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**ENGINEERING COMPLIANT, SMALL DIAMETER
VASCULAR PROSTHESIS FOR BYPASS GRAFTING**

JEAN-MARC BENEDIKT BEHR

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2018

תכנון תותב גמיש בעל קוטר קטן לשימוש במעקפים במערכת הלב

חיבור על מחקר

לשם מילוי חלקי של הדרישות לקבלת התואר דוקטור לפילוסופיה

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ENGINEERING COMPLIANT, SMALL DIAMETER VASCULAR PROSTHESIS FOR BYPASS GRAFTING

JEAN-MARC BENEDIKT BEHR

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2018

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarized materials, and has not been submitted for a higher degree to any other University or Institution.

12.12.2019

Date

Jean-Marc Benedikt Behr

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

12.12.2019

Date

Prof. Subbu Venkatraman

Authorship Attribution Statement

This thesis contains material from 3 papers published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 4 was accepted by Macromolecular Bioscience and is going to be published soon.

The contributions of the co-authors are as follows:

- Prof. Subbu Venkatraman provided the initial project direction and edited the manuscript drafts.
- Prof. Eyal Zussman was the co-supervisor of this project.
- I prepared the manuscript drafts. The manuscript was revised by Dr. Scott A. Irvine and Prof. Eyal Zussman Prof. Subbu Venkatraman.
- I co-designed the study with Dr. Scott A. Irvine and Prof. Subbu Venkatraman and performed all the laboratory work at the School of Materials Science and Engineering.
- Most of the sample fabrication, mechanical testing and evaluation of the results were done by myself. I received assistance by Mrs. Chaw-Su Thwin, Dr. Ankur Harish Shah and Mr. Kraun Bae Min-Chul.
- Mrs. Chaw-Su Thwin fabricated the specimen tested *in vivo* in lupine.

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The contributions of the co-authors are as follows:

- Prof. Subbu Venkatraman provided the initial project direction and edited the manuscript drafts.

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- I prepared the conference abstracts. The abstracts were by Dr. Scott A. Irvine and Prof. Subbu Venkatraman.
- I gave an oral presentation at both conferences

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Date

Jean-Marc Benedikt Behr

Abstract

Mechanical mismatch between vascular grafts and blood vessels is a major cause of smaller diameter graft (< 6 mm) failure. The fabrication of a compliant small diameter prosthesis remains elusive, due to the idiosyncratic elastic properties of arterial wall tissue. Native blood vessels exhibit a pressure-dependent compliance (non-linear elasticity), which is a principal reason behind the decade-long search for a clinical solution for small diameter vessel replacement. Mimicking the non-linear elastic properties of arterial tissue is the major goal in this thesis and has been achieved over multiple steps.

Initial research involved the evaluation of several poly-L-lactide-*co*- ϵ -caprolactone (PLC) copolymers as candidate materials for the fabrication of small diameter grafts. Using these materials, tubular prostheses of 4 mm inner diameter were produced by dip-coating. *In vitro* static and dynamic compliance tests were conducted, using a custom-built apparatus. Mechanical testing revealed a compliance-dependence on the monomer composition and the wall-thickness (WT) of the PLC tubes. PLC 70:30 tubes of 150 μm WT were similarly compliant as the porcine arteries. These tubes were assessed in a three hours *in vivo* experiment in a lupine model as an aortic bypass on their implantation and function. The recorded angiogram showed continuous blood flow, no aneurysmal dilatation, leaks or acute thrombosis, indicating the potential for clinical applications.

In order to achieve non-linear elasticity, different approaches were pursued, such as adding a soft inner lining to a PLC tube or outer melt-drawn fibers. However, none of these methods produced satisfying results. Hence, a third strategy was followed up, by combining a soft dip-coated tube with a 3D-printed outer pliable mesh. In an initial test, single spring-like fibers were assessed on their tensile properties. All fibers showed clear signs of non-linear elasticity, displaying an increasing elastic modulus upon unfolding in the stress-strain diagrams (J-Curve). Among the all tested spring geometries, the semi-hexagonal (HEX) were the most satisfying. Based on the results from the tensile experiments, meshes of different designs were printed and wrapped

around a soft dip-coated PLC 70:30 tube to form a mesh-tube composite (JM-Tube). Initially, the dip-coated tube was not elastic enough to sufficiently unfold the mesh and moreover expanded between the mesh struts. Fiber unfolding was facilitated when using HEX-meshes with two spring sections that were separated by a middle glue strip. This configuration allowed a better stress transfer from the tube to the fiber to enable easier unfolding. A reduction of the WT of the PLC 70:30 tube from $\sim 100\text{ }\mu\text{m}$ to $\sim 55\text{ }\mu\text{m}$ made the tube sufficiently elastic so that it could expand easily to force the mesh fibers to unfold. The use of meshes with high fiber coverage minimized tubular inter-strut expansion. By combining these three advances, the JM-Tubes then displayed the desired non-linear elastic properties.

This work demonstrates arterial-like compliance properties can be achieved by combining a soft dip-coated tube with a pliable stiffer outer mesh. Further optimizations in the design are required, to improve the compliance matching to native arteries.

Lay Summary

Cardiovascular diseases (CVDs) remain a major cause of death worldwide, accounting for millions of deaths. The most common forms of CVDs are heart attacks and strokes; both are attributed to the pathogenesis of atherosclerosis, which is a continuous plaque build-up that leads into a narrowing of the inner diameter of the artery and eventually to thrombosis. Diseased sites frequently require replacement with synthetic blood vessels, in order to restore the blood flow. With expanded polytetrafluoroethylene and polyethylene terephthalate, there exist clinical solutions for the substitution of diseased arterial tissue. However, for small diameter artery (< 6 mm) replacement, both materials suffer blockage after a couple of months. This is attributed to a mechanical mismatch, i.e. a mismatch in radial expansion between the grafted artery and the synthetic vascular prosthesis (compliance mismatch). Therefore, an arterial substitute with matched compliance is required, in order to minimize the incidences of post-implantation blockages. However, up to this day, this presents an elusive task to researchers, as arterial tissue exhibits idiosyncratic elastic properties: at low blood pressure arteries are highly compliant (elastic) and become increasingly stiff when the pressure is raised. In this work, the aim was to fabricate a prosthesis with arterial-like compliance. In an initial set of experiments, different materials were tested to fabricate vascular grafts of 4 mm inner diameter via dip-coating. Poly-L-lactide-*co*- ϵ -caprolactone (PLC) polymers of different monomer ratios (L-lactide (LA) to ϵ -caprolactone (CL)) were used for tube fabrication and assessed on their mechanical properties. PLC polymers of roughly equal monomer feeds were more elastic, due to their predominantly amorphous microstructure. Conversely, predominantly LA- or CL-rich PLC polymers were stiff because of their high crystalline phase content. PLC 50:50 (50% LA and 50% CL) and 70:30 were suitable materials for the fabrication tubular grafts with high compliance in the range of arterial tissue. However, PLC 50:50 was found to be too weak and therefore PLC 70:30 was favored as tube material. The compliance of PLC 70:30 tubes could be tailored by the wall-thickness (WT), which revealed thinner tubes were more elastic than thicker ones. PLC 70:30 tubes of 150 μ m WT showed compliance in the range of porcine arteries (81% relative to the porcine artery, 100%). A three-hour animal test in a rabbit showed 150 μ m WT PLC 70:30 tubes remained mechanically stable and free of any defects such as needle tears, aneurysmal dilatation or wall rupture.

In order to produce vascular substitutes with non-linear elasticity, bi-layered dip-coated tubes of a soft inner lining and a stiffer outer layer were fabricated. None of these tubes showed arterial-like elastic properties. However, in principle the approach of a composite structure of a soft and stiff material to mimic the elastic properties of arteries was found to be favorable, but not by fabricating a bi-layered tube. Therefore, a different structure had to be considered and hence the winding of melt-spun fibers around a soft dip-coated tube was considered. Different parameters were tested, such as tube and fiber material combination, spinning speed and circumferential or helical fiber winding. The winding of stiffer material fibers around dip-coated tubes reduced the compliance, as did increased spinning speeds. The latter was due to stress-induced crystallization, which occurs with faster fiber winding. Slow spun PLC 70:30 fibers (at 100 rotations per minute) around a PLC 70:30 tube produced non-linear elastic prostheses. It is assumed the slow spinning may have led to fiber shrinkage and coiling of the polymer chains that first require unfolding, before they get stretched.

Another approach using a more deliberate composite structure of a 3D-bioprinted pliable stiffer outer mesh was considered, in order to obtain a non-linear elastic prosthesis. Single fiber tensile tests were carried out to verify, whether such pliable fibers could produce such a behavior. Four spring types, zig-zag (ZZ), semi-hexagonal (HEX), stair (ST) and sine-wave (S) were considered, and all provided easy fiber unfolding at low strains. Upon complete fiber straightening, the fiber material itself got stretched, which created a transition from low to high stiffness. This could be seen from a J-shaped Curve (J-Curve) in the stress-strain diagrams. Several geometrical designs were tested before meshes comprising such spring-like fibers, J-Curve Meshes (J-Meshes), were printed. Afterwards, composites of a soft dip-coated tube with an outer J-Mesh (JM-Tubes) were fabricated and evaluated on their mechanical properties during dynamic compliance tests. Combining a thin ($\sim 55\mu\text{m}$ WT) PLC 70:30 tube and dense HEX-mesh with two hoops (springs) per round, made of PCL or PLC 95:5, produced JM-Tubes with non-linear elastic properties. A clear transition from high to low compliance was observed for such JM-Tubes. This proves the viability of the JM-Tubes, but yet requires optimization in further work, in order to better match the compliance profile of native arteries.

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Abbreviations

2L-T – Two-layered (bi-layered) tubes
 ANOVA – Analysis of variance
 C – Compliance
 Cat – Caterpillar
 C_{dyn} – Dynamic compliance
 CE – Chain extender
 CL – ϵ -Caprolactone
 Col – Collagen
 C_{stat} – Static compliance
 CVD – Cardiovascular diseases
 d – Distance
 D – Diameter
 DI – Diisocyanate
 DM – Diamond
 E – Elastic Modulus
 E – Elastic modulus
 EC – Endothelial cell
 ECM – Extracellular matrix
 ePTFE – expanded Polytetrafluoroethylene
 F – Melt-spun fiber
 F_C – Fibers, circumferentially spun
 FE-SEM – field emission scanning electron microscope
 F_H – Fibers, helically spun
 FN - Fibronectin
 H - Hoop
 GA – glycolic acid / glycolide
 HDL – High density lipoprotein
 HEX – Semi-hexagonal
 HFA – Human femoral artery
 HFT – Helical fiber tube composites
 HSV – Human saphenous vein

HUVEC – Human umbilical vein endothelial cells
 ISO – International Organization for Standardization
 J-Curve – J-shaped stress-strain curve
 J-Mesh – J-Curve mesh
 JM-Tube – J-Curve mesh tubes
 LA – L-lactide/lactic acid
 LDL – Low density lipoprotein
 MD – Macro diol
 MFT – Melt-spun fiber composite tubes
 NACLAR - National Advisory Committee for Laboratory Animals Research
 NIH – Neo-intimal hyperplasia
 NO – Nitric oxide
 OxLDL – Oxidized low density lipoprotein
 P – Pressure
 PCG – Poly-ε-caprolactone-*co*-glycolide
 PCI – Percutaneous coronary intervention
 PCL – Poly-ε-caprolactone
 PDC – Poly-(diol-citrates)
 PDCC – Poly-1,10-decandiolcitrate
 PDDC – Poly-1,12-dodecanediolcitrate
 PEG - Poly(ethylene glycol)
 PET - Poly(ethylene terephthalate)
 PGA – Poly(glycolic acid)
 PGS – Polyglycerol-sebacate
 PHA – Poly-hydroxy-alkanoate
 PHB – Polyhydroxybutyrate
 PHBHHx – 3-hydroxyhexanoate
 PHC – Poly-1,6-hexanediolcitrate
 PLA – Poly-L-Lactide
 PLC – Poly-L-lactide-*co*-ε-caprolactone
 PLGA – Poly-lactide-*co*-glycolide (glycolic acid)
 POC – Poly-1,8-octanediol-citrates
 PTFE - Polytetrafluoroethylene
 PU – Polyurethane

PVA - Poly(vinylalcohol)

Q – Flow rate

R – Strain recovery [chapter 4]

R – Round (number of wraps of the J-Mesh around the dip-coated tube) [chapter 6]

RA – Radial artery

RPM – Rotations per minute

RT – Room temperature

S – Sine wave

SA – sebacatic acid

SEM – Scanning electron microscope

SG – Stent graft

SIS - Small/sub-intestinal submucosa

SMC – Smooth muscle cell

ST – Stair

SV – Saphenous vein

T – Tube

WT – Wall-thickness

V – Voltage

ZZ – Zig-zag

ϵ_E – unfolding strain (experimental)

ϵ_T – unfolding strain (theoretical prediction)

$\dot{\epsilon}$ – Creep rate

Chapter 1

Introduction

This introduction chapter provides information about cardiovascular diseases, the currently available treatments and their limitations. The importance of mechanical property matching between small diameter vascular prostheses and the grafted arteries is discussed in this chapter. The structure of arterial walls is explained in brief, in order to understand their idiosyncratic mechanical properties. Furthermore, this chapter provides an overview of this dissertation, the hypothesis and objectives of this work, as well as the findings and outcomes.

1.1 Background

1.1.1 Atherosclerosis

Cardiovascular diseases (CVD) remain a leading cause of morbidity worldwide, accounting approximately 29% of deaths. 222 per 100'000 Americans suffering from CVD died in 2013 [1], while Europe accounts 4.35 million CVD-related deaths per year [2]. Around 1.4 million patients in the US were in need of a vascular prosthesis in 2010, of which 397'000 underwent bypass surgery and 954'000 coronary artery interventions [1].

Among CVD, atherosclerosis is the most prominent. It is caused by the accumulation of low density lipoprotein (LDL) within the subendothelium [2, 3]. In 1856 Rudolph Virchow [4] proposed arterial wall injury being the cause of atherosclerosis [5]. More than 100 years later, in 1977, Ross et al. [5] formulated the “response-to-injury hypothesis”, which has been refined since then [6].

Atherosclerosis may occur due to many factors; the major ones that contribute to an increased risk are: Age, gender, smoking, high sugar and diets rich in saturated fat, stress, excessive alcohol consumption, obesity, lack of exercise etc. [2, 7]. Age is one of the most important risk factors. While young individuals exhibit low risk of developing atherosclerosis (3% for 30-34 year old males), the prevalence of diseased people is tremendously higher for elder people (21% for males aged between 60-64 years) [8]. As fatty streaks develop into plaques over time, the risk of developing a pathologic condition is increasing with age [9]. Males exhibit higher risks of suffering from atherosclerosis than females. It has been proposed that women are less prone, due to their higher levels of high-density lipoprotein (HDL) and estrogen. The latter is assumed to have a preventive effect, as the prevalence of atherosclerosis increases in post-menopause women [10]. Some ethnic groups are more likely to suffer from atherosclerosis than others, e.g. due to different physiology and/or socioeconomic status [1, 2, 11, 12]: According to the American Heart Association, atherosclerosis was more commonly diagnosed among Afro-Americans in 2016, whereas Hispanics were the least prone [1].

Originally being considered as a western disease alongside diabetes mellitus, atherosclerosis is nowadays increasingly diagnosed in developing countries [13-15]. A primary factor is the shift in diets, which have become richer in fats and sugar [13, 15]. Other important factors are increased life-expectancy, due decreased mortality rates of contagious diseases and physical inactivity [2, 15, 16].

The pathogenic pathway of atherosclerosis is initiated by an injury to the endothelial layer [2, 3]. Also known as the *intima*, the endothelium is the innermost layer of arterial walls. It consists of a monolayer of endothelial cells (ECs), which form a non-thrombogenic sheet, a basal lamina and a subendothelial space [2, 17, 18]. Endothelial injuries can be caused through multiple factors such as high LDL concentrations, free radicals (in particular reactive oxygen species), infectious diseases (e.g. herpes virus), endogenous inflammatory signals (cytokines), shear stress at sites of turbulent blood flow etc. [2]. The latter reason explains why atherosclerosis is more prevalent at arterial bifurcations (e.g. carotid bifurcation) [19]. A dysfunctional endothelium allows the penetration and trapping of lipids, in particular LDL, into the arterial wall [2, 3]. Furthermore, a damaged endothelium is procoagulant, which leads platelets to aggregate at the intima [2, 3, 20]. Initial platelet adhesion to “von Willebrand Faktor” at the injury site is mediated by the GPIb-IX-V complex [21]. Once adhered, the platelets become activated and start to release various inflammatory factors such as proteases, chemokines (e.g. RANTES) and vasoactive substances. The hence caused inflammation attracts T-lymphocytes and monocytes from the blood to enter the sub-endothelium. The signals released by the platelets promote the monocytes to mature into macrophages [2, 22].

The initiated inflammatory response is the attempt of clearing the intima from harmful species (e.g. LDL) that are responsible for endothelial injury. If effective removal fails, the inflammation triggers the migration and proliferation of smooth muscle cells (SMCs) to the subendothelial space (Figure 1.1) [3]. This leads into the formation of an intermediate lesion (“*atherogenesis*”) and intimal thickening. Consequently, the arterial wall gets remodeled by proliferative SMCs through thickening. This is compensated by radial dilation, remaining the lumen unaffected [3].

Figure 1.1: Pathogenesis of Atherosclerosis. The LDL penetrating the intima gets oxidized in the subendothelial space and ingested by macrophages. Continuous intake of OxLDL by macrophages induces foam cell formation and later necrosis (reproduced with permission from [23], copyright Massachusetts Medical Society).

In the subendothelial space present LDL undergoes oxidation (acetylation), mediated through SMCs and ECs [10]. Oxidized LDL (OxLDL) is cytotoxic, thus induces endothelial injury and dysfunction. OxLDL can be recognized and ingested by macrophages, through their scavenger receptor [3, 10, 24, 25]. With continuous OxLDL-uptake, macrophages turn into foam cells. This stage is viewed as the starting point of atherosclerosis. The presence of foam cells together with the T-lymphocytes leads into the formation of lesions, sometimes also addressed as “fatty streaks” or atheroma, which later may either progress or regress [2, 25]. As OxLDL has an upregulating effect on the scavenger receptor activity of the macrophages, its ingestion remains persistent [26]. Due to the exaggerated cumulation of OxLDL the foam cells become necrotic. This leads into the formation of a more complex lesion (necrotic core), as the disrupted foam cells release their cytoplasmic content, which include modified lipids and pro-inflammatory growth factors. SMCs attracted from the media layer proliferate and form a fibrous cap by depositing extracellular matrix (ECM). Growing plaques lead into intimal thickening and protrude into the lumen [2, 3].

Atherosclerotic individuals often remain asymptomatic of angina pectoris with luminal narrowing up to 50-70% [27]. At the final stage of atherosclerosis, the affected arteries may experience a critical stenosis (blockage), due to excessive plaque growth, or plaque rupture. Latter is the most observed complication associated with atherosclerosis. Plaques become vulnerable due to continuous exposure of their shoulder region to stress, fibrous cap thinning, degradation of its matrix by macrophages or sprouting and thrombosis of micro-vessels [20, 25]. Plaque rupture leaves a highly thrombogenic surface behind, which causes platelet adhesion and activation. Once activated, they aggregate at the site of the wall injury, forming a thrombus, which may interrupt the blood flow. Consequently, the surrounding tissue will suffer from ischemia (oxygen depletion) [3, 20, 25]. The occurrence of atherosclerosis in the coronary artery may cause myocardial infarction, while the occurrence in the brain may lead to a stroke. Both can be extremely debilitating or even fatal [10].

Plaque rupture usually marks the ultimate failure and final stage of atherosclerosis. However, it does not necessarily result into thrombosis and occlusion, as the plaque has a chance of re-stabilizing. However, a larger plaque will be left behind and the consequences of further rupture will be more severe [2].

1.1.2 Revascularization Techniques

As in the late stage of atherosclerosis patients suffer from heart attacks or ischemia, the blood flow at the diseased artery needs to be restored [3, 17]. This is either realized via balloon angioplasty followed by stenting or graft bypassing [17, 28-31]. In percutaneous coronary intervention (PCI) a balloon is delivered to the diseased site via catheter. The catheter is usually inserted from the radial or femoral artery. Upon expansion of the balloon, the narrowed artery gets inflated and its lumen restored, allowing an unhindered blood flow [32]. Although early bare metal stents reduced the risk of restenosis in comparison to balloon angioplasty, they still display high incidences of re-occlusion. With the introduction of drug-eluting stents, such events became even rarer. Nevertheless, there remains a large number of patients, who still experience complications and need to go for re-intervention [33, 34]. PCI is a less invasive technique than artery grafting and exhibits much lower in-hospital mortality rates, which makes it a preferred method of treatment [31]. However, despite its clinical use

since decades, stenting cannot yet fully avoid re-stenosis or late post-surgery thrombosis [33, 34].

Alternatively, atherosclerosis may also be treated by bypass grafting, where the diseased blood vessel is replaced by either autografts, allografts, xenografts or synthetic prostheses [17, 31, 35-39]. The most commonly used autologous vessels for grafting are the thoracic artery, radial artery (RA), gastroepiploic artery and saphenous vein (SV), whereby latter remains today's golden standard [38, 40]. Although the use of SV for bypassing remains a popular choice, roughly 10-40% [38] of the patients do not have suitable tissue, due to diameter mismatch or lack in available healthy tissue [41]. Deb et al. [42] found a five years patency rate of 88% for RA and 80.3% for SV grafts. Although being in clinical use, SV or RA grafts do not guarantee absolute success, as more than 50% of the patients require to go for re-intervention after ten years [40, 43]. Allografts from human cadavers are susceptible to aneurysmal dilatation, calcification or thrombosis. Cryopreserved allogenic grafts exhibit lower patency rates than autologous tissue and may furthermore cause an immune response [44, 45]. However, the problem of allograft immunogenicity can be overcome through decellularization [46]. Like allografts, xenografts (tissue from different species) are potentially immunogenic, which is the reason why they are commonly decellularized [35, 38, 39]. However, at this stage, the *in vivo* performance of xenografts remains disappointing. High failure rates are associated with immunogenic residuals from the decellularization process, procoagulant production, thrombosis, etc. [47, 48]. Furthermore, the remaining collagen network is thrombogenic, which requires strategies such as heparinization [38, 49]. Although xenografts at this stage display such drawbacks, research has been carried out in the recent years, in order to improve their performance [38, 48, 50].

1.1.3 Synthetic Vascular Prostheses

Despite progress over the last decades with the use of stents or SV grafts, there is yet no golden standard that meets all requirements for guaranteeing absolute long-term patency [30, 33, 40, 51, 52]. Hence, intensive research in the development of synthetic grafts has been carried out [51, 53-55]. Artificial grafts made from expanded Polytetrafluoroethylene (ePTFE) and Poly(ethylene terephthalate) (PET) have been in clinical use since decades [51]. Large diameter artery grafting (> 8 mm) using ePTFE

or PET prostheses has led to satisfying results: ePTFE aortic grafts exhibit five year patency rates of 91-95% [56, 57] and PET as aortic bifurcation replacement grafts display a similar rate of 93% [56]. Both materials are non-degradable and mechanically stable over a duration of up to more than ten years, which makes them suitable for implantation [51, 53, 58]. ePTFE and PET prostheses experience adsorption of fibrin and platelets, which can be countered by modifying the luminal surface and anti-coagulation treatment [53, 55, 59].

Although a clinical solution for the replacement of large diameter arteries (> 8 mm) through ePTFE and PET prostheses has been established, their performance as small diameter (< 6mm) vascular prostheses remains poor [28, 35, 53, 55, 60-62]. They occlude within few weeks after implantation because of thrombosis and neo-intimal hyperplasia (NIH) [28, 35, 63]. While early graft failure is associated with acute thrombosis, long-term patency is principally attributed to a mismatch in mechanical properties, referred to as a compliance mismatch [17, 28, 35, 53, 64, 65]. The reason why materials like ePTFE and PET perform well as large diameter artery replacement is due to the high blood flow, which prevents thrombosis [35, 45, 51, 66]. The blood flow in such vessels is nearly laminar, which acts suppressive towards the development of NIH [25, 45]. Originally raised as a hypothesis by Baird et al. [67] in 1976, the issue of compliance mismatch has widely gained acceptance since then [68].

Compliance describes the ability of a material to deform elastically under applied stress [69]. In case of blood vessels/tubular structures, compliance (C) is defined as the degree of diametric distension ($\Delta D/D_0$) in response to pulsatile increases of pressure (ΔP). In the medical field, it is usually expressed in percent diameter expansion normalized by 100 mm Hg [49, 65]:

$$C = \frac{\Delta D/D_0}{\Delta P} \cdot 100\% [\% \cdot \text{mm Hg}^{-2}] \quad \text{Equation 1.1}$$

Compliance mismatch is caused by differences in the material stiffness between the vascular prosthesis and the grafted artery [65, 70]. This manifests itself in different radial expansion rates (Figure 1.2), which may induce a turbulent blood flow [65, 71]. As a consequence, the arterial tissue around the anastomosis gets remodeled, in order to match the wall-shear stress [71]. In case of poorly compliant vascular prostheses, the

intima at the recipient artery thickens. It is initiated by surgical injury to the endothelium at the anastomosis. On long-term scales, intimal thickening is primarily promoted by a compliance mismatch [65]. SMCs from the media switch their phenotype from contractile to proliferative and migrate to the intima, where they secrete large amounts of collagens to build ECM. The attracted inflammatory cells release growth factors, which promotes SMC proliferation. The newly formed intima (neo-intima) is highly disordered, due to the inability of the SMCs of organizing their secretory products. With continuous progression, more material accumulates in the neo-intima, which reduces the luminal cross-section and eventually leads into graft occlusion. This process is known as NIH [25, 72, 73]. Vascular prostheses that are overly compliant induce an adaptive outward modelling of the recipient arteries [71, 74].

Figure 1.2: Examples of vascular substitutes with compliance mismatch (green and blue). Stiff implants (green) expand their lumen insufficiently, compared to native arteries (red). The opposite is the case for too soft materials (blue).

Compliance matching is one of the key issues and sometimes addressed as a “holy grail”, for guaranteeing long-term success of vascular prostheses after implantation [64, 75-77]. However, the compliance issue is more complex than just targeting a single value: Like other biological tissues, such as skin, ligaments or tendons, arterial walls have the characteristic of being highly elastic at low strains and becoming increasingly rigid upon stretching [78-82]. This translates into high compliance at low blood pressure, while at high luminal pressure radial distension of arteries is reduced [82-84].

1.1.4 Blood Vessel Architecture

In order to understand the origin of the mechanical properties of arteries, it is important to look at its structural composition. The arterial wall comprises three layers: The *intima*, *media* and *adventitia* (Figure 1.3). The intima is the innermost sheet and consists of a monolayer of EC seated on a thin collagen-rich basal lamina, which is referred to as *endothelium* [17, 18, 85]. It plays an important role, as it is a non-thrombogenic layer that separates the arterial tissue from the blood. Additionally, it allows molecule diffusion (e.g. oxygen and nutrients) from and into the bloodstream [17, 85]. Large diameter arteries, such as the aorta, contain a *sub-endothelium* in addition [85]. With a total thickness of roughly 1.2-1.5 μm [85], the endothelium is very thin and thus of insignificant mechanical relevance in healthy arteries. In NIH or atherosclerosis this layer however thickens and becomes mechanically relevant [18, 85].

Figure 1.3: Structure of the arterial wall. The intima is innermost layer that faces the blood stream, the media is the elastic layer and adventitia at the outside provides structural reinforcement (reproduced with permission from [18], copyright Kluwer Academic Publishers).

Beneath the intima lies the media, the thickest layer in arteries. It is separated from the intima and adventitia by an internal and external elastic lamina. In between, the media consists of a complex network of elastin, collagen fibrils and SMCs, organized into helically, nearly circumferentially oriented lamellar units [18, 85]. Elastic arteries comprise multiple such elastic lamellar units of SMCs, which are separated by 3 μm thick elastin-rich layers, which are similar to the internal and external lamina. [85]. In muscular arteries, which are more abundant in the peripheral vascular system, the media contains more layers of SMCs than elastic lamellar units [85]. Elastic laminae, SMCs and elastin and collagen form a helix, which is close to circumferential alignment, due to its small pitch. Mechanically, the media represents the most significant layer within the arterial wall [18]. The media is furthermore capable of regulating vaso-activity, which is mediated through SMCs. They can regulate the blood pressure in response to several signaling molecules (e.g. nitric oxide (NO), prostaglandin E_2 , angiotensin II) through altering the blood vessel diameter [86-88]. However, SMCs also play an important role in vascular diseases: While in healthy arteries, the SMCs in the media are of contractile phenotype, which regulate mechanical function, in diseased arteries they switch to a proliferative phenotype. SMCs of the latter phenotype e.g. contribute to the build-up of an atherosclerotic plaque [3, 25, 86, 87, 89].

The outermost layer, the adventitia comprises about 10% of the arterial wall. It consists mainly of fibrocytes, fibroblasts and thick, wavy collagen bundles and elastin fibers. At low luminal pressure, the adventitia does not contribute much to the stiffness, due to the wavy configuration of the elastin and collagen fibers. The stiffness of the arterial walls at low blood pressure is mainly given by the media. With continuous unfolding of the elastin and collagen fibers, caused by an increase in the blood pressure, the stiffness of the arterial walls augments until they are fully straightened out, see Figure 1.4 [18, 90, 91]. The initial moderate increase in elastic modulus (E) is predominantly due to the unfolding of the soft elastin fibers ($E = 0.2\text{-}0.5 \text{ MPa}$ [90]), which is complete at around 80 mm Hg [81]. The steep increase in stiffness is provided by the collagen fibers ($E = 50\text{-}100 \text{ MPa}$ [90]), which fully align at 120 mm Hg [84, 90]. This non-linearity in elasticity (J-Curve) gives the adventitia a protective role in the prevention of aneurysmal dilatation or rupture, due to hypertension {Holzapfel, 2000 #24; Singh, 2015 #252; Holzapfel, 2000 #42}.

Figure 1.4: a) The stress-strain curve of arterial wall tissue, indicating non-linear elasticity. b) The waviness of elastin and collagen fibers unfold with increasing blood pressure, which create a non-linear elastic response upon elongating. Once straightened out, they add stiffness, rendering the blood vessels less compliant [84].

The progression from low to high modulus of arterial walls can be translated into a transition from high to low compliance, see Figure 1.5 [81, 82, 91]. It is fully reversible and an important characteristic of the arterial walls. From Figure 1.5 it can be seen ePTFE and PET (Dacron) are too stiff and do not possess the non-linear elasticity feature of arteries and veins. Thus, at low blood pressure the mismatch in compliance is even greater. This is the reason behind the poor patency rates of various synthetic vascular prostheses [65, 82]. In this work, the transition from high to low compliance is paid most attention to and aimed to be mimicked.

Figure 1.5: Compliance change with increasing blood vessels for arteries (red), veins (blue), compliant poly(carbonate)polyurethane (CPU, green), PET (Dacron, brown) and ePTFE (black). The compliance of arteries and veins is high at low blood pressure, while CPU, PET and ePTFE are much stiffer. With increasing blood pressure, the compliance of arteries and veins gets continuously reduced, becoming more similar with PET and ePTFE. The compliance mismatch at low pressures is more severe, compared to at higher levels [45, 82] (reproduced with permission from [45], copyright Elsevier).

1.2 Hypothesis

The compliance and non-linear stress-strain behavior of native small diameter arteries can be matched through fabricating a vascular prosthesis composite of an elastic tube made with a designed stiffer outer mesh construct.

1.3 Objectives and Scope

The aim of this work was the fabrication of a synthetic vascular prosthesis that can match the compliance and mimic the non-linear elastic behavior of native arteries. These grafts were chosen to be made from biodegradable elastomers of elastic moduli in the range of arterial tissue. High attention was paid to the co-polymer Poly-L-lactide-co- ϵ -caprolactone (PLC), which properties can be altered through the monomer ratio of L-lactide (LA) to ϵ -caprolactone (CL) [92-94]. Hence, PLC polymers of different monomer composition were tested, in order to find the most suitable material to work with.

A reproducible dip-coating method that allows the fabrication of tubular vascular grafts of uniform wall-thickness (WT) was established. The protocol has been optimized to make it suitable for all used materials and enabling the fabrication of tubes with WT from 100 – 250 μm .

As no “off the shelf” device for compliance measurement is readily available, a custom-built setup that fulfils the ISO analysis [95] requirements has been established. The setup was designed to allow simultaneous recording of the vascular graft’s diameter and the luminal pressure, for compliance determination. The apparatus was used to study the compliance behavior in a static and dynamic mode.

A bi-layered composite structure for synthetic tubular grafts was considered, in order to mimic the mechanical properties of arterial tissue. The tubes were consisting of a soft inner lining and a stiff outer shell and were fabricated through dip-coating. They were tested on their compliance properties. High attention was paid to the occurrence of non-linear elasticity. In addition, the bi-layered tubes were assessed on their creep behavior, strain recovery and kink-resistance.

Another strategy to achieve non-linear elastic vascular prostheses was pursued by winding melt-spun fibers at the outside of a dip-coated tube. These tubes were analyzed on arterial-like compliance behavior. Hence, various tube and fiber materials combinations and fiber spinning morphologies (fiber diameter and circumferential or helical winding) were tested.

As third approach to fabricate a prosthesis with compliance properties similar to arteries was followed by combining a soft dip-coated tube with a pliable outer 3D-bioprinted mesh (J-Mesh). The fabrication and mechanical characterization of these composite tubes comprised the major part of this thesis. The J-Meshes contained transversal fibers with pliable springs (hoops) that can unfold at low modulus. In a first set of experiments, the pliable fibers were analyzed on their tensile properties. The effects of fiber material, strut thickness and hoop geometry on the mechanical properties were evaluated. In parallel, computer simulations of fiber stretching were carried out using Finite Element Method (FEM) software. In a second set of experiment, the composite tubes (dip-coated tube with pliable outer mesh, JM-Tubes) were tested in the compliance apparatus. Of primary interest was the behavior of the J-Mesh during unfolding and its effect on the tube's compliance.

An *in vivo* experiment was carried out in a rabbit (short-term), in order to test a dip-coated PLC tube on its surgical properties (ease of handling and suture retention) and mechanical stability (e.g. not producing aneurysms or sutural bleeding). A second set of animal tests was performed in two pigs during two weeks, evaluating the *in vivo* performance of the JM-tubes.

1.4 Dissertation Overview

The aim was to design non-linear elastic properties of a vascular graft rather than using random scaffolding approaches to obtain arterial-like compliance. Therefore, several steps were carried out. First, the mechanical properties of various PLC polymers of different monomer rations, in order to find an optimal material for a dip-coated tubular vascular graft. After a suitable material was found to create a tubular prosthesis with satisfying compliance, adventitial-like outer structures were fabricated to induce non-linear elastic properties to the tubular grafts. Eventually, a novel design of a composite

graft of an elastomeric tube and a pliable stiffer outer mesh was developed. The designs of the meshes were optimized, to obtain non-linear elastic properties.

1.5 Findings and Outcomes

In a first step, a dip-coated vascular graft with a compliance matched to native arteries was developed by testing various PLC polymers of different monomer ratios. PLC 70:30 displayed satisfying mechanical properties: low creep, compliance in the range of native arteries and good kink-resistance. Furthermore, 150 μm WT PLC 70:30 tubes exhibited good surgical properties and performed well in a short-term *in vivo* experiment. Non-linear elasticity could be achieved by adding a fibrous outer layer to a PLC 70:30 tube. Melt-spun fibers showed occurrence of non-linear elasticity but the novel developed composites of an elastomeric tube with a stiffer pliable outer mesh (JM-Tubes) promised better control over the compliance. The designs of the JM-Tubes were optimized over various experiments so that they exhibited non-linear elasticity. Although the JM-Tubes require further optimization, the control over the compliance was thought to be greater than for the melt-spun fiber tubes, since the mechanical properties of the meshes can be designed through the spring geometry.

1.6 Novelty of the Thesis Work

The aim of this thesis was the fabrication of a vascular prosthesis that mimics arterial-like compliance. The focus was set on the composite structures of dip-coated tubes with pliable outer meshes as described in Chapter 6. There are a few other studies that pursued the same goal [96-102]. Although these studies managed to demonstrate the occurrence of non-linear elasticity, some of their approaches appear rather empirical than engineered. These studies fabricated hydrogels, electrospun composites or combination of the both. They lack reproducibility in their patterns or the ability of designing the compliance. They neither include any study on geometrical modulus [96, 98, 101].

Other publications that focus on the study of geometrical designs to alter the mechanical properties strongly differ in their designs from the here proposed “J-Curve Mesh Tubes” (JM-Tubes) [81, 97, 100, 103]. The closest designs from previous published work

include Bezuidenhout's [102] vascular graft with an adventitial fabric, Chen et al.'s [97] bi-layered tubes with a stiffer crimped outer layer and Loewen's [103] warp-knitted ElaGrafts. The latter study analyzed the impact of knitting parameters on the geometry and the resulting tunable non-linear elastic properties [103].

This work proposes a composite of a soft tube with a pliable stiffer outer mesh (JM-Tubes). The latter is a 3D bio-printed structure that consists of spring-like fibers. A major advantage of 3D-printing is its ease of customization and precision [104, 105]. This allowed to select a set of geometrical parameters to study their effect on the tensile properties of these fibers. This study could demonstrate that all chosen parameters have a significant impact on the unfolding properties of these spring-like fibers. We were able to conclude that this enables a high control over tailoring the mechanical properties of the meshes. Therefore, the JM-Tubes show a great potential for the fabrication of patient-specific vascular prostheses with appropriate diameter and compliance.

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Chapter 2

Literature Review

This chapter summarizes the early and recent efforts in the development of vascular prostheses. Various techniques and materials (synthetic and natural) for vascular graft fabrication are listed in this chapter. This review presents publications on vascular substitutes, with a focus on studies dealing with compliance.

2.1 Vascular Prostheses – Early Research

Vascular surgeries date back in the 18th century, where Hallowell and Lambert repaired a damaged brachial artery through suturing [1]. In 1948 Kunlin carried out a vascular surgery using a reserved saphenous vein (SV) graft as femoropopliteal bypass. This surgery was a milestone, as SV grafts remain up to this day the golden standard for vascular substitutes. Surgical application of synthetic vascular prostheses started in 1952, where Vorhees [2] implanted a Vinyon “N” tube as aortic graft in a canine experiment. Only two years later, in 1954, the first human trial was practiced, using the same type of prosthesis as abdominal aorta replacement [1].

During the 20th century the two principal polymers used for synthetic vascular grafts were discovered: Polytetrafluoroethylene (PTFE) and Polyethylene terephthalate (PET, Dacron) were synthesized by DuPont in 1937 and 1950 respectively [1, 3, 4]. Both materials are highly crystalline and hydrophobic, properties that make them resistant towards hydrolysis, which is desired for long-term applications [1, 3]. PTFE does not radially dilate over time, whereas PET grafts do, but only rarely causes complications. Both materials present a non-thrombogenic surface, which is a principal reason for their wide clinical use up to today. PET was applied for the first time as arterial substitute in 1957 as woven fiber tube [1, 5]. The use of PTFE in vascular surgery started in the early 1960s, where it was used as mitral heart valve replacement [3, 6]. A major advance was the development of expanded PTFE (ePTFE, Goretex) in 1969 by Gore [1]. The unique properties of ePTFE with its non-degradability, non-clotting and non-fouling surface (due to the high electronegativity) made this material suitable for vascular prostheses [1, 3].

ePTFE and PET have since been in constant use, predominantly as large diameter (> 8 mm) arterial substitutes [7-12]. The development of small diameter vessel prostheses remains a challenge up to this day [13, 14]. For a long time, the reasons behind the failure of ePTFE and PET for small diameter replacement were unknown, until Baird [15] proposed the occurrence of compliance mismatch. His hypothesis became widely accepted, despite remaining untested until 1986 [15, 16]. This discovery impacted on the development of vascular prostheses, where importance is given to match the mechanical properties of the grafts to native arteries [5, 17].

2.2 Vascular Prostheses - Recent Advances

Early vascular substitutes were biostable and designed to be non-clotting. Recent vascular graft development encounters compliance matching, luminal surface modification for EC-attachment and biodegradability. Synthetic materials used as vascular prostheses present a potentially thrombogenic luminal surface, which may cause complications after implantation. This risk may be lowered by surface modification, which enables EC-attachment and the formation of a confluent monolayer [18-20]. The use of biodegradable materials allows tissue ingrowth and remodeling by cells, which is not possible with biostable materials [21].

2.2.1 Fabrication Techniques

Melt-Extrusion

Melt-extrusion is one of the most widely used processing methods in industry. More than half of all plastic products are manufactured by melt-extrusion. This involves plastic bags, sheets, pipes etc. [22]. Melt-extrusion is also applied in pharmaceutical industry, e.g. for the fabrication of synthetic vascular tissue [22, 23].

The extrusion machine is fed with raw material, which gets heated and moved towards the die by a single or two rotating screws [22, 24]. The shape of the final product is given by the die used. Melt-extrusion is a continuous process, which makes it to a high output rate technology [22]. However, as a result of operating at high temperatures, melt-extrusion of polymers may lead to thermal degradation, which lowers the molecular weight [25].

Electrospinning

Electrospinning was discovered by Raleigh in 1897 and found its application in biomaterial fabrication around the beginning of the 21st century. In the past decade its potential for the fabrication of synthetic scaffolds has been recognized, and numerous studies and patents have emerged since [8, 21].

Electrospinning produces scaffolds with randomly deposited fibers from a liquid jet in a high-voltage electric field. A polymeric solution is forced through an electrified spinneret via syringe pump towards a grounded collector. Passing the capillary, the polymer solution gets charged and forms a jet, due to the electrostatic repulsive force that exceeds the surface tension [8, 21, 26]. In the high electric field (typically 10-30 kV [8]), the descending polymer jet becomes unstable and whips at high frequencies [8, 21]. This itself induces additional straining, due to bending and stretching [21, 26]. Simultaneously with the whipping, the solvent evaporates, turning the jet into a fiber. Additionally, the fast whipping leads into random deposition of thin fibers (3 nm to 5 μm [26]) on the collecting substrate, where they get grounded and the solvent evaporates [8]. Figure 2.1 summarizes the electrospinning process

The diameter and pattern of the deposited fibers are affected by various parameters, such as voltage, needle size (spinneret size), flow rate, gap distance (distance between the spinneret and the collector), type of collector, collector rotation speed (in case of a rotating collecting substrate) polymer, solvent, polymer concentration (viscosity) and molecular weight of the polymer [8, 26].

Figure 2.1: Electrospinning process, showing a liquid polymer jet being pushed through the nozzle. The polymer jet at the spinneret gets charged due to the high voltage applied. While dropping on the collection substrate, the generated electric field causes the jet to whip at high frequencies. The fast whipping causes a random fiber deposition on the substrate. The pattern

of the deposited fibers is affected by the flow rate, voltage, gap distance (d) etc. [26, 27] (reproduced with permission from [27], copyright WILEY-VCH).

Electrospun scaffolds are three-dimensional and porous structures that resemble the extracellular matrix (ECM). A great advantage of such scaffolds is their penetrability by host's cells, which enables remodeling [28]. Furthermore, electrospinning allows the inclusion of biomolecules of even eukaryotic cells, enabling the engineering of bioactive tissues [29].

Dip-coating

Dip-coating enables the fabrication of films on substrates of various cross-sectional shapes [30]. Films are produced by a single or multiple immersions of a substrate into a suspension or solution [31, 32]. With every withdrawal from the suspension/solution, an additional layer deposits on the substrate. The substrate can be functionally coated, for example with a protective or conductive layer or the substrate may serve as a template for the fabrication of hollow samples [30, 31, 33, 34]. The latter approach can be used to produce tubular structures, such as vascular substitutes [31, 35].

The film thickness can be controlled through various parameters, which include withdrawal speed (higher speeds leads to more material deposition), solution viscosity (polymer, molecular weight, solvent and/or concentration) and number of dips [30, 31, 36]. Vascular prostheses have been fabricated via dip-coating and have been used for *in vitro* and *vivo* testing [31, 35].

Melt-Spinning

In melt-spinning (also known as melt-drawing), fibers of molten polymers are wound around a rotating substrate [35, 37-39]. A polymer is heated above its melting temperature in a heated spinneret. Once the polymer is molten, it is drawn by gravity. The pulling force of the rotating collector shapes the dropping polymer mass into a fiber. With ongoing spinning, more polymer is being pulled through the spinneret [37, 38].

In contrast to electrospinning, melt-drawing is less random, as the wound fibers are aligned circumferentially around the substrate. Therefore, the patterns created by melt-spinning are more reproducible than electrospun scaffolds [37]. Another major difference is the advantage of melt-drawing being a solvent-free technique, which reduces potential toxicity and does not require additional drying steps [37-39].

The morphology and mechanical properties of the fiber can be tailored through the spinning speed of the rotating collector. Increased rotation speeds apply a higher tensile force to the descending polymer fiber, resulting in the deposition of thinner fibers. In addition, the stronger tensile force leads to a longitudinal alignment of the polymer chains, which facilitates the formation of crystalline domains, rendering the fibers stiffer [38, 39]. Higher spinning speeds also reduce the linear density of fibers on the substrate. The same is the case, if the polymer is heated to higher temperatures. However, there is a risk of fiber fusion upon deposition, as they may not cool down sufficiently [37].

Although the produced fibrous patterns are more reproducible than in electrospinning, melt-spinning has drawn less attention from cardiovascular researchers, as it does not enable the fabrication of ECM-like tissues [37]. Studies that involve melt-spinning in the development of vascular grafts, use this method for the fabrication of composite structures [35, 40].

3D-Printing

3D-printing was reported first by Charles Hull in 1986 under the name “stereolithography”. In the recent years, this technology has drawn increasing attention from researchers [41, 42]. 3D printing enables the manufacture of complex geometrical structures, which has been used for the fabrication of medical devices, such as blood vessels, cartilage, heart valves etc. [41]. Recently, a new technology of 3D-printing has evolved in the biomedical field, known as “3D-bioprinting”, which involves biological materials, cells, biomolecules etc. In vascular research, 3D-bioprinting has been used to produce tubular grafts or patterning of scaffolds, for example to improve mechanical properties or cell alignment [42-45]. The production of ECM-like structures using 3D-

bioprinting remains still a challenge [41]. However, with electrospinning and decellularization of native tissue, established alternatives for the production ECM-like structures already exist [13, 45].

2.2.2 Popular Synthetic Materials in Bioengineering

Various materials are used in the research for the development of vascular prostheses. Many publications are found on poly-urethanes (PUs), Polyglycolicacid (PGA), Poly-lactide-*co*-glycolide (PLGA), Poly-L-Lactide (PLA), Poly- ϵ -caprolactone (PCL), Poly-L-lactide-*co*- ϵ -caprolactone (PLC), Poly-hydroxy-alkanoates (PHA) and Polyglycerol-sebacate (PGS) [7, 8, 13, 31, 46-48]. Initially PLA, PLGA and PGA gained high attention. However, they are brittle, prone to plastic deformation and overly stiff, thus lacking suitability for the production of compliant blood vessel substitutes [48, 49]. Hence, the attention has shifted more towards elastomeric materials like PUs, PCL, PLC, or even natural materials like collagen, elastin and decellularized ECM [45, 48].

Poly- ϵ -Caprolactone

PCL is a semi-crystalline polyester with high toughness and flexibility. It is a biodegradable polymer that decomposes via hydrolysis or enzymatic degradation. In comparison to PLA it takes longer for PCL to degrade, making it suitable for long-term implantations. PCL has a low melting point (57°C) and glass transition temperature (-61°C), which makes it soft and rubbery at room temperature [50]. Because of its high biocompatibility, PCL has been used in many studies of vascular grafting in vitro and in vivo [9, 50-52]. The structure of PCL is shown in Figure 2.2:

Figure 2.2: Structure of PCL. The repeat units are connected by an ester-bond (adaption from [53]).

Sevostyanova et al. [51] fabricated 2 mm diameter vascular substitutes with a wall-thickness (WT) of 100 μm of PCL via electrospinning. The vascular grafts were assessed on their blood compatibility, elasticity and *in vivo* performance. As controls commercially available “KemAngioprotez” prostheses were used. The electrospun PCL tubes have shown to be more elastic than the controls, but hemocompatibility tests revealed the adherence of platelets. The PCL tubes were implanted into rats to graft the abdominal aorta for six weeks. The grafts allowed cell infiltration and their inner surface was covered with ECs. The zones of anastomosis showed signs of neo-intimal hyperplasia (NIH). No compliance tests were carried out, to assess whether NIH was associated with a mechanical mismatch between the PCL tubes and the grafted arteries.

In a similar study reported by Mrowczynski et al. [28], electrospun PCL grafts were implanted into pigs for one month. The electrospun tubes had a luminal diameter of 4 mm and a WT of 500 μm . As controls ePTFE prostheses were used. The PCL tubes have shown good surgical properties and did not undergo mechanical failure during the entire implantation period. The patency at the end of the study was 78% for the PCL grafts, compared to 67% for the ePTFE grafts. The PCL graft allowed cell ingrowth and endothelialization. One of the grafts failed due to thrombosis, which was attributed to a mechanical kink. The occurrence of NIH was associated to a mechanical mismatch between the graft and the arteries. However, no compliance data was available to support this conclusion.

Electrospun vascular substitutes of 2 mm inner diameter and 650 μm WT were fabricated and tested by de Valence et al. [9]. Long-term *in vivo* studies (up to 18 months) were carried out in rats. The grafts performed well in the first 6 months, showing endothelialization of the scaffold. No thrombosis or mechanical failure was recorded, and NIH occurred to a limited extent only. The following 12 months were marked by cellular regression and calcification. *In vivo* compliance measurements were carried out, which revealed a mismatch between the prosthesis and the grafted arteries: The PCL tube’s compliance was 37% of that of the grafted arteries (100%).

Vascular electrospun PCL/chitosan grafts of 1 and 5 mm diameter were assessed on their *in vivo* performance by Fukunishi et al [54]. The 1 mm prostheses were implanted

into three mice as “infrarenal abdominal aorta interposition conduits”, whereas the 5 mm diameter vascular substitutes were used for grafting the carotid artery in six sheep. All 1 mm grafts remained patent, while patency for the 5 mm prostheses was 67% after six months. The grafts were degraded to large parts, with 9.1% of the initial mass remaining. Histological analysis showed elastin and collagen deposition, endothelialization and circumferentially oriented contractile smooth muscle cells (SMCs). Indeed, the 1 mm diameter grafts performed well in the mice model, by maintaining patency and neither showing signs of thrombosis nor aneurysmal dilatation. However, when larger animals (dogs, sheep or pigs) are used the outcome may differ as that have higher physiological similarity with humans [55-57].

Poly-L-Lactide-co- ϵ -Caprolactone

PLC has drawn high attention from scientists in cardiovascular research due to its biocompatibility, degradability and mechanical tunability [48, 58, 59]. The molecular structure is shown in Figure 2.3:

Figure 2.3: Synthesis and structure of PLC. PLC is synthesized by a ring-opening copolymerization (adaption from [59, 60]).

It is a segmented copolymer, consisting of hard L-lactic acid (LA)- and soft ϵ -caprolactone (CL)-rich domains [61]. The homopolymers of both monomers are stiff, because they are highly crystalline. PCL is moderately stiff ($E = 250$ MPa [58]), whereas PLA is comparatively very rigid ($E = 1720$ MPa [58]). Both of its monomers (LA and CL) are immiscible and tend to phase separation. Therefore, doping LA-rich blocks with the co-monomer CL and vice versa leads into the disruption of the crystalline domains. The resulting PLC polymers are more amorphous and thus softer [61]. Extensive research on the mechanical properties of PLC polymers with different monomer feed ratios has been carried out. It was found that PLC with LA contents of 30-40% are very soft and mechanically weak, while PLC polymers with 50-70% LA are elastomeric. Other PLC polymers (with more than 70% or less than 30%) are stiff, as they have higher degrees of crystallinity, see

Figure 2.4 [58, 59]:

Figure 2.4: Stress-strain curve of a) melt-pressed PLA, PCL and PLC films [58] and b) electro-spun PLC fibers: $\diamond$ PLA, $\circ$ PLC 70:30, $\square$ PLC 50:50, Δ PCL [59]. PLC copolymers of rather equal LA:CL ratios (PLC 40:60 and 50:50) are highly elastic and very soft, while PLA (named

as PLLA in a), PCL and PLC 20:80 are stiffer (reproduced with permission from [58], copyright John Wiley & Sons, Inc; and from [59], copyright Elsevier).

The use of PLC as compliant vascular grafts has been reported in many studies [12, 17, 60, 62]. It exhibits high biocompatibility and has been used successfully in clinical trials [48, 59, 63, 64].

Inoguchi et al. [17] fabricated electrospun tubes made from PLC 50:50 and evaluated the effect of the WT on the mechanical properties. The tubes had inner diameters of 2.3 mm and WT of 50, 115, 215 and 340 μm . Thin-walled PLC grafts have shown to be more compliant ($18.7 \pm 11.2\% \cdot \text{mm Hg}^{-2}$) than those with thick walls ($2.0 \pm 0.2\% \cdot \text{mm Hg}^{-2}$). The greatest difference was found between grafts of 50 and 100 μm WT. However, compliance measurements were carried out by applying pressure intervals of 45 to 90 mm Hg, which is below the required testing level of 80 to 120 mm Hg as described in the ISO 7198:2016 [65].

Vascular prostheses of electrospun PLC 50:50 with 0.1% collagen I coating were fabricated by Jang et al. [66] and tested *in vivo*. The bi-layered sheets were seeded with fibroblasts and rolled up to produce lamellar tubes of 4 mm diameter. Thinner sheets allowed better mass transfer and permeability than thicker ones. Thus, cell proliferation was better for tubes produced from thin sheets. These tubes were cultured in a bioreactor under static and dynamic conditions. Culturing under dynamic conditions provided a more cell friendly environment, which was observed from stronger increases in the cell count and burst strength after 4 weeks of culturing. Static culturing showed only minor improvements. *In vivo* experiments in mice corroborated the benefits of using tubes made from thin sheets. Cell proliferation on 200 μm thick sheet was high and remained stable over four weeks. In comparison, the number of cells on 300 and 500 μm thick sheets initially largely increased but was followed by a regression after two weeks.

Bi-layered tubular grafts of PLC were fabricated by Kim et al. [67] using gel-spinning and salt-leaching techniques. The effect of WT (1, 1.1, 1.3, 1.4 and 1.5 mm) on the compliance and burst strength was evaluated. Dynamic compliance tests revealed thin-

walled tubes were more compliant but tended to burst at lower pressures than thicker grafts. Grafts of 1 and 1.3 mm WT were coated with collagen/fibronectin (col/FN) and then implanted into dogs for 4 weeks. The tubes of 1.3 mm WT showed thrombosis and occlusion due to a compliance mismatch. The more elastic 1 mm WT tubes showed signs of thrombosis, but no severe occlusion.

Poly- ϵ -Caprolactone-*co*-Glycolide

Poly- ϵ -caprolactone-*co*-glycolide (PCG) has recently gained the attention of scientists and has been used in the research on vascular substitutes [48]. Like PLC, it has tunable mechanical properties, which depend on the monomer feed ratio of CL to glycolide (GA) [59, 68]. PCG polymers of equal monomer feeds have shown to be elastomeric ($E = 23$ and 60 MPa for PCG 35:65 and 40:60 [68, 69]) [49, 68, 69]. It was observed that PCG 50:50 reached strains up to 250%, whereas PLGA could be stretched up to only 10-15%. Furthermore, PCG 50:50 recovers high deformations very well (96% recovery after elongation to 230%). In addition recovery after exposure to cyclic strains of 20% during six days has been 96% [49], compared to PLGA that recovered by only 85% when strains of 3% were applied [49].

An implantation study of an SMC seeded scaffold in mice confirmed the biocompatibility of PCG. Alongside its elastomeric properties, PCG can be seen as an attractive material for sutures and tissue engineering. For the use as vascular prostheses, the fast hydrolytic degradation of PCG (50% mass loss in phosphate-buffered saline [PBS] after six weeks [48, 49]) might be advantageous, as it may lead to rapid remodeling and hence lower the risk of NIH [70]. Further studies have to clarify, whether the degradation and tissue remodeling rate are appropriate for the use as vascular grafts [71].

Yoo et al. [72] demonstrated the potential of PCG as suture material. In vivo experiments showed occurrence of inflammation after two days, but not after 91 days. From these results it was inferred, the PCG sutures were already fully degraded after

91 days, hence no inflammation was left behind at this point. Furthermore, cytotoxicity and biocompatibility tests were positive. These outcomes were found to be promising to use PCG as a material for biodegradable sutures.

Poly-Hydroxy-Alkanoates

Poly-Hydroxy-Alkanoates (PHAs) comprise a family of biocompatible polyesters, which are synthesized by over 60 microorganisms [50]. PHAs consist of multiple hydroxyl-acid unit monomers with side chains of typically 3 to 14 carbon atoms in length. While PLA and PGA comprise two hydroxy-acid units, PHAs consist of three to six units. The most used PHAs consist of 3 of such units. Their mechanical tunability lies in the choice of side chain-length and distance between the ester linkages in the backbone. Short-chained PHAs (propyl- to pentyl-) tend to be crystalline and rigid, while long-chained PHAs (side chains of 6-14 carbons length) are soft. There are two groups of bacteria that either produce short (*Alcaligenes eutrophus*) or long-chained PHAs (*Pseudomonas eleovorans*) [50, 73]. PHAs, e.g. polyhydroxybutyrate (PHB) and 3-hydroxyhexanoate (PHBHHx), are commercially available, available by various companies at cheap prices of a few US Dollars per kilogram [74].

Qu et al. [47] evaluated PHA films of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) on their potential as biomaterial for vascular substitutes. PHBHHx films were fabricated via solvent casting and coated with FN after plasma treatment. FN-coated and pristine PHBHHx films were seeded with human umbilical ECs (HUVECs) and rabbit aorta SMCs and evaluated on their bioactivity. FN-coatings have shown to better promote HUVEC proliferation. The scaffold allowed them to form a confluent monolayer within 3 days only. However, FN-coatings only poorly supported proliferation of SMCs, as they could not even form a sub-confluent layer. The study concluded FN-PHBHHx being a suitable material as luminal lining for vascular prostheses.

Poly-Glycerol-Sebacate

Poly-glycerol-sebacate (PGS) is a copolymer of glycerol (GL) and sebacic acid (SA). Its synthesis was first reported by Wang et al. in 2002 and hence, it is still a relatively new polymer [75]. Both monomers (GL and SA) are biocompatible and can be metabolized. PGS is synthesized through a polycondensation reaction and requires further cross-linking (i.e. post-polymerization, under high vacuum at temperatures greater than 100°C), in order to improve its mechanical strength. A low density of cross-links is required for preserving the elastomeric character of PGS, as it would otherwise turn rigid [75]. Like other polyesters, PGS undergoes a surface mediated hydrolytic degradation *in vivo*. Surface mediated degradation leads to a slow and gradual decrease in mass and mechanical strength, in comparison to bulk degradation, which is marked by abrupt loss in mechanical strength. [46]. Increasing the density of the ester bonds hinders enzymatically controlled degradation (e.g. through lipases), which makes gradual hydrolysis the primary mechanism of decomposition [46, 76, 77]. Hydrolysis is a favored degradation mechanism as it is surface- instead of bulk-mediated and leads into a gradual loss in mechanical strength [46, 78].

PGS is a soft elastomeric polymer with moduli ranging from 25-1200 kPa, comparable to the stiffness of myocardial tissue or ligaments (20-500 kPa) [46, 76]. This polymer has the great advantage of tailorable mechanical properties through various parameters: Cross-linking temperature (curing temperature), cross-linking time (curing time) and ratio of GL to SA. Longer curing times and exposure of the pre-polymer to higher temperature lead to a higher degree of cross-links, which renders the polymer stiffer. However, the density of cross-links affects the degradation only to a minor extent [76]. Furthermore, incorporating more GL increases rigidity of PGS copolymers [46].

Animal tests in rats have also shown that the material is fully resorbed *in vivo* within 60 days [79], and no scar tissue formation at the implantation site was observed [46, 50]. As PGS is normally synthesized without any catalyst or additives, its degradation does not release any toxic products [46, 48]. Further biocompatibility tests have shown PGS serves as a good substrate for EC and SMC adhesion and proliferation. Together with its mechanical properties, PGS is an attractive material for vascular prostheses [46, 50].

Compliance measurement on small diameter vascular substitutes was carried out by Crapo et al. [80]. Porous PLGA and PGS tubes were seeded with SMCs and incubated for 10 days. Elastin and collagen deposition were found in both tubes, which increased their strength significantly. The PGS grafts were similarly compliant to porcine carotid arteries and exhibited high elasticity with complete strain recovery. In contrast, PLGA tubes were stiffer than the porcine arteries and showed the occurrence of plastic deformation. The burst pressure of the SMC-seeded PGS tubes increased with longer incubation times. After 10 days it was 27 ± 19 mm Hg, which is still well below the physiological blood pressure of 80-120 mm Hg [81] and bursting strength of human saphenous veins (HSV) of 1599 ± 877 mm Hg [82]. The porosity of the grafts was assumed to be the main reason behind the low burst pressure.

The hemocompatibility of PGS was compared, by Motlagh et al. [83] to that of glass, ePTFE and PLGA. Platelet adhesion and aggregation as well as the expression of inflammatory signals (IL-1 β and TNF α) were assessed. These tests revealed PGS was superior to the other materials: Platelet adhesion to PGS was low (28% relative to glass [100%]), whereas it was higher for ePTFE (61%) and PLGA (100%). Moreover, human monocytes (THP-1) on PGS expressed much less IL-1 β and TNF α than on ePTFE and PLGA. This study underlines the benefits of using PGS for the as blood contacting material.

Poly-Diol-Citrates

Poly-(diol-citrates) (PDCs) comprise a family of cross-linkable polyesters with elastomeric properties. The three carboxyl groups of citric acid react with the hydroxyl groups of the aliphatic diol monomers through a polycondensation reaction. Among the aliphatic diols, octanediol is the largest monomer that is water soluble. PDCs are biocompatible, inexpensive and have non-toxic degradation products, which makes them attractive for biomaterial research.

Poly-1,8-octanediol-citrates (POCs) are synthesized in a two-step polymerization: An initial polycondensation of octanediols with citric acid, followed by a post-polymerization. The second process is time consuming, which takes a few days up to

two weeks [50, 76]. Other considerable PDCs are poly-1,6-hexanediolcitrate (PHC) poly-1,10-decandiolcitrate (PDCC) and poly-1,12-dodecanediolcitrate (PDDC) [31, 76].

PDCs degrade via hydrolytic cleavage of their ester bonds. In case of POC, the rate increases if the molar ratio of octanediol to citrate is high or the degree of cross-linking is low [50]. POCs were shown to be a suitable substrate for EC attachment, in comparison to ePTFE, and confluent layers were achieved in tissue culturing [4, 84, 85]. It is assumed that POCs are superior to PGS in biomedical applications, because of their better mechanical properties [50].

Dip-coated tubular scaffolds of POC and PDDC were fabricated by Yang et al. [31]. The tubes were biphasic, consisting of an inner non-porous POC lining and an outer porous PDDC shell. Burst pressure, compliance and biocompatibility were evaluated. Burst pressure of 250 and 1000 mm Hg were measured for POC and PDDC grafts (increasing burst pressure with WT from 150 to 500 μm). Compliance was measured only for PDDC tubes, which was ~ 22.5 for thin and $\sim 32\% \cdot \text{mm Hg}^{-2}$ for thicker tubes. The scaffolds allowed tissue ingrowth and supported differentiation of ECs and SMCs. The study underlined the potential of such biphasic scaffolds as use for tissue engineered blood vessels.

Segmented Poly-Urethanes

Polyurethanes (PUs) are one of the widest used biomaterials today. PU was discovered in 1930 and its application in biomedicine began thirty years later. Doubt was raised about the suitability of PU in the 1970s due to the identification of toxic degradation products, such as aromatic diamines [48, 86]. However, since the 1990s the biocompatibility of PUs has been greatly improved, thus resurrecting interest in PU as a biomaterial. PUs consist of two classes: Thermoplastics and thermosets, where the first is a lot more widely used as elastomeric biomaterial [48].

Thermoplastic PUs are soft-hard segmented polymers consisting of three units: Macrodiol (MD), diisocyanate (DI) and chain extenders (CE). The synthesis of

thermoplastic PUs is carried out in two steps: A prepolymer formation (oligomer) through MD reacting with DI and a later connection by CE to obtain PU. The hard segments in PUs are formed by DI and the CE and the soft segments consist of MD (Figure 2.5). The hard segments are either crystalline or glassy and reinforced through established hydrogen bonds, whereas the soft segments are either semi-crystalline or amorphous. The mechanical properties of PUs can be tailored by the choice of monomers or altering the composition (ratio of hard to soft segment content) [48].

Figure 2.5: Structure of segmented thermoplastic PUs with crystalline hard and soft segment regions, which are either semi-crystalline or amorphous (reproduced with permission from [87], copyright Elsevier).

When DIs are selected to form the hard segment, the resulting PUs are normally quite resistant to degradation, while aliphatic DIs enable degradation *in vivo*. The degradation of the soft segments can be accelerated by increasing their length and hydrophilicity [48]. Recently, the development of biodegradable PUs has gained increasing attention. The introduction of degradable sequences into the backbone has been the key for that. Although there is a large set of biodegradable PUs, not all are biocompatible. PUs featuring aromatic DIs release toxic degradation products, whereas aliphatic DI PUs are biocompatible, due to their non-toxic degradation products [48]. Commonly, their backbone sequences are 6-hexamethylene DI, 4,4-dicyclohexylmethane-DI and isophorone-DI. The polymers PCL, PLA or PHAs are widely used as soft segment components.

Current research also includes the use of peptides or amino acids as CEs, to enable degradation (e.g hydrolytic degradation) [87, 88]. The principal decomposition mechanism for these PUs is hydrolysis, which may be accelerated by hydrolytic enzymes. The degradation rate can vary due to the polymer composition, indeed, it can be tuned by the choice of monomers, degree of crystallinity (less crystalline domains leads to faster degradation), molecular weight and density of hydrogen bonds [87]

In a study carried out by Guan et al., poly(ester-urethane)ureas based on PCL diols, 1,4-diisocyanatobutane and putrescine or lysine ethyl ester as CE were synthesized. The PUs showed elastomeric properties and were biodegradable. Choosing lysine ethyl ester as CEs turned the PUs more susceptible to degradation, displaying mass loss of ~40-60% after 56 weeks in 37°C PBS. On the other side, putrescine CE-based PUs were more resistant, losing only ~5% of their mass in the same duration [89].

Other workers chose to utilize biostable (long-lasting) materials in the development of a prosthetic graft with long-term compliance [90]. Examples of biostable PUs used in vascular prosthesis development are poly(carbonate-urea)urethane “Myolink™”, Poly(carbonate urethanes) “Carbothane®” (with 4,4'-Methylene dicyclohexyl DI soft segments) and “Chronoflex®” (Methylene diphenyl DI as soft segment) [90-92]. These polymers are suitable for the fabrication of compliant vascular grafts, due to their high elasticity. Mechanical testing according to ISO 7198:2016 [65] resulted in compliance values of 2.4 and 3.8 %·mm Hg⁻² Hg for Chronoflex and Carbothane respectively. Chronoflex showed a burst strength of 1680 mm Hg, which is similar to the one of saphenous veins (1599 ± 877 mm Hg [82]), whereas Carbothane failed at pressures of 1330 mm Hg [91]. Although these polymers are considered as biostable, they undergo slow hydrolytic degradation and oxidation *in vivo* [92-94]. A long-term *in vitro* hydrolytic degradation study of Mishra et al. [92] found a mass loss of ~10% over 50 weeks at a constant exposure to a temperature of 37°C.

Seifalian et al. [90] carried out eight long-term *in vivo* tests in canine, using a compliant graft made from Myolink. After 18 months, four grafts were explanted, for evaluation signs of short-term degradation. The remaining four grafts were harvested after 36 months and showed no changes in the compliance compared to pre-implantation. Furthermore, none of the grafts showed signs radial distension, chemical breakdown or

degradation. However, Myolink was later found to be unsuitable for clinical applications as biostable vascular prosthesis, due to surface thrombosis and evidence of *in vivo* degradation [93].

2.2.3 Natural Materials

Vascular grafts fabricated using natural biomaterials have gained popularity in the recent years [7, 95, 96]. High attention is paid to elastin and collagen, as they are components of natural arteries [45, 97]. Other important materials are fibrin, chitosan, silk-fibroin, small/sub-intestinal submucosa (SIS, i.e. cell free collagen) [98-100]. Natural polymers are admired for their very high biocompatibility, degradability and good cell adhesion properties [45, 101].

Catto et al. [98] fabricated 1.5 mm and 4.5 mm diameter silk-fibroin tubes by electrospinning. Burst pressure and compliance were estimated based on circumferential tensile testing. The estimated compliance (by taking the radial strain between the stress points that refer to 80 and 120 mm Hg) of the 1.5 mm diameter tubes was about 180% relative to that of a rat aorta (reference, 100%). The larger diameter tubes (4.5 mm) were less compliant than rat aorta (~60% relative to it), but more compared to ePTFE. Burst pressure was estimated based on the ultimate tensile strength of the tubes, which was 344 mm Hg for the 1.5 mm and 773 mm Hg for the 4.5 mm diameter grafts. Post-implantation (dorsal tissue in rats) histological analysis, revealed the presence of few macrophages. However, no T-lymphocytes were detected, which indicated no immune reaction was caused by the implantation of the grafts. In their earlier work, 1.5 mm silk-fibroin tubes were implanted for seven days as abdominal aortic grafts in Lewis rats and remained free of thrombosis and mechanical failures [102]. Indeed, the results seem promising, yet strong conclusions from estimating burst strength and compliance cannot be drawn, as compliance is normally determined under dynamic conditions and burst strength through pressurized testing. An *in vitro* characterization in accordance to ISO 7198:2016 [65], would make their results more comparable to other studies.

In a study by Liu et al. [103], silk fibroin was used to fabricate bi-layered tubes with an inner lining containing heparin and a highly porous outer layer. The compliance of the tubes was $2.3\text{--}2.5\% \cdot \text{mm Hg}^{-2}$, which was lower than for human HSV $3.4\% \cdot \text{mm Hg}^{-2}$ [104]. HSV remain currently the golden standard, but have a lower compliance of $\sim 74\%$ [105] relative to human femoral arteries (100%) [106, 107]. The compliance of synthetic vascular substitutes has to be closer matched to native arteries in comparison to HSV. Failure of HSV grafts has been attributed to remaining compliance mismatch, despite it is much smaller as compared for ePTFE prostheses [105, 108]. Burst pressure of the silk fibroin tubes was 779–806 mm Hg, about half of that of HSV [82]. In comparison to previous silk fibroin tubes, burst strength was improved, but yet has to be matched to or exceed the value of HSV. Suture retention strength was claimed to be better than for HSV [104]. *In vitro* platelet adhesion-, plasma recalcification- and hemolysis testing demonstrated good hemocompatibility. *In vitro* culturing of amniotic fluid-derived stem cells demonstrated a continuous proliferation up to 12 days and cytocompatibility tests showed good results.

Collagen type I (isolated from monomeric rat tail tendon) and elastin-like polymer (LysB10) were used to produce a nanofibrous graft composite [96]. Bi-layered sheets of collagen and elastin-like protein were rolled up to a multi-lamellar tube (1.3 and 4 mm diameter). The tubular grafts were assessed on their compliance and burst strength. In comparison to literature values, their grafts were more compliant than SV, but less than arteries. Burst strength was half of that reported for SV [82]. After 2 weeks *in vivo* testing in rats (grafting the aorta), the explanted 1.3 mm diameter prosthetic aortic grafts showed patency and no aneurysmal dilatation, thrombus formation or NIH was detected [96].

Fibrin gel tubes with entrapped human dermal fibroblasts were fabricated by Syedain et al. [109]. Six tubes were cultured in a bioreactor under pulsatile flow. Over time, the fibroblasts remodeled the gel and deposited circumferentially aligned collagen fibers and other ECM components. Burst strength increased with ongoing duration of culturing from ~ 300 mm Hg after two weeks up to ~ 1000 mm Hg for four weeks incubation. Compliance of 4 mm grafts after 7 weeks culturing was $\sim 4.5\%$ and $\sim 2.5\% \cdot \text{mm Hg}^{-2}$ for 2mm diameter tubes after 5 weeks, in comparison to ovine femoral

arteries with $\sim 3.5\% \cdot \text{mm Hg}^{-2}$. Suture retention of the fibrin tubes (with PLA sewing rings) was similar to that of human internal mammary arteries.

Roeder et al. [100] fabricated vascular substitutes of 5 and 8 mm diameter of porcine SIS and evaluated their mechanical properties. The compliance of the 5 mm tubes ranged from 7.25 to 17% $\cdot \text{mm Hg}^{-2}$, while it 18-23.75% $\cdot \text{mm Hg}^{-2}$ for the 8 mm grafts. In their comparison with literature values, it was claimed the SIS grafts are more compliant than HSV, but half as much as canine carotid arteries and one third of human carotid arteries. Burst pressure was 3517 mm Hg, similar to native internal mammary artery [82]. Although the results seem promising, compliance measurement was not carried out in accordance to ISO 7198:2016 [65] and no animal tests were carried out in this study.

2.2.4 Decellularized Tissues

Decellularization of natural tissue is carried out to obtain scaffolds with the architecture and mechanical properties of ECM. The process of decellularization removes antigenic material such as cells. Since the first development in the 1960s, decellularized vascular tissues have gained interest and also become commercially available, they have however, not shown any evident advantage over synthetic vascular prostheses [45, 110].

In a study reported by Conklin et al. [106], porcine carotid arteries were decellularized for vascular graft studies. The decellularized tissue was surface modified with immobilized heparin, in order to reduce thrombogenicity. Static compliance measurements revealed the decellularized arteries exhibit similar mechanical properties as untreated porcine carotid arteries. *In vivo* test in canine were carried out by bypass grafting the carotid artery. After two months, the lumen was covered by ECs and the graft wall was inhabited by SMCs.

Zhou et al. [111] coated decellularized canine carotid arteries with heparin and seeded them with endothelial progenitor cells for bioreactor culturing. Large scale *in vivo* tests were carried out, implanting seeded and unseeded scaffolds into 20 dogs as carotid

artery bypass grafts. After three months, the decellularized vessels were explanted. The bioreactor cultured decellularized arteries showed complete endothelial recovery and no signs of thrombosis. The controls of unseeded scaffolds showed signs of endothelial dysfunction and the occurrence of NIH. Patency rate for the seeded grafts was 95% after three months, compared to 60% for the controls.

Hybrid scaffolds of decellularized aortic rat tissue and outer electrospun PCL sheets were developed by Gong et al. [112]. The outer PCL sheet was added for structural reinforcement, as the decellularization processes commonly reduce the mechanical strength of vascular tissue and lead into accelerated elastin degradation. SEM analysis of the decellularized tissue confirmed damages in the media. The hybrid tissue-PCL vessels and pristine decellularized aortas were implanted into Sprague-Dawley rats as abdominal aorta interposition grafts for six weeks. No stenosis or thrombosis was found for either of the groups. The addition of the outer PCL layer reduced the penetration of extravascular inflammatory cells and preserved the integrity of the decellularized tissue. The explanted controls showed damages to the fiber structure.

2.3 Efforts in Achieving a non-linear elastic vascular Prosthesis

Arteries exhibit idiosyncratic mechanical properties, due to their three-layered structure. As the stiffness increases under tension, arteries display a reversible transition from high to low compliance with increasing blood pressure [97, 113, 114]. Many studies aimed to match the compliance of vascular grafts with HSV and HFA but their measurements were only carried out at physiological pressure (80-120 mm Hg) [17, 31, 100, 103, 109]. In order to match the compliance to native arteries, vascular prostheses are required to display a similar profile over the entire physiological blood pressure range (up to 220 mm Hg [115]). This requirement is crucial, as compliance is very high at low luminal pressure and gets reduced with increasing blood pressure [65, 114, 116].

Bezuidenhout et al. [117] patented a vascular prosthesis made of a polymeric tube with outer fabric sock. The sock consists of a chain-like structure that displays a non-linear elastic stress-strain response (J-Curve). The inner tube was designed to be porous, in order to allow tissue ingrowth. Finite element method modeling was carried out on this

system, as well as *in vitro* static and dynamic tests [118, 119]. The fabric sock reinforced tubes, however, yet have to be tested *in vivo*.

A bi-layered vascular prosthesis with a soft inner lining and a stiff crimped outer layer was designed by Chen et al. (Figure 2.6) [120]. The structural design of the tube allowed the soft inner layer to expand, while the crimped shell straightens out and only later contributes to an increase in stiffness. Compliance was initially high at low luminal pressures, whereas a decrease set in above pressures of roughly 80 mm Hg. The compliance compared to PET prostheses was higher at low pressures and less at high blood pressures. However, in comparison to native arteries, the compliance was lower overall. The design was found to be promising but requires further optimization. Although the tubes displayed non-linear elasticity, Singh et al. [114] state the bi-layered structure may produce poor kink-resistance, which is unfavorable for clinical applications.

Figure 2.6: Scheme of the mechanical behavior of the bi-layered crimped tube by Chen et al. [120] under increasing blood pressure. At low pressure, the layer is crimped and unfolds upon increasing pressure, until it gets fully in contact with the soft inner tube and restricts it from further expansion [114] (reproduced with permission from [120], copyright Taylor & Francis).

Singh et al. [121] developed a PU covered caterpillar-inspired stent-graft (Cat-SG), which is based on a knitted structure. Their prosthesis consists of soft and hard segments, where the soft segment allows longitudinal contraction and extension, as well as radial expansion at low modulus (Figure 2.7). With increased luminal pressure, the

soft segments expand their lumen, until they reach the diameter of the hard segments. Up from that point, mechanical contribution of the hard segments sets in. The inclusion of the soft segments provided high kink-resistance to the Cat-SG tubes. Radial expansion between 60-160 mm Hg was shown to be in the range of natural arteries (human aorta). No *in vivo* studies have yet been carried out for these Cat-SG tubes [114].

Figure 2.7: Structure of the Cat-SG grafts fabricated by Singh et al. [121]. The upper image a) shows the mesh only with its soft (blue) and hard (orange) segments, whereas the lower image b) shows the Cat-SG graft when it is covered with PU (reproduced with permission from [121], copyright Elsevier).

In a further study, a similar approach was chosen, by creating a multi-segmented mesh tube with hard (Nitinol and polyester) and soft regions (PU) as external vein graft support. Four different mesh types with varying lengths of soft and hard segments were analyzed on their mechanical properties (Figure 2.8). Compliance tests were carried out, by inserting a silicon balloon into the mesh grafts. A non-linearity in the compliance was found; all grafts showed high initial radial expansion, whereas at higher pressure they became stiffer. The decrease in compliance was more noticeable for grafts with long segments (4/4 samples) [122]. The meshes were found to be flexible during bending, which is beneficial for surgical applications. Further work is planned on addressing long-term *in vitro* behavior, in particular fatigue. The external mesh supported vein grafts have not been used for animal testing so far.

Figure 2.8: Knitted soft/low modulus (LM, white) and hard/high modulus (HM, grey) segmented meshes for external vein graft support developed by Singh et al. [122]. Four designs with different segment lengths were analyzed (reproduced with permission from [122], copyright Elsevier).

2.4 Questions to be answered based on Literature

Most of the listed publications discuss how to fabricate a compliant vascular prosthesis by employing a single material. In order to mimic arterial-like mechanical properties, a composite structure (soft/hard material compounds) should be considered. This work addresses the question, whether composites with structures inspired by the arterial wall may be the solution to obtain a vascular prosthesis non-linear elastic properties [97, 113, 114, 123].

2.5 PhD in Context of Literature

Most of the studies described in this literature review chapter deal with the fabrication of vascular prostheses using biodegradable materials (natural or synthetic). Furthermore, many of the listed studies are mechanical, aiming to match the compliance of native arteries. However, most of the here named publications attempted to match the compliance of arteries at physiological pressure (80 -120 mm Hg) only. Arteries display an idiosyncratic property of a reversible transition from high to low compliance upon pressure increase [97, 113, 114]. Therefore, compliance matching of vascular

prostheses to native arteries, requires them to display non-linear elastic properties [65, 114, 116].

The current project addresses the issue of mimicking the idiosyncratic compliance properties of native arteries. Inspired from the architecture of arterial walls, several composite structures were fabricated, in order to obtain a non-linear elastic prosthetic graft. In comparison to existing literature on non-linear elastic graft fabrication (section 2.3), this work followed different approaches, in order to be able to design the compliance properties.

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Chapter 3

Experimental Methodology

In this chapter the methods for specimen fabrication and characterization are presented. The vascular prosthesis fabrication involves techniques as dip-coating, melt-spinning and 3D-bioprinting. Imaging characterization was carried out, in order to verify the quality control of the tubes and melt-spun fibers. Mechanical testing, in particular dynamic compliance measurement, was performed, to verify and match the properties of the specimen to native arteries.

3.1 Materials used

The materials and suppliers used in these experiments are listed below: Poly(vinyl alcohol) (PVA, 80% hydrolyzed, M_w 9'000-10'000 Da), poly(ethylene glycol) (PEG, M_w 6000 Da), poly- ϵ -caprolactone (PCL, M_n 80'000 Da) were supplied by *Sigma Aldrich*; poly-L-lactide (PLA, IV 6.5) by *Polysciences Inc.*; poly-L-lactide-co- ϵ -caprolactone (PLC) 95:5, PLC 85:15 and PLC 70:30, sold as PLC 95:17, PLC 85:16 and PLC 70:15 respectively, were supplied by *Corbion Purascorb*. PLC 50:50 (AP124, M_n 100'000-200'000 Da) and 40:60 (AP145, M_n 100'000-200'000 Da) were purchased from *Akina Inc.* Additional PLC 50:50 was purchased from Huizhou *Huizhou ForYou Medical Devices Co., Ltd.* As solvents, ethanol (Tech Grade, Aik Moh/64-17-5, 95% + 5% methanol) and chloroform (Tedia, amylene stabilized, CS1332-001) were used. Aortic branches from porcine for control study were freshly obtained, with permission, from *Primary Industries* slaughter house in Singapore.

3.2 Dip-coated Tube Fabrication

3.2.1 Single Material Tubes

Tubular vascular grafts were fabricated in a two-step dip-coating method (Figure 3.1). Metallic rods of 4 mm diameter were first coated with PVA and in a second step with either PLC or PCL. The rods were fixed at their top of a motorized rotator (IKA, RW 20 Digital) and coated by manual lifting and draining of a PVA solution (15 w/Vol% in 50:50 water-ethanol) under rotation (300 rotations per minute, rpm), see Figure 3.1a) [1-3]. After the PVA solution was drained, the rods kept rotating for a further 5 minutes, in order to achieve uniformly thick layers. Afterwards, the rods were clamped and left for 15 minutes to dry, before they were transferred into the oven (60°C) for another 30 minutes.

Figure 3.1: Fabrication of dip-coated tubes: a) drain-coating of metallic rods with PVA b) immersion into PLC or PCL solution c) gradient minimization through up-side-down placing and subsequent horizontal rotation and d) soaking in water and following removal of the tube from the metallic rod.

After coating the metallic rods with PVA, they were immersed twice into a PLC or PCL solution (see specifications in Table 3.1) using a dip-coating machine (Nima Technology). The immersion speed was set to 300 mm/min, while withdrawal was slower (120 mm/min), see Figure 3.1b.

After the second immersion, the rod was turned up-side-down and inserted into a sample holder (Figure 3.1 c) for a designated time (Table 3.2). This step minimized wall-thickness (WT) gradients along the tube. For all tubes of WT greater than 120 μm , the rods were required to be coated with one additional layer to further increase the thickness. For the fabrication of 120 μm WT tubes, the rods were instead clamped for 1 minute at their top for additional quality control (minimization of longitudinal WT gradients) and then inserted into a custom-built horizontal slow rotating machine (10 rpm) for 5 minutes. Horizontal rotation was necessary, in order to guarantee WT-uniformity around the circumference (Figure 3.1c).

Table 3.1: Solution preparation parameters with varying concentration (w/v %) and amounts of PEG for different PLC compositions. Soaking time refers to the dissolving period of the PVA in water.

Polymer Type	PLC 40:60	PLC 50:50	PLC 70:30	PLC 85:15	PCL
Polymer	15%	15%*	15%**	13%	14%
PEG	0.20%	0.20%	0.60%	0.85%	0.95%
Chloroform	42 mL	42 mL*	42 mL**	50 mL	45 mL
Soaking Time	4 days	4 days	3 days	2 days	3 days

*A concentration of 15% was chosen for PLC 50:50 purchased from *Akina* and 14% for the polymer supplied by *Huizhou ForYou Medical Devices*. **For the fabrication of 150 μm WT PLC 70:30 tubes, a polymer concentration of 14.2% was chosen (7.5g in 45 mL chloroform). For PLC 70:30 tubes of other WT (120, 200 and 250 μm) the polymer concentration was 15% (7.5 in 42 mL).

Table 3.2: Dip-coating protocols for PLC 70:30 grafts with different WT.

Wall-Thickness (WT)	120 μm	150 μm	200 μm	250 μm
1. Immersion into PLC Solution	2 dips	2 dips	2 dips	2 dips*
2. Turning Rods up-side-down	1 min	30 sec	5 min	10 min
3. Horizontal Rotation	10 min	-	-	-
4. Immersion into PLC Solution	-	1 dip	1 dip	1 dip*
5. Turning Rods up-side-down	-	30 sec	30 sec	30 sec
6. Horizontal Rotation	-	10 min	10 min	10 min

*For the fabrication of 300 μm WT PLC 50:50 tubes, three immersions were required at points 1 and 4 for the polymer purchased from *Akina*, while for the polymer from *Huizhou ForYou Medical Devices* two immersions were required at points 1 and 4. The PLC 50:50 rods fabricated with the polymer purchased from *Akina* were immersed and withdrawn at speeds of 240 mm/min, while *Huizhou ForYou Medical Devices* immersion speeds of 300 mm/s and withdrawal speeds 150 mm/s were chosen for the polymer from *Huizhou ForYou Medical Devices*.

Afterwards, the rods were placed up-side-down into a sample holder and left overnight for solvent evaporation. The following day, they were transferred into a vacuum oven (room temperature, RT, 25°C) and dried for another 24 hours.

After solvent removal, the WT thickness was determined for each sample. For precise measurement, a CCD micrometer (Keyence, LS-7030-MT, $\pm 2 \mu\text{m}$ accuracy and 0.3-30 mm detection range) was used. The diameter of the dip-coated tube was measured at 10 points along the length. Subtracting the diameter of the PVA-coated rod from the average tube diameter equaled 2x the WT.

If the addition of outer structures to the dip-coated tubes (sections 3.3 and 3.4) was not required, the rods were soaked in water for several days (for details, see Table 3.1), in order to dissolve the PVA sacrificial inner layer, enabling an unencumbered removal (Figure 3.1d).

After the tubular grafts were removed from the metallic rod, they were dried in a vacuum oven (RT) for 1 hour and then glued to luer locks. An epoxy adhesive (Alteco 3-ton) was used to fix the tubes at their ends to the luer fittings (Nordson medical, MLRL025-1 and MLSL025-1)

3.2.2 Bi-Layered Tubes

Tubes consisting of a soft inner lining and a stiff outer shell were fabricated for later evaluation on non-linear elastic compliance properties. The inner lining was fabricated according to Table 3.1 ($\sim 120 \mu\text{m}$ WT). After the soft polymer lining has dried sufficiently, the rods were coated with one layer of the stiff polymer. Table 3.3 lists the polymer combinations that have been used for the fabrication of bi-layered tubes:

Table 3.3: Specifications of the bi-layered tubes.

Material	Samples			WT [μm]
Inner lining	PLC 50:50	PLC 50:50	PLC 70:30	~ 120
Shell	PLC 70:30	PCL	PCL	~ 100

3.3 Melt-Spun Composite Tube Fabrication

As an alternative to the fabrication of bi-layered tubes, a layer of ~ 70 - $100\ \mu\text{m}$ WT melt-spun fibers were wound around dip-coated PLC tubes of ~ 120 - $150\ \mu\text{m}$ WT. A custom-built melt-spinning machine was used for this purpose, see

Figure 3.2. Metallic rods were fixed at a rotator (Stuart, stirrer SS10) and spun at either 100 or 500 rpm. PLC or PCL polymer was heated above its melting point (150°C for PLC and 70°C for PCL). The polymer mass dropping from the melt-holder spinneret was attached manually (by tweezer) to the rotating PLC tube; this marked the onset of the spinning process. The spinneret was moved for- and backwards, in order to allow an even coverage of melt-spun fibers on the dip-coated tube. Table 3.4 summarizes the spinning conditions for the individual sample types.

Figure 3.2: a) Melt-spinning machine with rotating metallic rod carrying the PLC tube. A fiber gets drawn down from the orifice at the bottom of the melt-holder and is wound around the PLC tube under the rotational force. b) Close-up of the melt-holder with the fiber being pulled by the rotational force of the tube.

The rods were left at room temperature for three hours after melt-spinning and then soaked in water for 1-2 days. The post-soaking protocol was the same as described in section 3.2.1.

Figure 3.3: Melt-spun composites of dip-coated tubes at the inside (blue) and outer fibers (white). The fibers were either a) wound circumferentially [F_C] or b) helically (cross-spun) [F_H].

Table 3.4: Fabrication parameters for melt-spun composite tubes.

Tube	Fiber	Spinning Speed [rpm]	Spinneret Speed [mm/s]	Spinning Time [min]
PLC 50:50	PLC 50:50	500	0.7	1
PLC 50:50	PLC 70:30	100	0.7	5
PLC 70:30	PLC 70:30	100	0.7	5
PLC 70:30	PLC 70:30	100	20	5
PLC 70:30	PLC 70:30	100	30	5
PLC 70:30	PLC 70:30	500	0.7	1
PLC 70:30	PCL	100	0.7	5

For circumferential fiber deposition (F_C , Figure 3.3a), speeds of 0.7 mm/s were applied. Melt-spinning of helically wound fibers around a dip-coated tube [$T+F_H$] (Figure 3.3b) was carried out with a different melt-spinning machine that allowed fast spinneret travelling speeds (up to 30 mm/s). Two travelling speeds for the spinneret were used: 20 and 30 mm/s, see Table 3.4.

3.4 3D-bioprinted Mesh-Tube Composites

Composites of soft dip-coated tubes and outer 3D-bioprinted meshes were fabricated with the aim of mimicking the non-linear elastic behavior of natural arteries. The design of the meshes was inspired by cardiovascular stents and were characteristic of their pliable features, which should provide a J-Curve behavior. Thus, these bioprinted

meshes were named as “J-Meshes” and the composite of dip-coated tubes with outer J-Mesh “JM-tubes”.

3.4.1 Printing of Pliable Fibers

Zig-Zag versus Semi-Hexagon Spring Geometry

In a first set of experiments, spring-like fibers of different designs were printed. They all had the characteristic of including pliable hoops [4]. Such designs were chosen, in order to initially allow the fiber to unfold at low elastic modulus, before it gets fully straightened, where it would initiate a stiff response. As hoop geometries, four different designs were chosen and compared based on their printing quality and tensile properties. These hoops were either printed with zig-zag (ZZ), semi-hexagons (HEX), stair (ST) or sine (S) features (see Figure 3.4a).

Figure 3.4: a) Spring (hoop) types for the pliable fibers: Zig-zag (ZZ), semi-hexagons (HEX), stairs (ST) and sine-waves (S). b) Examples of ZZ- and HEX-fibers with different length to amplitude ratios.

Continuous ZZ and HEX fibers were printed with different length to amplitude ratios (L/A, hoop angles) and their tensile properties evaluated. ZZ and HEX fibers with L/A ratios of 1x to 10x (1:1 mm up to 10:1 mm), as shown in Figure 3.4b), were printed from a 12% PLC 95:5 solution in chloroform on a glass slide. Early optimization experiments of printing with 30G needles have shown a printing speed of 10 mm/s and

applied pressure of 0.1 MPa was suitable for obtaining well defined fibers. The effect of hoop angle and spring designs were evaluated on their tensile properties in tensile experiments (see section 3.7.2). In a second set, ZZ and HEX fibers with the L/A ratios of 1-10x were printed from PCL and the effect of material choice evaluated.

HEX versus ST- and S-Spring Geometry

The mechanical properties of the HEX-fiber design were compared against the ST- and S-spring geometries (Figure 3.4a). Hence, fibers with an L/A ratio of 3x and 1 hoop (1H) were printed from PCL (12% in chloroform) at 10 mm/s and 0.1 MPa.

Segment Length

The effect of segment length (scaling factor) on the tensile properties of PCL fibers was evaluated on HEX 1x designs. Scaling factors of 1 (1:1 mm), 2 (2:2 mm) and 4 (4:4 mm) were chosen. The total length of the fiber was kept constant. Hence, HEX-1x-2 fibers contained only half as many (4H) hoops as 1x-1 (8H) and 1x-4 four times less (2H) than 1x-1 respectively. Examples are shown in Figure 3.5:

Figure 3.5: Example of three HEX-1x fibers with different scaling factors of 1, 2 and 4. The total length was kept constant, whereas the number of hoops was reduced proportionally to the scaling factor.

Effect of Number of Hoops

HEX-1x-1 fibers were printed from PCL with a varying number of hoops (H) from 1 to 5 (Figure 3.6). The mechanical properties were assessed in a tensile experiment. Furthermore, the strains at which the fiber unfolding induces a stiff response, ε_E , were determined. The strains, ε_T , at which complete unfolding is assumed for an ideal fiber were calculated using equation 3.1.

Figure 3.6: HEX-1x fibers with varying number of hoops.

$$\varepsilon_T = \frac{L_{\max} - L_0}{L_0} \cdot 100\% = \frac{L_0 + H \cdot \left(2 \cdot \sqrt{\frac{l^2}{4} + h^2} - l \right) - L_0}{L_0} \cdot 100\%$$

$$= \frac{H \cdot \left(2 \cdot \sqrt{\frac{l^2}{4} + h^2} - l \right)}{L_0} \cdot 100\% [\%], \quad \text{Equation 3.1}$$

where L_0 is the length of the printed fiber, $L_{\max}$ the total length of the fiber after unfolding, l the step-length, h the step-height and H the number of hoops. For a 1x-1 fiber l and h equal 1 mm. The fibers were printed at a total length of 40 mm and a gap distance of 30 mm was chosen for tensile testing. Thus, L_0 was set to 30 mm and equation 3.1 becomes:

$$\varepsilon_T = \frac{H \cdot \left(\sqrt{\frac{5}{4}} - 0.5 \right)}{15} \cdot 100\% [\%] \quad \text{Equation 3.2}$$

Effect of Fiber Width

PCL 0.5x-1-2H fibers (1:2 mm) with different widths (W1, W2 and W3) were printed (Figure 3.7). W1 fibers consisted of normal single stranded fibers, whereas W2 fibers were double and W3 fibers triple stranded. Between the strands a gap distance of 200 μm was chosen, as it equals the smallest distance the 3D printer could move. At this distance the fiber strands were able to fuse, which generated thicker fibers, see Figure 3.7:

Figure 3.7: Example of HEX-1x-2 fibers of different widths: W1, W2 and W3. W2 and W3 fibers were printed with double and threefold widths of W1.

Rounding of Hoops

HEX-1x fibers were printed with different degrees of waviness (rounding), see Figure 3.8. The corners of the hoops were smoothened, in order to obtain a more curved spring. The fibers later were assessed on their mechanical properties.

Figure 3.8: Examples of rounded HEX-fibers (black and blue) in comparison to a normal HEX-fiber.

3.4.2 Mesh Printing

Initially, various ZZ-meshes (50x50 mm) were printed with continuous designs. However, it was soon revealed that the ZZ-hoops, due to their high number, are not able to unfold, as the tube would have to grow to a very high diameter ($> 7\text{ mm}$). Later, two major changes were made: The ZZ-spring geometry was no longer considered, and a HEX-pattern chosen instead and the mesh length was adjusted to the circumference of the tube (e.g. for a tube with 4.3 mm diameter the circumference equals 13.5 mm) to fit one wrap.

Based on equation 3.1, reasonable dimensions for the hoops of the mesh were chosen. Earlier compliance tests of thin and soft PLC tubes revealed diametric expansion upon pressure increase up to about 6 mm (details are shown in section 6.4). The diameter required to fully unfold the mesh was estimated using equations 3.3 and 3.4:

$$L_{\max} = L_0 + H \cdot 2 \cdot \left(\sqrt{\frac{l^2}{4} + h^2} - \frac{l}{2} \right) \quad \text{Equation 3.3}$$

$$D_{\max} = \frac{L_{\max}}{\pi} \quad \text{Equation 3.4}$$

Meshes were similarly to the fibers, printed from a 12% PCL solution on a glass slide. Printing speeds of 15 and 10 mm/s were used for 27G and 30G needle tips respectively. Meshes printed with a 27G needle consisted of two layers, whereas those produced using 30G size tips were printed with three layers. After printing was completed, the glass slides with the meshes were placed into a vacuum oven (RT) for 24 hours for solvent removal. Afterwards, the hoops of the bioprinted meshes were painted blue (permanent marker, M), in order to allow a better display during the mechanical characterization.

Single-Hoop Meshes

Equations 3.3 and 3.4 suggest using small number of hoops, long step-length and low step-heights to enable complete mesh unfolding at low radial expansions of the tube. Hence, in a first set, J-meshes with one hoop were printed according to Table 3.5 and Figure 3.9:

Table 3.5: List of single hoop HEX-meshes with different angles and fiber width.

Mesh Type	Full Name	Step Length [mm]	Step Height [mm]	Vertical Fiber Gap [mm]
A1	HEX-0.5x	1	2	2.25
A2	HEX-1x	1	1	2.25
B1	HEX-1x	1	1	1.25
B2	HEX-2x	2	1	1.25
B3	HEX-4x	4	1	1.25

The meshes consisted of multiple fibers and two glue strips at the edges (red lines, Figure 3.9). The glue strips were the sites where the mesh was fused to the dip-coated tubes.

Figure 3.9: a) Scheme of an A1 and b) of a B1-mesh. The vertical gap between the fibers was reduced, leading into a higher fiber density.

Double Hoop Meshes

In a second set, meshes with two hoops and three glue-strips were printed (

Figure 3.10. The meshes were separated into two halves by a middle glue-strip. Each half contained one spring-section. The parameters of the printed meshes are summarized in Table 3.6:

Table 3.6: List of the second series of mesh printing. All meshes contained 2 hoops, but the spring types and L/A-ratio were altered.

Mesh Type	Full Name	Spring-Type	Step Length [mm]	Steep Step [mm]	Step Height [mm]	# Hoops
C1	HEX-0.5x-2H	HEX	1	N/A	2	2
C2	HEX-1x-2H	HEX	1	N/A	1	2
C3	HEX-1x-2-2H	HEX	2	N/A	2	2
C4	HEX-2x-2-2H	HEX	4	N/A	2	2
D1	ST-0.5x-2H	ST	0.5	N/A	2	2
D2	ST-1x-2H	ST	0.5	N/A	1	2
D3	ST-2x-2-2H	ST	2	N/A	2	2
E1	S-0.5x-0.5x-2H	S	1	1	2	2

Figure 3.10: Examples of the printed meshes: a) C1- mesh (HEX), b) C3-mesh (HEX), c) D1-mesh (ST) and d) E1-Mesh (S).

Grid- and Two-Round Meshes

Based on the results from the mechanical tests on the single- and double-hoop meshes and computer simulations of the fibers, a third series of meshes were printed. The results have shown that the tube may expand between the struts and the struts undergo bending, if their length is too high (see section 6.4). To increase the fiber coverage on the tube and thus to reduce inter-strut expansion, grid meshes and two-round meshes were printed (Figure 3.11). The grid meshes consisted of one narrow hoop section per round, whereas the other larger zone comprised a dense grid. The hoop sections were designed to be narrow, in order to minimize fiber bending during unfolding. The grid section was included, in order to reduce inter-strut expansion of the tube.

Table 3.7: List of grid- and two-round meshes.

Mesh Type	Full Name	Scaling Factor
F1	HEX-0.5x-1H-2R-Grid	1
F2	HEX-1x-2-1H-2R-Grid	2
G1	HEX-0.5x-2H-2R	1
G2	HEX-1x-2-2H-2R	2

Two-round HEX-meshes were printed, which contained a vertical offset between the first and the second layer of wrapping. The vertical offset was chosen to be half of the strut spacing, in order to allow a better fiber coverage on the dip-coated tube (Figure 3.11 b and c). lists all the tested meshes.

Figure 3.11: Examples of grid-meshes (a) and two-round meshes (b and c). a) F1-mesh, b) G2-mesh and c) 2D projection of a G2-mesh on a tube surface (10x10 mm section) with the second layer of mesh (green) occupying the space between the struts of the first round (black).

Diamond Grid Meshes

A fourth set of meshes with designs based on the advances and findings from the third series was printed. The meshes were printed with two rounds that contained each of two sections similar to the grid meshes. One half contained a spring section, the other comprised a dense crossing diagonal (diamond, DM) network. The inclusion of a DM-section was supposed to eliminate tubular inter-strut expansion. A second modification of the third set of meshes was to horizontally displace the hoop section of the second wrap. The purpose was to improve the fiber coverage of the tube at the hoop section, in order to achieve a more even stress distribution. This was aimed to shift the load to the spring sections, which should facilitate mesh unfolding at lower pressures. An example

of a HEX-diamond mesh is shown in Figure 3.12a and the 2D projection of the mesh on a dip-coated tube in Figure 3.12b.

Figure 3.12: a) Scheme of the H1 diamond mesh. The mesh contains two rounds (shown in black and green) with each of the two sections (hoop and DM sections). Among these two sections per round, one contained the pliable hoops, whereas the other section consisted of a dense network (crossing diagonals, diamond pattern, DM). b) 2D projection of a H1-mesh on a tube surface, showing the second layer of mesh (green) occupies the space between the struts of the first round (black). The hoop and DM-section of the second round were swapped, in order to guarantee dense fiber coverage.

H1 meshes were printed with three different polymers PCL, PLC 95:5 and PLA, in order to evaluate the effect of material choice. A list of the printed diamond meshes is shown in Table 3.8.

Table 3.8: List of the printed DM meshes of three different materials.

Mesh Type	Full Name	Step Length [mm]	Step Height [mm]	Mesh Material
H1	HEX-2x-2H-2R-DM	2	2	PCL
H1	HEX-2x-2H-2R-DM	2	2	PLC 95:5
H1	HEX-2x-2H-2R-DM	2	2	PLA

Low WT Two-Round JM-Tubes

Same as for the DM-mesh JM-Tubes, a WT of $\sim 55 \mu\text{m}$ was chosen to be combined with a two-round mesh tube. Two mesh designs were used: G2 HEX-1x-2-2H-2R and G3 HEX-2x-2-2H-2R. The tested JM-Tube types are summarized in Table 3.9:

Table 3.9: List of the printed two round meshes with different mesh materials.

Mesh Type	Full Name	Step Length [mm]	Step Height [mm]	Mesh Material
G2	HEX 1x-2H-2R	2	2	PCL
G3	HEX-2x-2H-2R	2	2	PCL
G3	HEX-2x-2H-2R	2	2	PLC 95:5
G3	HEX-2x-2H-2R	2	2	PLA

3.4.3 Mesh-Tube (JM-Tube) Fabrication

Composites of dip-coated tubes and 3D-bioprinted meshes (JM-Tubes) were fabricated through solvent binding (fusion). A thick PCL glue (20% in chloroform) was dispensed (by the 3D printer at 5 mm/s) on the anchor glue-strip at the end of the mesh (see Figure 3.13). A metallic rod with a dip-coated tube was then attached longitudinally to the glue-strip and held in position for several seconds (up to 30 seconds), in order to allow the mesh and the tube to fuse. Afterwards, another line of glue was dispensed on the next glue-strip and the tube was rolled up. Same as for the initial attachment at the anchor glue-strip, the tube was held in position for a while. The procedure was continued for all the remaining glue-strips. The length of the meshes was adjusted to the dip-coated tube diameter so that it exactly fits around the circumference. Hence, the sealing glue strip could be fused to the anchor line. After sealing the mesh, the tube was put into a vacuum oven (RT) for two hours, in order to remove residual solvent from the fusion sites.

Figure 3.13: Scheme of the two single components, the dip-coated tube and the 3D-bioprinted mesh (two round HEX-mesh) and the tube mesh composite on the right. The glue-strips (sites of fusion) are indicated by the red lines.

After vacuum drying, the tubes were soaked in water, in order to dissolve the PVA to allow an unencumbered removal from the metallic rod.

3.4.4 In-Situ Printing

Zig-Zag Meshes

ZZ-meshes were printed directly on a rotating PLC tube with a 12% PCL solution from a 30G needle tip. The printer head was moving sideward by 2 mm, forming 3 hoops. The printing speed v_{print} was adjusted to the rotating speed of the rod, see equation 3.5:

$$v_{\text{print}} = \frac{2 \cdot h \cdot H}{T_{\text{rot}}}, \quad \text{Equation 3.5}$$

where h is the amplitude, H the number of hoops and T_{rot} the time required for one full rotation. As T_{rot} was ~ 3.9 seconds, v_{print} was set to 1.5 mm/s. The printing process is shown in Figure 3.14:

Figure 3.14: Illustration of in-situ printing of ZZ-fibers by horizontal forward- and backward motion of a printing head on a rotating dip-coated tube.

The fibers were printed with three ZZ-hoops with step heights of 2mm and an L/A-ratio of 2.2. Tube WT of 55 and 100 μm were chosen. A ZZ-spring geometry was chosen, as the San-Ei-Tech robot was unable to print HEX-, ST- or S-fibers.

Nose Meshes

Three alternative mesh types to the pliable spring J-Meshes were printed, of which all contained vertically free-standing hoops. For that, lines of a thick PVA solution (20% in 50:50 water-ethanol) were printed longitudinally and dried for three hours. Afterwards, PCL fibers were directly printed onto the rotating tube as shown in Figure 3.15a. The mesh-tube composites were dried for 24 hours in a vacuum oven (RT), in order to remove residual chloroform from the printed fibers. The following day, the tubes were soaked into water for 48 hours, allowing the PVA to dissolve.

For comparison, PLC 70:30 [T+F, 100 rpm] tubes were fabricated, winding the melt-drawn fibers around the PLC tubes PVA-line coated (Figure 3.15b) and a mesh of straight fibers was wound around a PVA-line coated PLC 70:30 tube (Figure 3.15c). The melt-spinning process parameters were same as in Table 3.4.

Figure 3.15: a) Fabrication of nose-fiber tubes by in-situ printing. b) Melt-spinning of fibers around a rotating tube with a thick PVA line to form free-standing hoops. c) Fusion of a stiff-mesh (without pliable fibers) to a dip-coated tube with a longitudinal PVA line to create a vertical hoop.

3.5 Computer Simulations

Finite Element Method (FEM) modeling was carried out using Autodesk Nastran-In-CAD and Inventor software. HEX-, ST- and S-fibers with a total length of 7 mm were modeled in Inventor software. A hypothetical elastic modulus of 30 MPa was chosen for the experiments. The latter equaled the value for PCL, while the others were hypothetical values. In addition to the spring geometries and elastic moduli, the fibers were modeled with three different cross-sections: square, filleted square and circular. Examples are shown in Figure 3.16:

Figure 3.16: Three cross-sectional shapes were chosen for the spring-like fibers: a) circular, b) square and c) filleted square. The width of the fibers was set to 0.15 mm and a fillet radius of 0.05 was chosen for the filleted square.

After the fiber modelling was complete, FEM analysis was carried out in Nastran-In-CAD software. Static analysis was chosen as simulation method, whereby the software offers the choice to run it in linear or non-linear mode. Either of the methods is chosen based on criteria, under which the test is supposed to be run. Linear analysis is carried out under following criteria:

- The software assumes a linear stress-strain relationship, i.e. Hooke's law
- No variation of boundary conditions during the simulation, i.e. no change in magnitude of load nor the direction it is applied to
- The change in stiffness can be ignored, because the induced displacement is small

Because of the geometry of the HEX-, ST- and S-fibers, the stress-strain relationship was expected to be non-linear, similar as it is the case for wavy collagen fibers [5]. Therefore, "Non-linear static analysis" was chosen as simulation method. The mode of analysis is carried out under the assumptions listed below:

- The relationship between stress and strain is not linear
- The stiffness of the material changes upon deformation [6]

Under "mesh-setting", a mesh-size of 0.14 mm was chosen (mesh size in Nastran-In-CAD refers to the size of the finite elements and is not to be confused with the size of the 3D-bioprinted mesh). The fiber was fixed at one end, while the other one was loaded with a tensile force of 0.03 N. Once these parameters were set, the analysis was run. If no successful analysis could be carried out, the program required corrections in the pre-setup. The analysis provided videos of the fiber unfolding, as well as a colored stress map, indicating the areas of high stress-concentration.

3.6 Imaging Analysis

3.6.1 Light Microscopy

The 3D-bioprinted fibers and tubular cross-sections of porcine aortic branch arteries and dip-coated tubes were analyzed using a light microscope. The images were obtained using a Leica M165-FC stereoscopic microscope at magnifications of 2 to 12x and Nikon Eclipse E600 POL microscope at magnifications of 4 and 10x. Both microscopes allowed lighting from above and below.

Sample preparation for cross-sectional imaging of the dip-coated tubes required cryoslicing. Small tubular ring segments (max 5 mm length) were cut with scissors and embedded into a gel (O.C.T. Compound, Sakura, Tissue-Tek) and stored for at least one hour at -150°C. Prior to freezing, air bubbles were removed manually, using tweezers, in order to improve the slicing quality. Afterwards, the gel was taken out of the container and mounted on a slicer holder. Cryo-sectioning was carried out in a Leica slicing machine (CM 1950) at -25°C. Sheets of 10-30 µm thickness were cut. The high WT of the porcine arteries (600 µm) allowed specimen preparation through direct cutting, using scissors.

Specimen for cross-section analysis of the bioprinted fibers were not prepared in the cryoslicing machine, due to the tiny sample size (~240×20×20 µm) that would have been produced, which would have made handling difficult. Hence, for microscopical fiber cross-section analysis (for fiber height determination), the fibers were quenched with liquid nitrogen and then cut with a microblade. The fibers were then stuck by tape to a Teflon cube. Photos of the top view on the fiber cross-sections were taken for width measurement.

Image capturing was carried out in *Leica LAS X* software. Mobile lamps were used for improving the lighting, in order to take optimal pictures for later WT determination in *ImageJ* software.

3.6.2 Scanning Electron Microscopy (SEM)

Micrographs of the surfaces of dip-coated tubes, melt-spun- and the mesh-tube composites were obtained via scanning electron microscopy (SEM) and field emission scanning electron microscopy (FE-SEM). Square 4×4 mm sections of the tubes were cut and stuck to a carbon tape on a SEM sample holder. To improve the grounding, thin strips of copper tape were adhered onto the samples. The specimen were sputtered with gold for 40 seconds at a current of 18 mA in a JEOL JFC-1600 (for FE-SEM) at 18 mA and 20 mA in a SPI-Module Sputter Coater (for SEM) machine respectively.

Microscopical analysis (Jeol JSM-6360A and Jeol JSM-6340F for field emission SEM) was carried out at a voltage of 5 kV (to allow destruction-free imaging) and spot sizes between 7 and 14. The secondary electron detector was used for topographical image analysis. Surface analysis micrographs of the dip-coated tubes, melt-spun composites and JM-Tubes were taken at magnifications of 50-10'000x. High magnifications were applied for pore analysis before and after mechanical testing.

3.7 Mechanical Testing

3.7.1 Tensile Testing of Films

Stress-strain diagrams were obtained from tensile testing of solvent casted and melt pressed PLC 50:50, 70:30, 85:15 and 95:5, PLA and PCL films. Dumbbell-shaped specimens were prepared by punching the films.

The samples were analyzed using a tensile testing machine (Instron® 5567) with a 500N load cell. The dumbbell specimen were strained at a rate of 3 mm/min until failure (break). The stress-strain diagrams were plotted with *OriginPro 9.0* software.

3.7.2 Tensile Testing of Fibers

The bioprinted fibers (listed in section 3.4.1) were assessed on their tensile properties using an Instron® 5567 machine with a 10N loadcell. A strain rate of 3 mm/min was

applied and testing was stopped manually after plastic deformation set in. OriginPro 9.0 software was used for creating the stress-strain diagrams.

The unfolding degree, at which the fibers induce stiffness was determined through the intercepts of ϵ_E at the unfolding progression $U(\epsilon)$ curve. The latter curve is a function of the tensile strain ϵ , which is defined in Equation 3.5:

$$U(\epsilon) = \frac{\epsilon}{\epsilon_T} \cdot 100\% = \frac{\frac{L-L_0}{L_0}}{\frac{L_{max}-L_0}{L_0}} \cdot 100\% = \frac{L-L_0}{L_{max}-L_0} \cdot 100\% [\%] \quad \text{Equation 3.6}$$

3.7.3 Compliance Testing

A custom-made apparatus was built, which allowed compliance study of vascular substitutes according to the *International Organization for Standardization* (ISO) 7198:2016 guidelines [7]. The system was improved over time, in order to better meet the ISO 7198:2016 requirements [7]. The setup could be used for static and dynamic compliance testing but required different equipment. The details are given in the paragraphs below.

Static Compliance

The apparatus shown in Figure 3.17 was used for static compliance testing. The sample was attached at its proximal end to a syringe (BD plastic, 10 mL) and at its distal site to a digital pressure gauge (WIKA, MRT-2500) and placed between the ranges of a CCD micrometer (Keyence, LS-7030-MT). Prior to testing, air bubbles were shunted through the tubing and the distal end sealed with a luer cap (Nordson Medical, FTLLP-1). The pressure was raised by a syringe pump (Spectra-Teknik Pte. Ltd, Fusion200) at a rate of 500 $\mu\text{L}/\text{min}$ to 150 mm Hg and held for 30 minutes (pressure was maintained against distension, by greater infusion). Pressure data was recorded with the software provided from WIKA (USBsoft2500), whereas the diameter signals from the CCD micrometer were retrieved from a data logger (Analog Oscilloscope Digiwave) using *Digilent waveforms* software.

Figure 3.17: a) Static compliance apparatus and b) a close-up showing the sample attached at both ends to the syringe and pressure sensor, placed between the ranges of an optical micrometer.

After 30 minutes, the pressure was released by removing the plug at the distal end. The relaxation behavior of the tube was studied for up to 60 minutes, depending on the specimen material. Based on the recorded diameter data, the creep rate $\dot{\epsilon}_{stat}$ and the strain recovery R were calculated, see equations 3.6 and 3.7:

$$\dot{\epsilon}_{stat} = \frac{\Delta D_c}{\Delta t} [\text{mm} \cdot \text{min}^{-1}] \quad \text{Equation 3.6}$$

$$R = \frac{D_{max} - D_{\infty}}{D_{max} - D_0} \cdot 100\% [\%] \quad \text{Equation 3.7}$$

,where ΔD_c is the diametric expansion due to creep, measured at different time interval Δt , D_{max} is the maximum diameter, D_{∞} is the diameter after complete strain recovery, and D_0 is the initial diameter at zero pressure.

Dynamic Compliance

A similar apparatus to the one shown in Figure 3.17a was used for dynamic compliance measurement. The setup included a water bath heating unit (Polyscience, temperature controller), in order to provide a physiological temperature of 37°C, a four-roller peristaltic pump (Masterflex Cole-Parmer, 7522-30), to generate cyclic pressure intervals, a roller clamp, to adjust the pressure level and a reservoir. A different pressure gauge was used (Argon Medical, RT2000), which could be linked to the data logger,

allowing simultaneous recording with the diameter signals. An illustration of the dynamic compliance testing apparatus is shown in Figure 3.18. A flow rate of 15 or 20 rpm was applied, in order to generate physiological heart beating frequency of 1 Hz and 1.33 Hz respectively. The dip-coated tubes were tested at three pressure levels: 50 (30-70), 100 (80-120) and 150 (130-170) mm Hg. For the melt-spun composite tubes and the JM-tubes, the compliance was measured at more pressure levels: 70 (50-90), 130 (110-150), 175 (155-195), 200 (180-220), 250 (230-270), 300 (280-320) and 330 (310-350) mm Hg. Dynamic compliance testing at multiple pressure ranges (≥ 3) is a requirement of ISO 7198:2016 [7], to verify the specimens on non-linear elasticity. At each level, the pressure and diameter signals were recorded for 30 seconds at 500 Hz, using Digilent Waveforms software. The dynamic compliance, denoted as C_{dyn} , was determined using Equation 1.1.

The use of the Argon Medical pressure sensor required calibration prior to every use. The signals from the pressure gauge and CCD micrometer were sent to the software in the form of voltage and had to be converted to pressure (P) and diameter (D) in the software. Equations 3.8 and 3.9 were used for signal conversion:

$$P = a \cdot C_1 + b \quad \text{Equation 3.8}$$

$$D = c \cdot C_2 - d \quad \text{Equation 3.9}$$

, where a and b were parameters retrieved from a linear curve fitting from the pressure sensor calibration, C_1 the voltage signal from the pressure gauge, c the sensitivity (usually 0.2 V for high accuracy, 1 V for low accuracy) and C_2 the voltage signal of the CCD micrometer and d the offset (typically 4 mm).

Figure 3.18: Schematic of the dynamic compliance testing apparatus.

Compliance was calculated using equation 1.1. ΔD equaled the difference between the maximum and minimum diameter within one pumping cycle. The creep rate during the dynamic experiment $\dot{\epsilon}_{\text{dyn}}$ was calculated based on equation 3.6, taking the difference between two diameter maxima of different intervals for ΔD_c (usually between the two maxima from the start and end of the measuring cycle, $\Delta t = 30\text{s}$).

After measuring the compliance of the JM-tubes, the pressure required to fully unfold the mesh fibers was determined. As the Argon Medical sensor was unable to detect pressures above ~ 330 mm Hg, the WIKA pressure gauge (upper limit of 2000 mm Hg) was included into the setup. Videos of the fiber unfolding and relaxation were taken, to assess the mesh performance and reversibility of elongation and recoiling. The videos were taken with a USB-microscope camera (Androstar) using *Amcap* software for recording.

3.7.4 Burst Strength Measurement

Pressurized burst-tests were carried out using the static compliance setup shown in Figure 3.17a. Like in the static compliance test, the tubular specimen was attached to a syringe pump and a pressure sensor. The distal end of the pressure gauge was sealed with a luer lock. The testing conditions were in accordance to ISO 7198:2016 [7]. The tubes were infused with water at a rate of 500 $\mu\text{L}/\text{min}$ and the pressure increase recorded through *USBsoft2500* software. The pressure was increased until the testing specimen underwent mechanical failure, which was marked by spontaneous leakage or aneurysmal dilatation. The burst-strength was indicated by the peak pressure of the recorded pressure-time diagrams.

3.7.5 Kink Resistance Measurement

Kink resistance testing was carried out in accordance to ISO 7198:2016 [7], see Figure 3.19a. The samples were filled with water, using a 10 mL syringe (Terumo), and were attached to the pressure sensor. The distal end of the pressure sensor was sealed with a luer cap. The pressure was raised to 100 mm Hg and the samples were assessed on their kink resistance through bending around cylindrical objects of varying radii (5, 9, 15, 20, 29 and 35 mm). Any noticeable signs of buckling (Figure 3.19b and c) were deemed a failure of kink resistance at that mandrel radius [7].

Figure 3.19: a) Kink-resistance testing setup with a flexible sample showing no signs of buckling. b) Failure of a tubular conduit due to narrowing and c) kinking during bending.

3.8 Animal Testing

Two animal tests were carried out by Innoheart Pte. Ltd. in Singapore. The *in vivo* tests were performed in accordance to the National Advisory Committee for Laboratory Animals Research (NACLAR) guidelines.

A first *in vivo* assessing a dip-coated tube on its short-term performance was carried out in one rabbit during three hours. 300 μm WT PLC 50:50 and 150 μm WT PLC 70:30 tubes were passed to the surgeons for prior suturing testing. Eventually, a PLC 70:30 tube of 150 μm WT with a length of 3 cm was implanted into one rabbit for aortic bypass grafting. For 3 hours following implantation *in vivo*, the prosthesis was analyzed on leakage, immediate thrombosis or formation of aneurysms and an angiogram was recorded. The rabbit was given Ketamine (10-15mg/kg, IM) and Domitor (0.1-0.2mg/kg, IM) for anaesthesia and maintained on Isoflurane (1-3% via endotracheal tube) during the procedure. Heparin (80-100IU/kg, IA) was given prior to percutaneous vascular intervention for patency assessment via fluoroscopy.

A second set of *in vivo* experiments were carried out in porcine for two weeks. Two JM-Tubes (PLC 70:30 [T] + PCL S-1x-2H-1R [M]) of 4.0 mm inner diameter were used for right carotid artery grafting (via end-to-end anastomosis) in two pigs. The first pig was given 1mg of nitroglycerin intra-arterially and 100mg of lidocaine was dripped topically on the anastomosed ends to relieve anastomotic vaso-constriction. The second pig received 1mg of nitroglycerin (intra-arterially), to reduce the anastomotic vaso-constriction. 40mg of clexane was given subcutaneously immediate post-implantation and post-operatively as per study plan. At the end of the study, the animals were sacrificed and the prostheses explanted. The JM-tubes were analyzed on leakage, thrombosis or aneurysmal dilatation. An angiogram was recorded immediately after implantation and after two weeks (prior to animal sacrifice), to verify the blood flow, leakage due to suturing errors and occurrence of occlusion.

3.9 Summary

Tubular vascular prostheses made of biodegradable PLC or PCL were fabricated via dip-coating. Outer structural reinforcements were added, in order to obtain a non-linear elastic composite. These structures were fabricated by coating a soft tube with a stiffer outer layer the addition of melt-spun fibers or a pliable outer mesh. The meshes were fabricated via 3D-bioprinting. Several mechanical testing methods were used to assess the properties of the different PLC polymers. Tensile testing of solvent cast films or static/dynamic compliance testing of PLC tubes carried out, to choose the most suitable material for the dip-coated tube. Pliable fibers were assessed on their elastic properties in computer simulations (FEM analysis) and during tensile testing, whereas the mechanical performance of the JM-Tubes was studied in dynamic compliance tests.

Animal tests were carried out in order to assess the implantability of pristine PLC- and JM-Tubes. Angiograms were recorded subsequent implantation and at the end of the study, to verify the prostheses on occurrence of mechanical failure and uninterrupted blood flow.

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Chapter 4

Dip-Coated Tubes

Mechanical mismatch between vascular grafts and blood vessels is a major cause of smaller diameter graft failure. To minimize this mismatch, several poly-L-lactide-co-ε-caprolactone (PLC) copolymers were evaluated as candidate materials, to fabricate a small diameter graft. Using these materials, tubular prostheses of 4 mm inner diameter were fabricated by dip-coating. In vitro static and dynamic compliance tests were conducted, using custom-built apparatus, featuring a closed flow system with water at 37°C.

4.1 Methods

Dip-coating was carried out as described in section 3.2. Mechanical properties assessment, tensile-, kink- and compliance tests followed the protocols given in section 3.7.

When preparing the control porcine aortic branch arteries for dynamic compliance testing (4-5 mm diameter, 4 cm length), the fascia was first removed. The arterial vessels were then secured at both ends to luers (two FTLB220-1 luer locks linked to MTLL025-1, Nordson Medical) using nylon cable ties (150 mm length, 4 mm width).

4.2 Fabrication Optimization

Initially, dip-coated tubes with approximately 180 μm wall-thickness (WT) were produced by four times immersion into a 15 w/Vol% PLC solution in chloroform. Gradients in the WT in longitudinal and circumferential direction were very noticeable.

Major improvements were achieved by splitting the fabrication process into two parts: An initial coating of PLC with two layers followed by drying for some time (see Table 3.2) and one later additional immersion (for $\text{WT} > 120 \mu\text{m}$). This allowed the film of the first two layers to partially dry out, which resulted in lower longitudinal WT gradients. The up-side-down placing during the drying furthermore allowed the thicker polymer mass at the bottom to flow backwards, resulting in a more even coating.

Circumferential WT gradients were minimized through horizontal rotation after completion of the dip-coating process. This post-coating treatment could be done only after the dipping procedure was complete. Various experiments have shown that dip-coating after horizontal rotation created a lot of bubbles, which are considered as fabrication defects.

4.3 Mechanical Tests

4.3.1 Tensile Properties of Films

The stress-strain curves and respective tensile data of PLC films are shown in Figure 4.1 and Table 4.1. Poly-L-lactic acid (PLA) and PLC 95:5 were very strong, but brittle as they broke at low elongations of 36 ± 22 and $6 \pm 3\%$ (Figure 4.1a). PLC 85:15 and poly- ϵ -caprolactone (PCL) had higher fracture toughness, as they failed only at strains of $291 \pm 35\%$ and $526 \pm 52\%$. PLA only and PLC 95:5 predominantly consists of L-lactic acid (LA), a monomer that introduces stiffness and brittleness. The small portion of ϵ -caprolactone (CL, the monomer of which PCL is made of) in PLC 85:15 adds softness and elasticity. The tiny fraction of 5% CL in PLC 95:5 seems to be insufficient to disrupt the formation of crystalline LA-domains, whereas in PLC 85:15, the higher availability of CL rendered the polymer noticeably softer [1]. DSC tests have confirmed the crystallinity of PLC 85:15 was lower than in PLC 95:5.

Figure 4.1: a) Tensile test results for PLA, PCL, PLC 95:5 and 85:15. PLA and PLC 95:5 showed a high tensile strength, but were very brittle compared to PCL and PLC 85:15. b) Stress-strain curves for PLC 70:30 and 50:50. Both materials showed high fracture toughness, by only breaking at elongations of greater than 800%. PLC 70:30 was classified as elastomeric, whereas PLC 50:50 is a highly deformable plastic polymer. Much lower stresses were required to elongate both films as for the other materials shown in a).

Table 4.1: Tensile test results of PLA, PLC and PCL films (n = 3).

Material	Elastic Modulus [MPa]	Ultimate Tensile Strength [MPa]	Elongation at Break [%]
PLA	1550.7 ± 224	32.6 ± 2.7	36.4 ± 22.4
PLC 95:5	1348 ± 424.6	25 ± 14	7.1 ± 3.4
PLC 85:15	162.7 ± 25.1	15 ± 0.3	291.6 ± 3.5
PLC 70:30	6 ± 1.8	3.4 ± 0.3	792.7 ± 130.5
PLC 50:50	2.0 ± 0.1	0.5 ± 0.02	761 ± 143.1
PCL	272 ± 10	26 ± 1.4	527 ± 52

4.3.2 Dynamic Compliance Testing Setup

The compliance apparatus allowed detection of ΔD of $\sim 2 \mu\text{m}$ and commonly provided pressure intervals (ΔP) of ~ 29 -40 mm Hg. Both numbers are related to the equipment: The CCD micrometer's accuracy is $\sim 2 \mu\text{m}$, whereas the pressure interval is affected by the peristaltic pump and the tubing used.

The effect of the flow rate of the peristaltic pump was analyzed on three samples. No significant difference was found between the compliance measured at both flow rates (15 and 20 rpm). This indicates the viscoelastic properties of PLC do not lead to significant differences in compliance between flow rates of 15 to 20 rpm (see Appendix Figures A.3 and A.4).

4.3.3 Effect of Monomer Ratio on Tube Properties

Static Compliance

The static compliance test results are summarized in Figure 4.2 and Table 4.2. PLC 50:50 (Figure 4.2b) and 70:30 (Figure 4.2a) were relatively soft and exhibited high compliance ($C_{\text{stat}} = 0.94 \pm 0.3\%$ and $3.52 \pm 0.7\% \cdot \text{mm Hg}^{-2}$), whereas PCL and PLC 85:15 (Figure 4.2a) were the stiffer ($C_{\text{stat}} = 0.2 \pm 0.06\% \cdot \text{mm Hg}^{-2}$ and $0.18 \pm 0.1\%$). The findings corroborated the tensile test results from Figure 4.1. The reasons behind the differences in mechanical properties are the same as explained in section 4.3.1.

Figure 4.2: Radial strain versus time curves from the static compliance tests of different PLC-based tubes. (a) PLC 85:15, 70:30 and PCL, (b) PLC 50:50. The section of the first 30 minutes represents the tubes radial strain during exposure to constant pressure. The section upon 30 minutes shows the strain-recovery of the tubes due to pressure release. ($n \geq 3$ for all grafts).

The inability of the grafts to completely recover from dilatation following the release of the internal pressure was observed for all the grafts. Creep occurred in all the grafts made from each material, which is indicated by the sections of linear increase in radial strain in Figure 4.2. The radial strain curves of PLC 50:50, 70:30, 85:15 and PCL can be divided into five sections: An instantaneous elastic response, followed by retarded elastic deformation, linear creep, instantaneous recovery and delayed recovery. In viscoelasticity theory such a material behavior is described by the *Burgers model* [2]. Instantaneous and retarded elastic deformation are a result of the luminal pressure increase from 0 to 150 mm Hg, whereas linear creep occurred during the exposure to constant pressure of 150 mm Hg. The instantaneous and retarded elastic deformation are fully recoverable, while the dilatation due to creep remains permanent.

Table 4.2: Static Compliance test results for dip-coated tubes ($n \geq 3$ for all grafts). There was a statistical similarity between the compliance of PLC 85:15 and PCL, the recovery was similar for all polymer groups, whereas the creep values of PLC 70:30, 85:15 and PCL were similar.

Material	Static Compliance (C_{stat}) [$\% \cdot \text{mm Hg}^{-2}$]	Strain Recovery (R) [%]	Creep Rate ($\dot{\epsilon}_{\text{stat, stat. Experiment}}$) [$\mu\text{m/min}$]
PLC 50:50	3.52 ± 0.7	80.5 ± 5.6	37.1 ± 12.9
PLC 70:30	0.94 ± 0.3	75.2 ± 3.8	0.4 ± 0.2
PLC 85:15	0.20 ± 0.06	82.2 ± 11.9	0.2 ± 0.08
PCL	0.18 ± 0.1	79.2 ± 12.1	0.06 ± 0.02

It can be noticed from Figure 4.2b that PLC 50:50 showed a very distinct behavior from the other PLC tubes. The polymer displayed a steep increase in strain over time ($\dot{\epsilon}_{\text{stat}} = 37.1 \mu\text{m/min}$). Furthermore, the tubes were almost able to fully recover ($R = 80.5\%$) despite their large deformation (Figure 4.2b and Table 4.2). This implies the linear increase in radial strain over time combines elastic deformation (instantaneous and retarded) and non-recoverable creep. While maintaining the pressure level at 150 mm Hg, the PLC 50:50 tubes kept expanding radially, which led into a decrease in pressure. Hence, the syringe pump had to continuously infuse the PLC 50:50 tubes at rates of 50 $\mu\text{L/min}$ (compared 1-5 $\mu\text{L/min}$ for PLC 70:30 tubes), which further enhanced the tubes' radial expansion. This induced a significant wall-thinning and resulted in a continuous increase in wall-stress. We found the increase in wall-stress was linear, from which we infer to have induced a recoverable elastic deformation (instantaneous and retarded) in addition to the expansion due to creep for the PLC 50:50 tubes (Appendix Figure A.3). Although profoundly elastic, the PLC 50:50 tubes were far from suitable, as they suffered from low burst pressure, excessive wall-thinning and radial growth. Indeed, the tubes were prone to spontaneous leakage during prolonged (30 minutes) static testing.

Dynamic Compliance

The dynamic compliance experiments (Figure 4.3) substantiated the trend observed for the static compliance test. PLC 50:50 tubes were the most compliant with $C_{\text{dyn}} = 1.56 \pm 0.32\% \cdot \text{mm Hg}^{-2}$ and were insignificantly different from explanted porcine aortic branch arteries ($C_{\text{dyn}} = 1.56 \pm 0.43\% \cdot \text{mm Hg}^{-2}$). However, during exposure to

physiological cyclic pressure (80 - 120 mm Hg) the diameter gradually distended at a rate of $\dot{\epsilon}_{\text{dyn}} = 87 \mu\text{m}/\text{min}$, while the porcine arteries maintained their diameter. Furthermore, PLC 50:50 tubes were prone to spontaneous leakage and excessive dilation during the dynamic experiment, which required the WT to be set to 300 μm and reduce the PEG concentration to 0.2%. PEG concentrations of greater than 0.2% increased the occurrence of spontaneous leakage drastically.

In comparison to PLC 50:50 and 70:30, PCL tubes were rigid, displaying the lowest measured compliance among all tested materials ($C_{\text{dyn}} = 0.12 \pm 0.02\% \cdot \text{mm Hg}^{-2}$). No significant difference between PCL and PLC 85:15 was found. The variance in compliance (static and dynamic) within the same specimen group may be attributed to polymer batch-to-batch- and WT-variation (more about influence of WT, see 0) [3, 4].

Figure 4.3: Dynamic compliance boxplot of the dip-coated tubes made of PLC polymers of different monomer composition. ($n \geq 4$ for all grafts, brackets indicate no significant difference).

For further work, PLC 85:15 and PCL were no longer considered as tube material, due to the high mismatch in compliance in comparison to the porcine arteries.

Burst Tests

The results of the burst tests are summarized in Table 4.3. PLC 85:15 and PCL tubes were very strong, i.e. capable of resisting pressures beyond 2000 mm Hg. The pressure

gauge range was up to 2000 mm Hg (2.6 bar). Except for a single sample that displayed severe aneurysm, the failure of PLC 50:50 tubes in the burst tests were marked by the appearance of spontaneous leakage. The failure of PLC 70:30 tubes was due to minor aneurysmal dilatation at the proximal or distal sites (Appendix Figure A.4).

Table 4.3: Burst test results for the tubes of different PLC polymers ($n \geq 3$ for all grafts). The difference between all groups was significant. No statement of significance could be given for PLC 85:15 and PCL, as the pressure gauge could not detect values above 2000 mm Hg.

Material	PLC 50:50	PLC 70:30	PLC 85:15	PCL
Burst Pressure [mm Hg]	256 ± 11	1168 ± 99	> 2000	> 2000

Kink Resistance Tests

The kink-test results demonstrated the kink resistance of the tubes depends, like the compliance, on the monomer composition of the PLC polymers (Table 4.3). Thus, the tubes with high compliance displayed better kink resistance. The best kink resistance was recorded for PLC 50:50 tubes, which only had noticeable kinking at a mandrel radius of 9 mm. PLC 85:15 in comparison and the PCL tubes kinked at radii of approximately 35mm (Table 4.4). It has been shown previously that kinking of vascular grafts during surgical implantation led into thrombosis [5]. This underlines the importance of this mechanical property. A kink resistance test has a typical range of bending radii of 10 to 100 mm [6]. The PLC 50:50 and 70:30 tubes kinked at 9 and 15 mm respectively, which is in the higher end range of kink resistance.

Table 4.4: Kink-test results for single material dip-coated tubes. The numbers indicate the quantity of samples failing at the given bending radii ($n \geq 6$ for all grafts).

Material	Radius of Kinking [mm]				
	9	15	20	25	> 35
PLC 50:50	6				
PLC 70:30		3	3	1	
PLC 85:15					6
PCL					6

4.3.4 Effect of Wall-Thickness on Tube Properties

Compliance

From the static and dynamic tests, it was found that the compliance of the tubes can be tailored through altering the WT. As shown in Figure 4.4 and Table 4.5 for PLC 70:30, compliance decreased with increasing WT. The highest compliance was recorded for tubes of 120 μm WT ($C_{\text{stat}} = 4.79 \pm 2.0 \% \cdot \text{mm Hg}^{-2}$ and $C_{\text{dyn}} = 2.35 \pm 0.64 \% \cdot \text{mm Hg}^{-2}$), whereas tubes of 250 μm WT were markedly stiffer ($C_{\text{stat}} = 0.94 \pm 0.3 \% \cdot \text{mm Hg}^{-2}$ and $C_{\text{dyn}} = 0.77 \pm 0.12 \% \cdot \text{mm Hg}^{-2}$). It was noticed that the compliance decrease was considerable from the 120 WT to the 150 μm WT tubes (values for 150 μm WT tubes = $C_{\text{stat}} = 1.63 \pm 0.30 \% \cdot \text{mm Hg}^{-2}$ and $C_{\text{dyn}} = 1.26 \pm 0.20 \% \cdot \text{mm Hg}^{-2}$). Interestingly, PLC 70:30 tubes 150 μm WT had a compliance closest to that of the porcine aortic branch arteries ($C_{\text{dyn}} = 1.61 \pm 0.36 \% \cdot \text{mm Hg}^{-2}$). No significant difference was noticed between these two groups.

Figure 4.4: (a) Radial strain plotted versus time during the static experiment of PLC 70:30 tubes with varying WT. The first 30 minutes show the radial strain at constant inner pressure, while the other part shows the recovery after pressure release. (b) Boxplot of the dynamic compliance of PLC 70:30 tubes of different WT. Porcine aortic branch arteries were chosen as control. ($n \geq 5$ for all grafts, brackets indicate no significant difference).

Table 4.5: Static Compliance test results for PLC 70:30 tubes with different WT ($n \geq 3$ for all grafts). There was a significant difference between the compliance of the 120 μm WT with the other three groups. The compliance of the 150, 200 and 250 μm WT were statistically similar. Recovery and creep were statistically similar for all groups.

Wall-Thickness (WT)	Static Compliance (C_{stat}) [$\% \cdot \text{mm Hg}^{-2}$]	Strain Recovery (R) [%]	Creep Rate ($\dot{\epsilon}_{\text{stat, stat. Experiment}}$) [$\mu\text{m}/\text{min}$]
120 μm	4.79 ± 2.1	78.4 ± 5.6	2.7 ± 2.1
150 μm	1.63 ± 0.3	73.6 ± 2.7	1.3 ± 0.7
200 μm	1.25 ± 0.3	76.3 ± 4.5	0.9 ± 0.3
250 μm	0.94 ± 0.3	75.2 ± 3.8	0.4 ± 0.2

Similar as discussed in section 4.3, none of the tubes was capable to completely recover the radial deformation during the static test. The rate of creep was dependent on the WT (Table 4.5), being the highest for the 120 μm WT tubes ($\dot{\epsilon}_{\text{stat}} = 2.7 \mu\text{m}/\text{min}$) and the lowest for 250 μm WT tubes ($\dot{\epsilon}_{\text{stat}} = 0.4 \mu\text{m}/\text{min}$). The largest difference was noticed between 120 and 150 μm WT tubes. No significant changes were observed for the strain recovery, which was for all tubes between 70-80% (Table 4.5).

An earlier study by Inoguchi *et al.* [7], found a strong dependency of the compliance on the WT of electrospun PLC 50:50 tubes ($18.7\% \cdot \text{mm Hg}^{-2}$ for 49 μm WT and $2\% \cdot \text{mm Hg}^{-2}$ for 336 μm WT tubes). The reason behind the tunability of the compliance through the WT lays in the cylinder stress σ_{cyl} , which is inversely proportional to the WT τ , see:

$$\sigma_{\text{cyl}} = \frac{P \cdot r}{\tau} \quad \text{Equation 4.1,}$$

where P is the pressure and r the mean radius of the tube. Hence, thinner tubes experience larger stresses at constant inner pressure than tubes of thicker WT [8]. This may explain the increased compliance and creep rate of thin-walled tubes in comparison to the grafts with larger WT [2].

It is inferred from the large increase in compliance, a reduction of the WT below 150 μm may cause the tubes to undergo plastic deformation or fatigue (Figure 4.4 and A.5 and Table 4.5).

This experiment showed how the compliance of PLC 70:30 tubes can be tuned through changing the WT. Increases in compliance were achieved through choosing a thinner WT for dip-coated tubes. However, it was shown that the WT should not be less than 150 μm , as the tubes will experience plastic deformation and fatigue.

Burst Tests

The burst pressure of PLC 70:30 was dependent on the WT (Table 4.6): Thin walled tubes (120 μm WT) failed at low pressures of 584 ± 41 mm Hg, whereas thicker 250 μm WT tubes did at 1168 ± 99 mm Hg.

Table 4.6: Burst test results for the PLC 70:30 tubes of different WT. The difference between the burst pressures of the four groups were significant ($n \geq 3$ for all grafts).

Material	120 μm	150 μm	200 μm	250 μm
Burst Pressure [mm Hg]	584 ± 41	769 ± 9	887 ± 1	1168 ± 99

Although the burst strength of 150 μm WT PLC 70:30 tubes was 769 ± 9 mm Hg, it was more than double the highest blood pressure ever recorded in humans (370 mm Hg) [9]. 250 μm WT PLC 70:30 (1168 ± 99 mm Hg), PLC 85:15 (> 2000 mm Hg) and PCL (> 2000 mm Hg) exhibited better burst strengths than the 150 μm PLC 70:30 tubes, but were remarkably less compliant than the porcine arteries. Hence, a compromise between high compliance and high burst strength had to be found, which was satisfying for 150 μm WT PLC 70:30 tubes. These tubes showed good suture retention strength and the *in vivo* test showed no acute mechanical failure (e.g. leakage, aneurysmal dilatation).

4.3.5 Bi-layered Tubes

Compliance

The aim of fabricating two-layered dip-coated tubes (2L-T) was to create a composite structure with non-linear elastic features similar to arterial tissue, by adding a soft inner lining, mimicking the elastin-rich media and the outer shell taking the role of the adventitia layer [10-12].

With the addition of an inner lining of PLC 50:50 to PLC 70:30 tubes, a compliance of $C_{\text{dyn}} = 1.0 \pm 0.15\% \cdot \text{mm Hg}^{-2}$ (Figure 4.5) were recorded. In comparison to pure PLC 70:30 tubes with $250 \mu\text{m}$ WT ($C_{\text{dyn}} = 0.77 \pm 0.12\% \cdot \text{mm Hg}^{-2}$), the compliance was increased by 79% and 37% respectively. When selecting PLC 50:50 or PLC 70:30 as an inner lining for a pure PCL tube dynamic compliance remained the same (Figure 4.5).

Figure 4.5: Dynamic compliance boxplot for two-layered tubes (2L-T) with added single material tubes (T) for comparison. Brackets indicate statistically similar groups ($n \geq 3$ for all grafts).

The bi-layered tubes showed increased compliance compared to mono-layered PCL and PLC 70:30 tubes. Presumably, this is a result of the reduced WT of the stiff outer layer and the presence of a soft material as an inner lining. Hence, compliance can be increased by maintaining WT and decreasing the amount of stiff polymer with a soft inner lining. PLC 70:30 tubes of $\leq 120\mu\text{m}$ WT were too weak without the 50:50 inner lining. Similar to the mono-layered tubes, the bi-layered tubes lacked any reversible transition, as found for the porcine artery, of high to low compliance with increasing luminal pressure (Figure 4.6).

The bi-layering produced a desired increase in compliance of the tubular grafts but did not introduce any reversible transition from high to low compliance over the physiological pressure range reminiscent of the natural artery (Figure 4.6). The design of adding a soft inner lining to a dip-coated tube shows potential regarding protection from creep and serves as a good platform for further modifications. Optimizations regarding the structural design have to be considered, in order to create vascular grafts with the arterial-like non-linearity in compliance.

Figure 4.6: Compliance-pressure diagrams of the bi-layered tubes and the porcine artery (control, black). The porcine artery showed a reversible transition from high to low compliance upon pressure increase, whereas for the PLC 50:50+PCL and PLC 70:30+PCL [2L-T] tubes it remained unaffected. The PLC 50:50+70:30 [2L-T] tubes showed a slight increase in compliance when the mean pressure was elevated.

Kink Resistance Tests

Furthermore, kink resistance was not improved by the addition of soft inner lining of either PLC 50:50/70:30 to the PCL or the PLC 50:50 to the 70:30 tube (Table 4.4 and Table 4.7). Based on the results, it can be inferred, kink behavior is mainly determined by the stiffer outer layer, unlike a combination of both materials.

Table 4.7: Kink-test results for two-layered dip-coated tubes (2L-T). The numbers indicate the quantity of samples that failed while being bent around items given radii ($n \geq 3$ for all grafts).

Material	Radius of Kinking [mm]		
	20	25	> 35
PLC 50:50+70:30	5	3	
PLC 50:50+PCL			3
PLC 70:30+PCL			3

4.3.6 Dynamic Compliance Assessment

Two types of the fabricated grafts had a compliance that was close to the one of the porcine arteries: PLC 50:50, 150 μm WT PLC 70:30. Figure 4.7a displays their compliance values normalized to the tested porcine artery value. PLC 50:50 showed a very close compliance match to that of porcine arteries. When analyzing these results alongside literature values [13], it can be noticed that expanded Polytetrafluoroethylene (ePTFE) is considerably stiffer than a human femoral artery (HFA) (Figure 4.7b). In addition, Human saphenous veins (HSV), the current golden standard comparison for vascular grafting, has been recorded as having a compliance 74% of that of HFA [13-15].

Figure 4.7: a) Compliance values of PLC 70:30 (150 μ m WT) and PLC 50:50 tubes expressed relative to those of porcine arteries (porcine arteries designated as 100%). The three samples had no significant difference. b) Compliance values of ePTFE and human saphenous veins (HSV) expressed relative to those of human femoral arteries (HFA, designated as 100%) [13].

Although these studies are not directly comparable, as compliance does vary across artery type, species and age [13], the high compliance mismatch between ePTFE and HFA (Figure 4.7b) and the closer compliance matching between PLC tubes and porcine arteries (Figure 4.7a) demonstrated here, it can be assumed the PLC tubes are considerably more elastic than ePTFE. Furthermore, it can be inferred from Figure 4.7a and b the 150 μ m WT tubes have a compliance similar to that recorded for a HSV [16-18].

4.4 Animal Test

As PLC 50:50 and 70:30 displayed the most suitable mechanical characteristics to produce a compliant vascular prosthesis tube, both materials were considered for suture retention testing. Unfortunately, PLC 50:50 formed soft tacky surface, the tubes of which, when handled, became sealed when the luminal surface stuck together. Furthermore, the tubes were easily torn and unsuitable for implantation. Therefore, a 4 mm diameter PLC 70:30 tube (150 μ m WT) was fabricated for testing by a contract research organization (Innoheart, Singapore), to assess handling ability and surgical implantation. One PLC tube was anastomosed as an end-to-end trans-aortic bypass in a rabbit model. The PLC 70:30 tubes could be handled by the surgeon without collapse or noticeable damage and could be easily trimmed to the required size for the implantation site. The tube allowed suturing without forming overly large holes or tears

from the needle punctures. The tube furthermore supported interrupted suturing to the host vasculature to form a sound anastomosis. When the clamps were removed and circulation was re-established and trans prosthetic blood flow was observed without leakage as observed by angiogram (Figure 4.8)

Figure 4.8: (a) A 4mm diameter PLC 70:30 tube was implanted as an aortic graft in a lupine bypass model for three hours. (b) Angiogram showing successful anastomotic suturing with no detectable leakage. The diameter of the implanted graft was 3.8 ± 0.5 mm (shown measurement bar).

The dip-coated tube has demonstrated good surgical implantation ability characteristics for *in vivo* studies. This was not a long-term proof of principle study, since there are additional requirements of a synthetic vascular graft not addressed in this study. Even though PCL and PLC have been described as having good hemocompatibility [19-22], they are not an ideal surface for blood contacting devices. Without any modification PCL/PLC surfaces will absorb protein over time and allow platelet adhesion [21, 23, 24]. Several studies involving PCL or PLC implantation have also included combinations of anti-coagulant and anti-platelet treatment [5, 25, 26]. Further *in vivo* tests should be carried out in large animals, e.g. in canine or porcine and be of longer duration (4 to 6 weeks), in order to evaluate the tubular grafts on their patency rate. Indeed, there is no significant difference in the compliance at physiological blood pressure (80-120 mm Hg) between the 150 μ m WT PLC 70:30 tubes and the porcine arteries. However, arterial compliance is a non-linear property that is very high at low blood pressures and strongly reduced during hypertension. The 150 μ m WT PLC 70:30 tubes did not demonstrate such a characteristic, which requires further modification to match the compliance over the entire physiological blood pressure range.

4.5 Conclusions

Tubular grafts of 4 mm inner diameter of PLC polymers of different monomer compositions were fabricated through dip-coating. The mechanical properties, such as compliance and creep were tested under static and dynamic (physiological conditions) and the kink resistance was determined. We have shown the compliance, creep and kink resistance of PLC tubes are dependent on the monomer feed ratios of LA to CL. We also demonstrated the compliance of PLC 70:30 tubes can be tailored by the WT.

The implantation of a PLC 70:30 tube of 150 μm WT in one rabbit worked successfully, being free of any mechanical failure. For future *in vivo* studies larger animals, i.e. pigs, should be considered and the duration of the study should be increased to 2-6 weeks.

This work shows PLC is a suitable material for the fabrication of compliant tubular grafts. In particular PLC 70:30 with a WT of 150 μm has demonstrated good properties regarding compliance (80% match to porcine arteries), low creep, suture retention, ease of handling and *in vivo* performance. This work suggests PLC 70:30 as a material for further work to fabricate tubular grafts with non-linear elastic features (arterial-like compliance). A first attempt was carried out by fabricating bi-layered dip-coated tubes. No arterial-like compliance was observed, which was attributed to the structural design. The approach by combining a soft and a stiff material was assumed to be the right strategy to be pursued in further work, however, a different structure for the stiffer outer layer has to be considered.

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Chapter 5

Melt-Spun Fiber Composite Tubes

In the previous chapter, dip-coated tubes of various PLC polymers were assessed on their compliance. It was found that PLC 50:50 and 70:30 were suitable materials to match the compliance of arterial tissue. However, none of the tubular prostheses exhibited non-linear elastic properties, a requirement for guaranteeing long-term patency in vivo [1, 2]. An arterial-like compliance behavior was tried to be mimicked by fabricating bi-layered dip-coated tubes. However, the desired properties could not be obtained by that approach. Studying the arterial wall structure, the combination of a soft and stiff material was assumed to be the right strategy, but a different structural design needs to be considered [3-5]. A different approach was followed up by adding a melt-spun fibrous layer [F] to a soft dip-coated tube [T]. PLC 50:50 and 70:30 tubes, both specimen with high compliance were reinforced with outer melt-spun fibers made of different materials. These composites of a dip-coated tube and melt-spun fibers [T+F] were tested and evaluated on their compliance and non-linear elasticity. The effect of spinning speed and fiber orientation (circumferential or helical winding) on the mechanical properties was analyzed.

5.1 Methods

Dip-coating and melt-spinning followed the protocols from sections 3.2.1 and 3.3. SEM imaging was carried out as described in section 3.6.2. In addition to the fiber width measurement in ImageJ, the winding angle was determined.

5.2 Topographical Analysis

Melt-spinning of fibers was successful for all material combinations (as summarized in Table 3.4) tested, see Figure 5.1. The fiber diameter was dependent on the spinning speed used during melt-drawing: The thickest fibers were spun at low mandrel rotation speeds of 100 rpm with an average width of ~50-70 μm . In contrast, fast spinning at 500 rpm produced thin fibers with diameters of ~20-40 μm . Similar findings were reported by Tan et al. [6, 7]: Faster rotation speeds during melt-drawing created thinner fibers but increased the degree of crystallinity. Latter increases the mechanical strength of the fiber.

The melt-spun PLC 50:50 fibers (Figure 5.1a) did not exhibit sharp edges compared to melt-drawn PLC 70:30 or PCL fibers (Figure 5.1c-e). The PLC 50:50 fibers appear fused. The reason behind the partial fusion may be the high poly dispersity index (PDI) of 1.53 and partial degradation, which makes melt-spinning difficult as gummy fibers get formed. For one set of PLC 50:50 tubes with outer PLC 70:30 fibers, it was observed that fibers were mostly vanished or at best p recognizable despite fusion (see Appendix Figure A.8). The PLC 70:30 polymer in the melt-holder and on the tubes appeared yellowish, which was attributed to degradation [8]. Therefore, the partial fusion of the PLC 50:50 fibers may be due to the high PDI or degradation. Melt-spinning always worked best, when freshly obtained polymer was used. In the case of PLC 50:50, the polymer batch from Akina could not be reproduced nor was the polymer obtained from Huizhou ForYou Medical Devices spinnable, which made it not possible to fabricate a new set of PLC 50:50 [T+F] tubes.

Figure 5.1: Micrographs of melt-spun fiber composite tubes: a) PLC 50:50 [T+F, 500 rpm], b) PLC 50:50 [T] + 70:30 [F, 100 rpm], c) PLC 70:30 [T+F, 100 rpm], d) PLC 70:30 [T+F, 500 rpm] and e) PLC 70:30 [T] + PCL [F, 100 rpm].

Melt-drawing of PCL portrayed a challenge, as fiber rupture occurred frequently. Spinning at 500 rpm was not possible, as the fibers ruptured only a few seconds subsequent initiation of the winding process. Hence, PCL fibers could only be spun at 100 rpm, which itself was accompanied by difficulties but still remained possible. It is assumed that either the molecular weight of PCL (80'000 Da) may not have been sufficiently high or the PDI was too high (also partially due to degradation, similarly to the degraded PLC 70:30 fibers, initial PDI of the PCL is < 2 [9]) to enable problem-free melt-spinning [10].

Fast transversal motion of the spinneret led to the deposition of helical (or cross-spun) fibers on the dip-coated PLC 70:30 tubes, see Figure 5.2. As expected, higher traveling speeds increased the winding angle: 11° for 20 mm/s and 20° for 30 mm/s [11]. In comparison to circumferential spinning, the helical winding did not significantly affect the fiber width.

Figure 5.2: SEM micrographs of helically wound fibers produced from transversal spinneret speeds of a) 20 mm/s and b) 30 mm/s. Faster motion of the spinneret increased the winding angle (7.5° For 20 mm/s and 13° for 30 mm/s).

5.3 Compliance Tests

5.3.1 Physiological Compliance

The benefits from reinforcing the soft dip-coated tubes with outer melt-spun fibrous layers were assessed during dynamic compliance tests. The compliance was measured at different pressure levels, to verify the melt-spun fiber composite tubes (MFTs) on non-linear elasticity.

The dynamic compliance results from the MFTs are shown in Figure 5.3. The compliance of MFTs with slow (100 rpm) spun fibers of PLC 50:50 or 70:30 was higher in comparison to those with fast drawn (500 rpm) fibers. The increase in stiffness for fast-spun PLC fibers has been studied in detail by Tan et al. [6, 7]. PLC 50:50 [T+F, 500 rpm] MFTs showed reduced compliance, against the assumptions based on the results in section 4.3.3. It can be inferred from Figure 5.1a the partial fusion of fibers led to a reduction in compliance. Although the WT was the same as for the pure PLC 50:50 dip-coated tubes (300 μm), the outer melt-spun layer may have added more

stiffness, due to crystallization while and after the melt-drawing. The MFTs of PLC 70:30 [T] + PCL [F] were very little compliant as expected based on the results from the bi-layered tubes (see section 4.3.5).

Figure 5.3: Dynamic compliance test results of the MFTs at physiological pressure of 80-120 mm Hg. Brackets indicate statistical similarity between the groups ($n \geq 3$ for all samples, except for PLC 70:30 [T] + PCL [F, 100 rpm] $n = 2$).

In comparison to the PLC 50:50+70:30 bi-layered tubes the PLC 50:50 [T]+70:30 [F] MFTs were more compliant. The reason behind this finding was assumed to be the fibrous structure of the “outer layer”, which was less restrictive than the dip-coated PLC 70:30 outer shell of the bi-layered tubes. However, the dip-coated tube was too soft and the fibrous outer layer incapable of providing sufficient protection from excessive dilatation.

5.3.2 Pressure-dependent Compliance

The plots of the compliance versus mean pressure of the MFTs is shown in Figure 5.4 and Figure 5.5. The porcine arteries exhibited high compliance at low pressures (< 80 mm Hg), which continuously decreased upon pressure elevation. Above 150 mm Hg, the compliance changes were no longer significant (Figure 5.4). The MFTs made of PLC 50:50 [T+F] showed a linear compliance. It is assumed that the partially fused

fibers and post-spinning crystallization have created a stiffer outer layer (similar to the bi-layered tubes) around the soft inner dip-coated PLC 50:50 lining. This may explain their low compliance and resistance to increased pressures.

PLC 50:50 [T] + 70:30 [F] tubes showed increasing compliance over the physiological blood pressure range. It is inferred from the curves in Figure 5.4 that the PLC 70:30 fibrous layer was not sufficiently reinforcing the dip-coated PLC 50:50 tube and hence unable to prevent excessive dilatation (fatigue). Furthermore, the PLC 50:50 used for these MFTs was from a different supplier (ForYouMedical), while PLC 50:50 [T+F, 500 rpm] were made from polymer purchased by Akina (AP 124, batch could not be reproduced by the supplier). PLC 50:50 tubes (300 μm WT) made from the polymer from Akina and ForYouMedical were compared on their compliance. It could be shown that the tubes made from PLC 50:50 supplied by Akina were on average 60% less compliant and more resistant to spontaneous leakage and aneurysmal dilatation. The polymer from Akina was favored due to its superior compliance properties, but the synthesis of that batch could not be reproduced. For better comparison PLC 50:50 [T+F, 500 rpm] tubes were aimed to be fabricated by the polymer supplied from ForYouMedical, but the polymer was not melt-spinnable.

Figure 5.4: Compliance versus mean pressure plots of the MFTs. The porcine arteries show a strong decrease in compliance. Among the MFTs only PLC 70:30 [T+F, 100 rpm] show a slight decrease in compliance. This was observed for 60% of the tested samples. PLC 50:50 [T] + 70:30 [F] samples showed an increase in compliance and massive dilatation. At pressures above 120 mm Hg they underwent aneurysmal dilatation or showed spontaneous leakage ($n \geq 3$ for all samples).

An arterial-like compliance was observed for ~67% of the tested PLC 70:30 [T+F, 100 rpm] MFTs. In comparison, only 25% of the PLC 70:30 [T+F, 500 rpm] MFTs showed such a behavior. It was initially assumed that the decreasing compliance of the PLC 70:30 [T+F, 100 rpm] MFTs may not be a result of the melt-spun fibers but of an oval cross-section of the tubes. However, some MFTs showed an initial increase in compliance between pressures of 50 to ~70 mm Hg, which was then followed by a regression. Indeed, the initial increase in compliance may be due an oval cross-section (thoroughly discussed in section 6.4.1, see Figure 6.16). It was assumed the cross-section became circular upon expansion. The reason for the non-linearity of the compliance of PLC 70:30 [T+F, 100 rpm] MFTs may be a result of the slow spinning at 100 rpm. The latter is assumed to have produced a more coiled polymer chain configuration within the fiber. A similar structural arrangement for slowly spun PET fibers was proposed by Heuvel et al. [12]. Furthermore, the slow spinning led into a looser fiber winding, which is inferred to have allowed post-spinning shrinkage (the ability of PLC 70:30 fibers to shrink was observed for the melt-spun PLC 70:30 [T+F, 100 rpm] tubes with free-standing hoops, see section 6.5). This may have caused additional recoil of the polymer chains. The coiled configuration of the polymer chains within the melt-spun fibers may be the reason, why they have create a non-linear elastic behavior. If that would have been the case, the polymer chains within the fibers would act similarly to the wavy collagens in arteries, which align into the tensile direction upon stretching to induce stiffness [3, 5, 13].

PLC 70:30 [T+F, 500 rpm] and PLC 70:30 [T] + PCL [F, 100 rpm] tubes showed occurrence of a loop formation during compliance testing. When exposed to higher luminal pressures, loop formation was accelerated. It is inferred from post-testing SEM analysis that these tubes underwent longitudinal creep (Appendix Figure A.7). The pores became elliptic and were all oriented into the same direction, which supports the assumption of longitudinal creep. This phenomenon was observed to an only minor extent for tubes with softer fibers at the outside, such as for the PLC 70:30 [T+F, 100 rpm] tubes. The longitudinal creep may be a result of the dip-coated tube's inability to transfer the radial strain to the stiffer outer fibers. Since the fibers were not fused to the tube, this may instead lead into partial expansion in longitudinal direction.

Figure 5.5: Compliance versus pressure diagrams of PLC 70:30 [T+F_C, 100 rpm] tubes. Three compliance curves are shown in this diagram that clearly highlight the occurrence of arterial-like elasticity. In total 6 specimen were tested and 67% exhibited a decreasing compliance upon pressure elevation (n=6).

5.4 Helical Melt-spun Fiber Composite Tubes

The compliance versus mean pressure curves of the helical fiber tube composites (HFT) are shown in Figure 5.6. It is not clear, whether the addition of cross-spun fibers produced a decreasing compliance. The compliance of the HFTs fabricated at medium spinneret traveling speeds (PLC 70:30 [T+F_H, 100 rpm, 20 mm/s]) remained rather linear upon pressure increases.

A slightly different behavior was noticed for the HFTs produced from fast transversal spinneret speeds. 60% of the PLC 70:30 [T+F_H, 100 rpm, 30 mm/s] tubes showed an initial increase in compliance, followed by a minor decrease above pressures of 100 mm Hg, see Figure 5.6b. It is assumed the high winding angle of these helical fiber tube composites (HFT) may be responsible for this compliance behavior. At low luminal pressures, the contribution of the fibers to the mechanical behavior may be low in comparison to the [T+F_H, 100 rpm, 20 mm/s] tubes. Hence, the tube becomes increasingly compliant until pressures of ~100 mm Hg, where a regression sets this may be due to partial circumferential alignment of the fibers. However, at pressures above

150 mm Hg, still no complete circumferential alignment was observed; the fibers were far from orienting into circumferential direction. This may be due to insufficient tubular expansion, which is required to align the helical fibers into transversal direction. The mild decrease in compliance for the PLC 70:30 [T+F_H, 100 rpm, 30 mm/s] HFTs may be attributed to a similar fiber response as observed for the PLC 70:30 [T+F_C, 100 rpm] tubes. However, from the helical winding, it is inferred the stiff response from the fibers is reduced and would become more noticeable upon circumferential alignment. Initial increases in compliance may be attributed to an oval cross-section as discussed in section 5.3.2 (more brief discussion is provided in section 6.4.1), especially between pressures of 50-70 mm Hg.

Figure 5.6: Compliance versus mean pressure diagrams of PLC 70:30 [T+F_H] HFTs. a) compliance of HFTs produced from medium (20 mm/s) and b) fast transversal spinneret speeds (30 mm/s). Compliance of [T+F_H, 100 rpm, 20 mm/s] HFTs remained rather unaffected, whereas slight decreases above 100 mm Hg were noticed for [T+F_H, 100 rpm, 30 mm/s] (n=3 for all samples).

Other materials shall be tried for further work: A softer material for the dip-coated tube that is more resistant to plastic deformation than PLC 50:50, whereas the fiber material may be in the stiffness range of PCL to PLC 95:5.

5.5 Conclusions

After the bi-layering of dip-coated tubes has shown no non-linear elasticity, a fibrous outer layer structure, consisting of melt-drawn fibers, was considered. Melt-drawing

was successful for all material combinations, with the exception of PLC 50:50 purchased from Huizhou ForYou Medical Devices that was not spinnable. In comparison to the bi-layered dip-coated tubes, choosing melt-spun fibers as reinforcing outer layer structure better preserve the compliance of the underlying dip-coated tube. Furthermore, PLC 70:30 [T+F_C, 100 rpm] tubes showed clear occurrence of non-linear elasticity, by exhibiting a decreasing compliance upon luminal pressure increase. It is assumed the slow spinning of PLC 70:30 produces a coiled configuration of the polymer chains within the fibers. This was supposed to have created a non-linear elastic behavior for the PLC 70:30 [T+F_C, 100 rpm] tubes. The fact that non-linear elasticity was much less frequent and pronounced for the faster spun fiber tubes of PLC 70:30 [T+F_C, 500 rpm], supports this assumption. The addition of helically wound fibers did not produce any clear arterial-like compliance behavior. Replacing PLC 70:30 as tube material and choosing a much stiffer polymer for the helically wound fibers, may produce a more desired arterial-like compliance. Although the fabrication of MFTs was quite reproducible, a more deliberate pattern for the outer fibrous structure has to be considered. Also, the issue of loop formation deserves more attention in further work. 3D-bioprinting of a pliable stiffer outer mesh was chosen as alternative to the melt-spun fibrous layer.

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Chapter 6

Mechanical Assessment of Mesh-Tube Composites (JM-Tubes)

The design of bi-layered dip-coated tubes did not provide non-linear elastic properties as desired. Hence, a fibrous structure for the outer reinforcing layer was considered, in order to obtain arterial-like mechanical properties. The addition of melt-spun fibers at the outside of a soft dip-coated tubes had several benefits in comparison to the bi-layered dip-coated tubes. The compliance of the soft dip-coated tube could be better preserved by the melt-spun fibers at the outside. In addition, even non-linear elasticity was observed for grafts produced from slowly spun fibers. The fabrication of such composites (dip-coated tubes with outer melt-spun fibers) was quite reproducible, however, a more deliberate structure for the outer reinforcing layer was considered, to gain more control over the elastic properties. In order to obtain such arterial-like elasticity, composites of a soft dip-coated tube with a pliable stiffer outer mesh (JM-Tubes) were fabricated. The meshes comprised pliable fibers, inspired from stent designs that provide non-linear elasticity [1]. JM-Tubes of various material combinations and mesh designs were assessed on their compliance. The role of the mesh was studied during mechanical testing, in order to improve its unfolding and subsequent restrictive behavior.

6.1 Methods

6.1.1 Fiber Printing

Fibers of PLC 95:5, 85:15, 70:30, PCL and PLA were printed as described in section 3.4.1. Fiber printing was carried out on thick (8 mm) 110 x 300 mm glass slides using 30G needle tips. The printing speed was set to 10 mm/s and a pressure of 0.1 MPa was applied. The fibers consisted of three layers, with a vertical offset of 0.05 mm.

6.1.2 Mesh Printing and JM-Tube fabrication

Mesh printing usually followed the standard procedure as described in sections 3.4.1 and 3.4.2: a 27G size needle tip was used for printing and the speed and the pressure were set to 15 mm/s and 0.1 MPa respectively. PLA meshes had to be printed at 0.15 MPa due to the high viscosity (inherent viscosity was 6.5 dl/g). The meshes were fabricated by depositing two layers of fiber with a vertical offset of 0.1 mm.

For the thin-walled (50-60 μm) JM-Tubes, dip-coating of PLC 70:30 was carried out at immersion- and withdrawal speeds of 300 and 180 mm/s into and from a 15 w/Vol % solution (in chloroform). The WT of 50-60 μm was achieved through singular immersion of the PVA-coated metallic rods into the PLC 70:30/chloroform solution.

PCL and PLC 95:5 meshes were printed from solutions with concentrations of 11.9 w/Vol % (in chloroform) and PLA meshes from 4 w/Vol % (in chloroform) solutions.

6.1.3 Mechanical Testing

Tensile testing of singular fibers was carried out as specified in 3.7.2, and dynamic compliance measurement followed the protocol given in section 3.7.3 (flow rate was for all specimen 15 rpm, while it was 20 rpm for the arterial tissue). The 100 μm WT JM-Tubes were attached to MTLL013-1 and MLRL025-1 (Nordson Medical) luer locks, whereas the 55 μm WT tubes had to be attached to smaller ones (Nordson Medical, FTLB220-1 and MTLL013-1). According to ISO 7198:2016 [2], to assess a

vascular graft on non-linear elasticity, compliance testing should be conducted under dynamic conditions at three pressure ranges at least. In order to comply with this requirement, the JM-Tubes were tested at multiple (> 5) pressure ranges of 50 mm Hg (~ 30 diastolic and ~ 70 mm Hg systolic) up to 300 mm Hg (280 – 320 mm Hg). A second cycle of compliance measurement was carried out at least at three pressure ranges, 30-70, 80-120 and 130-170 mm Hg, to verify the reversibility of the compliance transition of the JM-Tubes. The latter step was an important measure to take, as some samples revealed compliance reduction at low pressures happened due to inflation of an oval tube to adopt a circular cross-section. This issue is thoroughly discussed in section 6.4.1.

After the compliance measurements were completed, the pressure required to straighten out the mesh fibers, P_U , was determined. For that, the same apparatus as for the dynamic compliance tests was used. The OPT pressure sensor was unable to detect pressures above 300 mm Hg, which required the inclusion of the WIKA pressure gauge (MRT-2500) into the setup (detection up to 2000 mm Hg). A flowrate of 10 RPM was applied and the tubing near the reservoir compressed by a ruler clamp to increase the pressure. Pressure and diameter recording was carried out at rates of 500 Hz. Videos to study the unfolding and recoiling behavior of the meshes were taken using an Andonstar microscope and AMCAP software. The pressure required to unfold the mesh, P_U , was estimated by the peak pressure. Additionally, the recorded pressure and diameter signals of these dynamic steady pressurized tests were converted into a stress-strain diagram, to assess the nature of the elastic response (occurrence of a J-Curve).

6.2 Tensile Properties of Fibers – Computer Simulation

6.2.1 Effect of Cross-Section

HEX-fibers of different cross-sections, square, filleted square and circular, were compared against each other in a 2D and 3D simulated tensile experiment (see Figure 3.16 in methods section 3.5). It was hypothesized that the cross-sectional shape may have an effect on the mechanical properties of the fibers. The von Mises stresses were higher for all cross-sections in the 3D scenario (Figure 6.1). FEM Analysis of a square

fiber in the 3D tensile simulation was not possible and attempts to resolve were unsuccessful.

Figure 6.1: Maximum von Mises stresses occurring in HEX fibers of different cross-sections in 2D and 3D tensile testing simulations.

The lowest maximum von Mises stress occurred within the square fibers, compared to the filleted square and circular fibers. The latter two displayed zones of high stress-concentration at the hinges of the HEX-hoops. The circular or filleted square cross-section showed a more even stress distribution throughout the entire fiber with (Figure 6.2). It is inferred from the areas of high stress concentration of square cross-section fibers around the hinges that they are the most prone to break. Furthermore, Figure 6.2 reveals the hinges of the HEX-fibers do not act as ideal joints, which would allow unfolding into fully straightened lines. Instead, the fiber undergoes bending at the corners and leaves visible hinges behind (see the corners in Figure 6.2 with the red zones). High stress-concentration was observed at the corners, as the fibers unfold due to bending at their joints. Hence, one site of the fiber at the corner experiences tensile stress (red areas), while the opposite site is compressed [3-7]. As these stresses increase steadily during unfolding, it is expected that the fibers would provide a stiff response prior to complete straightening.

Figure 6.2: Unfolded HEX-fibers with a) square and b) circular cross-section. The red areas mark centers of high stress-concentration, which was more noticeable on square fibers. Dark blue areas indicate very low stress-concentration, which were observed on the diagonal segments of the square HEX-fiber. The circular HEX-fiber shows very few areas of very low and very high stress-concentration, indicating the stresses are more evenly distributed throughout the entire fiber.

6.2.2 Effect of Hoop Angle

Three HEX-fibers of different length to amplitude ratios (L/A -ratios, step-length was fix 1.0 mm) 0.14x (85.96° hoop angle), 0.5x (75.96°) and 0.82x (65.96°) were compared on their tensile properties in 2D and 3D simulations. It could be shown fibers with steep hoops (low length to amplitude L/A ratio, i.e. short step-length relative to the large step-height.) require more stress to unfold, whereas blunt hoops (high L/A ratio, i.e. longer step-length relative to the step-height.) straightened out more easily

(Figure 6.3). Among these three hoop angles, the HEX-0.14x fiber required the highest strains to unfold, which was attributed to the large step-height of ~ 7 mm. Based on equation 3.3, fibers with low L/A-ratios (large hoop angles) unfold into longer lines than ones with high L/A-ratios (smaller hoop angles). Minimal differences in the 2D simulations were noticed between HEX fibers with L/A-ratios of 0.5 and 0.82x, whereas for the 3D scenario, additional stress was required for blunt hoops. The simulation has shown both fiber types unfold at low strains compared to the 0.14x fibers (85.96°), which were only straightened out at high strains and thus required larger stress. This analysis suggests, ease of unfolding is optimal for fibers with L/A-ratios of ~ 0.5 x. Any further increase or decrease in the L/A-ratio would require larger stresses.

Figure 6.3: Maximum von Mises stresses occurring in HEX-fibers printed at different L/A-ratios (hoop angles).

6.3 Tensile Properties of Fibers – Experimental

6.3.1 Effect of Length to Amplitude Ratio (L/A)

The 3D-bioprinted fibers were first assessed on their tensile properties, in order to detect any non-linear elastic behavior. Several design parameters and materials were tested, to evaluate their effect on the mechanical properties. In a first set of experiments, the tensile properties of PLC 95:5 zig-zag (ZZ) fibers of different L/A-ratios from 1x

(1:1 mm length:amplitude) to 10x (10:1 mm length:amplitude) were analyzed. The stress-strain curves of ZZ- and HEX-fibers are shown in Figure 6.4:

Figure 6.4: Stress-strain curves of continuous PLC 95:5 ZZ fibers with different L/A ratios at the hoops. A J-Curve was obtained for all fiber types. Unfolding occurred at very low elastic modulus, until a stiff response set in after the fibers were straightened sufficiently. The part of the stiff response was linear, until a kink set in, where plastic deformation set in. Fibers with sharp tips were unfolding at higher strains than those with blunt tips. Furthermore, the stiff response is strongly increased for fibers with blunt hoops, compared to fibers with sharp tips (n=3 for all samples).

All fibers exhibited non-linear elasticity, which is indicated by the J-shaped stress-strain curves (J-Curves). The tensile properties of these fibers are assumed to be structural properties, as the material (PLC 95:5) and printing procedure were the same for all fibers. The lower the L/A-ratio (i.e. steeper hoops) of the fibers, the more strain was required for the stiff response to set in. ZZ-10x fibers were unfolding at low strains, as they contained less hoops than the other fibers and because of their blunt tips. This required minimal stretching, in order to straighten the ZZ-10x fibers out.

ZZ-1x and -2x fibers were not tested, as they showed fused hoops, due to polymer accumulation at their sharp tips. An example is shown in Figure 6.5a. This problem could be overcome by changing the spring geometry to HEX (semi-hexagon). The

inclusion of a bridge at the tips, was a major structural improvement, as it increased the angle at the tip, which minimized polymer accumulation at the joints (corners). This enabled printing of very steep hoops with L/A ratios of 2x, 1x down to even 0.14x (1:7 mm length to amplitude). Hoops of HEX-1x and -2x fibers showed no noticeable polymer accumulation at the tips (Figure 6.5b), while for ZZ-fibers (Figure 6.5a) it was clearly visible:

Figure 6.5: Images of the fibers taken by a stereoscopic microscope (Leica M165-FC). a) PLC 95:5 ZZ-1x-2 fiber (lighting from below) and b) PCL HEX-1x-1 fiber (lighting from above using mobile lamps). Although at this stage, the fiber printing was not optimized, HEX-fibers visibly showed better printing quality, which becomes more evident, when comparing the hoop corners. The inclusion of a horizontal bridge to separate the diagonal segments, has increased the angle, which reduced polymer accumulation (green circle). The tips of the ZZ-fibers are fused, showing polymer accumulation (red circle). For the HEX-fibers polymer accumulation was nearly absent.

Tensile tests of HEX-fibers showed similar results (Figure 6.6) as for the ZZ-fibers. In comparison to the ZZ-fibers, the stiff response of the HEX-fibers occurred at lower stresses. Since the fibers were printed with continuous designs, the ZZ-fibers contained more hoops over the same length, due to the absence of the horizontal bridge. For both fiber types, ZZ and HEX, the stiff response was more pronounced when the L/A-ratio was increased. This corroborates the findings discussed in section 6.2.2. The reason behind this may be a more even stress-distribution within the fiber, as blunt hoops are more likely to unfold into straight lines, whereas fibers with steep springs showed more bending as it was predicted in the FEM analyses. It can be inferred from the increased

stress-concentration at the corners (as it was shown in the computer simulations in section 6.2), the stiff response was caused predominantly from the corners, rather than the entire straightened fiber. This may explain the softer response of the fibers with low L/A-ratios, as well as their lower modulus during the stiff response.

Figure 6.6: Stress-strain curves of PCL HEX fibers printed of different L/A ratios. The stiff response is decreased towards lower L/A ratios, whereas the fibers unfold at higher strains. All fibers were printed with continuous designs (n=3 for all samples).

This experiment was a proof of concept, confirming the choice of printing fibers with hoops (ZZ- and HEX-designs), is suitable to create structures with non-linear elasticity. Furthermore, it was shown the unfolding and stiff response can be controlled by altering the L/A-ratio.

6.3.2 Effect of Hoop Geometry

In a third set, the tensile properties of fibers with HEX-, ST- and S-hoop geometries were compared. HEX-fibers showed the steepest increase in the stress upon straightening (Figure 6.7). ST-fibers showed a smaller slope, which may be due to its different unfolding mechanism. The geometry of the ST-fibers leads into diagonal unfolding, unlike for HEX- and S-fibers, which undergo linear stretching. S-fibers required the largest strains to unfold into straight lines. One S-hoop consists of three diagonal segments that unfold into straight lines, HEX-hinge consists of two, whereas ST-fibers only possess one. This may explain, why S-fibers required the most and ST-

fibers the least strain to unfold. Table 6.1 summarizes the theoretical calculations of the unfolding strain ε_T (strain at which the fiber is fully straightened out) and the experimental strain values ε_E , at which the stiff response was initiated. For all spring types, the stiff response occurred at unfolding degrees of $\sim 68\%$, indicating no full extension (100%) is required as theoretically assumed. This shows, the joints of the fibers may not act as ideal springs.

Figure 6.7: Stress-strain curves of PCL-3x-1H fibers with different hoop geometries ST, S and HEX ($n=3$ for all samples).

Table 6.1: Theoretical and experimental strains required to initiate a stiff response for pliable fibers of different spring geometries. The experimental values were lower than theoretically predicted. The stiff response occurred at unfolding degrees of $\sim 45\%$.

Stiff Response	HEX	ST	S
Theoretical ε_T [%]	2.01	1.00	4.03
Experimental ε_E [%]	1.35 ± 0.1	0.72 ± 0.1	2.8 ± 0.5
Relative $\varepsilon_E / \varepsilon_T$ [%]	66.83	70.96	69.31

6.3.3 Effect of Fiber Material

In a third set of tensile experiments, HEX-fibers of different materials (PCL, PLA and PLC 95:5, 85:15 and 70:30) were compared against each other. The effect of material

choice on the unfolding and stiff response was then evaluated. PLC 70:30 fibers were strongly adhering to the glass slides and lost their shape during detaching, which made it impossible to test them. The removal of PLC 85:15 fibers was also critical. On the other side, removal was easy for PCL, PLA and PLC 95:5. As expected from the tensile tests of solvent cast films, PLC 95:5 was stiffer than PCL (Figure 6.8). Stiffer materials would be preferred, in order to obtain a noticeable transition from high to low compliance for the JM-Tubes.

Figure 6.8: Tensile properties of HEX-fibers made from PLC 95:5 (darker colors) and PCL (brighter colors). PLC 95:5 was stiffer. The stiff response set in at lower strains, which was more noticeable for larger L/A-ratios ($n=3$ for all samples).

Figure 6.9: PCL S-fiber being completely unfolded and b) recoiled PCL S-fiber after stress removal. The shape of the S-spring could be recovered to a large part, which was not possible for PLC 95:5 of PLA. The photos were taken using a Sony α 290 camera.

Albeit PLC 95:5 would be a favorable choice, its shape memory was poor after stress release. In contrast, PCL fibers were capable of retaking their coiled stage shape, see Figure 6.9.

6.3.4 Effect of Segment Length

PCL HEX-1x fibers of different scaling factors (1,2 and 4) were printed and their mechanical properties assessed in a tensile experiment, see Figure 6.10. Although the total length of the fiber was kept constant (through adjusting the number of hoops according to the scaling factor; eight hoops for the 1x-1 fibers [1x-1-8H], 1x-2-4H and 1x-4-2H), ϵ_E was increased for fibers of larger scaling factors.

Figure 6.10: Stress-strain curves of PCL HEX-1x fibers of different scaling factors 1, 2 and 4. The scaling factor has an effect on the unfolding strain and the stiff response. Larger scaling factors delay the unfolding but also increase the stiffness of the straightened fiber. The blue line shows the progression of the unfolding degree with further straining (based on theoretical calculations using Equation 3.2). The curve was created using Equation 3.6; because the maximum length $L_{\max}$ of the three fiber types was equal, the unfolding degree was consequently

the same for them. Complete fiber straightening is reached at strains of $\sim 32\%$. The yellow, orange and red lines indicate the unfolding degrees of the fibers at which the stiff response sets in. Fibers of large scaling factors require higher strains to induce stiffness ($n=3$ for all samples).

Furthermore, the stiff response appeared more sudden for HEX fibers of greater scaling factors. The stiff response for the three fiber types tested, occurred at different degrees of fiber unfolding: HEX 1x-1-8H fibers required an unfolding degree of only $\sim 45\%$ (yellow line), whereas HEX 1x-4-2H fibers (red line) displayed a stiff response at unfolding degrees of $\sim 82\%$. As mentioned in section 6.3.1, HEX-fibers of small L/A-ratios can be printed at much better precision than ZZ-fibers. However, their corners do neither behave as ideal joints. For fibers with shorter diagonal segments (i.e. lower scaling factors), the distance between the accumulation centers at the corners in relation to the total segment length is short. In comparison, fibers of larger scaling factors, such as HEX-1x-4-2H, this distance relative to the diagonal length is greater. In addition to that, due to the larger segment length, the diagonals would contain more areas of low stress concentration. This is hypothesized to have provided a more ideal unfolding behavior, which may explain why ϵ_E was the largest among the tested fiber types. FEM simulations may provide further clarification.

6.3.5 Effects of Number of Hoops

Based on equation 3.1 it was assumed the inclusion of additional hoops would increase the total length of the pliable fiber and hence require more strain to initiate a stiff response. Therefore, the unfolding properties of HEX fibers with a varying number of hoops from 1 to 5 were evaluated in tensile experiments. As predicted by equation 3.1, ϵ_E is shifted towards higher strains for an increasing number of hoops (Figure 6.11). The inclusion of additional hoops requires more diagonal segments to unfold into straight lines, which leads to an increase in the total fiber length. Table 6.2 lists the values of ϵ_T and ϵ_E and shows the unfolding degree at which the stiff response occurred (ϵ_E/ϵ_T) was not significantly affected by the number of hoops. Hence, all fibers showed a stiff response at unfolding degrees of $\sim 32\%$ (100% equals complete fiber straightening, i.e. ϵ_T), as for the HEX-fibers with a scaling factor of 1 (see Figure 6.10).

This indicates the findings in section 6.3.4 are predominantly a result of the scaling factor rather than the number of hoops.

The modulus during the stiff response was decreased, when more hoops were included. As mentioned in sections 6.3.2 and 6.3.4, the fiber unfolding is not ideal. With every inclusion of an additional hoop, the portion of not fully straightened bits in relation to the total fiber length is increased. This may explain the decreased elastic modulus of HEX-3x-5H fibers (and the HEX 1x-1-8H fibers in section 6.3.4) during the stiff response.

Figure 6.11: Stress-Strain Curves of PCL HEX-1x fibers with varying hoop number. More hoops increase the unfolding strain, but reduce modulus of the stiff response ($n=3$ for all samples).

Table 6.2: Theoretical and experimental strains required to initiate a stiff response for HEX-fibers of varying number of hoops. The experimental values were lower than theoretically predicted. The stiff response occurred at unfolding degrees of $\sim 32\%$.

Stiff Response	1H	2H	3H	4H	5H
Theoretical ε_T [%]	4.12	8.24	12.36	16.48	20.6
Experimental ε_E [%]	1.58 ± 0.24	2.64 ± 0.08	3.87 ± 0.12	2.23 ± 1.16	6.87 ± 0.48
Relative $\varepsilon_E / \varepsilon_T$ [%]	38.35	32.04	31.31	24.27	33.35

6.3.6 Fiber Width

The effect of the strut width on the mechanical properties of HEX-fibers was studied in tensile experiments. The tensile properties of HEX-fibers are affected by the strut width. More stress is required to unfold thicker fibers, while the stiff response sets in at lower unfolding degrees, see Figure 6.12 and Table 6.3:

Figure 6.12: Stress-strain diagrams of HEX-fibers (PCL-HEX-0.5x-2H) of different strut width. The stiff response occurred at higher strains for thinner fibers ($n=3$ for all samples). As explained in section 6.3.4, equations 3.2 and 3.6 were used to determine the degree of fiber unfolding upon increase in strain (blue line). The helper lines (yellow, orange and red) were drawn to obtain the experimental strains ϵ_E at which the stiff response occurs.

As fiber unfolding is mediated by a bending mechanism at the joints, it is assumed that more stress is required for thicker fibers. Bending theory predicts the sites that are further away from the neutral line experience more strain, which may explain the findings [3-7]. The increased moduli of the stiff response for the thicker fibers may arise from bending at the corners, rather than stretching of the straightened fiber. For the latter, the measured stress would be independent from the cross-sectional area [3,

6-8]. This assumption may be supported by the decreasing values of ϵ_E , measured for thicker fibers (Table 6.3).

Table 6.3: Theoretical and experimental strains required to initiate a stiff response for the HEX-0.5x-2H fibers of different width. The experimental values were decreasing towards larger strut sizes.

Stiff Response	W1	W2	W3
Fiber Width [μm]	233	427	646
Theoretical ϵ_T [%]	20.82	20.82	20.82
Experimental ϵ_E [%]	8.39 ± 0.17	6.98 ± 0.18	5.36 ± 0.04
Relative ϵ_E / ϵ_T [%]	40.30	33.53	25.74

It can be concluded thicker struts for the J-Meshes would turn the JM-Tubes less compliant at low pressures. Hence, increasing the fiber width may not be a favorable choice. Instead, fabricating J-Meshes of stiffer materials with a strut width of 233 μm (W1) may be a better option.

6.3.7 Rounded Hoops

The tensile properties of fibers with rounded (smoothened) hinges were compared to normal HEX-fibers. The stress-strain diagrams are shown in Figure 6.13. The results show no significant changes regarding the stiffness of the straightened fiber. Printing smoothened hoops did not provide additional ease of unfolding. The elastic moduli of the soft region remained unaffected. The strain at which the stiff response occurred was affected to a smaller degree: for Smooth-1 it was about 5%, compared to ~6% for normal HEX-fibers.

Figure 6.13: Tensile properties of normal and smoothened HEX-0.5x-2H fibers. Smoothing of the corners did not improve ease of unfolding, neither was the stiff response affected. The strain where the stiff response sets in ϵ_E was only slightly affected ($n=3$ for all samples).

It was assumed the rounding of the corners would minimize the areas of high stress concentration at the hinges that appear in normal HEX-fibers as shown in Figure 6.2 and enable easier unfolding. However, when analyzing the stress-strain diagrams in **Error! Reference source not found.**, no clear benefits from rounding the hoops become apparent, nor was the stiff response of the fibers affected. The reason why no differences during the unfolding could be detected may lie in the load cell used. A 10 N load cell was used for single fiber testing. The use more sensitive load cell may provide further clarity. Since there was no clear difference in the mechanical properties of the three tested fiber types, rounding of the hoops was not considered for later mesh printing.

6.4 Compliance of JM-Tubes

6.4.1 Single Hoop Meshes

After a thorough study of the elastic behavior of the pliable fibers, JM-Tubes were fabricated with mesh designs based on the findings from the singular fiber tensile tests. In this first set, JM-Tubes with single hoop meshes (see Figure 3.9) were tested. The purpose of including only one hoop was to achieve complete mesh unfolding at low

radial expansion of the underlying tube. The compliance test results are summarized in Figures 6.14 and 6.15. All JM-Tubes showed occurrence of decreasing compliance between pressures of 50 and 150 mm Hg.

From Figures 6.14 and 6.15 it can be seen, the compliance curves of A1-A2 and B1-B3 were distinct from the meshless PLC 70:30 100 μ m WT tube. The latter showed a progression in compliance upon luminal pressure increase, in contrast to the JM-Tubes. Steady increase in compliance for 100 μ m WT tubes is due to the same reasons as explained in section 4.3.4. The compliance of the JM-Tubes showed slight decreases or increases at low to medium pressures (50-120 mm Hg). It is assumed, the different types of JM tubes display a similar behavior at elevated pressures (usually above 150 mm Hg), showing a moderate increase in compliance. In particular for the JM-Tubes with A1/A2 meshes, the increases in compliance were noticeable above pressures of 150 mm Hg, while for the B1-B3 meshes this phenomenon was more suppressed. The reduction of the vertical gap between the struts may have been the reason for this, as the tubes were more densely covered with fibers. The compliance data shown in Figures 6.14 and 6.15 highlight the J-Meshes fulfil their role as a protective structure to prevent excessive dilation and fatigue of the underlying tube.

Figure 6.14: Illustrations of the designs of the a) A1 and c) A2 mesh. Compliance of JM-Tubes with single hoop meshes b) A1 and d) A2 and e) controls (grey: meshless PLC 70:30 tube and black: porcine aortic branch artery). The compliance of JM-tubes was slightly decreasing from low to medium pressures (50 to 120 mm Hg). In contrast, the meshless tubes (grey) underwent fatigue and showed steady increase in compliance towards higher pressures (brackets indicate statistical similarity, $n=3$ for all samples).

Figure 6.15: Illustrations of the designs of the a) B1, c) B2 and e) B3 mesh. Compliance of JM-Tubes with single hoop meshes b) B1 and d) B2 and f) B3. Similar as for the A-mesh JM-Tubes, the compliance of the B-mesh JM-tubes was slightly decreasing from low to medium pressures (50 to 120 mm Hg, $n=3$ for all samples).

Although the fabrication procedure was the same within one group of JM-Tubes, the compliance curves of the three specimens are not necessarily similar. This may be due to four reasons:

1. The JM-Tube may have been oval
2. Differences in the tightness of the mesh grip
3. Crystallization post-dip-coating and -printing may have led to sample to sample variation
4. Location of the LED hitting the specimen tube surface for compliance measurement.

It was commonly observed that tubes showed slightly oval cross-sections, which required inflation to become circular. In the compliance-pressure plots this can be seen from initial increases in compliance or steep decreases in compliance. Depending how the tube was placed in the testing apparatus it either expanded at high rates under cyclic pressure (Figure 6.16b) or only little (Figure 6.16c). If the oval tube was placed upright so that the semi-major axis would be aligned (or nearly aligned) in vertical direction, luminal pressure increases caused a shrinkage, see Figure 6.16a and Appendix Figure A.8. This was avoided by placing the sample adequately as shown in b) and c). In both cases, the compliance mostly decreased moderately above pressures of ~100 mm Hg.

Figure 6.16: Inflation of oval tubes depending on how they are being placed in the compliance testing apparatus: a) The tube is placed with the semi-major axis oriented in vertical direction. Upon inflation the diameter shrinks until the tube's cross-section has become circular. b) Tube is placed with the semi-minor axis being oriented in perpendicular direction. Inflation of the tube causes the detected diameter to increase. The tube is initially getting inflated, so its cross-section becomes circular, before material stretching sets in at higher pressures. c) Neither of the axes is aligned vertically. The compliance either moderately increased or decreased upon inflation. d) Compliance-pressure curves of two B1-JM-Tubes. A second measuring cycle (run)

was carried out, in order to verify the reversibility in compliance change over pressure. The curves from the second run show compliance at low pressure is reduced in comparison to the first run, but above pressures of ~100 mm Hg, the curves were overlapping.

Although quality control during mesh wrapping has been optimized, full reproducibility may not be guaranteed. Slight variations while fusing the mesh to the tube, may either produce a tighter or looser grip. The latter may also justify high compliance values detected at low pressures. Secondary runs of compliance testing support the assumptions listed in point 1 and 2. Figure 6.16d shows the compliance at low pressure has been decreased in comparison to the first run. Since the dip-coated tubes were exposed to pressures up to 300 mm Hg during the first run, it underwent creep (full relaxation was not possible, see section 4.3.3), which may have left behind a more circular cross-section and led to a tighter grip of the mesh. Upon pressure increase, the differences between the first and second run diminished, which was usually observed above ~70 mm Hg.

Variation in mechanical properties due to different crystallization in polymers during printing was reported previously [9]. This may apply to the dip-coated tubes and the meshes and be the cause of the mechanical differences [10].

Depending on the location, where the LED of the CCD micrometer hits the tube surface during compliance measurement, the tube may expand at different rates. This is a result of the presence of the mesh, which did not provide uniform pressure distribution throughout the composite tube. Tube expansion was high near the middle between two struts, while it was comparatively low near the mesh fibers. A schematic explanation is shown in Figure 6.17:

Figure 6.17: Schematic view of a JM-Tube during dynamic compliance testing. The tube in between the fibers of the J-Meshes is not restricted and hence expanding at higher rates (yellow and green zone), compared to the node points (red region). Tube expansion near the fibers (yellow zones) was moderate, while in the middle (green zone) it was the highest. Hence, compliance measurement is highly dependent on the location on the JM-Tube. It was tried to be minimized during setting up the test but could not be fully avoided.

When comparing the JM-Tubes of groups A and B, two major findings were observed: Reducing the spacing between the struts and increasing L/A -ratios rendered the JM-Tubes less compliant. Especially a reduction of the inter-strut space had a strong influence, which can be seen when comparing the compliance curves of the A2- and B1-JM-Tubes.

The addition of a pliable mesh around a soft dip-coated tube has shown to reduce the compliance (Figures 6.14 and 6.15) and moreover provide protection against excessive dilation. The latter could be seen in Figure 6.18; all JM-Tubes were more resistant to bursting than 100 μm WT PLC 70:30 tubes. Furthermore, the unfolding pressure does not indicate bursting of the JM-Tubes, but the mesh spring having straightened out:

Figure 6.18: Pressures required to unfold the different meshes of the JM-Tubes (light grey) and burst pressure (red cross) of the meshless PLC 70:30 100 μm tubes (dark grey). The burst pressure of the controls was measured the same way as the unfolding pressure for the JM-Tubes. JM-Tubes with low L/A-ratio were unfolding at lower pressures, despite consisting of a steep hoop, whereas meshes with blunt hoops required higher pressures. The B-JM-Tubes show an increased degree in fiber coverage, i.e. lower vertical strut spacing, which renders them stiffer. Brackets with * indicate statistically significant difference ($n=3$ for all other samples).

The trend of the measured mesh unfolding pressures summarized in Figure 6.18 contradicts the theoretical predictions by equations 3.3 and 3.4. According to both equations, mesh-tubes with blunt (large L/A-ratio) hoops are required to grow to smaller diameters than such with steeper ones (low L/A-ratio) and are supposed to unfold at lower pressures. However, as discussed in section 6.3.1, tensile tests revealed fibers with large L/A-ratios require more stress to unfold than those with low ones. The results shown in Figure 6.18 corroborate the stiffer behavior of high L/R-ratio fibers observed during tensile testing (section 6.3.1). Near complete mesh unfolding, it was observed that the expansion of the mesh was accelerated (not bursting). This may explain the large standard deviation in the measured unfolding pressures.

The single hoop meshes showed difficulty in unfolding, which was assumed to be due to two factors: Insufficient stress transfer from the glue line to the hoops and inter-strut expansion of the dip-coated tube. In this composite design, the hoop of the JM tubes is located at the opposite site of the glue line. From the videos and Figure 6.18 it is inferred the stress is not accordingly transferred from the glue line to the hoop, resulting in an insufficient force pulling the spring from both sides to unfold the hoop. Furthermore, it is assumed that the force generated onto the hoop is rather vertical than circumferential, because the glue line was located at the opposite site of the tube. This may have caused the fiber to be “imprinted” into the PLC tube. In addition, the space between the fibers was sufficient to allow the tube to expand, which could be observed in the videos. This may explain the large differences between the theoretically assumed and measured mesh unfolding pressure.

Although no compliance reduction was observed upon progressing mesh unfolding, it is inferred the meshes have instead created a primary decrease in compliance at low pressures. Initial fiber unfolding may have caused a primary stiff response, which led to a compliance reduction as it was observed for most of the samples above 70 mm Hg. This primary stiff response may be a result of the initiation of fiber unfolding and friction between the fiber and the tube surface. Based on the compliance data shown in **Error! Reference source not found.**, it is assumed the initial decreases are not solely a result of oval tube inflation and lose mesh grip. The compliance test results show the meshes fulfil their role to provide an arterial-like compliance behavior. However, a secondary compliance reduction due to fiber unfolding, as it was observed during tensile testing, has yet to be achieved.

6.4.2 Double Hoop Meshes

The single hoop JM-Tubes revealed the inability of the meshes to fully straighten out at physiologically relevant pressures. Insufficient stress transfer from the glue-line to the hoops was hypothesized to be the obstacle preventing easy mesh unfolding. Hence, JM-Tubes with meshes consisting of two hoop sections separated by an additional glue

line in the middle were fabricated (as shown in

Figure 3.10). Furthermore, meshes of different spring geometries HEX, ST and S were compared.

The compliance-pressure diagrams of JM-Tubes with HEX- (C), ST- (D) and S- (E) meshes are shown in Figure 6.19 and Figure 6.20. Although C2, C3 and C4 JM-Tubes showed some signs of arterial-like compliance, it is not clear, whether it is a result of a non-linear elastic mechanical behavior. As explained in the previous section, the decrease in compliance may be due to a primary stiffening response from the initiated fiber unfolding. The C1, D1 and E1 meshes may have been too flexible/soft, to create any noticeable primary stiffening response at the tested pressures. Like the single hoop mesh JM-Tubes, all tested JM-Tube specimens were more resistant to excessive dilation under increasing pressure than the meshless PLC 70:30 100 μm WT controls (grey). Inter-strut expansion was observed for all tubes (C1-C4, D1-D3 and E1), indicating this issue has yet to be solved. As discussed in section 6.4.1, the spacing between the mesh fibers was too big, which allowed the tube to locally „bulge“ out.

The D2 and D3 JM-Tubes only show a minor decrease in compliance. This may be due to the different unfolding mechanism of the ST-fibers. The fibers would straighten out

into diagonal lines, in comparison to the HEX-hoop, which completely flattens. It is assumed that the initiation of unfolding and friction between the fibers and the tube did not create an arterial-like stiff response as observed for the C2-C4 mesh tubes.

HEX- and S-mesh unfolding resulted in nearly straight fibers, whereas ST-fibers were unfolding into diagonals. For further work, HEX-fiber meshes were considered, as they showed reasonable expansion upon pressure increase, although mesh-designs require further optimization. The ST-designs were not chosen due to the diagonal unfolding and S-designs, because of their large spring length, which requires high radial expansion of the underlying tube to straighten out.

Figure 6.19: a), c), e) and g) Designs of the double hoop HEX-meshes (C1-C4). b), d), f) and h) Compliance of JM-Tubes with HEX-meshes (C1-C4). Most of the tubes showed a mild decrease/increase in compliance up to pressures of 100 mm Hg. This may be attributed to an oval cross-section of the JM-Tubes at low pressures, as well as a primary stiffening response from the mesh. The C1-meshes create no stiff response within the mesh-tube composite, while

C2 and C3 meshes did. C4 JM-Tubes showed an increase in compliance above a pressure 250 mm Hg, indicating fatigue. All JM-Tubes show better resistance to excessive dilation than the meshless PLC 70:30 100 μm WT tubes (n=3 for all samples).

Figure 6.20: a), c), and e) designs of the double hoop ST- and g) of the double hoop S-meshes. b), d), f) and h) Compliance of JM-Tubes with ST- and S-meshes (D1-D3 and E1). Most of the

tubes showed mild decrease/increase in compliance up to pressures of 150 mm Hg, which may be due to an oval cross-section of the JM-Tubes at low pressures. All JM-Tubes show better resistance to excessive dilation than the meshless PLC 70:30 100 μ m WT tubes (n=3 for all samples).

The double hoop mesh tubes showed an improved unfolding behavior under continuous pressure increase in the dynamic compliance apparatus. The inclusion of a second hoop allowed the JM-tubes to better radially expand upon pressure increase and fiber unfolding was facilitated. An example of the mesh unfolding of a C1-JM-Tube is shown in Figure 6.21:

Figure 6.21: a) JM-Tube with C1-mesh exposed to 30-70 mm Hg intervals (mean pressure 50 mm Hg) of cyclic pressure during dynamic compliance testing, showing the coiled fibers. b) Same JM-Tube exposed to high pressure of < 400 mm Hg, showing the fibers being nearly unfolded into straight lines. After pressure release, the fibers returned to their original shape as shown in a). Some hoops were colored blue for better display. Also visible in b) is the tube bulging (blue box) due to inter-strut expansion.

Figure 6.22 shows the required pressures to completely unfold the mesh fibers into straight lines. Comparing these results with Figure 6.18, the unfolding pressure was no longer increasing for meshes with higher L/A-ratios. In fact, the C4-mesh (HEX-2x-2-2H) required the least pressure as theoretically assumed based on equations 3.3 and 3.4. The pressures required to fully extend the C1-C3, D2 and E1 meshes were similar. Theoretically, it should be descending, as large L/A-springs require lower strains. However, comparing the unfolding pressure value with the burst pressure (under dynamic testing) with the meshless PLC 70:30 100 μ m WT control tubes, it may be

assumed the mesh tubes were instead undergoing fatigue at pressures above 450 mm Hg. Aneurysmal dilatation was observed for the JM-Tubes with the D3 meshes.

Figure 6.22: a), b) and c) Unfolding pressures of the HEX- C1-C4, (light grey), ST- D1-D3 (blue), S-JM-tubes E1 (red) and burst pressure of the meshless PLC 70:30 100 μ m WT tubes (dark grey, red cross) (brackets with asterisks indicate statistical difference between the specimen (* indicates difference between the same JM-Tube group, e.g. within C-meshes; ** indicates significant difference between two different mesh type groups, e.g. between C and D meshes; n=3 for all samples).

6.4.3 Grid- and two-round Meshes Tubes

The double hoop meshes showed improved fiber unfolding and were less restrictive during the coiled stage. However, inter-strut expansion remained an unsolved issue. Hence, grid (F) and two-round meshes (G) (as shown in Figure 3.11) that would provide a denser fiber coverage in the JM-Tube composite were printed.

The compliance-pressure diagrams of the F- and G-JM-Tubes are shown in Figure 6.23. In the low-pressure region (< 100 mm Hg), it was observed that the compliance either decreased or increased, which was attributed to an oval cross-section of the tubes and/or loose mesh grip. Some compliance curves, in particular for the F1 and G1 JM-Tubes, show positive or negative peaks. It is assumed to be a result of the increased fiber density, which caused difficulty in compliance measurement, as discussed in section 6.4.1. From the recorded compliance values, it is inferred that the tube diameter was either measured on the fiber or on the tube surface itself. The location where the laser beam of the CCD micrometer hit the tube surface may have been shifting due to tubular expansion. As explained in Figure 6.17, the fibers act as node points, i.e. regions where the tube is the least compliant. Hence, measuring either on the fiber or on the tube surface provides different compliance values. This issue was less observed for the A-E-mesh tubes, as it was easier to carry out the compliance tests by measuring the diameter on the tube surface (between the fibers).

The G-meshes have shown to be less restrictive than the F-meshes, which allowed the underlying tube to expand easier. When the expansion of the meshless control tube (grey) was compared to the porcine arteries, it was revealed that it was less elastic. This may be overcome by choosing a softer material for the dip-coated tube or reducing the WT.

Figure 6.23: Designs of the grid meshes: a) F1 HEX-0.5x-1H-1R and b) F2 HEX-1x-2-1H-1R. Compliance of the JM-Tubes: b) F1 , d) F2 mesh tubes and e) controls (grey: meshless PLC 70:30 100 μm WT tubes and black: porcine artery). Compliance measurement was accompanied by difficulties, as the diameters either were measures on the fiber or tube surface. It is assumed the expansion of the tube may have caused small dislocations, so the laser beam either hit the fiber or tube surface. For JM-Tubes with flatter hoops, i.e. F2, this phenomenon did not occur (n=3 for all samples).

Figure 6.24: Designs of the two-round double hoop meshes: a) G1 HEX-0.5x-2H-2R and b) G2 HEX-1x-2-2H-2R. Compliance of the JM-Tubes: b) G1 , d) G2 mesh tubes and e) controls (grey: meshless PLC 70:30 100 μm WT tubes and black: porcine artery). Compliance measurement was accompanied by difficulties, as the diameters either were measures on the fiber or tube surface. It is assumed the expansion of the tube may have caused small dislocations, so the laser beam either hit the fiber or tube surface. For JM-Tubes with flatter hoops, i.e. G2, this phenomenon did not occur (n=3 for all samples).

Figure 6.25: Unfolding pressures of the grid mesh- (grey) and two-round mesh JM-tubes (red). Meshless PLC 70:30 tubes with a WT of 100 μm were chosen as control and exhibited burst at pressures of ~ 400 mm Hg. The brackets indicate statistically similarity ($n=3$ for all samples).

Figure 6.25 shows the unfolding pressures of the grid and two round mesh tubes. The pressures of the F2 and G2 meshes were similar, albeit the F2 meshes only contained one hoop. This showed the F-meshes were more restrictive and did not show complete unfolding at lower pressures as it would be expected. Once more, it could be confirmed meshes with larger L/A-ratios provide more stiffness and showed complete unfolding only at higher pressures, despite having shorter springs.

6.4.4 Diamond Mesh-Tubes

For this fourth set of JM-Tubes, amendments in the composite structure were made to fix most of the issues that had remained unsolved so far:

- Insufficient radial expansion of the JM-tubes at low pressure $\rightarrow$ reduction of WT
- Inter-strut tube expansion $\rightarrow$ printing of very dense meshes
- Lack of stiff response from the mesh under unfolding $\rightarrow$ different mesh materials

Hence, the WT of the dip-coated tube was reduced to $\sim 50\text{-}60$ μm , in order to increase the radial elongation. Furthermore, inter-strut expansion was hindered by including a mesh section of high density of crossing diagonal fibers (diamond, DM, sections, see

Figure 3.12). Additionally, different mesh materials, PLC 95:5 and PLA were analyzed on their stiffness contribution upon unfolding.

Figure 6.26: a) Mesh design of the H1-DM mesh. Compliance of H1-DM meshes of different materials: b) PCL, c) PLC 95:5 and d) PLA. Arterial-like compliance (black curve) was observed for the JM-Tubes with a PLA mesh (red). d) Compliance of the controls (grey: meshless PLC 70:30 100 μm WT tubes and black: porcine artery). The meshless tubes were highly compliant and underwent mechanical failure (proximal, distal or spontaneous leakage). Their compliance was increasing very fast, indicating excessive dilation and fatigue. On the other side, the arteries showed a reduction in compliance upon pressure increases, demonstrating non-linear elasticity (n=3 for all samples) (n=3 for all samples).

The JM-Tubes with PCL and PLC 95:5 also displayed decreasing compliance, however, to a lesser degree than the PLA-JM-Tubes (Figure 6.26). The compliance at high pressures for PCL and PLA meshes was similar, whereas PLC 95:5 meshes were providing the highest amount of stiffness. The steep decreases at low pressures (50-100 mm Hg) in compliance can be partially accounted to an oval cross-section and/or loose mesh grip. In addition to that, primary stiffening from the initiation of fiber unfolding and differences in the mesh grip are assumed to have contributed to these findings. Inter-strut expansion was nearly eliminated; it was not noticed during exposure to high stresses. This shows the DM-meshes were dense enough to create an even stress-distribution throughout the entire tube. However, Figure 6.26 shows the JM-Tubes were visibly more rigid than the meshless tube, which indicates the DM-meshes were too restrictive, especially at low pressures (50-100 mm Hg).

For these mesh types (H1), the material choice had an effect on the compliance properties of the JM-Tubes. PCL meshes acted the least restrictive, whereas PLC 95:5 meshes were the most. This corroborates the findings from sections 4.3.1 and 6.3.3. Albeit PLC 95:5 and PLA were stiffer than PCL, they were prone to fiber rupture above pressures of 200 mm Hg; especially around 300 mm Hg, it occurred very frequently. This shows, PCL is still superior regarding its elastic properties and shape memory. Due to frequent fiber rupture and occurrence of spontaneous leakage at pressures around 300 mm Hg, mesh unfolding pressure could not be recorded.

6.4.5 Thin-walled two Round Mesh Tubes

Although a reduction in the WT of the dip-coated PLC 70:30 tube to ~55 μm increased compliance, the addition of the DM-meshes was found to be too restrictive. Thus, two round meshes were considered instead, as they have shown satisfactory unfolding and compliance behavior. Using a thin-walled PLC 70:30 tube was assumed to enable mesh unfolding at physiologically relevant pressures. In addition, high fiber coverage and material change were considered to enable the mesh to take a progressively restrictive role during unfolding. HEX-1x-2-2H (G2) and even denser HEX-2x-2-2H (G3) mesh were chosen for this last set of JM-Tubes. The vertical fiber spacing for the

G3 mesh was 1.25 mm (high fiber density), whereas it was 2.25 mm (medium fiber density) for the G2 meshes. The compliance test results of the G3-JM-Tubes are summarized in Figure 6.27, while the results for the G2-Tubes are shown in the appendix (Figure A.9):

Figure 6.27: a) Illustration of the G3- HEX-2x-2-2R mesh. Compliance of JM-Tubes of 50-60 μm WT with the G3-meshes made of different materials: (b) PCL, c) PLC 95:5 and d) PLA). All samples showed either initial increases or unchanged compliance at pressures between 50-150 mm Hg, followed by a noticeable regression when the pressure was increased beyond 100-150 mm Hg. In particular for the PLC 95:5 meshes, the changes in compliance were very noticeable. The results indicate the compliance transition is a created by the mesh unfolding

and its subsequently initiated restriction. The compliance of the pristine meshless PLC 70:30 55 μm WT tube (grey) and the artery (black) is shown in e). The meshless PLC 70:30 tube showed a strong progression in compliance upon pressure increase and underwent mechanical failure at pressures between 150 and 200 mm Hg, which was attributed to fatigue. In contrast to the meshless PLC 70:30 tubes, the arteries displayed compliance reduction upon pressure increase ($n=3$ for all samples).

Indeed, the meshes produced a non-linear elastic compliance behavior, which was clearly visible for the PCL and PLC 95:5 mesh JM-Tubes (Figure 6.27a and b). For the JM-Tubes with PCL G3-meshes, the compliance decrease was initially moderate (up to 100 mm Hg), followed by a steady decrease. The JM-Tubes with PLC 95:5 meshes became more compliant, showing the underlying PLC 70:30 tube could easily expand, whereas at roughly 150 mm Hg a decrease set in. The PLA meshes showed no clear contribution towards a non-linear elastic behavior. The reason may be the strut thickness ($\sim 540\ \mu\text{m}$). However, the struts of the PLC 95:5 meshes were thicker (about $620\ \mu\text{m}$) and a regression in compliance was visible. The unfolding of PCL was the easiest and is assumed based on the findings from section 6.3.3 to be a result of the lower elastic modulus. Furthermore, the low width of the fibers ($\sim 380\ \mu\text{m}$) is also thought to have contributed to the ease of unfolding. In section 6.3.6, it was shown thinner fibers unfold easier than thicker ones. The absence of stiff response of PLA is thus assumed to be due to friction between the fibers and the underlying dip-coated tube, which may have hindered the fibers from easy unfolding.

Based on Figure 6.27, the PLC 95:5 meshes were more reinforcing than the PCL meshes, which can be seen from the stronger and more sudden compliance reduction. This corroborates the findings from the tensile testing of solvent cast films (section 4.3.1) and singular HEX-fibers (section 6.3.3). Regarding the stiffness, PLC 95:5 would be the preferred material compared to PCL. However, albeit the PLC 95:5 meshes produced a non-linear elastic behavior, they were unable to recoil after pressure release (from 300 to 50 mm Hg). A second compliance measurement cycle was carried out, which revealed these JM-Tubes were more compliant than in the first run. This was attributed to the inability of the mesh to recoil and protect the underlying dip-coated tube. This may explain the high compliance measured. When approaching pressures of 250 mm Hg, a regression in compliance set in as the tube diameter has grown

sufficiently to get in contact with the PLC 95:5 mesh (Appendix, Figure A.10). This may further support the assumption that JM-Tubes with G3 PLC 95:5 meshes indeed show a clearly non-linear elastic behavior.

Testing of the PCL G2 meshes did not produce any non-linear elastic behavior. It is inferred from the results the vertical strut spacing of the G2 mesh was already too large (2.25 mm), which may not have created a uniform pressure distribution.

In conclusion, this set has shown the fabrication of thin walled JM-Tubes with G3-meshes produces a non-linear elastic vascular prosthesis. The PCL and PLC 95:5 meshes showed the most evident occurrence of arterial-like behavior. Although the PLC 95:5 mesh was unable to recoil, these results proof the concept of the JM-Tubes is the right strategy to achieve non-linear elasticity.

6.5 In-situ Printed Tubes and Nose Mesh Tubes

As alternatives to the pliable fiber mesh tubes, nose mesh tubes (N-Tubes) and in-situ printed ZZ-fiber tubes (IP-Tubes) were fabricated. N-tubes and IP-Tubes showed no occurrence of arterial-like compliance. It is assumed based on the study of Chen et al. [11] that N-Tubes would show a reversible transition from high to low compliance upon pressure increase. As only one free-standing hoop was included, it may be assumed that the hoop was not high enough to allow the tube to expand its lumen at low pressures easily (< 100 mm Hg). Furthermore, the inclusion of additional hoops may be beneficial, as it would facilitate tubular expansion until the mesh gets fully in contact with the tube. The vertical hoops of the similarly fabricated melt-spun tubes got lost during soaking in water for dissolving the PVA line and underlying PVA layer. After the PVA line was dissolved the melt-spun PLC 70:30 fibers shrunk and created a tight grip to the dip-coated tube. The mechanical properties were similar as for the slow spun PLC 70:30 [T+F, 100 rpm] tubes.

IP-Tubes have shown several drawbacks in comparison to the JM-Tubes, which may be primary due to the complete fusion of the PCL fibers to the PLC 70:30 tube. The fibers had no freedom to unfold. This may have created a local PCL over layer, similar

as for the bi-layered tubes (see section 4.3.5). Furthermore, high fiber coverage could not be achieved, without avoiding fiber to fiber fusion. As a result, inter-strut expansion was very noticeable. Figure 6.28 shows the IP-Tubes became increasingly elastic, similar to the meshless control tubes. Although direct printing of PCL fibers reduced excessive dilatation, it could not eliminate it.

While N-Tubes have potential for further improvements and may be interesting for future work, the IP-Tube design may not be suitable for the fabrication of a vascular prosthesis with arterial-like compliance behavior.

Figure 6.28: Compliance of the IP ZZ 2.2-3H tubes. The tubes were highly compliant and became increasingly soft upon pressure increase (n=2).

6.6 In Vivo Test in Porcine

Two *in vivo* tests using 4.0 mm inner diameter JM-Tubes with S-2x-1x-2H-2R meshes were carried out in porcine. Both tubes exhibited good surgical properties, allowing the surgeon ease of handling. Their implantation was successful, not showing any failure such as needle tears or anastomotic bleeding and the specimen were capable of supporting an uninterrupted blood flow (Figure 6.29a). At the end of the study (after two weeks), the pigs were sacrificed and the tubes explanted. The tubes showed no

signs of aneurysmal dilatation or damages at the anastomosis, indicating good mechanical stability during two weeks of *in vivo* testing. However, the tubes showed signs of thrombosis, which can be seen from in the narrowed lumen of the grafted ends of the carotid artery Figure 6.29b and the clot (marked area) in Figure 6.30b.

Figure 6.29: Angiogram a) before implantation of the JM-Tube showing the carotid artery and b) subsequent JM-Tube implantation. The angiogram in (b) showed the lumen of the implanted S-mesh JM-Tube remained intact and the prosthetic graft was capable of supporting an uninterrupted blood flow. However, the artery at the anastomosis showed vaso-constriction. Nitro-glycerin and lidocaine were given to relieve the vaso-constriction. After 14 days as shown in c), thrombosis occurred at the proximal end, which can be seen from the interrupted blood flow (yellow circle).

Figure 6.30: a) Implantation of ‘specimen 1’ of 5.0 mm inner diameter JM-Tube with an S-2x-1x-2H-2R mesh. The graft lumen remained intact and no anastomotic bleeding was observed. b) Explanation of ‘specimen 1’, showing signs of thrombosis due to the presence of blood clots, while c) shows the same graft after removal of the clots.

In conclusion, one graft occluded within one week and another one remained patent up to two weeks with signs of thrombosis. Short-term failure is mostly attributed to acute thrombosis, whereas long-term patency reduction is related to a compliance mismatch [12-15]. Hence, it can be assumed that compliance mismatch was not the determining factor of graft failure. The compliance of the JM-Tubes $2.1 \pm 0.5 \text{ } 4\% \cdot \text{mm Hg}^{-2}$ is not much different from the porcine arteries ($1.56 \pm 0.43\% \cdot \text{mm Hg}^{-2}$, i.e. 34% difference), which supports this assumption. Furthermore, 6 mm ePTFE grafts have shown patency rates of 62% [16] after two years implantation in human, compared to 23% [17] for 1 mm grafts after two weeks. ePTFE is a stiff material and its compliance is much lower (about 73% less) than human femoral artery [18]. However, ePTFE is electronegative and its surface is non-thrombogenic. Generally, the luminal surface of any synthetic vascular graft is potentially thrombogenic. The risk can be reduced by surface modification that allow endothelialization or heparinization [19, 20]. Our group has

been working on surface modification of PLC polymers in a separate project, in order to facilitate endothelial cell (EC) attachment.

Indeed, the JM-Tube prostheses require improvement regarding *in vivo* patency. However, this animal study may be partially seen as a success, as the 150 μm WT tubes with S-meshes did not undergo any mechanical failure. It was previously assumed the low WT may be delicate, as ongoing creep may later result in aneurysmal dilatation, which has not been the case. The mesh fibers served as a good protection and themselves remained intact during the entire duration of this *in vivo* study (no fiber breaking observed).

6.7 Conclusions

Shifting from melt-spun fibers to spring-like fibers fabricated by 3D-bioprinted improved the reproducibility, as the patterns were more deliberate. Furthermore, the choice of the four spring designs ZZ, HEX, ST and S, provided non-linear elastic tensile properties. This work showed how high control over the elastic properties could be achieved by altering the L/A-ratio, number of hoops, segment length (scaling factor), fiber width and hoop geometry. Choosing different polymers such as PCL or PLC 95:5 highlighted the elastic properties of the fibers (unfolding and stiff response) are strongly affected by the material. Combining a soft dip-coated tube with a 3D-bioprinted mesh, consisting of such spring-like fibers, was chosen as approach to create a vascular prosthesis with non-linear elastic features. Initially, no non-linear elasticity could be detected on the JM-Tubes, due to tubular inter-strut expansion, insufficient compliance of the underlying dip-coated tube and mesh design. These issues could be overcome by: reducing the vertical strut spacing to obtain a denser fiber coverage on the tube, to achieve better stress distribution, a reduction of the WT of the dip-coated tube to render it more compliant and including two hoop sections per layer of mesh. G3-JM-Tubes showed clear signs of non-linear elasticity, which highlights the concept of combining a soft tube with a pliable stiffer outer mesh is working. Yet, the non-linear elastic properties of the JM-Tubes are required to better match the compliance of native arteries, which can be achieved by further mesh design optimization and the choice of different materials.

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Chapter 7

Discussion

The development of a synthetic vascular prosthesis with a matched compliance remains an elusive task [1-4]. Hence, this work mainly focused on mechanical aspects, in particular the compliance and non-linear elasticity, of the fabricated and characterized tubular prostheses. The purpose of this work was to design the compliance of vascular prostheses rather than randomly scaffolding it. A composite structure of a dip-coated tube with a pliable outer mesh was proposed to achieve a vascular substitute with an engineered compliance. The compliance properties could be controlled by the mesh patterns.

7.1 Dip-coated Tubes

Early work mainly involved the establishment of a compliance testing apparatus and reproducible sample fabrication technique. As there is yet no shelf-off equipment available for compliance testing, a self-made customized apparatus was built that allowed characterization according to the guidelines of ISO 7198:2016 [5]. The setup enabled the recording of pressure and tube diameter at high accuracy. Pressure level (systolic and diastolic) and flow rate (frequency, heart rate) were regulated during compliance characterization. A reproducible dip-coating protocol for the fabrication of tubular vascular substitutes made of various Poly-L-lactide-co- ϵ -caprolactone (PLC) polymers and different wall-thicknesses (WT) was established. Throughout optimization steps, such as “turning over”, drying time and horizontal rotation, longitudinal and circumferential gradients in the WT could be minimized. There are other well-established fabrication methods, such as electrospinning or melt-extrusion that could have been chosen instead. However, dip-coating was continued, since a reproducible protocol was established that provided sufficient tunability over the WT of the tubular grafts. Melt-extrusion is known to provide high output rates and reproducibility, however, it is accompanied by degradation [6, 7].

Dip-coated tubes and solvent cast films made of PLC of various monomer ratios (L-lactic acid [LA] to ϵ -caprolactone [CL]) were assessed on their mechanical properties, such as tensile properties, static and dynamic compliance, burst strength and kink resistance. All tests found PLC polymers with rather equal monomer feeds, such as PLC 50:50 and 70:30 were soft in comparison to predominantly LA or CL rich materials (like PLC 85:15, 95:5, poly-L-lactide (PLA) and Poly- ϵ -caprolactone (PCL)). Tensile tests pointed out the brittleness of PLA and PLC 95:5; on the other side PLC 50:50 and 70:30 were soft and highly elastic. Static compliance testing revealed all tested PCL and PLC tubes undergo non-recoverable creep. The creep rate was higher for softer polymers.

Dynamic compliance testing showed 300 μm WT PLC 50:50 and 150 μm WT PLC 70:30 tubes were similarly elastic as porcine arteries. Indeed, PLC 50:50 (purchased from Akina) performed very well in the dynamic compliance test but displayed major drawbacks during static compliance testing and burst strength (purchased from Huizhou ForYou Medical Devices) measurement. Furthermore, the PLC 50:50 tubes did not pass

suture retention testing. This led to the conclusion that PLC 70:30 was the most suitable polymer, as it passed suture retention testing and displayed satisfying compliance, burst strength and low creep rates. In a relative comparison, 150 μm WT PLC 70:30 tubes displayed a compliance of 81% of that porcine arteries, whereas human saphenous veins (HSV) the current golden standard for clinical application has a relative compliance of 74% to a human femoral artery [8-10]. Comparing the burst strength of the 150 μm WT PLC 70:30 tubes (769 ± 9 mm Hg) with HSVs (1599 ± 877 mm Hg) or radial artery (3196 ± 1264 mm Hg) [11], they were noticeably less resistant to bursting. However, the 150 μm WT PLC 70:30 tubes were capable of resisting pressures that exceeded double the highest blood pressure ever recorded in humans (370 mm Hg) [12].

Due to the satisfying compliance-, burst- and suture retention test results, one 150 μm WT tube was chosen for a three hours animal test in rabbit, in order to assess its short-term mechanical performance *in vivo*. The PLC 70:30 tube did not undergo mechanical failures such as anastomotic bleeding, aneurysmal dilatation or wall rupture and furthermore was able to provide an uninterrupted blood flow. The good performance of 150 μm WT PLC 70:30 tubes made it a preferred choice for the further work carried out on the fabrication of a non-linear elastic vascular prosthesis.

Indeed, PLC 70:30 displayed satisfying mechanical properties regarding compliance, burst-strength, kink-resistance and implantability, but it lacked a major feature of arterial wall tissue: non-linear elasticity. The compliance of PLC 70:30 remained widely unchanged upon luminal pressure increases, unlike the porcine arteries that displayed a decreasing compliance. Hence, various composite structures of a soft dip-coated PLC 70:30 tube and outer features were fabricated and assessed on their nature of elasticity. Bi-layered dip-coated tubes were fabricated, where a soft inner lining was added to a stiffer outer material, while keeping the total WT at ~ 220 μm . Creep rate and kink-resistance were found to be predominantly determined by the stiffer outer layer, while compliance was increased. However, these bi-layered tubes did not provide any non-linear elastic behavior. Yet, there are no reported studies of a vascular prosthesis with arterial-like elastic properties that consist of two bulky layers. All in all, it was found the concept of having a soft-stiff material composite tube was the right strategy, but structural changes were required, in order to achieve the desired arterial compliance properties.

7.2 Melt-Spun fiber Tube Composites

The results found for the bi-layered dip-coated tubes showed the stiffer outer layer should not consist of a completely covering solid layer. Hence, a fibrous outer layer structure was considered. Composites of soft dip-coated tubes [T] with melt-spun fibers [F] were fabricated. The effect of material combination (tube and fibers), spinning speed and fiber winding (circumferential or helical) on the mechanical properties were tested. The addition of stiffer fiber materials to the dip-coated tube led to a decrease in compliance. In particular, the addition of PCL fibers to PLC 70:30 tubes produced poorly compliant composites.

Helical fiber winding, by increasing the transversal spinneret motion speed to 30 mm/s, provided tubular grafts with minor signs of non-linear elasticity. A moderate stiff response above pressures of 100 mm Hg followed an initial increase in compliance for two out of three samples. It is assumed the high winding angle of the fibers (13°) allowed the dip-coated tube to easily expand before the fibers progressively align into circumferential direction, which would cause a stiff response.

The effect of spinning speed (mandrel rotation) on the mechanical properties of the tube fiber composites was studied: high speeds of 500 rotations per minute (rpm) produced stiff vascular prostheses, whereas composite tubes with slowly spun fibers (100 rpm) were more compliant. PLC 70:30 [T+F, 100 rpm] reached a physiological compliance of 60% of porcine arteries (100%) and furthermore displayed non-linear elasticity.

It is assumed the slow spinning of PLC 70:30 produced non-linear elastic fibers, which may consist of coiled polymer chains that can straighten out upon stretching [13, 14]. The compliance behavior was not fully reproducible, a few tubes displayed linear elasticity (33%, 2/6). The reproducibility was found to be an issue of tube adopting an oval profile at low pressures, differences in fiber deposition during melt-drawing and post-spinning crystallization [14-17].

Melt-spinning allowed the tuning of the mechanical properties of the vascular prosthesis and deposition pattern of the fibers through the spinning speed (fiber winding speed), transversal spinneret motion and tube/fiber material combination. However, winding

melt-spun fibers around a dip-coated tube was found to not be a powerful approach to engineer biomimetic compliance, as post-spinning processes such as crystallization and the molecular arrangement cannot be controlled [13, 14].

7.3 Pliable Mesh-Tube Composites (JM-Tubes)

The lack of reproducibility in achieving non-linear elastic properties was approached by the fabrication of an outer structure with a more deliberate pattern. This was thought to be more effective to design compliance properties of vascular prostheses. Thus, 3D-bioprinted meshes, with designs inspired by stents, were fabricated. The meshes were designed to consist of spring-like fibers that unfold at low modulus before they are fully straightened out. In initial experiments, singular fiber tensile testing was carried out, in order to verify their non-linear elastic properties. The occurrence of a J-Curve in the stress-strain diagrams of fibers printed with zig-zag (ZZ), semi hexagon (HEX), stair (ST) and sine-wave (S) springs confirmed the choice of such designs was the right strategy to mimic the wavy adventitial collagen fibers (Figures 1.3 and 3.4). Several geometrical factors such as hoop angle (length-amplitude ratio, L/A-ratio), scaling factor, number of hoops, fiber width and rounding of hoops, which may have a significant influence on the mechanical properties of the fibers were analyzed.

Higher L/A-ratios and the inclusion of more hoops shifted the stiff response of the fiber to higher strains, as it increased the total length of the fiber. Fibers with larger scaling factors and thinner struts were unfolding easier. As single factor, rounding of the hoops (smoothing) did not significantly affect the tensile properties. All tensile experiments revealed the stiff response from the fiber occurs before complete straightening. This was attributed to the corners (hinges), which do not function as ideal joints. The unfolding is mediated by bending, which causes compression at the outer site of the corners and simultaneously generated tensile force at the inner site [18-22]. The stiff response occurred between unfolding degrees of 40-85%, where 100% refers to complete straightening. Factors that strongly affected that number were scaling factor and fiber width. The stiff response of thin fibers and ones with hoops of large scaling factors occurred at high unfolding degrees.

The brief study on the tensile properties of singular fibers (section 6.3) shows their properties can be tuned by several geometrical factors, highlighting the ability of designing the mechanical properties of these spring-like fibers. In comparison to the melt-drawn PLC 70:30 fibers spun at 100 rpm, 3D-bioprinting of pliable fibers enables a much greater control over the non-linear elastic properties.

The confirmation of non-linear elasticity of the spring-like fibers allowed proceeding to the fabrication of a vascular composite prosthesis. These composites (J-Curve Mesh Tubes, JM-Tubes) consisted of a soft dip-coated tube and a 3D-bioprinted pliable stiffer outer mesh. 3D-bioprinted meshes of spring-like fibers were wound around a dip-coated tube. Five different sets of JM-Tubes were tested on their dynamic compliance properties and compared against meshless PLC 70:30 tubes and porcine arteries.

The optimization of the JM-Tube design presented a challenge, as unexpected behaviors emerged: difficulty of mesh unfolding and tubular inter-strut expansion. The first issue was overcome by switching from single hoop meshes to double hoop meshes, as the latter showed improved unfolding. It was assumed to be a result of better stress transfer from the tube to the fibers. Inter-strut expansion of the underlying tube could be minimized by increasing the fiber density of the mesh, which was realized by reducing the vertical fiber spacing and wrapping two round meshes around the tubes. Combining both led to a better stress distribution within the underlying dip-coated tube, which noticeably reduced inter-strut expansion. Reducing the WT of the PLC 70:30 tube from ~100 to ~50-60 μm , rendered the tubes more compliant and allowed the mesh to unfold sufficiently so that it could induce a stiff response within the physiological blood pressure range.

PCL and PLC 95:5 meshes provided a clear change in compliance, showing both materials are suitable. Although PLC 95:5 provided more reinforcement than PCL, it did not display satisfying shape memory, i.e. not enabling the fibers to recoil upon pressure release. In contrast, PCL exhibited good shape memory (Figure 6.9).

The *in vivo* performance of S-mesh JM-Tubes was studied in two pigs for two weeks. Both tubes showed good surgical properties, allowing ease of handling and suture retention. Furthermore, they remained mechanically stable and their lumen was intact

over the entire duration of the experiment. No anastomotic bleeding, spontaneous leakage, wall rupture or aneurysmal dilatation was observed for either of the samples. However, both pigs suffered from thrombosis. The occurrence of thrombosis within two weeks is considered as an acute complication that is usually attributed to lack of luminal surface modification, poor endothelialization and insufficiently effective anti-coagulation medicine [1, 2, 23-27]. The long-term patency is largely affected by the compliance, which requires *in vivo* tests of longer durations (>6 weeks [28]) [1, 2, 25]. Since the JM-Tubes at this stage caused acute thrombosis, they require strategies to improve blood contacting properties such as e.g. luminal heparinization or pre-endothelialization [26, 27, 29, 30].

This work proved that a combination of a soft dip-coated tube with a stiffer pliable outer mesh is a valid strategy towards fabricating vascular prostheses with arterial-like compliance behavior. With all the studied geometrical factors that allow tunability of the tensile properties, this work underlines the benefits of using such pliable fibers to engineer a biomimetic vascular prosthesis. Singh et al. [31] reported a caterpillar-like structure as vascular substitutes (more details in section 2.3). Indeed, arterial-like elastic properties were achieved, but the designs show a few drawbacks, which may lead to failure *in vivo*: the lumen of the graft was rough instead of smooth and the caterpillar-like structure did not provide a uniform diameter. While a smooth lumen is crucial to reduce the risk of acute thrombosis, a non-uniform diameter of the vascular graft may create a turbulent blood flow, conferring an increased risk for occlusion [32-35]. Furthermore, the caterpillar structure may not produce isotropic mechanical properties along the length of the graft. Indeed, the JM-Tubes displayed minor inter-strut expansion of the tube, which also shows the mechanical properties of the tubes are yet not fully isotropic. However, comparing both tube types, the JM-Tubes show residual anisotropy on a small scale (due to inter-strut expansion of the tube as shown in Figure 6.17 and Figure 6.21), whereas for the caterpillar-like tubes the non-uniformity is on a macroscopic scale. The same would be the case for the Nitinol-polyester mesh tubes (section 2.3) reported by Singh et al [36]. The use of 3D-bioprinted fibers are also thought to offer better ability to design non-linear elastic properties. The crimped bi-layer design of Chen et al. [37] (see section 2.3) exhibited non-linear elastic properties. However, their design may lead into low kink-resistance, due to the crimped solid outer layer, whereas the J-Meshes with its individual fibers are assumed to be less prone to

induce kinking [38]. The latter is inferred from the structural design of the JM-Tubes, in which the mesh fibers are not fused to the tube and thus would not negatively affect the underlying tube during bending. Furthermore, from the tunability of the tensile properties of the pliable fibers, it is inferred the kink-resistance properties of the J-Meshes can be also controlled by the mesh designs, an assumption that needs to be confirmed in future work.

Currently clinically available solutions remain ePTFE and PET grafts, which both tend to fail if used as small diameter artery replacement [2, 25, 27, 39-41]. A clinically successful study was carried out by Shin'oka et al. [26], where PLC 50:50 coated Poly(glycolic acid) woven fabrics (18 mm diameter) were implanted as cavopulmonary connections. The study was carried out over 3-6 months, showing no incidence of graft occlusion. It remains an open question, whether these woven tubes could be used to replace arteries of small diameter (< 6 mm diameter) or low flow state (below-knee). In these arteries, failures are commonly associated with compliance mismatch [42]. A currently advanced decellularized vascular prosthesis is being tested in clinical trials by Humacyte Inc. [43, 44] Their grafts are synthesized by human SMCs in a bioreactor and later decellularized. Clinical trials showed a rate of 28% for primary and 89% for secondary patency (after intervention [45]) [44], which is seen as very promising. In comparison to the JM-Tubes, their vascular grafts are not fabricated but grown. This may not provide reproducibility and customization at the same level as the here fabricated JM-Tubes. Furthermore, bioreactor culturing is effort- and time consuming, which raises doubts about industrial upscaling. In contrast, the fabrication of the JM-Tubes takes 4 days and multiple tubes can be produced in only one day.

This work proves the concept of the JM-Tubes exhibiting non-linear elastic properties. Future work would include optimizations in the design and material combination, in order to better match the compliance of native arteries.

7.4 Outstanding Questions

This work showed non-linear elastic properties could be achieved by combining an elastomeric tube with a pliable stiffer outer mesh. The designs yet have to be optimized to match the compliance of native arteries, as it is a crucial requirement to guarantee long-term patency *in vivo* [4, 23, 46-49].

A phenomenon not being addressed in this work was the friction between the fibers and the tube, as well as the effect of the cross-section of the fibers on unfolding in *in vitro* experiments. It yet remains unclear, whether a round cross-section of the fibers would significantly reduce the friction between both components and allow an easier fiber unfolding. Furthermore, the effect of creep of the JM-Tubes has neither been addressed in this work. It is thought that materials with minimal creep may provide more desirable mechanical behavior, an assumption, which has to be validated in further work. Another important mechanical property, for which the JM-Tubes were not assessed for, is the kink-resistance. Yet, it is not clear, whether the JM-Tubes exhibit high kink-resistance, similar to the pristine PLC 70:30 tubes. It is assumed that the kink-resistance of the tubes can be improved by altering the mesh structure.

Functional cellularization with vascular cells was not addressed in this study. However, it is known to improve the functionality of an implanted vascular prosthesis. It remains an open question, whether the pliable fibers would allow radial orientation of seeded smooth muscle cells, which would support the preservation of their contractile phenotype [50]. Another unsolved question is the occurrence of thrombosis during both *in vivo* experiments in the two pigs. It yet is only assumed the graft failure was a reason of lack of endothelialization or heparinization [27, 29]. Also *in vivo* tests of longer duration would be interesting, once strategies for overcoming thrombosis are included, to evaluate the benefits of the non-linear elastic compliance properties of the JM-Tubes.

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Chapter 8

Conclusions

This chapter summarizes the work carried out described in this thesis, as well as its outcomes. This includes the established protocol for dip-coating of tubes, choice of tube material and the fabrication and properties of the proposed composites for non-linear elastic vascular prostheses.

In this thesis, small diameter vascular prostheses were fabricated. The main focus of this work was the development of a synthetic vessel that mimics the pressure-dependent arterial compliance behavior. It was hypothesized that such a behavior could be mimicked through fabricating a composite prosthesis, consisting of a soft tube with a stiffer outer mesh.

In an initial step, tubular conduits were fabricated using dip-coating. Different Poly-L-lactide-co- ϵ -caprolactone (PLC) polymers and Poly- ϵ -caprolactone (PCL) were used as materials for the dip-coated tubes. The materials were evaluated on their tensile properties, as well as on their static and dynamic compliance behavior. PLC 50:50 (50% L-lactic acid and 50% ϵ -caprolactone) and 70:30 tubes were found to be profoundly compliant, but the PLC 50:50 tubes underwent excessive creep. In contrast, PLC 70:30 was found to be a suitable material due to its satisfying compliance and low creep. The compliance of PLC 70:30 tubes was tailored by a reduction of the wall-thickness (WT), which resulted in 150 μm being close to the compliance of porcine arteries at physiological pressure. It was therefore used as a platform for further modifications.

The non-linear elasticity of arterial walls arises from their composite nature [1, 2]. Inspired from the wall-architecture of arteries, three different composite structures were fabricated and evaluated on their nature of elasticity: bi-layered dip-coated tubes, melt-spun composite tubes and J-Curve-Mesh-Tubes (JM-Tubes). The first type of composite showed no occurrence of non-linear elasticity during the compliance test, indicating the design was not sufficiently sophisticated. In a second step, composites of dip-coated PLC tubes with outer melt-spun PLC/PCL fibers were fabricated. Different spinning parameters were tested out (spinning speed and fiber winding angle). For only one design convincing results were found: Slowly spun PLC 70:30 fibers (100 rpm) on PLC

70:30 tubes showed in 67% of the cases arterial-like compliance. The result was positive but the melt-spun composite tubes lacked the ability to design the compliance properties.

In a third last set, composites of a dip-coated PLC 70:30 tube with outer pliable 3D-bioprinted meshes (JM-Tubes) were fabricated. The meshes consisted of spring-like fibers, of which tensile properties could be altered through the type of spring, hoop angle, scaling factor, number of hoops and fiber width. Tensile experiments of all tested fibers revealed the occurrence of non-linear elasticity. This indicated the reproducibility of non-linear elasticity was very high. In a further step, meshes of such spring-like fibers were fabricated using 3D-bioprinting. The meshes were wound around PLC 70:30 tubes and fused to it at their glue-strips. After several optimization steps – reduction of the strut distance, hoop section/glue strip configuration and reduction of the WT of PLC 70:30 tubes – eventually, clear signs of non-linear elasticity were observed during dynamic compliance testing. The mesh fibers were unfolding at physiologically relevant pressures (around 200 mm Hg), simultaneously creating a reduction in compliance. The compliance curve of these JM-Tubes were not overlapping with those of the porcine arteries, which indicates that further optimization is required to guarantee a close match. Due to the large set of geometrical parameters that allow the tailoring of the structural modulus of the JM-Tubes, these composites may have the potential for a future application as patient-specific prostheses.

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Chapter 9

Future Work

This chapter lists work, which has not been addressed in this thesis that may be interesting for further work. The control over the non-linear elastic properties of the JM-Tubes requires further improvement. Several additional structural modifications of the J-Curve-Mesh-Tubes are suggested in this chapter. Other non-mechanical aspects include cellular work, e.g. endothelialization of the luminal surface and smooth muscle cell-seeding of the tubular grafts at the outside.

In this work, vascular prostheses were fabricated, and their designs optimized through multiple steps, in order to obtain non-linear elastic mechanical properties (J-Curve). Mechanical testing was primarily limited to dynamic compliance measurement, whereby a customized apparatus was built that allowed characterization according to ISO 7198:2016 [1]. Poly-L-lactide-*co*- ϵ -caprolactone (PLC) 70:30 (70% L-lactic acid [LA], 30% ϵ -caprolactone [CL]) was shown to be a suitable polymer for the fabrication of vascular prostheses with compliance in the range of native arteries. Although PLC 70:30 provided satisfying compliance, it also showed occurrence of non-recoverable creep, post-crystallization (after dynamic testing) and low burst pressure at WT < 150 μ m. For future research a change of tube material would be a suitable option to address these issues. Soft biostable polyurethanes may serve as a suitable alternative, in order to fabricate tubes with high compliance that are less prone to plastic deformation (burst, aneurysmal dilatation). This would be of great interest for the mesh-tube composites (JM-Tubes), which are required to exhibit high compliance at low luminal pressures. Among polyurethanes, a large set of elastomeric polymers is available. Interesting candidates for the tube would be CarbothaneTM, QuadrathaneTM, ChronoflexTM, ChronopreneTM etc. [2-4]. Dynamic compliance tests have shown PLC 70:30 tubes often either insufficiently expand under increasing pressure or, if a low WT was chosen, they were prone to plastic deformation.

Although compliance matching is a crucial requirement for the long-term patency of vascular prostheses, future work should pay attention to the reversibility of high to low compliance transition and of mesh unfolding/recoiling. This work could prove that the JM-Tube designs fulfill their purpose of generating non-linear elasticity. However, further research on this topic needs to achieve a reversible transition from high to low compliance, i.e. complete unfolding and subsequent recoiling of the mesh after pressure release. It is assumed that the design of the JM-Tube has been optimized to a satisfying stage, while the search for an ideal material combination for the tube and the mesh needs to be given priority in future work. Elastomeric materials with low creep should be used for the tube, whereas the fibers may still consist of PCL or any other stiff elastomer. The current tube materials are either not elastic enough (PLC 70:30) or undergo massive non-recoverable creep (PLC 50:50). Although Poly- ϵ -caprolactone (PCL) displayed the best shape memory among all tested mesh materials (PLC 70:30, 85:15, 95:5 and PLA), it soon undergoes plastic deformation after complete unfolding. An ideal mesh material

would combine the shape memory of PCL with the stiffness of PLC 95:5. For the assessment of mesh unfolding/recoiling through cyclic pressurized tests, a custom-built pulsatile pump that provides pressure intervals of ~80 mm Hg would serve as suitable equipment. The pressure interval can be adjusted through the choice of syringe and the volume of water.

Although the G3-JM-Tubes showed a greatly reduced tubular inter-strut expansion, it has not yet been eliminated; different approaches may be taken into consideration. This may include the use of meshes with multiple rounds (> 2) and their dispersion into a hydrogel. The mesh would no longer be fused to the tube and thus act as a freestanding cage. Covering the soft tube with a hydrogel layer and a dispersed J-Mesh may also even out stress distribution. Furthermore, friction between the mesh struts and the underlying tube may no longer be a problem after dispersing the mesh within a hydrogel. This would give increased freedom to both the mesh and the tube, which better resembles the idealized behavior of the two components within the JM-Tube composite.

The mechanical behavior of the JM-Tubes was optimized through various changes in design and yet requires further improvement, in order to match arterial compliance over the physiological blood pressure range. Further finite element method (FEM) modeling may be of great help in understanding the mechanical behavior of the fibers during unfolding. In addition, the effect of structural changes may be better understood. FEM testing of the fibers in the JM-Tube composite may provide accurate information on its mechanical behavior, as it would better match the *in vitro* dynamic compliance test. FEM modeling of the 3D scenario may also highlight major differences from single fiber testing (*in silico* [FEM] and *in vitro* [tensile]). As an auxiliary tool, design of experiment (DOE) may be taken into further consideration [5]. DOE may help to improve the understanding of the significance of each parameter on the mechanical properties of the tube. Although it is a powerful tool, the complexity of the system may make its use difficult, which is the reason why no DOE was carried out in this work.

As alternative to mesh printing from polymer solutions, filament printing may be considered, since it is a solvent-free technique [6, 7]. In addition, fibers printed from filament are circular, which may change the mesh unfolding properties within the JM-Tubes. It is hypothesized that the circular cross-section may produce less friction during

fiber torsion (as they would roll on the tube surface) and hence allow smoother unfolding. This may further improve the control over the mechanical properties of the mesh, when the length to amplitude (L/A) ratio and the segment length (scaling factor) are altered.

Melt-spinning of fibers around a soft dip-coated tube has shown some benefits regarding non-linear elasticity. The composites with the most promising reversible high to low compliance transition over the physiological blood pressure was PLC 70:30 [T+F_C, 100 rpm]. 67% of the specimens displayed such a behavior. Improved non-linear elasticity may be achieved by replacing the PLC 70:30 tube with a softer material and stiffer cross-spun fibers (e.g. of PCL). Although the PLC 70:30 [T+F_H, 100 rpm] did show a mild stiff response upon luminal pressure increases, the non-linear elastic properties can still be further improved by choosing different materials for the dip-coated tube and melt-spun fibers. Like the JM-Tubes, a softer material for the dip-coated tube and a stiffer polymer for the melt-spun fibers should be used for further studies. As melt-spinning may produce partially-fused fibers (minor adhesion or fusion was observed on a couple of samples), filament winding may offer a suitable alternative [8, 9]. The patterns may be more controllable and the fibers on the surface of the tube would have better mobility. The fabrication of tubes with melt-spun circumferential PLC 70:30 fibers with vertical hoops was not possible, as they shrunk after the sacrificial PVA line was dissolved. The design is promising, but the PLC 70:30 fibers should be replaced by non-shrinking materials (e.g. with PCL or polyurethanes).

Compliance mismatch has been reported to be a major reason behind low patency rates of vascular prostheses *in vivo* [10-12]. Besides mechanical aspects, a confluent endothelial monolayer is another principal requirement for guaranteeing long-term patency after implantation [10, 13, 14]. PLC and PCL do not serve as ideal substrates for cell adhesion, due to their hydrophobicity [14-17]. Efforts to endothelialize PLC tubes have been published previously [16, 18, 19]. The same goal was pursued by our group, which involved chemical modification of the inner surface of a dip-coated PLC 70:30 tube, in order to allow cross-linking to gelatin. This was shown to create a suitable substrate for endothelial cells. Once a reproducible strategy has been set up, the JM-Tubes should be endothelialized prior to implantation for *in vivo* testing. This may strongly reduce the incidence of acute thrombosis and enable a long-term animal study.

This would be essential to quantify the contribution of non-linear elasticity (compliance) of the JM-Tubes to their long-term patency.

In a previous study by our group, smooth muscle cells (SMCs) were cultured on PLC 70:30 tubes, with melt-spun PCL fibers [20]. The channels between the fibers were populated by the SMCs and allowed them to align circumferentially. This enabled the preservation of their contractile phenotype. A similar approach may be followed up with helically melt-spun fiber tubes [T+F_H] or the JM-Tubes. The channel width between the fibers is a critical parameter in SMC alignment, which is optimal around 10 μm . Regarding the JM-Tubes, this would require the printing of denser meshes. Filament-printing or the use of multi-round meshes (as described in sections 3.4.2, 6.4.3 and 6.4.5) may be a suitable option for this. Cell-culturing in a bioreactor under dynamic conditions should also be taken into consideration. Cyclic pressurization of the tubular prostheses has shown to stimulate SMCs to maintain their contractile function [21]. The JM-Tubes or melt-spun composite tubes may be cultured with SMCs in a bioreactor. Once such a reactor procedure is established and provides reproducible results, co-culturing of SMCs with endothelial cells may be considered.

In summary, future work may take different directions, which either focusses on improving the understanding of the mechanical behavior of the JM-Tube and optimizing its design, addressing the reversibility of the mesh unfolding/recoiling or moving towards tissue engineering. As this work has led to major advances in the behavior of the JM-Tube, the first two suggested directions should be given priority.

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APPENDIX

Figure A.1: Tensile testing data of the different PLC polymers: a) elastic modulus, b) ultimate tensile strength and c) elongation at break. Brackets indicate statistical similarity ($n = 3$).

Figure A.2: a) Average kink-radii of the different PLC tubes ($n \geq 6$, errors are zero for all tested materials, except for PLC 70:30; kink radii of PLC 85:15 and PCL > 35 mm) and b) average kink-radii of the 2L-Ts (kink radii of PLC 50:50+PCL and PLC 70:30+PCL > 35 mm, $n \geq 3$).

Figure A.3: Dynamic compliance boxplots for three samples tested at different flow rates (15 and 20 rpm). The difference between the values was found to be significantly different. However, the compliance was increased for the second measurement cycle. Therefore it is assumed the increasing compliance is rather a result of creep and not flow rate.

Figure A.4: a) Dynamic compliance test results for a 200 μm WT PLC 70:30 tube tested during cycles 1 and 2 at 20 rpm and cycles 3 and 4 at 15 rpm (the compliance measurement was carried out for 30 seconds for each cycle). One-way-analysis of variance (ANOVA) showed the compliance values for all four cycles were statistically significantly different. As discussed in sections 4.3.3 and 4.3.4, this may be a result of creep. b) Compliance test results of a PLC 70:30 tube of 150 μm WT. The tube was tested three times during cycles of 30 seconds measurement. The compliance has increased continuously. The difference between cycles 2 and 3 was found to be statistically significant. The reason for this finding is also assumed to be creep. Brackets indicate no statistically relevant difference was found according to One-way-ANOVA.

Figure A.5: Change of wall-stress of PLC 50:50 and 70:30 tubes over time during the static compliance experiment. PLC 50:50 showed a continuous increase, while the wall-stress remained the same for the PLC 70:30 tubes. The increase in wall-stress for the PLC 50:50 tubes was attributed to the excessive dilatation/creep and wall-thinning.

Figure A.6: Aneurysmal dilatation of a PLC 70:30 tube at the distal site during burst pressure testing.

Figure A.7: Plot of the compliance versus intraluminal pressure of a thin-walled (120 μm) and a thick-walled (250 μm) PLC 70:30 tube.

Figure A.8: Melt-spun PLC 70:30 fibers on a PLC 50:50 tube. The PLC 70:30 in the melt-holder cup was partially degraded, as it appeared in a yellowish color. The resulting melt-spun fibers did not show sharp edges and often were fused to each other.

Figure A.9: a) Loop formation of a PLC 70:30 [T+F, 500 rpm] tube. b) SEM picture of a PLC 70:30 [T] + PCL [F, 500 rpm] tube before compliance testing. The pores of the PLC 70:30 tubes appeared circular. c) SEM micrograph of a PLC 70:30 [T] + PCL [F, 500 rpm] tube surface after dynamic compliance testing. The pores adopted an elliptical shape, showing orientation into one direction. The pores did not orient perpendicularly or parallel to the melt-spun fibers. Due to these findings it was assumed the loop formation as shown in a) is a result of longitudinal creep of the PLC 70:30 tube.

Figure A.10: Tube shrinkage was observed during compliance testing, which was attributed to an oval cross-section and upright placing as shown in Figure 6.16a. The shrinkage can be recognized by the diameter maxima (blue curve) that occur at the diastolic pressure (black curve).

Figure A.11: Compliance of JM-Tubes of $\sim 55 \mu\text{m}$ WT and G2-meshes. The G2 meshes had a vertical fiber spacing of 2.25 mm in comparison to 1.25 mm for the G3 meshes. In comparison to the densely covered G3 JM-Tubes, which showed non-linear elasticity, the reduced fiber density for the G2-mesh $\sim 55 \mu\text{m}$ WT tubes and the lower L/A-ratio may explain the absence of an arterial-like behavior.

Figure A.12: Compliance of the JM-Tubes with PLC 95:5 G3 meshes of the first and second runs: a) specimen 1, b) specimen 2 and c) specimen 3. The first runs are the same as shown in Figure 6.27b. The second runs (blue curves) show the compliance at low pressure is generally higher than in the first run. Upon pressure increase the compliance measured during the second run approaches the values from the first run. Above 200 mm Hg, the curve from the second run approaches the pathway of the first run. This was attributed to the reduced shape memory of PLC 95:5 fibers; they were not able to fully recoil, in comparison to PCL fibers, which nearly fully returned to their original coiled state.