by gel permeation chromatography (GPC) (Roediger Agencies cc, Analytical Laboratories, Stellenbosch, SA). The system consisted of a Waters 486 tuneable absorbance detector set to 260 nm; Waters 510 HPLC pump; Waters 410 differential refractometer; and a Thermo Separations Products Spectra Series AS100 auto sampler. Five columns and a pre-column filter were used with the column oven set to 30 °C. Polystyrene standards were used for calibration. Data analysis was done on DPSS Win GPC Scientific v4.02. Samples were dissolved in THF (1 mg/ml) at 37°C with sonication for 20 min. Pump flow rate was set to 1.06 ml/min. The volume of the samples injected was 180 µl.

### 3.3. Mechanical tensile testing

All samples were tested within 24 hours after retrieval, from degradation medium or animal model. Samples were submerged in a phosphate buffer solution (PBS) heated to 37 ± 2°C; clamped; and pulled whilst logging force and displacement data at 0.01% strain intervals.

The tensile testing rig consists of the Instron 5544 uni-axial tensile tester with 10 N load cell (Instron, Norwood, USA); control PC with Merlin logging software; water bath with temperature controller; and custom clamps, as displayed in Figure 3.7.

![Figure 3.7. Instron tensile testing set-up with custom built, temperature controlled water bath.](image)

Prior to testing, rectangular sample dimensions were determined in the following way:

1. Thickness for *in vitro* from stereo microscope and with a calliper for *in vivo*,

...
2. Width with calliper before clamping, and
3. Length with calliper after clamping.

Testing protocol:

Samples were clamped in the top clamp, submerged into the heated PBS, and fixed in the bottom clamp. Approximately 2 mm was clamped on each side. Strain rate was consistent for all studies at 9.6% per second. This was based on 8%/100 mmHg compliance; blood pressure range of 80-120 mmHg; a heartbeat of 60 BPM; and systole of 1/3 of cardiac cycle. Samples underwent five preloading cycles of 8-12% strain, followed by a final load to test termination. This was either at sample failure or upon reaching a load of 7.4 N (load limit to protect the 10 N load cell with 260 g clamp). Figure 3.8 shows a sample (circled in red), clamped for testing.

![Figure 3.8. Tensile test sample in Instron, circled in red, between two clamps prior to testing.](image)

Force displacement data was logged in comma separated variable files and processed with a custom Python script. Processing included separating cyclic from final load data, calculating stress, elastic modulus, and averaging data within various groups. Engineering stress was reported.

3.4. *In vivo* tissue ingrowth and degradation

In order to determine the effect that tissue has on the mechanics of the electrospun scaffold, three *in vivo* animal studies were conducted. The common procedures to all are outlined first, followed by study specific details. All animal experiments were approved by the institutional review board of the University of Cape Town and were performed in accordance with the National Institutes of Health (NIH, Bethesda, MD) guidelines. Male Wistar rats were used for all studies.

All animal studies made use of established Wistar rat models. Animals were obtained from the UCT Research Animal Facility one day prior to surgery to allow for acclimatisation. Animal housing and surgery were at the same location. Surgical procedures were performed under sterile conditions. The
procedure details preparation, implantation and explantation for each of the studies are discussed in the following three sections

3.4.1. Study 1: Tissue ingrowth in biostable scaffold in subcutaneous rat model

The aim of this study was to assess the tissue ingrowth in a 4-week time span into electrospun, biostable Pellethane® scaffolds in a subcutaneous rat model in order to better prepare for and assess the DegraPol® study. The objectives were to obtain the data as an independent study; to provide a guideline and estimates for a similar, larger DegraPol® study; and to serve as a comparison with the DegraPol® study.

3.4.1.1. Scaffold material and manufacture

Pellethane® solution was prepared as described in Section 3.1. Scaffolds were spun with the parameters outlined in Table 3.1 below and cut into 9×18 mm samples, 55 circumferential and 40 axial samples. Of these, 20 were used for the non-implant control group.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>15%/weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>6m l/h</td>
</tr>
<tr>
<td>Voltage</td>
<td>13 kV</td>
</tr>
<tr>
<td>Spinning mandrel</td>
<td>25 mm</td>
</tr>
<tr>
<td>Spinning distance</td>
<td>250 mm</td>
</tr>
<tr>
<td>Rotation speed</td>
<td>1200 RPM</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>8</td>
</tr>
<tr>
<td>Total samples</td>
<td>95</td>
</tr>
</tbody>
</table>

3.4.1.2. Morphological scaffold characterisation

Scaffold morphological characterisation was as described in Section 3.2, except that no molecular mass was determined as the material is considered biostable.

3.4.1.3. Mechanical tensile testing

Tensile testing was conducted as outlined in Section 3.3.

3.4.1.4. Sample implantation and explantation procedures

Fifteen rats were used with five samples implanted per animal. Figure 3.9 below shows a flow chart summarising the study design, followed by details of the procedure.
1) Preparation
The rat was put under light anaesthesia using isoflurane (5%; oxygen 1.5 l/min) in an inhalation chamber, after which the area for incision was carefully shaved using an electric razor and sterilized with iodine. Following this the rat was placed on operating table, warmed to 37 °C by underneath heating pad and the nose cone fitted to rat. Oxygen flow rate was set to 1.5 l/min while administering 1.5% Isoflurane. Buprenorphine (0.05 mg/kg) was administered subcutaneously after which the rat was placed in the prone position on operating table for the graft implant procedure.

2) Graft Implant procedure
Five longitudinal incisions of 1 cm in length were made, 2 on one and 3 on the other side of the dorsal midline. From each of these incisions, a 2 cm deep subcutaneous pocket was blunt dissected for implantation (See Figure 3.10). One scaffold sample was placed per pocket. The incisions were then closed with silk 4/0 sutures, using interrupted sutures in a sub-cuticular fashion, with a buried knot. The isoflurane was switched off whilst a 1.5 l/min oxygen flow was maintained for an additional 3-5 min. After this the rat was kept warm in its cage by placing it on a heating pad. A post-operatively, 0.05 mg/kg Buprenorphine dose was delivered subcutaneously, after which the animal was observed during recovery from anaesthetics in left lateral position until it mobilised on its own.

Figure 3.9. Pellethane® subcutaneous model experimental design.
Another 0.05 mg/kg Buprenorphine dose was administered subcutaneously the morning after the procedure. Rats were housed individually for one week, after which they were kept 5 per cage.

![Figure 3.10. Schematic for Pellethane® subcutaneous samples, showing incisions (dashed) and approximate sample positions (dotted).](image)

3) Explant procedure
Explants occurred at fixed times, namely 7, 14 and 28 days after implantation. Prior to sample retrieval, rats were killed whilst under general anaesthesia by inhalation of 5% halothane in air. Death was ensured with a cardiac injection of 1 ml saturated KCl solution. All cardiac punctures and delivery of KCl were carried out by a qualified animal technician from the UCT Animal Unit. The samples with surrounding tissue were excised and processed for mechanical and histological analysis.

3.4.2. Study 2: Tissue ingrowth in biostable scaffold in circulatory rat model
The aim of this study was to assess the tissue ingrowth in a 4-week timespan into electrospun, biostable Pellethane® scaffolds placed in a descending aorta rat model. The objectives were to obtain the data as an independent study; and to make a comparison with the Pellethane® subcutaneous study.

3.4.2.1. Scaffold material and manufacture
Pellethane® solution was prepared as described in Section 3.1.1. Scaffolds were spun on a 2.2 mm diameter mandrel with the parameters outlined in Table 3.2 and cut into 10 mm long cylindrical samples.
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Table 3.2. Spinning parameters for Pellethane® circulatory model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>15%/weight</td>
</tr>
<tr>
<td>Flow rate</td>
<td>4.8 ml/h</td>
</tr>
<tr>
<td>Voltage</td>
<td>18 kV</td>
</tr>
<tr>
<td>Spinning mandrel</td>
<td>2.2 mm</td>
</tr>
<tr>
<td>Spinning distance</td>
<td>25 cm</td>
</tr>
<tr>
<td>Rotation speed</td>
<td>9600 RPM</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>18</td>
</tr>
<tr>
<td>Total samples</td>
<td>50</td>
</tr>
</tbody>
</table>

3.4.2.2. Morphological scaffold characterisation
Scaffold morphological characterisation was as described in Section 3.2, with no molecular weight determination as the material is considered biostable.

3.4.2.3. Mechanical tensile testing
There were some variations in the mechanical testing when compared to flat, rectangular samples. Samples were tested only in a circumferential fashion as depicted in cross section in Figure 3.11. Samples were held by two pins (Figure 3.11 left) and then preloaded such that the scaffold walls were near parallel but the measured load less than 0.01 N (Figure 3.11 right). Gauge length was determined by Instron crosshead movement from a zeroed position.

Cyclic loading was as described earlier, but the test endpoint was set to 25% strain or 0.7 N, in order to prevent damage to the fragile pin holders.

![Figure 3.11. Schematic cross section of tubular samples for circulatory model, showing preloading and determined gauge length.](image)

The wall thickness and sample width was determined from images taken by stereo microscope as depicted in Figure 3.12. Test sample thickness was twice the measured wall thickness.
3.4.2.4. Sample implantation and explantation procedures

Thirty rats were used with a single sample implanted per animal. Figure 3.13 shows a flow chart summarising the study, followed by details of the procedure.

1) Preparation

The rat was put under light anaesthesia using isoflurane (5%; oxygen 1.5 l/min) in an inhalation chamber, after which the area for incision was carefully shaved using an electric razor and sterilized with iodine. Following this the rat was placed on operating table, warmed to 37 °C by underneath heating pad and the nose cone fitted to rat. Oxygen flow rate was set to 1.5 l/min while administering 1.5% Isoflurane. Buprenorphine (0.05 mg/kg) was administered subcutaneously after which the rat was placed in the supine position on operating table for the graft implant procedure.
2) Graft Implant procedure

Implantation was started with invasive surgery through a midline laparotomy incision to expose the aorta using aseptic technique. After this, a 20 mm piece of aorta distal to the renal vein and artery beyond the iliac bifurcation was dissected and cleaned. One millilitre Heparin solution (50 Units/kg) was injected into the iliolumbar vein as part of standard procedure for vascular anastomosis. The graft was then cut to the required length and all the air removed from graft using a saline solution. Once the graft was prepared the vessel was clamped (proximal followed by distal) at least 15 mm apart, and a 10 mm section of the aorta removed. The exposed aortic lumens were washed with Heparin solution (50 Units/kg). The distal end of the graft was then sutured with 12 - 15 interrupted sutures using 9-0 Nylon. Prior to application of the proximal suturing, the graft was filled with saline solution, after which the proximal anastomosis was sutured. The proximal clamp was released first to fill graft with blood, after which it was clamped again with a hand-controllable clamp. The distal clamp was then slowly released and the hand-controllable clamp used to control the bleeding. After bleeding had been contained (no more haemorrhaging) the graft was assessed for an additional 5 minutes. Once deemed stable, the isoflurane concentration was lowered to 0.5% while maintaining a 1.5 l/min oxygen flow rate. The abdomen was closed using 2/0 Ethibond in two layers of continuous suture. The rectus abdominis was closed in a continuous manner from subxiphoid process inferior to
the supra-pubic region. Once closure of the abdominal sheath was completed, the closure of the skin was performed in a continuous manner superiorly and the superior knot was buried at the superior end of the wound. This closure ensured that the animal is unable to loosen the sutures by gnawing. Buprenorphine (0.05 mg/kg, subcutaneous) was administered twice daily for three consecutive days.

Figure 3.14. Schematic for Pellethane® circulatory samples, showing the section which was dissected and cleaned (between black dashed lines) and the section of removed aorta where the graft was implanted (between blue dashed lines).

3) Explant procedure
Explants occurred at fixed times, namely 7, 14 and 28 days, after implantation. The rat was taken from its cage and put under light anaesthesia using Isoflurane (5%; oxygen 1.5 l/min). The abdominal area was carefully shaved using an electric razor and the rat placed on the operating table. Anaesthetic was administered with a nose cone with the oxygen set to 1.5 l/min while administering 1.5% Isoflurane. The aorta was then exposed by means of invasive surgery through midline incision using aseptic technique and the graft exposed from adhesion. Intravenous injection of heparin (100IU) was given via the inferior vena cava after which 3 minutes passed before exsanguinating the rat via an incision in the inferior vena cava. After this, wait for apnoea and open chest via sternotomy to insert 20G cannula through apex of heart into the aorta. After flushing the aorta with 200 ml, 0.9% saline solution, the graft was retrieved.

3.4.3. Study 3: Tissue ingrowth into and degradation of scaffold in subcutaneous rat model
Study 3 directly addresses the first aspect of the overall project aim, namely investigating the mechanical changes in biodegradable DegraPol® DP30 during implantation in an animal model. These
results were further used for the second aspect of the overall project aim, namely in developing a constitutive material model to computationally assess the potential use of the scaffolds for arterial grafts, discussed in Section 3.5.

The aim of this study was to assess the tissue ingrowth into electrospun, biodegradable DegraPol® DP30 and biostable DegraPol® DP0 scaffolds, placed in a subcutaneous rat model; and to obtain measures of the changes in scaffold mechanical properties in a 4-week *in vivo* time span. The objectives were to obtain the data as an independent study; and to make a comparison with the Pellethane® subcutaneous study.

### 3.4.3.1. Scaffold material and manufacture

Electrospun scaffolds were manufactured and cut as described in Section 3.1, with electrospinning details given in Table 3.3 below.

<table>
<thead>
<tr>
<th>Table 3.3. Spinning parameters for DegraPol® subcutaneous model.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
</tr>
<tr>
<td><strong>Voltage</strong></td>
</tr>
<tr>
<td><strong>Spinning mandrel</strong></td>
</tr>
<tr>
<td><strong>Spinning distance</strong></td>
</tr>
<tr>
<td><strong>Rotation speed</strong></td>
</tr>
<tr>
<td><strong>Number of grafts (DP0 / DP30)</strong></td>
</tr>
<tr>
<td><strong>Total samples (DP0 / DP30)</strong></td>
</tr>
</tbody>
</table>

### 3.4.3.2. Morphological scaffold characterisation

Scaffold morphological characterisation was as described in Section 3.2.

### 3.4.3.3. Tensile testing

Tensile testing is outlined in Section 3.3, with some variations implemented for this study. Cyclic testing was conducted for n=5 of the T=0 day samples and at no other time points. This was due to a concern that material weakening due to degradation could lead to sample loss during the latter stages of the study. All cycling was conducted as described in Section 3.3, whilst non-cyclic testing comprised a single load from gauge length to 16% strain at 9.6%/min.
3.4.3.4. Sample implantation and explantation procedures

Thirty rats were used with six samples implanted per animal. Figure 3.15 below shows a flow chart summarising the study.

Each animal received 6 implants, 3 biodegradable and 3 biostable samples. Of each of these three, one was for mechanical tensile testing in the fibre direction, one for tensile testing across the fibres and one for histology.

![Flow chart summarising the study](image)

**Figure 3.15. DegraPol® subcutaneous model experimental design.**

1) Preparation

The rat was put under light anaesthesia using isoflurane (5%; oxygen 1.5 l/min) in an inhalation chamber, after which the area for incision was carefully shaved using an electric razor and sterilized with iodine. Following this, the rat was placed on operating table, warmed to 37 °C by underneath heating pad and the nose cone fitted to rat. Oxygen flow rate was set to 1.5 l/min while administering 1.5% Isoflurane. Buprenorphine (0.05 mg/kg) was administered subcutaneously after which the rat was placed in the prone position on operating table for the graft implant procedure.

2) Graft Implant procedure

Five longitudinal incisions of 1 cm in length were made, 2 on one and 3 on the other side of the dorsal midline. From each of these incisions, a 2 cm deep subcutaneous pocket was blunt dissected for implantation (See Figure 3.10). One scaffold sample was placed per pocket. The incisions were then closed with silk 4/0 sutures, using interrupted sutures in a sub-cuticular fashion, with a buried
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knot. The isoflurane was switched off whilst a 1.5 l/min oxygen flow was maintained for an additional 3-5 min. After this the rat was kept warm in its cage by placing it on a heating pad. A post-operatively, 0.05 mg/kg Buprenorphine dose was delivered subcutaneously, after which the animal was observed during recovery from anaesthetics in left lateral position until it mobilised on its own. Another 0.05 mg/kg Buprenorphine dose was administered subcutaneously the morning after the procedure. Rats were housed individually for one week, after which they were kept 5 per cage.

Figure 3.16. Schematic for DegraPol® subcutaneous samples, showing incisions (dashed) and approximate sample positions (dotted).

3) Explant procedure

Explants occurred at fixed times, namely 7, 14 and 28 days, after implantation. Prior to sample retrieval, rats were killed whilst under general anaesthesia by inhalation of 5% halothane in air. Death was ensured with a cardiac injection of 1 ml saturated KCl solution. All cardiac punctures and delivery of KCl were carried out by a qualified animal technician from the UCT Animal Unit. The samples with surrounding tissue were excised and processed for mechanical and histological analysis.

3.4.4. Histology and histology image processing

After explanting, samples were fixed in a 10% formalin solution for two days after which they were placed in a 70% ethanol solution for storage. Samples were processed for histology and microscope slides prepared with 4µm slices. Hematoxylin and eosin stain was used to determine tissue ingrowth.
3.4.5. Quantifying tissue ingrowth

H&E slides were used to quantify tissue ingrowth by classifying area in the images as open space or tissue. Images were captured with a Nikon eclipse 90i (Nikon, Tokyo, Japan) and processed with VIS Visiopharm Integrator System (Visiopharm, Hørsholm, Denmark). The scaffold outline was selected on the image as the region of interest which was then segmented into tissue and open space, from which tissue ingrowth per histology slide was calculated. Full segmentation involved three phases, depicted in Figure 3.17: a) the original image; b) pre-processing by means of Visiopharm’s H&E pre-set, highlighting colour ranges specific to the stain; c) segmenting by means of an untrained k-means clustering technique into nuclei, cytoplasm and ECM, and open space; and d) post-processing to group all tissue and to remove small artefacts.

Electrospun fibres did not stain, and were thus counted as open space. To correct for this, the total area of each image was adjusted by the study’s respective scaffold porosity, as calculated in Section 3.2.3. As an example, if a scaffold was 80% porous, the total area was reduced by 20% prior to calculating the ingrowth percentage.
3.5. Finite element modelling of the graft

Finite element modelling was used to assess the material behaviour of DegraPol® DP30 with \textit{in vivo} tissue ingrowth and material degradation when used in a physiological simulation. Modelling the material in a simple, cylindrical graft section was done to estimate behaviour under physiological pressures, specifically taking note of:

- Radial deformation due to axial stretching
- Circumferential strain with the internal pressure
- Compliance data in the 80-120 mmHg range
- Mises Stress
- Strain

All FEM work was conducted in Abaqus CAE® 6.13-1 (Dassault Systèmes, Providence, RI, USA). This section describes the various components of this process.

3.5.1. Geometry

The model geometry represented the mid-section of a vascular graft, thus eliminating the effect of the anastomosis and other geometric complications. This allowed for using an axis-symmetric partial cylindrical geometry, i.e. a wedge segment of the cylinder, as depicted in Figure 3.18. Numerical stability due to model size was investigated by a variety of cylinder segment sizes, and is discussed in 3.5.6 below. Following this, a final model comprising a 0.1 mm long, 1° wedge angle was used. The graft wall thickness was 0.35 mm and the internal diameter 6 mm.

![Diagram of cylindrical graft segment with annotations](image)

\textbf{Figure 3.18. Cylindrical graft segment used in finite element analysis, showing a) a large, 90° wedge, and b) a magnified view of the final geometry used in the FEA study.}
3.5.2. Constitutive material model

A constitutive model developed by G. Limbert and described in detail in Limbert et al. (2016), was used to simulate the scaffold mechanics. The model is based on the Arruda and Boyce 8-chain model (Arruda and Boyce 1993).

The modified 8-chain material model is governed by the strain energy of the bulk material, \( \psi_{\text{bulk}} \) and the fibre chain, \( \psi_{\text{chain}} \), given below in terms of the invariants \( (I_1, I_3, I_4) \)

\[
\psi_{\text{bulk}} = c_1 \left( I_1 - 3 + \frac{1}{\beta} (I_3^{\beta} - 1) \right)
\]

\[
\psi_{\text{chain}} = c_2 \left( \frac{1 + \frac{I_4 \xi_1^2 + (I_1 - I_4) \xi_2^2}{\xi_1^2 + 2 \xi_2^2}}{I_4 \xi_1^2 + (I_1 - I_4) \xi_2^2} + 2 \frac{\xi_1^2 + 2 \xi_2^2}{I_4 \xi_1^2 + (I_1 - I_4) \xi_2^2} - 3 \right)^{\frac{p}{2}} - 1
\]

where \( c_1, c_2, p \) are constitutive parameters,

\( \beta \) is a parameter for constitutive material compressibility, and

\( \xi_a \) and \( \xi_b \) are the initial cell dimensions for the 8 chain cell.

The constitutive material model is only partially phenomenological, and as such the constitutive parameters \( c_1, c_2, p \) and their values do not have physical meaning. The ratio \( \xi_a / \xi_b \) characterises the degree of anisotropy of the unit cell in the 8-chain material model, and thus also reflects physical anisotropy, whilst \( \beta \) indicates material compressibility.

The fibre and bulk strain energy is then combined, with \( \phi_{\text{bulk}} \) and \( \phi_{\text{chain}} \) as the bulk and fibre volume fractions, and the material model is written as:

\[
\psi(I_1, I_3, I_4) = \phi_{\text{bulk}} \psi_{\text{bulk}}(I_1, I_3) + \phi_{\text{chain}} \psi_{\text{chain}}(I_1, I_4)
\]

The material model (Eq. 3-7) was then implemented as an Abaqus UMAT and used for the graft FEM models.

DegraPol® DP30 uni-axial tensile test data from Study 3 was used to identify constitutive parameters by means of a constrained, non-linear global hill-climbing algorithm (Global Optimization 9, Loehle Enterprises, Naperville, IL, USA) within the Mathematica® 9 environment (Wolfram Research Inc., Champaign, IL, USA). The objective function used for the parameter identification compared the analytical solution to the tensile test results. Parameter identification was conducted by G. Limbert who developed the constitutive model (Limbert 2014).
Table 3.4 below lists the UMAT material parameters for the four experimental implant durations, T=0, 7, 14 and 28 days. Fibre orientation $V^1_i$ was set as to reflect circumferential fibre alignment within the Abaqus model.

### Table 3.4. UMAT material parameters of the four experimental implant durations for the DegraPol® DP30 subcutaneous model.

<table>
<thead>
<tr>
<th>In vivo time (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>28</th>
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<td>$c_1$</td>
<td>Constitutive parameter 1</td>
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<td>35.31143</td>
<td>53.99337</td>
</tr>
<tr>
<td>$c_2$</td>
<td>Constitutive parameter 2</td>
<td>15.49679</td>
<td>11.69837</td>
<td>348.3756</td>
</tr>
<tr>
<td>$p$</td>
<td>Constitutive parameter</td>
<td>0.044319</td>
<td>0.029299</td>
<td>0.484927</td>
</tr>
<tr>
<td>$\beta$</td>
<td>parameter for compressibility</td>
<td>368.5635</td>
<td>138.3654</td>
<td>1306.409</td>
</tr>
<tr>
<td>$\xi_a / \xi_b$</td>
<td>Ratio of the dimensions of the unit cell</td>
<td>1.157256</td>
<td>1.165500</td>
<td>1.066834</td>
</tr>
<tr>
<td>$\phi_{chain}$</td>
<td>Volume fraction of the fibre phase</td>
<td>0.998168</td>
<td>0.998212</td>
<td>0.997321</td>
</tr>
<tr>
<td>$\phi_{bulk}$</td>
<td>Volume fraction of the isotropic bulk phase</td>
<td>0.001831</td>
<td>0.001788</td>
<td>0.002679</td>
</tr>
</tbody>
</table>

#### 3.5.3. Finite element mesh

The finite element mesh was created by specifying the number of element seeds across the wall thickness and allowing Abaqus to define the axial and circumferential seeding based on element aspect ratio. The number of seeds across the wall was adjusted for the mesh verification study. The simple geometry resulted in a structured mesh, as depicted in Figure 3.19 with two elements across the wall thickness. The material model used limited the element type to linear, hexahedral elements with hybrid formulation (C3D8H).

![Figure 3.19. Meshed section of finite element graft model, showing a) a large, 90° cylinder wedge, and b) a magnified view of the final geometry used in the FEA study.](image-url)
3.5.4. **Boundary conditions on FEM graft model**

Boundary conditions were set in a cylindrical coordinate system within Abaqus CAE, with the graft axis aligned with the \( z \)-axis. Figure 3.20 below depicts the boundary conditions.

![Figure 3.20](image)

The cylinder end surfaces (a) and (b) were constrained on the \( z \)-axis, with surface (a) fixed at zero translation and surface (b) displaced by 10\% of the model length, simulating axial stretch, as described in Section 3.5.5. The length-wise surfaces (c) and (d) were fixed along the angular axis and left free to move in the radial direction and \( z \)-axis, creating an axisymmetric model.

3.5.5. **Graft model loading**

The simulation was split into two steps, the first to simulate axial pre-stretch of the graft in situ, the second step served to apply a luminal pressure. To mimic the natural stretch in arteries, an axial displacement boundary condition was applies to one of the graft end in order to achieve 10\% axial strain (Holzapfel et al. 2000).

In the second step, a uniformly distributed pressure was applied to the internal, luminal, surface, as shown in Figure 3.21. The pressure was increased linearly from 0 to 200 mmHg, (0-26.6 kPa), covering the physiological blood pressure range and passing into hypertension.
3.5.6. Mesh and geometry verification

In order to establish the effect of geometry and mesh density on the results, a variety of model configurations were simulated, and the radial compliance and von Mises stress used as measures.

Mesh density verification was done in two phases, prior and after geometry verification. As the geometry is homogeneous, the number of elements across the wall thickness for a 0.35mm wall was used as the mesh density measure. For the first step, a 2 mm long, 30° wedge was used. The number of elements implemented across the wall thickness was: 1, 2, 3, 4, 5, 6, 7, and 8.

Subsequently geometric verification was performed, where both graft length and wedge angle were considered. Eight elements were seeded across the wall for the six configurations, listed in Table 3.5.

<table>
<thead>
<tr>
<th>Graft length</th>
<th>Wedge angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mm</td>
<td>30°</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>30°</td>
</tr>
<tr>
<td>5.0 mm</td>
<td>30°</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>1°</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>5°</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>90°</td>
</tr>
</tbody>
</table>

Based on the results from the geometric validation, the mesh density was investigated further, using a considerably finer mesh since the axisymmetric geometry allowed for a very small wedge to be
modelled. The geometry used was a 0.1 mm, 1° wedge. The number of elements across the wall was varied between 8, 10, 20, 30, 40 and 50, respectively. The results from this were used to decide on a mesh density.

3.5.7. Post-processing of FEA results

Post processing and data output were done with a Python script. Data extracted included the minimum and maximum von Mises stress in the graft and the radial displacement of the luminal surface, recorded after axial preloading without luminal pressure, and with 80, 120 and 200 mmHg luminal pressure. The radial displacement was used to calculate the circumferential strain, and subsequently circumferential compliance (C) was calculated by:

$$C = \frac{r_2 - r_1}{r_1} \times \frac{10^4}{P_2 - P_1} \% / 100mmHg \quad (3-8)$$

where $P_1$ and $P_2$ are the luminal pressures at the lower and upper bound, in mmHg, and $r_1$ is the inner graft radius at pressure $P$.

Compliance for the pressure range of 80-120 mmHg as well as the entire 0-200 mmHg was calculated. Circumferential strain at 200 mmHg was also recorded to assess whether the strain exceeded 16%, as the material model was only validated between 0 and 16% strain.

3.5.8. FEM graft evaluation

Mimicking arterial physiological behaviour, specifically compliance in the normal physiological 80 to 120 mmHg pressure range is important for graft success. Graft behaviour was modelled at each experimental in vivo time point to investigate changes due to scaffold degradation and tissue ingrowth. Current ePTFE and PET grafts wall thickness range between 0.35 mm for specifically thin grafts and 0.65 mm for larger varieties (Anonymous 2012). The smaller calibre grafts start at 5 mm in inner diameter, and range to large calibre grafts greater than 30 mm, not covered in this research project.

Simulations were performed for a graft with 6 mm internal diameter and a wall thickness of 0.35, 0.50 and 0.65 mm, for each degradation time point investigated in the in vivo studies. Similarly, the effect of the graft diameter was investigated for 5, 6, 7, and 8 mm diameter grafts with a wall thickness of 0.35 mm.
Circumferential strain and compliance, as well as maximum and minimum Mises stress from the various model configurations were compiled in order to assess the use of electrospun DegraPol® DP30 as a potential scaffold for small calibre vascular grafts.

3.6. Statistical analysis

Mean values and standard deviation were reported and comparisons made by one way ANOVA, implemented in Statistica (StatSoft Inc., Tulsa, Ok, USA). Statistical significance was accepted as $p<0.05$. Significance of comparisons between values at various time points were made by means the Tukey-Kramer Honestly Significant Difference test, with $p<0.05$ accepted as significant. No outliers were removed.
4. Results and Discussion

Firstly, this chapter states the results of the three animal studies. Scaffold morphology, histology and tensile properties are described for each study, followed by the discussions and observations of each study. Thereafter an overall discussion is provided, focusing on comparisons between the various studies.

Secondly, computational results of the FEM study are described. This includes model verification, the geometry variation study and detailed results for the chosen geometry, followed by the discussion of the results drawing a comparison to the relevant animal study.
4.1. Study 1: Biostable scaffold in subcutaneous rat model

This study constituted the *in vivo* assessment of electrospun biostable Pelletane® scaffolds in a subcutaneous rat model.

4.1.1. Scaffold morphology

Scaffold morphology includes fibre diameter and dispersion obtained from SEM images and porosity measured by liquid displacement. Figure 4.1 shows examples of SEM images and Table 4.1 summarises the corresponding morphological properties. General observations were that the fibres were long, mostly uniform, and highly aligned. Fairly low fibre merging was observed, as shown in Figure 4.1 c, where fibres curving around each other were crossing without merging together. High magnification also showed a dimpled fibre surface.

![SEM images of electrospun Pelletane® for subcutaneous rat model showing: a) x100 magnification; b) x750 magnification and c) x3500 magnification.](image)

Figure 4.1. SEM images of electrospun Pelletane® for subcutaneous rat model showing: a) x100 magnification; b) x750 magnification and c) x3500 magnification.
Table 4.1. Morphological properties of Pellethane® scaffolds for subcutaneous rat model.

<table>
<thead>
<tr>
<th>Property</th>
<th>n</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>6</td>
<td>85.4 ± 2.15%</td>
</tr>
<tr>
<td>Fibre diameter</td>
<td>3</td>
<td>6.7 ± 2.4 µm</td>
</tr>
<tr>
<td>Dispersion (goodness)</td>
<td>6</td>
<td>12.4 ± 4.0° (0.98 ± 0.01)</td>
</tr>
<tr>
<td>Width</td>
<td>66</td>
<td>8.9 ± 0.7 mm</td>
</tr>
<tr>
<td>Thickness</td>
<td>66</td>
<td>1.5 ± 0.5 mm</td>
</tr>
<tr>
<td>Length</td>
<td>66</td>
<td>8.1 ± 2.1 mm</td>
</tr>
</tbody>
</table>

4.1.2. Implantation and explantation procedures

Fifteen animals were used during the study. Figure 4.2 shows images from the explant procedure: a) the five implanted scaffolds, enclosed in a collagen capsule just below the skin; b) close-up of sample implants, illustrating how some samples got folded; c) sample after excess tissue had been removed. Where possible, folded samples were used for histological analysis. Small folds on the ends, such as in Figure 4.2 (c), were simply clamped in the Instron jaws as these samples tended to still fail in the centre, implying that the folding had a limited effect on test results.

Figure 4.2. Pellethane® subcutaneous model explants showing samples encapsulated below the skin (a and b) and an excised sample with minor folding in the corners (c).
Observation during explant was that T=7 days samples were heavily encapsulated, but the surrounding tissue was not well attached to the scaffold. With T=28 days, the capsule had diminished and samples had more tissue adhesion, making it harder to remove excess tissue without potentially damaging the scaffold.

### 4.1.3. Histological analysis

H&E stained histology slides were imaged and used to measure tissue ingrowth over time. Ingrowth values and example images for each of the time points are shown in Figure 4.3.

<table>
<thead>
<tr>
<th>Time</th>
<th>H&amp;E image</th>
<th>Ingrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td><img src="image1.png" alt="Image" /></td>
<td>65 ± 13%</td>
</tr>
<tr>
<td>14 Days</td>
<td><img src="image2.png" alt="Image" /></td>
<td>87 ± 4%</td>
</tr>
<tr>
<td>28 Days</td>
<td><img src="image3.png" alt="Image" /></td>
<td>96 ± 2%</td>
</tr>
</tbody>
</table>

Figure 4.3. H&E histology images from Pellethane® subcutaneous model, showing tissue ingrowth over time.
4.1.4. Mechanical properties of the scaffold

Samples underwent five 8-12% strain pre-cycles, after which they were tested either to failure or a maximum load of 7.4 N. This cyclic range was selected to represent physiological blood pressure cycling of 80 to 120mmHg of an artery with a 10%/100 mmHg compliance. Figure 4.4 shows cyclic test data for one circular and one axial sample at T=14 days. Circumferential, in-fibre testing is presented in green and axial, cross-fibre in red. This convention was maintained throughout the thesis.

From the data summarised in Figure 4.5, stress at 12% and 16% strain, as well as elastic modulus at 6% and 12% strain were extracted and are presented in Figure 4.6.

Data-points on plots describing values along the degradation timeline (such as Figure 4.6) include a small horizontal offset between data-point within each time increment to enable differentiation between overlapping points.
Figure 4.5. Pellethane® subcutaneous model stress-strain curves for each implantation time point.
4. Results and Discussion

4.1.5. Discussion

The ingrowth of tissue into the scaffolds was very rapid, with 65 ± 13% by 7 days. Subsequently, ingrowth slowed down and reached 96 ± 2% by 28 days, nearly filling all open space around the fibres.

The anisotropy of the scaffold created by the fibre alignment is clearly illustrated by the difference between circumferential and axial tensile results. The high degree of fibre alignment results in very low tensile strength in the axial scaffold direction compared to the circumferential scaffold direction prior to tissue ingrowth.

There are three key observations from the tensile test results:

1) A decrease in circumferential stiffness between 14 to 28 days (see Figure 4.6)

The fibres were highly aligned prior to implantation, causing the material’s anisotropic mechanical behaviour. If the degree of fibre alignment decreases with increasing tissue ingrowth over time, the scaffold will behave more mechanically isotropic. One way in which this may happen involves the effect of the tissue on the scaffold fibres. Tissue ingrowth into the porous scaffold may disrupt the fibre position, displacing the fibres over time as tissue populates the scaffold. This assumption is
supported by the very low degree of merging of fibres to each other, allowing for easier displacement of individual fibres relative to each other than with fibres merged. Displacement of fibres through ingrowing tissue is mostly expected in the axial scaffold direction owing to the circumferential alignment of the fibres. This proposed mechanism is illustrated in Figure 4.7. Four fibres (black) of an as-spun scaffold are displaced by ingrowing tissue (red). During the early phase of ingrowth, fibre alignment may be affected, but the scaffold-tissue construct still has a loose arrangement with space for movement and local re-alignment of fibres. With higher degrees of tissue ingrowth at a later implant time point, the scaffold-tissue construct is more compacted and there is very little remaining space for fibres to realign when the scaffold sample is stretched.

![Figure 4.7. Fibre alignment changes and scaffold shortening due to tissue ingrowth: a) as-spun scaffold; b) low degree of tissue ingrowth at shorter implant duration; c) high degree of tissue ingrowth at longer implant duration. (Fibres are shown in black, tissue is shown in red).](image)

This change in local fibre alignment will shorten the scaffold in the circumferential direction as the fibres, of fixed length, are being displaced to follow a longer path around the ingrowing tissue.

Although the tensile testing gauge length could not be directly compared between time points, as it was greatly affected by changes in clamping, there was a 22% decrease in gauge length from T=14 and T=28 days. Considering the consistency of the original sample length for the entire study (18 ± 0.5 mm), this decrease, which was also qualitatively observed during sample handling post-explant, supports the proposal that tissue ingrowth into the electrospun scaffold did alter fibre alignment. Verifying fibre alignment of explanted samples was unfortunately not feasible as tissue ingrowth prevented visual inspection.

This shortening of the scaffold is slightly different from that observed by Van Vlimmeren et al. (2012) in tissue engineered heart valves, where tissue compaction was observed to initiate when scaffolds lost their mechanical integrity. As Pellethane® is biostable, the mechanical integrity of the scaffold itself did not change during the four week experiment. The main cause for the two effects is,
however, the same: ingrowing tissue exerts forces on the scaffold, which in turn modifies the geometry.

A further cause for the decreased scaffold stiffness may be related to the capsule surrounding the scaffold in vivo. As noted in Section 4.1.2, the capsule diminished over time in vivo. Complete elimination of this capsule could not be verified visually, however, a thicker capsule for the early time points may have contributed to higher stiffness measured, compared to the lower stiffness measured at later time points.

2) An increase in axial stiffness and stress with increasing implant duration

A continuous increase in the stiffness observed in axial direction of the scaffold (Figure 4.6, red curve) led to the increase in stresses. The considerable increase of the stress at 16% strain by 940% (p < 0.05) between T=0 and T=28 days is associated with a very low stiffness in the axial scaffold direction prior to tissue ingrowth, due to predominant alignment of fibres in circumferential direction. Based on this low pre-implant stiffness, the ingrowing tissue increases the axial stiffness considerably. The continuous increase in axial stress (T=0 to 7 days: 298%; T=7 to 14 days: 94%, T=14 – 28 days: 35%) is as such ascribed to the progressive ingrowth of tissue as observed from histology (Figure 4.3).

The increase in stiffness in axial scaffold direction is further supported by the considerations of decreasing fibre alignment. As fibre alignment becomes more random and mechanical anisotropy decreases, the ratio of stiffness in fibre and cross-fibre direction of the scaffold approaches unity. This causes an increase in axial stiffness with implant duration, as there is a greater contribution by the fibres than at T=0 days.

3) Changes in stress-strain behaviour with increasing implant duration

There was a change in shape of the stress-strain curves with the incorporation of tissue in the scaffold. At T=0 days, the stress-strain curve is fairly linear, based only on the mechanical and structural properties of the fibrous scaffold. From T=7 days onwards, the curves indicate non-linear stiffening in the 0-12% strain range (Figure 4.5), as is generally associated with tissue (Holzapfel and Ogden 2010, Kurane et al. 2007, Vito and Dixon 2003, Wu et al. 2012 ). This change from linear to non-linear elastic behaviour is associated by a decrease in modulus at 6% strain from T=0 days to 7 and 14 days of 41% and 29% respectively, and an increase in modulus at 12% strain of 11% and 41%, respectively.

These changes in the stress-strain behaviour can be attributed to the contribution of the ingrowing tissue to the mechanical behaviour of the scaffold. An additional factor may be the local changes in fibre alignment already discussed. The new tissue at T=7 and 14 may be modifying the scaffold
morphology (Figure 4.7 b), lowering the scaffold stiffness in the circumferential direction at low strains, yet with enough space remaining around the fibres to straighten with greater deformation.

The largest change in elastic modulus is observed in the circumferential direction. Elastic modulus at 16% strain at T=28 days is significantly lower than at any other time point (see Figure 4.6). This decrease in stiffness resulted in the previously discussed lower stress at T= 28 days.

4.2. **Study 2: Biostable scaffold in circulatory rat model**

This study constituted the *in vivo* assessment of electrospun biostable Pellethane® grafts in an aortic, i.e. circulatory, rat model.

4.2.1. **Scaffold morphology**

Scaffold morphology includes fibre diameter and dispersion obtained from SEM images and porosity measure by liquid displacement. Figure 4.8 shows examples of SEM images used for quantifying fibre alignment (a) and measuring fibre thickness (b). The general observation was a fairly random mesh with no obvious alignment and a high variation in fibre thickness. As with the Pellethane® subcutaneous samples, little fibre merging was observed, however, the fibre surface was much smoother with dimpling only being observed in some of the fibres on some of the scaffolds.

Table 4.2 summarises the scaffold morphological properties, and Figure 4.9 shows an electrospun scaffold prior to implantation.

<table>
<thead>
<tr>
<th>Property</th>
<th>n</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>18</td>
<td>82.04 ± 3.49%</td>
</tr>
<tr>
<td>Fibre diameter</td>
<td>3</td>
<td>10.5 ± 4.7 µm</td>
</tr>
<tr>
<td>Dispersion (goodness)</td>
<td>6</td>
<td>23.2 ± 9.5° (0.72 ± 0.28)</td>
</tr>
<tr>
<td>Cylinder length</td>
<td>33</td>
<td>8.3 ± 2.4 mm</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>33</td>
<td>0.9 ± 0.4 mm</td>
</tr>
<tr>
<td>Tensile gauge length</td>
<td>33</td>
<td>2.7 ± 2.1</td>
</tr>
</tbody>
</table>
Figure 4.8. SEM images of electrospun Pellethane® for circulatory rat model showing a) x100 magnification, b) x750 magnification and c) x3500 magnification.

Figure 4.9. Pellethane® scaffold for circulatory rat model prior to implant.
4.2.2. **Implantation and explantation procedures**

Fifteen rats were used in the study. Figure 4.10 shows various images from the procedure, including: graft shortly after implantation (a); at the time of explant (b-d); the anastomosis (c); and the graft with excess tissue removed.

![Images of implantation and explantation procedures](image)

**Figure 4.10.** Pellethane® circulatory model implant and explant images a) newly implanted graft; b) graft prior to explant; c) explanted graft with anastomosis; d) explanted graft with most excess tissue removed and cut for histology and tensile testing.

4.2.3. **Histological analysis**

Figure 4.11 below shows examples from the H&E histology images, as well as the percentage tissue ingrowth measured for each time point.
<table>
<thead>
<tr>
<th>Time</th>
<th>H&amp;E image</th>
<th>Ingrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td>![Image]</td>
<td>65 ± 10%</td>
</tr>
<tr>
<td>14 Days</td>
<td>![Image]</td>
<td>74 ± 10%</td>
</tr>
<tr>
<td>28 Days</td>
<td>![Image]</td>
<td>78 ± 5%</td>
</tr>
</tbody>
</table>

Figure 4.11. H&E histology images from Pellethane® circulatory model, showing tissue ingrowth over time.

### 4.2.4. Mechanical properties of the scaffold

Samples underwent five 8-12% strain pre-cycles, after which they were tested either to 25% strain or a maximum load of 0.7 N. Figure 4.12 below shows the averaged stress-strain curves across the time periods (T=0, 7, 14, 28 days) with standard deviation. Pre-cycle data has been removed from the plots. From the data illustrated in Figure 4.12, stress at 12% and 16% strain, as well as elastic modulus at 6% and 12% strain were extracted and are presented in Figure 4.13.
Figure 4.12. Pellethane® circulatory model stress-strain curves for each implantation time point.
4.2.5. Discussion

Initial ingrowth into the scaffolds was very rapid, with $65 \pm 10\%$ by 7 days. From here, ingrowth slowed down and reached $78 \pm 5\%$ by 28 days.

The reported fibre dispersion was moderate for this study at $23 \pm 9\%$ ($180^\circ$ corresponds to a random distribution). Confidence in this measure was, however, low due to the low goodness factor of 0.72, meaning the Gaussian fit for dispersion is not accurate and that fibres were more dispersed than the measured $23^\circ$. This is further reinforced by a qualitative comparison of SEM images with other, highly-aligned scaffolds. During spinning, very high rotational speeds were required to obtain mandrel surface velocities comparable to larger targets. These high speeds introduced vibrations into the system, which negated the effect of the high rotational speed and led to high fibre dispersion.

Figure 4.13. Pellethane® circulatory model tensile strain and elastic modulus at various strains plotted against implantation time.
Tensile stress at 12% strain showed a significant increase from T=0 days to T=7 of 110%, and to T=28 days of 79%, but not to T=14 days. Stress at 16% strain for T=7 days was significantly higher than for any other time point.

Elastic modulus at 6% strain increased from T=0 to 7 days by 158% and from 0 to 28 days by 79%. Although the differences were not significant, the material showed stiffening from 6% to 12% strain for T=0, 7 and 14 days (39, 11 and 45% increase in elastic modulus, respectively), but the mean values at 28 days showed only a 0.1% difference, thus a very linear response.

The spike in mechanical stiffness at 7 days was unexpected. For all subcutaneous and the circulatory studies, the T=7 days samples displayed more surrounding tissue than at any other time point. Removing this tissue capsule without damaging the scaffold was more complex for the cylindrical circulatory samples than the flat subcutaneous samples. This excess tissue is the most likely explanation for the peak in stiffness. Disregarding the T=7 day time point, the stress at 12% and 16% strain increased gradually in vivo.

4.3. **Study 3: Biostable and biodegradable DegraPol® scaffold in subcutaneous rat model**

This study constituted the in vivo assessment of electrospun degradable DegraPol® scaffolds in a subcutaneous rat model. It directly addresses the first aspect of the overall study aim, namely investigating the mechanical changes in biodegradable DegraPol® DP30 during implantation in an animal model. The DegraPol® DP30 results were further used in developing a constitutive material model to computationally assess the potential use of the scaffolds for arterial grafts, which forms the second aspect of the overall research project aim.

4.3.1. **Scaffold morphology**

Scaffold morphology includes fibre diameter and dispersion obtained from SEM images, and porosity measured by liquid displacement. Figure 4.14 shows typical SEM images of the fibrous mesh, of both inner and outer surfaces of DegraPol® DP0 and DP30. Table 4.3 summarises the morphological properties of the DegraPol® scaffolds, including porosity, fibre diameter, dispersion and dimensions of samples for tensile testing.
### Table 4.3. Morphological properties of DegraPol® scaffolds for subcutaneous rat model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>DegraPol® DP0</th>
<th>n</th>
<th>DegraPol® DP30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>7</td>
<td>74.0 ± 3.2%</td>
<td>6</td>
<td>77.0 ± 2.7%</td>
</tr>
<tr>
<td>Fibre diameter</td>
<td>3</td>
<td>13.0 ± 2.2 μm</td>
<td>3</td>
<td>12.7 ± 4.1 μm</td>
</tr>
<tr>
<td>Dispersion (goodness)</td>
<td>6</td>
<td>9.6 ± 2.0° (0.97 ± 0.01)</td>
<td>6</td>
<td>17.8 ± 4.6° (0.98 ± 0.01)</td>
</tr>
<tr>
<td>Width</td>
<td>70</td>
<td>8.6 ± 0.5 mm</td>
<td>68</td>
<td>8.1 ± 0.5 mm</td>
</tr>
<tr>
<td>Thickness</td>
<td>70</td>
<td>1.2 ± 0.2 mm</td>
<td>68</td>
<td>1.2 ± 0.2 mm</td>
</tr>
<tr>
<td>Length</td>
<td>70</td>
<td>11.1 ± 1.6 mm</td>
<td>68</td>
<td>11.1 ± 1.5 mm</td>
</tr>
</tbody>
</table>

Figure 4.14. SEM images of electrospun DegraPol® DP0 and DP30 for subcutaneous rat model, showing x750 and x100 magnification of both the inner and outer surfaces.
Both DegraPol® DP0 and DP30 showed a high degree of alignment. The inner surfaces showed flattened fibres, indicating that the fibres were still wet upon impact with the mandrel. This is further illustrated by the fibres merging at places of contact, as shown in Figure 4.15. Various degrees of fibre merging were observed, though fibres remained distinct and did not merge into sheets of polymer. In addition to this, some DegraPol® DP30 scaffolds showed severed fibres, as in Figure 4.15 b).

![Figure 4.15](image)

*Figure 4.15. High magnification SEM images of electrospun DegraPol® DP0 (a) and DegraPol® DP30 (b) for subcutaneous rat model, showing the rough surface, fibre merging and severed fibres in (b).*

The weight average molar weight, Mw, as determined by GPC, is illustrated in Figure 4.16. For DegraPol® DP0, n=1 and only two time points (T=0 and 28 days) were used, as the material is considered biostable.

![Figure 4.16](image)

*Figure 4.16. Molecular weight of DegraPol® samples submerged in PBS up to 28 days.*

GPC results supported the claim of biostability of DegraPol® DP0 with only a small, negligible difference in molecular weight readings. DegraPol® DP30 values decreased significantly by 66% from 77.6 ± 5.7 kDa at T=0 to 26.1 ± 6.7 kDa at 28 days. Nearly half of this decrease occurred within the first 7 days, after which it continued more gradually (T=0-7 days: 51%; T=7-14 days: 19%; T=14-21 days: 16%; T=21-28 days: 14%, values expressed as portion of total decrease in molecular weight).
4.3.2. Sample implantation and explantation procedures

Thirty animals were used during the study — none died prior to the study endpoint. Figure 4.17 shows images from the explant procedure, with the samples prior (a) and after (b) explantation.

![Figure 4.17. DegraPol® subcutaneous model explants showing (a) six samples encapsulated below the skin and (b) an explanted scaffold (T=7 days) with excess tissue removed.](image)

Observation during retrieval of the implants was again that T=7 days samples were more encapsulated, but T=28 days samples had more tissue adhering to the scaffold than at earlier time points, making it more complex to remove excess tissue without potentially damaging the scaffold.

4.3.3. Histological analysis

Figure 4.18 below shows examples from the H&E histology images, as well as the percentage tissue ingrowth measured for each time point, firstly of DegraPol® DP0 and then DegraPol® DP30.
4: Results and Discussion

<table>
<thead>
<tr>
<th>Time</th>
<th>DegraPol® DP0 H&amp;E images</th>
<th>Ingrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td><img src="image" alt="DegraPol® DP0 H&amp;E image" /></td>
<td>31 ± 5%</td>
</tr>
<tr>
<td>14 Days</td>
<td><img src="image" alt="DegraPol® DP0 H&amp;E image" /></td>
<td>58 ± 14%</td>
</tr>
<tr>
<td>28 Days</td>
<td><img src="image" alt="DegraPol® DP0 H&amp;E image" /></td>
<td>83 ± 10%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>DegraPol® DP30 H&amp;E images</th>
<th>Ingrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Days</td>
<td><img src="image" alt="DegraPol® DP30 H&amp;E image" /></td>
<td>29 ± 11%</td>
</tr>
<tr>
<td>14 Days</td>
<td><img src="image" alt="DegraPol® DP30 H&amp;E image" /></td>
<td>55 ± 19%</td>
</tr>
<tr>
<td>28 Days</td>
<td><img src="image" alt="DegraPol® DP30 H&amp;E image" /></td>
<td>77 ± 4%</td>
</tr>
</tbody>
</table>

Figure 4.18. H&E histology images from DegraPol® subcutaneous models, showing tissue ingrowth over time.
4.3.4. Mechanical properties of the scaffold

Samples were tested either to failure or a maximum load of 7.4 N, with no pre-cycling applied. Figure 4.19 below shows the averaged stress-strain curves across the time study period (T=0, 7, 14, 28 days) with standard deviation. Circumferential, in-fibre testing is in green and axial, cross-fibre in red.

Figure 4.19. DegraPol® subcutaneous model stress-strain curves for each implantation time point.

To investigate the effect of tensile pre-cycling, 10 circumferential and 10 axial samples at T=0 days were subjected to five 8-12% strain cycles prior final loading. Figure 4.20 (upper row) repeats the
stress-strain curves of $T=0$ from Figure 4.19 to form a comparison with the data from pre-cycled samples (Figure 4.20 lower row)

These data were then used to calculate the cyclic scaling vector, expressed as $\sigma_{\text{pre-cycled}}/\sigma_{\text{non-cycled}}$ across the strain range, and is illustrated in Figure 4.21. Signal-to-noise ratio of tensile data at low strains was very low due to the low tensile forces. This resulted in potentially inaccurate scaling vectors for these low strains (<2% strain, greyed in Figure 4.21).

This cycling scaling vector was then used to modify the stress-strain data for the implanted scaffold by component-wise multiplication of the scaling vector and the stress-strain data (per strain data-
point) of each test (the individual tests averaged to form Figure 4.19). The average stress-strain curves were then recalculated and the result is shown in Figure 4.22. The effect at low strains was negligible.

![Figure 4.22. DegraPol® subcutaneous model stress-strain curves for each implantation time point with pre-cycling scaling applied.](image)

From this pre-cycling modified dataset, stress at 12% and 16% strain, as well as elastic modulus at 6% and 12% strain was extracted and is presented in Figure 4.23 and Figure 4.24, respectively. The
DegraPol® DP30 dataset presented in Figure 4.22 was further used for constitutive material characterisation, which is further discussed in Section 4.5.

Figure 4.23. DegraPol® subcutaneous model tensile strain at various strains, plotted against implantation time.
4.3.5. Discussion

Mean values for tissue ingrowth for DegraPol® DP0 were constantly, though not significantly, higher than that of DegraPol® DP30 (p>0.05). Tissue ingrowth increase was gradual throughout the study, with DegraPol® DP0 showing significant increments at each time step when compared to the previous. DegraPol® DP30 followed the same trend, however, only the increase from 0 to 7 and 7 to 14 days was significant (p<0.05).

4.3.5.1. DegraPol® DP0

DegraPol® DP0 scaffolds showed significantly higher alignment than DegraPol® DP30 (p<0.05), both with high goodness values for the Gaussian fits. Visual inspection of SEM images supported this. Scaffolds of both materials showed a certain degree of fibres merging due to solvent in the fibres at time of contact with the mandrel. The effect that fibre merging has on scaffold mechanical properties and tissue ingrowth would make for an interesting investigation. Too much fibre merging could, however, constrict interconnected ingrowth passages, which would hinder tissue ingrowth. This was not observed.
Tensile properties for DegraPol® DP0 and DP30 were within the same range, though the two materials showed different responses over implantation time. Circumferential results for DegraPol® DP0 showed a significant increase from T=7 to 14 days, and a significant decrease from T=14 to 28 days in all tensile measures, namely stress at 12% and 16% strain, as well as elastic modulus at 6% and 12% strain, summarised in Table 4.4. There was no significance when comparing these measures for axial samples.

Table 4.4. Changes in DegraPol® DP0 stress and elastic modulus from 7 to 14 and 14 to 28 days.

<table>
<thead>
<tr>
<th>Measure</th>
<th>T=7 to 14 days</th>
<th>T=14 to 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress at 12% strain</td>
<td>67%</td>
<td>-57%</td>
</tr>
<tr>
<td>Stress at 16% strain</td>
<td>78%</td>
<td>-49%</td>
</tr>
<tr>
<td>Modulus at 6% strain</td>
<td>63%</td>
<td>-52%</td>
</tr>
<tr>
<td>Modulus at 12% strain</td>
<td>87%</td>
<td>-46%</td>
</tr>
</tbody>
</table>

4.3.5.2. DegraPol® DP30

Tensile results of DegraPol® DP30 scaffolds showed a significant increase in stiffness in vivo. Elastic modulus at 6% strain remained constant (p>0.05), whilst modulus at 12% strain from T=7 to 14 days increased by 50% (p<0.05) for circumferential samples and by 213% for axial samples.

This increase in stiffness was not statistically reflected in the stress of circumferential samples, but was for axial samples. Stress at 16% for the first seven days remained constant (p<0.05), after which it increased by 296% to 14 days and remained there.

The increase in material stiffness past 7 days was most likely linked to tissue ingrowth. Although there was approximately 30% ingrowth by 7 days, it was not yet fully interconnected throughout the scaffold, and thus did not contribute much to the overall mechanics. As tissue continued to propagate, ingrown areas connected and thus formed greater structural strength.

In vascular grafts, consistent performance from implantation through healing and scaffold degradation is highly desired. In the early phase after implantation, the circumferential stiffness is due to the fibres contributing to the bulk of the stiffness. As scaffold fibres degrade, the graft stiffness will decrease, unless the tissue balances out the difference. Consistency observed in circumferential stiffness of DegraPol® DP30 scaffold (p>0.05) was thus of specific importance.

Previous in vitro studies showed that the loss in material strength of DegraPol® DP30 predominantly occurred in the first 14 days after implantation (Krynauw et al. 2011), which correlated with the molecular weight drop by 14 days. Assuming a similar trend for the scaffold in vivo, it holds that the tissue incorporation was contributing to the overall stiffness and balancing out the loss due to degradation. The stability in circumferential mechanical response contrasts with the work done by
Wu et al. (2012) where the grafts not implanted were significantly stiffer than the neovessels explanted after 90 days.

4.3.5.3. Effect of pre-cycling on DegraPol® DP0 and DP30 scaffolds

Tensile test results showed that pre-cycling had an effect on both the DegraPol® DP0 and DP30 scaffolds, though to a differing degree. When disregarding the initial 2% strain, the scaling showed definite trends for the two materials.

1) Pre-cycling of DegraPol® DP0

For axial DegraPol® DP0 testing, pre-cycled samples showed 43 ± 1% of the stress of non-cycled scaffolds in the 2 – 12% strain range. From 12% strain onwards, the cyclic factor decreased to 33%, indicating further weakening due to cycling. Looking at Figure 4.20, the stress-strain curve has a negative slope (negative modulus). This is an indication that the scaffold had started failing.

Circumferential DegraPol® DP0 samples did not display this constant difference between pre-cycled and non-cycled test data. Instead, pre-cycling stress values at 2% strain were 20% of non-cycled samples. This value gradually increased to 59% by 12% strain, after which it decreased to 47% at 16% strain, again indicating the onset of scaffold failure.

2) Pre-cycling of DegraPol® DP30

Pre-cycling had much less of a damaging effect on DegraPol® DP30. At 2% strain, pre-cycled axial samples showed 80% of the stress experienced by non-cycled samples, which increased to 96% by 12% strain and remained there up to 16% strain.

Pre-cycled circumferential DegraPol® DP30 stress values at 2% strain were 26% of non-cycled samples. This, however, increased drastically to 93% by 12% strain and, as for the DegraPol® DP30 axial samples, plateaued, this time at 97% by 13% strain.

With both materials, the effect of pre-cycling on tensile results was more consistent over the strain range for axial than for circumferential samples. Circumferential samples showed a large decrease in stress at low strain ranges, which increased with higher strains, up to the 12% end of the cycling range.

The DegraPol® DP0 scaffolds showed a more substantial weakening due to pre-cycling when compared to the DegraPol® DP30 scaffolds. DegraPol® DP0 scaffolds were significantly more aligned than DegraPol® DP30, with a mean dispersion angle of less than 10°, compared to >17° for DegraPol® DP30. DegraPol® DP30 scaffolds also showed more fibre merging than DegraPol® DP0, another possible factor.
Lee K et al. (2005) described similar behaviour in Pellethane® electrospun meshes. Firstly, they described how more fibre merging created a structure which better balanced a load than an otherwise similar structure with low merging. Low degrees of fibre merging led to a structure where the load was not as readily split among fibres, resulting in earlier failure of first individual fibres, and consequently the entire mesh. This correlated with the observation that DegraPol® DP30 fibres displayed more merging and higher tolerance to cyclic loading than the DegraPol® DP0 scaffolds. In addition to this, higher alignment led to individual fibres experiencing higher strains at low scaffold strain and thus failing earlier, as the fibres were already straight and thus did not need to first straighten out (Mauck et al. 2009). Again, the scaffold morphology and tensile results correlated, with the higher aligned DegraPol® DP0 scaffolds incurring more damage at lower strains, as reflected by the decrease in stress post 12% strain.

4.3.5.4. Changes in elastic modulus
Both DegraPol® DP0 and DP30 displayed a similar trend in changes to circumferential sample elastic modulus in vivo. Results for T=0 and 7 days showed a decrease in modulus between 6% to 12% strain (p>0.05), seen as material softening with deformation. From T=14 days, this effect reversed into stiffening between 6% and 12% strain, a result most likely due to the effect of ingrown tissue. As the scaffold became more populated, the mechanical strength of the tissue contributed more towards the overall strength, as well as the non-linear stiffening.

4.4. Overall study comparisons
There are various aspects to consider when comparing studies, including variations in scaffold morphology (alignment, fibre diameter, fibre merging), two different animal models, and differences in material. These all play a complex role in results and there are many interesting comparisons to be made.

4.4.1. Comparison of biostable Pellethane® scaffolds in subcutaneous and circulatory implant position
Looking at the subcutaneous and circulatory Pellethane® studies, the main difference was the use of a subcutaneous versus a circulatory model. Ingrowth into both was very rapid for the first seven days up to 65% ingrowth, after which the circulatory model slowed down, topping out at 78% ingrowth when compared to the subcutaneous model, which continued to rise to 96% by 28 days. Scaffolds in the circulatory model had 57% thicker fibres (10.5 ± 4.7µm vs 6.7 ± 2.4µm, p<0.05) — the difference most likely attributed to the different spinning target and rotational speed, as the uptake speed affects fibre thickness (Milleret et al. 2011). The circulatory model scaffold fibres were smooth and
lacked the dimpled surface texture which was present for the subcutaneous scaffolds. With comparable porosities, these two aspects could have contributed to deeper tissue penetration in the subcutaneous model. The difference in implant site was, however, the most likely cause, as subcutaneous samples were not directly loaded and were not clotted with blood at the time of implant.

From the tensile results, the most obvious difference was that the circulatory study showed much lower stress values for the same strains. The main contributing factor to this was lower fibre alignment in the circulatory scaffolds.

**4.4.2. Comparison of biostable DegraPol® DP0 and Pellethane® scaffold in subcutaneous implant position**

The Pellethane® and DegraPol® DP0 scaffolds assessed in the subcutaneous implant positions (studies 1 and 3) exhibited the highest degree of fibre alignment (dispersion of 12 ± 4° and 9 ± 2°, respectively). The tissue ingrowth was faster in the Pellethane® scaffolds than in the DegraPol® DP0 scaffolds, yet final ingrowth values at 28 days were similar (p>0.05).

DegraPol® DP0 showed higher stiffness than Pellethane® in the circumferential direction for all time points. In the axial direction, this only occurred at T=0 days.

The most noteworthy similarity between the two studies was a trend in circumferential stress over the *in vivo* time period (Table 4.5) for both materials, despite DegraPol® DP0 showing higher stiffness values for all time points. Possible contributions to this could include the similarities between the two studies which included: subcutaneous model, high fibre alignment and non-degradable materials. Table 4.5 illustrates this trend by listing, for the two studies, the changes in stress at 16% strain between consecutive time points.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Pellethane®</th>
<th>DegraPol® DP0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 7 days</td>
<td>-19%</td>
<td>-21%</td>
</tr>
<tr>
<td>7 to 14 days</td>
<td>+22%</td>
<td>+78%</td>
</tr>
<tr>
<td>14 to 28 days</td>
<td>-71%</td>
<td>-49%</td>
</tr>
</tbody>
</table>

The similarity was, however, limited to the circumferential scaffolds, as axial Pellethane® scaffolds showed a significant increase in stress at 16% strain by T=28 days, whereas DegraPol® DP0 only showed a small, insignificant increase during the entire *in vivo* period.
4.4.3. Tissue ingrowth during implantation period of all *in vivo* studies

Ingrowth data for all studies and materials are shown in Figure 4.25. All studies showed significant ingrowth from T=0 to 7 days. DegraPol® showed 54% less ingrowth than Pellethane® in the first seven days (p<0.05), after which the difference decreased to 8% by 28 days (p>0.05). Both DegraPol® DP0 and DP30 showed a significant increase from 14 to 28 days (43% and 40% respectively, p<0.05), which Pellethane® did not (8%, p>0.05).

![Figure 4.25. Tissue ingrowth into scaffolds for all studies.](image)

Although the ingrowth values for all three studies at T=28 days were similar (p>0.05), the mean value for subcutaneous Pellethane® was 16% higher than that of subcutaneous DP0; 25% higher than subcutaneous DP30; and 24% higher than circulatory Pellethane®. Looking at differences in scaffold morphology showed two possible contributions to this:

Firstly, subcutaneous Pellethane® fibre thickness was significantly lower than that of the other studies (% lower than Pellethane® circulatory: 36%; DegraPol® DP0: 48%, DegraPol® DP30: 47%). Scaffold diameter affects the ease of tissue attachment to the scaffold, as it plays the role of the natural ECM, which typically ranges in the 20-100 nm diameter range. Thinner electrospun fibres are thus favoured from a tissue regeneration point of view (Nisbet *et al.* 2009, Stella *et al.* 2010).

A second possible contribution to the differences in tissue ingrowth was that qualitative inspection of SEM images showed that DegraPol® scaffolds had more merged fibres than Pellethane®. Merged fibres limit effective pore size, thus potentially slowing and limiting ingrowth. This is illustrated in Figure 4.26, where a DegraPol® DP30 scaffold displays a very high degree of fibre merging and a Pellethane® scaffold with little merging. If the fibres were less merged, cells would be able to push them around, increasing the effective pore size. Merging severity as depicted in Figure 4.26 was, however, very limited. As the spinning rig was not climatically controlled, variations in ambient temperature and humidity were an unavoidable reality. Lower temperatures and higher humidity
decreases the rate of solvent evaporation, leading to higher degrees of fibre merging (Mauck et al. 2009).

Figure 4.26. SEM images of a) highly merged DegraPol® DP30 fibres and b) less merged Pellethane® fibres.

4.4.4. Graft manufacture

Electrospun Pellethane® and DegraPol® scaffolds were manufactured for three animal studies. Qualitative assessment showed some variation in scaffolds within a study group, shown in Figure 4.27. Some scaffolds showed more fibre merging due to the solvent not drying whilst others had many broken fragments.

Figure 4.27. Variance in resulting fibres with DegraPol® DP30 and same material spinning parameters.

Scaffold thicknesses for the various studies were: Pellethane® subcutaneous: 1.5 ± 0.5 mm; Pellethane® circulatory: 0.9 ± 0.4 mm; and DegraPol® subcutaneous: 1.2 ± 0.2 mm. This is much thicker than the 0.35 mm used for the FEM model. Spinning scaffolds this thin, and much thinner, is possible as the fibres are gradually deposited on the target whilst spinning. Scaffold thickness can thus be controlled by the duration of spinning. Handling of the scaffolds, specifically removal from the mandrel and implanting, will need further investigation and method development.

Fibre alignment with varying graft diameter also needs more investigation. Spinning on the larger 25 mm mandrel (Study 1 and 3) showed significantly lower fibre direction dispersion (thus higher
alignment) than the 2.2 mm mandrel of Study 2. In order to achieve high degrees of alignment on smaller targets, much faster rotational speeds are required, which can introduce more vibration which can in turn reduce alignment.

Electrospinning has an inherent randomness factor, which is magnified by poor system control. Although control of most of the spinning parameters was good, environmental control was lacking. Any improvement in the manufacturing can improve the consistency of scaffolds, which would in turn improve the ability to describe and compare the scaffolds and their mechanical behaviour.

4.5. Finite element modelling of the graft
The following section presents and discusses the results of the computational modelling study, including the constitutive material parameters, the mesh and graft geometry verification and the graft FEM model.

4.5.1. Constitutive material parameters
The analytically modelled and physical test stress-strain results of the DegraPol® scaffolds are shown in Figure 4.28. Table 4.6 lists the $R^2$ values for the constitutive model fit accuracy, for both the circumferential and axial data (Limbert 2014).

<table>
<thead>
<tr>
<th>In vivo time (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumferential</td>
<td>0.9991</td>
<td>0.9988</td>
<td>0.9989</td>
<td>0.9975</td>
</tr>
<tr>
<td>Axial</td>
<td>0.9874</td>
<td>0.989</td>
<td>0.9957</td>
<td>0.9909</td>
</tr>
</tbody>
</table>
Figure 4.28. DegraPol® DP30 experimental and analytical stress strain results at implantation timepoints, showing constitutive model behaviour compared to explanted scaffold.
4.5.2. Mesh and model geometry verification

The first phase of mesh density investigation did not show sufficient convergence by 8 elements across the wall. Beyond this, the model became excessively large for such simple geometry. Geometric verification, however, showed that changes in length and wedge angle hardly affected results. The six models used for geometric verification showed a compliance between 80 and 120 mmHg of $4.34 \pm 1.49 \times 10^{-5} \%/100 \text{ mmHg}$, and max von Mises stress of $0.348 \pm 5.86 \times 10^{-7} \text{ MPa}$, for variations in both length and wedge angle. Subsequent to the geometric validation, a smaller wedge was used to complete the mesh density verification, using up to 50 elements across the wall thickness. Figure 4.29 shows the results for the full mesh density study, including the initial and second phase.

![Figure 4.29. Stress and compliance as measures of mesh density verification.](image)

Based on the geometry and mesh verifications, a 0.1 mm long, 1° section of a cylindrical graft was used in the subsequent FEA models. A mesh density of 30 elements across the wall was used. This did not adversely affect computation time, as the model was still small with 960 elements and a computation time of ~20 seconds.

4.5.3. Graft FEM result

Results from the various FEM simulations are presented below. Figure 4.30 shows the von Mises stress of a 0.35 mm wall, 6 mm ID graft simulation with the graft lumen surface on the right.
Compliance is one of the main considerations for graft performance (Nisbet et al. 2009, Sarkar et al. 2007, Zilla et al. 2007). Figure 4.31 and Figure 4.32 show the compliance (measured on ID) from FEM simulation over the experimental time. Figure 4.31 shows the result of increasing the initial ID of the graft from 5 mm to 8 mm, whilst maintaining a 0.35 mm wall thickness.

Figure 4.31. Numerically predicted compliance versus implantation period for grafts with different inner diameters and a wall thickness of 0.35 mm.

Figure 4.32 shows the numerical prediction for graft compliance when maintaining the ID at 6 mm and increasing the wall thickness from 0.35 mm to 0.65 mm.

Figure 4.32. Numerically predicted compliance versus implantation period for grafts with different wall thickness and an inner diameter of 7 mm.
Table 4.7 summarises various measures of mechanical behaviour for a 0.35 mm wall, 6mm ID graft model.

Table 4.7. Changes in modelled DegraPol® DP30 graft behaviour based on in vivo degradation and tissue ingrowth.

<table>
<thead>
<tr>
<th>Time in vivo (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>28</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID (after axial preload) with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0mmHg (mm)</td>
<td>5.72</td>
<td>5.72</td>
<td>5.66</td>
<td>5.69</td>
<td>5.70 ± 0.02</td>
</tr>
<tr>
<td>80mmHg (mm)</td>
<td>6.05</td>
<td>6.04</td>
<td>5.96</td>
<td>5.93</td>
<td>6.00 ± 0.05</td>
</tr>
<tr>
<td>120mmHg (mm)</td>
<td>6.16</td>
<td>6.15</td>
<td>6.07</td>
<td>6.03</td>
<td>6.10 ± 0.05</td>
</tr>
<tr>
<td>200mmHg (mm)</td>
<td>6.38</td>
<td>6.34</td>
<td>6.26</td>
<td>6.21</td>
<td>6.30 ± 0.07</td>
</tr>
<tr>
<td>80-120mmHg Compliance (%/100 mmHg)</td>
<td>4.60</td>
<td>4.31</td>
<td>4.61</td>
<td>4.34</td>
<td>4.47 ± 0.14</td>
</tr>
<tr>
<td>0-200mmHg Compliance (%/100 mmHg)</td>
<td>5.72</td>
<td>5.42</td>
<td>5.26</td>
<td>4.63</td>
<td>5.26 ± 0.4</td>
</tr>
<tr>
<td>Maximum circ. strain at 200 mmHg (%)</td>
<td>6.26</td>
<td>5.69</td>
<td>4.28</td>
<td>3.56</td>
<td>4.90 ± 1.18</td>
</tr>
<tr>
<td>Min von Mises stress at 200 mmHg(MPa)</td>
<td>0.23</td>
<td>0.23</td>
<td>0.27</td>
<td>0.30</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Max von Mises stress at 200 mmHg(MPa)</td>
<td>0.30</td>
<td>0.30</td>
<td>0.34</td>
<td>0.35</td>
<td>0.32 ± 0.02</td>
</tr>
</tbody>
</table>

4.5.4. Discussion

The constitutive material was able to accurately represent the orthogonal uni-axial test data. Mean R² values for the data fit were 0.9986 ± 0.0006 for circumferential scaffolds and 0.9907 ± 0.0031 for axial scaffolds.

All measures except minimum von Mises stress; maximum circumferential strain at 200 mmHg; and the 0-200 mmHg compliance had standard deviations less than 10% of the mean value over the experimental time period. This indicated small variance in the predicted graft behaviour over the in vivo timeframe.

Compliance in the physiological range of 80 - 120 mmHg is an important measure for graft performance and remains fairly constant throughout the study. As compliance is a way of interpreting material stiffness, reviewing DegraPol® DP30 tensile data gives some insight into the limited changes observed in the modelled results. Figure 4.33 shows the elastic modulus for DegraPol® DP30 scaffolds from 2% to 14% strain for each of the experimental time points.
This highlights the similarity of the elastic modulus up to 8% strain across the *in vivo* study. As the maximum circumferential strain in the model at 200 mmHg only just exceeded 6% strain, the graft model was expected to behave consistently over all the time points. The variation in elastic modulus past 8% strain observed after 14 days *in vivo* would only have affected graft behaviour if the graft was loaded as to strain the material past 8%. This could be achieved through applying a higher internal pressure or by changing graft geometry. If the operating pressure range considered remained 80-120 mmHg, decreasing the wall thickness and / or increasing the internal diameter would have increased graft compliance, and thus led to a higher strain range. Compliance of 6.4 %/mmHg is needed for the graft to exceed 8% strain at 80 mmHg.

The compliance range of 3%/100 mmHg (6mm inner diameter, 0.65 mm wall thickness) to 5.5%/100 mmHg (0.35 mm wall thickness, 8 mm inner diameter) predicted with the DegraPol® DP30 scaffold with graft sizes comparable to clinical grafts was in an ideal zone between two extremes. On the one hand the scaffold was stiff enough to lend itself to implantation without external reinforcing, as required for more elastic polymers such as Pellethane® (Sirry *et al.* 2010). On the other hand, the compliance was greater than, and a closer physiological match than, that of many clinically used grafts, such as ePTFE (1.6 ± 0.2%/100 mmHg) and PET (1.9 ± 0.3%/100 mmHg) (Seifalian *et al.* 1999).

As the constitutive material was based on orthogonal uni-axial tensile tests, the effect of simultaneous bi-axial strain was not considered in the constitutive material characterisation. As the FEM model, and any physiological graft, included axial pre-stretch as well as internal pressure loads, bi-axial test data would have been invaluable to verify and improve on constitutive parameters (Holzapfel and Ogden 2010).
The modelled geometry was a simple cylindrical section. In reality, vessels are irregular and tissue ingrowth and the effect of surrounding tissue will cause similar irregularities in tissue engineered grafts. Although this model was a simplification of the complexity expected when using longer grafts for bypass surgery, it was a vital step in assessing material feasibility for the use of graft manufacture, as well as providing an estimate on required geometry for specific *in vivo* performance.
5. Summary

The aim of this project was to investigate the changes in mechanics occurring in biodegradable (DegraPol® DP30) electrospun scaffolds during implantation in animal models, and to computationally evaluate the suitability of the scaffolds for use as arterial grafts. In pursuing this aim, the changes in mechanics of biostable scaffolds (Pellethane® and DegraPol® DP0) in animal models were investigated as foundation studies.

In order to achieve this aim, the following objectives were defined:

a) To manufacture biostable and biodegradable scaffolds by electrospinning Pellethane®, DegraPol® DP0 and DegraPol® DP30
b) To implant and explant scaffolds in animal models, using both subcutaneous and circulatory rat models to generate tissue ingrowth
c) To characterise the scaffolds, both morphologically and mechanically, including fibre size and alignment, scaffold porosity, and mechanical strength
d) To develop and verify finite element models and assess computationally DegraPol® DP30 scaffold for use in vascular grafts

This summary chapter reviews the achievement of each of the above-mentioned objectives by linking various parts of the project and demonstrating the novel aspects of the research.

5.1. Scaffold manufacture

This objective required electrospun scaffolds to be manufactured for use in further objectives. Electrospun scaffolds were manufactured from biostable Pellethane® and DegraPol® DP0, as well as biodegradable DegraPol® DP30. After spinning, scaffolds were prepared for implantation into various animal models and subsequently analysed.

Scaffolds for each study displayed good consistency in morphology, indicating adequate control of the spinning process. Variations between studies were in part due to differences in material, as each material required slightly modified conditions for optimal spinning. Even the change from DegraPol®
DP0 to DP30 showed a need for parameter fine-tuning. The change in mandrel geometry for the circulatory study also brought changes in spinning requirements.

Spinning parameters were selected for stable spinning, yielding consistent results for each material and configuration. Achieving more consistent scaffolds for a variety of materials and geometries would require detailed spinning optimisation for each scenario.

5.2. **In vivo studies**

Three separate animal studies were undertaken to provide an environment for tissue ingrowth, all based on established albino Wistar rat models. Two studies considered biostable Pellethane®, one in a subcutaneous model and the second in an aortic circulatory model. The third study made use of a subcutaneous model for DegraPol® DP0 and DP30 scaffolds. All studies investigated three implant durations (time points), namely 7, 14 and 28 days.

Histological analysis showed consistent ingrowth over time within each study. Pellethane® scaffolds exhibited a faster ingrowth rate for the first 7 days compared to DegraPol®, as well as higher total ingrowth (p<0.05 up to 7 days, p>0.05 from 7 to 28 days).

5.3. **Scaffold morphology and mechanical properties**

Scaffold morphology, including fibre thickness, fibre alignment and porosity, was consistent within each study. Morphological differences of the scaffolds were, however, observed between the studies conducted. The scaffolds for assessment in subcutaneous implant positions (studies 1 and 3) exhibited significantly higher degrees of fibre alignment than the scaffolds used in the circulatory implant position. This difference was attributed to the dimension of the mandrel used as the electrospinning target, with a larger-diameter mandrel used for scaffolds for subcutaneous implantation compared to the scaffolds for circulatory interposition. A larger rotating spinning target results in higher velocities of fibre uptake, and thus more fibre alignment, compared to a target with a smaller diameter. In addition to this, electrospinning of Pellethane® produced scaffolds with thinner fibres and greater porosity compared to DegraPol® scaffolds.

Scaffold morphology plays a complex role in the mechanical behaviour of scaffolds. Despite the challenges arising from the morphological differences of the scaffolds used in the three studies, cross-study comparisons provided a number of noteworthy findings on the mechanical behaviour of the scaffolds.
One of the most promising outcomes was the stability of DegraPol® DP30 mechanics during the *in vivo* study. Circumferential direction scaffolds showed a remarkable consistency in stiffness even though the fibre material was losing strength due to degradation.

The lower elastic modulus of Pelletane® compared to DegraPol® was reflected in the scaffold mechanics by significantly lower stress during deformation. This was further emphasised by the thinner fibres and higher porosity (less material) of Pelletane® scaffolds compared to the DegraPol® scaffolds.

Both DegraPol® DP0 and DP30 showed similar trends in changes to material stiffness induced by cycling. Axial direction samples showed a fairly uniform reduction in stiffness across the strain range, whereas circumferential fibres showed a greater decrease at low strains. The main difference between the two materials was that DegraPol® DP0 did not perform well with pre-cycling displaying a major reduction in stiffness. This large decrease is indicative of major damage in the scaffold, proven by the fact that the cycled scaffold started to fail after 12% strain. DegraPol® DP30 in comparison performed very well with cycling. Both axial and circumferential direction samples only showed a small 5% decrease in stiffness by 16% strain which is promising for physiological applications with cyclic mechanical loading conditions. The poor cyclic performance of DegraPol® DP0 scaffolds is not necessarily only due to differences in the bulk materials. DegraPol® DP0 scaffolds were extremely aligned, giving rise to the scenario where most fibres are loaded in tension from very low strain, as opposed to a more gradual local rearrangement of fibres.

### 5.4. FEM studies

Finite element studies made use of a constitutive material model inspired by the behaviour of electrospun scaffolds in this research. A simplified cylindrical graft model was created and subjected to 0 – 200 mmHg pressure loads. Graft behaviour based on DegraPol® DP30 scaffolds at 0, 7, 14 and 28 days was modelled and specific measures recorded. Of main interest was vascular compliance across the implant duration range. Trends in results from finite element models correlated with expectations from tensile test data.

The finite element models provided the first proof of feasibility of using DegraPol® DP30 for electrospun vascular grafts. Scaffold deformation remained well below the strain threshold of 16% above which the constitutive model was not validated. Numerically predicted compliance for a vascular graft with a 6 mm inner diameter and 0.35 mm wall thickness remained nearly constant at $4.47 \pm 0.14% / 100 \text{ mmHg}$ over the 28 day implantation period. This indicates a vital balance between tissue ingrowth and degradation. In addition to this, the numerically predicted compliance for DegraPol® DP30 grafts was higher than that of ePTFE ($1.6 \pm 0.2% / 100 \text{ mmHg}$) and PET
(1.9 ± 0.3%/100 mmHg), showing an advancement towards improved matching physiological compliance.

5.5. Addressing the research project aim

The overall aim of investigating changes in the mechanics of electrospun scaffolds during in vivo studies and furthering this knowledge to evaluate the potential use of DegraPol® DP30 for vascular grafts was achieved.

Electrospun DegraPol® DP30 showed promising mechanical behaviour for vascular grafts. This project as a whole showed little change in graft compliance in the physiological pressure range over a 28 day period of material degradation and tissue ingrowth. Although compliance values were still lower than the ideal physiological values, it represented an improvement over commercially available PET and ePTFE grafts. Changes in scaffold morphology such as an increase in porosity, thinner fibres, and lower fibre alignment, as well as the basic formulation of the DegraPol® DP30 (to lower material bulk elastic modulus) are all possibilities to further improve vascular compliance.

5.6. Novel aspects of this research project

This research project comprises a number of aspects that have not been investigated by other researchers to date.

1. Exploration of fast degrading polyester-urethane for cardiovascular tissue regeneration

The project explores the mechanical suitability of biodegradable DegraPol® for the use in tissue engineered vascular grafts, which has not been done before. One of the main achievements was demonstrating consistent mechanical properties during in vivo scaffold degradation and tissue formation, thus obtaining a balance between the weakening due to degradation and the strength provided by new tissue. This balance is seen as one of the key achievements when investigating biodegradable implants and prostheses.

2. Paired in vivo assessment of the mechanics of biostable and degradable electrospun scaffolds based on the same polyester-urethane formulation to isolate the effects of material degradation and tissue formation.

The use of a biostable and a biodegradable polyester urethane scaffold, implanted concurrently in the same animal has not been done before. Using biostable DegraPol® DP0 and fast-degrading DegraPol® DP30 in such a comparative in vivo model highlights the differences in changes to scaffold mechanics during the in vivo healing process.
3. **Constitutive description of the effects of concurrent material degradation and tissue formation on mechanical properties and structural integrity of fast-degrading polyester-urethane scaffolds**

Characterising the mechanical behaviour of the DegraPol\textsuperscript{®} scaffolds with concurrent tissue formation and scaffold degradation with a constitutive model has not been done before. This translates the laboratory work into the computational field, opening up the application of the research for further use.

4. **Proof of feasibility of a fast-degrading polyester-urethane electrospun scaffold for tissue regenerative small and medium calibre vascular grafts mimicking arterial mechanics**

This research provided the first proof of feasibility of using fast-degrading DegraPol\textsuperscript{®} DP30 scaffolds for tissue regenerative small and medium calibre vascular grafts. Not only did the research demonstrate a balance between scaffold and tissue contribution to the overall strength during \textit{in vivo} degradation and tissue formation, but the mechanical response of the scaffold was also promising. The finite element modelling showed arterial compliance values better suited for small to medium sized arterial grafts than the current industry standard of ePTFE and PET.

5.7. **Limitations and recommendations**

There were several limitations in this research, listed here with recommendations on how to potentially address them in future work. Included are other general recommendations on furthering the research presented in this thesis.

1. **Consistent scaffold morphology would enable better comparative analysis**

Low consistency in scaffold morphology between the different studies limited the extent to which cross-study comparisons could be explored. Improved consistency may be achieved through improving the electrospinning process such as temperature and humidity control and minimising spinning target vibration.

2. **Bi-axial testing would closely mimic \textit{in situ} scaffold loading**

Orthogonal, uni-axial tensile testing of the scaffolds, as done in this research, limited the constitutive characterisation of the scaffold behaviour. One of the most substantial advances in characterising the mechanics of anisotropic materials is through bi-axial testing. Bi-axial testing mimics the \textit{in situ} loading conditions more closely thus enabling one to determine the mechanical properties of the scaffolds under these conditions. At the time of the research, there were, however, no testing facilities in the country which could conduct such testing.
3. **Limited histological investigation**

Histology was primarily used to identify the filling of empty spaces in the scaffold to quantify tissue ingrowth. High magnification histology, potentially using various other staining methods, could further the knowledge of the tissue density and cell diversity within the scaffolds.

4. **DegraPol® circulatory *in vivo* model would be recommended for future studies**

It would have been ideal to also evaluate DegraPol® in the circulatory model, however, this proved to be beyond the scope of the project. It would be a worthy component of future research in order to confirm and expand the computational framework.

5. **Validation of finite element models would be recommended for future studies**

Validating the finite element models of the grafts would be another valuable extension of the research. This could be done either with an *in vitro* compliance testing device, or alternatively with a specialised small animal ultrasound system to monitor graft diameter *in vivo*. 
6. Conclusions

Regenerative medicine and tissue regeneration are receiving increasing attention in treatment of a wide variety of diseases and conditions. Since biological tissues may exhibit different responses with different scaffold materials, it is important to investigate the dynamics of tissue ingrowth when considering a particular polymer for tissue engineering and regeneration in a specific application.

The current research project is the first, to the author’s knowledge, that investigated the suitability of the biodegradable polyester-urethane DegraPol® for the potential use in vascular tissue engineering and regeneration, with particular emphasis on the mechanical and structural integrity of the scaffolds and developing tissue constructs. The research was largely motivated as a result of the shortcomings of current small-diameter vascular grafts. The outcomes of this research can, however, also contribute to research in other areas of regenerative medicine targeting soft biological tissues and functional constructs. This may include the regeneration of cartilage, skeletal muscle, kidney tissue, skin, and heart valves, to mention only a few.

The ability to manufacture co-block polymers such as DegraPol® with a wide range of stiffness and degradation rate makes this class of materials very versatile and interesting for such a wide range of regenerative applications. Studies investigating the use of fast-degrading DegraPol® (DP30) in the regeneration of cartilage and skeletal muscle have been undertaken, however, the mechanical behaviour of the scaffolds during the regenerative process has yet to be addressed. The data and computational models developed during this research can be applied and extended to gain insight into the anticipated mechanical changes of other tissue engineered structures, and tailor scaffold materials and structures for optimal performance and long-term response.

As such, the current work can serve as a basis for further research. The large number of applications in tissue regeneration offers a multitude of possible extensions. Equally for tissue regenerative vascular grafts and the regeneration of other tissues and organs, the computational models, for example, can be expanded to phenomenologically describe the material degradation and tissue ingrowth. This will hopefully allow one to investigate regeneration processes that take place at different rates, and require scaffolds with different degradation rates than those observed in this
project. Further development can also address the effects that treatments to promote regeneration may have on the scaffold mechanics. Lastly, micro-structural computational models could be used to investigate scaffold-guided stem cell differentiation.
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