

École polytechnique de Louvain

Arterial grafts: in depth characterisation of structure and mechanical properties

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Most people say that it is the intellect which makes a great scientist. They are wrong: it is character. – Albert Einstein

Preface

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TABLE OF CONTENTS

Abstract	III
LIST OF FIGURES	V
LIST OF TABLES	IX
1 INTRODUCTION	1
2 STATE OF THE ART	3
2.1 Native tissue	3
2.1.1 Structure	3
2.1.2 Mechanical properties	4
2.2 Clinically applied synthetic vascular grafts	12
2.2.1 Structure	12
2.2.2 Mechanical properties	17
2.3 Novel grafts under research	20
2.3.1 Coatings for the vascular grafts	20
2.3.2 Composite, biosynthetic and totally engineered vascular grafts	20
3 PROBLEM STATEMENT, AIM AND OBJECTIVES	25
4 STRUCTURAL CHARACTERISATION	27
4.1 Materials and methods	27
4.1.1 Synthetic grafts and native tissue	27
4.1.2 Sample preparation	27
4.1.3 Micro-CT image acquisition	27
4.1.4 MicroCT image analysis	28
4.1.5 Statistical analysis	29
4.2 Results and discussion	29
4.2.1 Visual inspection	29
4.2.2 3D morphological analysis	34
5 MECHANICAL CHARACTERISATION	39
5.1 Materials and methods	39
5.1.1 Synthetic grafts and native tissue	39
5.1.2 Sample preparation	39
5.1.3 Planar biaxial tester	40
5.1.4 Strain map calculation	42
5.1.5 Stress calculation	44
5.1.6 Experimental protocol	45
5.1.7 Statistical analysis	47

TABLE OF CONTENTS

5.2	Results and discussion.....	47
5.2.1	Thickness measurements.....	47
5.2.2	Force and displacement	48
5.2.3	Stress	58
6	CONCLUSION	63
6.1	Structural characterization.....	63
6.2	Mechanical characterisation	65
6.3	Work methodology	68
7	FUTURE PROSPECTIVE.....	69
	APPENDIX A: Continuum Mechanics.....	71
	APPENDIX B: Standard Travels	73
	APPENDIX C: Polymers and Elastomers Overview	75
8	BIBLIOGRAPHY.....	77

Abstract

Motivation. Although polymer-based synthetic bypasses are substituting autografts and homografts in the clinical use, complications that affect their normal performance remain existing. However, the exact causes of this are still unknown. Although many studies aim to characterise the synthetic grafts as well as the native tissue in terms of their structure and mechanical properties, results are very diverse, leading to no uniform conclusions. For these reasons, the aim of this study was to define a workflow to assess, using the same methodologies, the structural and mechanical properties of all these materials. Thanks to a standardized comparative study between synthetic grafts and native tissue, a more robust and intelligent input for the design of an improved biomimetic synthetic graft could be achieved.

Materials and methods. Three types of synthetic grafts, Dacron, Gore-Tex and Vectra, have been characterised as well as native tissue. For the 3D structural characterisation, high-resolution microfocus computed tomography (microCT) was used. Strain-controlled planar biaxial tensile testing was used for the mechanical characterization. Forces and displacements of the samples were tracked during all the tests. Also, strain maps were generated to obtain the stresses.

Results and discussion. From the visual evaluation of the microCT images, the three native tissue layers within the homograft could be observed in an unprecedented manner. More detailed 3D analysis of these data is, however, still needed. For the synthetic grafts, a full 3D analysis was performed. For Dacron and Gore-Tex, a longitudinally orientation of the matrix was observed, providing a higher failure resistance in this direction. Dacron presented a corrugated structure, similar to the homograft lumen, which provides elasticity longitudinally. Vectra is composed of a foam in which a wire and a sheet are embedded, providing elasticity and a uniform distribution of the stresses. Endothelial cells fixation is anticipated to be more favourable in Dacron due to its higher specific surface area, followed by Gore-Tex and then Vectra. Vectra is the most porous material. Studying the foams, based on the Gibson-Ashby theory of cellular foams, it can be anticipated that Gore-Tex is the stiffest material of the three. However, a compromise between structure and pore thickness must be found to ensure the stiffness.

For measuring the thickness of the samples that were tested mechanically, optical images as well as the microCT images were applied. The latter ones were more accurate, since this measurement technique was not influenced by the residual stresses in the materials. The forces registered from the mechanical test demonstrated a large anisotropy in Dacron, Gore-Tex and the homograft. Dacron and Gore-Tex were stiffer in the circumferential direction, while the homograft was stiffer in the longitudinal. However, it should be noted that the exact orientation of the homografts samples still need to be checked. The anisotropy degree in Vectra was lower compared to the others. Viscoelasticity or damage in Dacron, Gore-Tex and the homograft samples was tracked. Dacron and Gore-Tex presented a low resistance to deformation in the longitudinal direction. Furthermore, it was observed that the circumferential direction acquired preconditioning state faster in all the samples, and that the homograft presented, at high strains, a clear elastomeric behaviour.

Conclusion. This study shows the need for a combined structural and mechanical characterisation of synthetic graft materials for vascular bypasses, together with native tissue. Only in this way, a direct comparison can be made, and a better understanding of the mismatch in functional behaviour between the synthetic materials and the native tissue can be obtained.

LIST OF FIGURES

Figure 1: Structure of an artery wall [9].....	3
Figure 2: Elastin and collagen effect in pressure-diameter relation of an artery. In the right, the graft shows the relation between the pressure of the blood flow and the variation that it provokes in the diameter, information needed to compute the compliance with the depicted formula. In the left the collages (blue) and elastin (green) fibres under different levels of pressure can be observed [7].....	4
Figure 3: Schematic plot indicating the Green Lagrange strains E_{11} and E_{22} in the (x_1, x_2) plane of an artery [49].....	5
Figure 4: Different combinations of strengths in biaxial testing (a) planar extension, (b) extension-shear, (c) extension-inflection [50]	5
Figure 5: Circumferential and longitudinal stress-stretch responses of samples from layer of a human left anterior descending coronary artery. A: adventitia. B: media. C: intima. The experimental data is represented with dots and compared with numerical results represented with continuous lines [82]	6
Figure 6: Comparison of pressure-dimeter curves between an artery (red) and a synthetic implant (blue) [7]	6
Figure 7: Stress-strain loading-unloading loop diagram of one tissue strip of the internal mammary artery. F1 and F3 are the mathematical curves obtained after Fourier analysis and synthesis of the periodic data using the first and the third Fourier functions [53]	7
Figure 8: Pulsatile Flow setting [33]	8
Figure 9: Steady Flow setting [33].....	8
Figure 10: Setup to measure pressure increase in an artery. A and B are the clamping points between the artery and the copper tubes through which the mercury is pumped. X and Y are pressure sensors. The copper pipes are clamped to rubber tubes in their ends. The pressure wave is set up by hitting or squeezing the left-hand rubber pipe at some distant spot [59].....	10
Figure 11: Customize tensile tester using surgical clamps attached to an artery strip of 15 mm [60]	10
Figure 12: Structural design patterns of a woven Dacron graft [7]	13
Figure 13: The structural design patterns of a knitted Dacron graft [7]	14
Figure 14: Electron microscopy of ePTFE node-fibril structure [22]	14
Figure 15: Corvita vascular graft structure, fibrillar polyurethane [45]	15
Figure 16: Vectra vascular access graft	15
Figure 17: Scanning electron microscopy of native MyoLink, compliant poly(carbonate-urea)urethane [46]	16
Figure 18: Experimental data force vs stretch for the three directions analysed. In the y axis the force applied is represented in Newton and the stretch in the x axis which is the increase of length divided by the initial length [29]	17
Figure 19: Schematic of the test bench used to generate the pulsatile hydraulic flow through the prosthetic conduit and wall distension measurement [55]	19
Figure 20: Dynamic compliance curves tested in 1.8-2.7 bar pressure range of textured Dacron (C1), Low-twisted Viscosuisse (C3) and Intermingled Diolen (C2) up to bottom presented [55]19	19
Figure 21: Variation of compliance vs. mean pressure for different conduits. The pulse pressure was 60 mmHg [88]	21
Figure 22: Segmented innovative graft design to improve the compliance [21].....	21
Figure 23: Scaffold-based tissue-engineered vascular graft manufacturing process [48]	22

Figure 24: Pioneering manufacturing process for TEVG, (a) sheet-based tissue engineering, (b) microtissue aggregation, (c) bioprinting [48].....	23
Figure 25: A: 3D view of 6mm diameter 5 cm length homograft. B: three orthogonal views of the homograft cross-section indicating the different layers, from inside to outside intima, media, elastic lamina and adventitia	30
Figure 26: Femoral artery histogram. The x-axis represents the amount of exposure, and the y-axis represents the incidence of pixels for each exposure level.....	30
Figure 27: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Dacron graft. B: three orthogonal views of the Dacron sample cross-section. In b.1 a detail of the fibres is presented	31
Figure 28: Three orthogonal views of a 6mm diameter and 2 cm length Dacron graft. In z.1 a zoomed image of the graft is presented wall in which the dispersion between the fibres can be observed.....	32
Figure 29: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Gore-Tex graft. B: three orthogonal views of the Gore-Tex sample cross-section	33
Figure 30: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Vectra graft. In orange the wire and the self-sealing middle layer are presented and the foamy structure in grey. B: Three orthogonal views of the Vectra sample cross-section indicating the different layers and structures composing the graft.....	34
Figure 31: Box plot of the specific surface ($1/\mu\text{m}$) for Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p<0.0001$).....	34
Figure 32: Box plot of the porosity (%) for Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (** $p<0.01$, **** $p<0.0001$)	35
Figure 33: Box plot of the volume fraction (%) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p<0.0001$).....	36
Figure 34: Box plot of the structure thickness (μm) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p<0.0001$).....	36
Figure 35: Box plot of the structure separation (μm) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p<0.0001$).....	37
Figure 36: Schematic of the foam structure of the materials aim of study. In blue the structure is represented while the pores are presented in white	37
Figure 37: A Dacron sample between two plates of known thickness	40
Figure 38: Dacron sample mounted in the biaxial tester with graphite speckles applied. Numbers 1,2,3 and 4 are the notation for the different loading cells. 1 and 2 will be considered x axis, the equivalent circumferential direction in the graft, and 3 and 4 y axis, the longitudinal direction	40
Figure 39: Biaxial tester set-up.....	41
Figure 40: Manta G-917 B camera, Allied Vision Technologies GmbH, Stadtroda, Germany.....	41
Figure 41: Schematic representation of the 2D-DIC principle. The left image presents the undeformed situation and, in the right, the deformed one. The red square indicates the subset size of point P [75].....	43

Figure 42: Systemic blood pressure (mmHg) registered in the main arteries concerning their distance from the left ventricle. Both systolic (maximum) and diastolic (minimum) pressures are presented [79].....	45
Figure 43: Box plot comparing the measure methods used to get the graft wall thickness (mm), Matlab and DataView. Vectra does not report a significant difference (ns) between each method, Dacron reflects a significant difference (***) $p < 0.001$, so does Gore-Tex (**** $p < 0.0001$)	48
Figure 44: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Dacron (D1) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step	49
Figure 45: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Gore-Tex (G4) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step	50
Figure 46: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Vectra (V14) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step	52
Figure 47: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Homograft (H5) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step	53
Figure 48: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Homograft (H5) sample. High strain protocol is presented with 30% loading step and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1	54
Figure 49: Box plot of the maximum force read in the load cell number 1 (left) and number 2 (right) for Dacron, Gore-Tex, Vectra and the homograft. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively. Both, Fmax1 (left) and Fmax2 (right), resulted significantly different ($p < 0.0001$) for all the materials except for Dacron and Vectra where not significant different (ns) was found	55
Figure 50: Box plot of the maximum force read in the load cell number 3 (left) and number 4 (right) for Dacron, Gore-Tex, Vectra and the homograft. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively. Fmax3 (left) did not registered a significance difference between Dacron and the homograft, while Fmax4 (right) did not found a significant difference between any of the materials.....	56
Figure 51: Box plot comparing the maximum forces recorded in each load cell for each material. In Dacron and Gore-Tex forces are significant different except between Fmax1-Fmax2 and Fmax3-Fmax4. In Vectra all were not significant different except between Fmax1-Fmax2 and in the homograft all forces were not significant different between each other	57
Figure 52: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Dacron (D1) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step.....	57
Figure 53: Cauchy stress (MPa) vs stretch (-) experimental responses in circumferential (σ_{11}) and longitudinal (σ_{22}) directions for a low strain protocol 2.5% strain, 1:2 ratio and 5 cycles	

(Loadingcycle1-4 for preconditioning) for synthetic grafts and native tissue. One significant sample of each of the materials have been selected. D1: Dacron. G4: Gore-Tex. V12: Vectra. H5: homograft	59
Figure 54: In the right representative stress/strain curves of PET films before (black) and after (red) coating with whey and whey removal by washing with Alcalase at 50°C for 2 h [90]. In the left, Dacron's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions	60
Figure 55: In the right representative stress/strain curves of ePTFE layers of different thickness (see legend) after uniaxial tensile test both in x and y direction are presented [91]. In the left, Gore-Tex's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions	60
Figure 56: In the right, representative stress/strain curves of cellulose nanofibrils (CNF) modified polyurethane foams (PUF) of different densities PUF0 0.059, PUF20 0.05 and PUF 0.051 all in gr/cm^3 [92]. In the left, Vectra's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions	61
Figure 57: 1 st Piola-Kirchhoff stress (MPa) vs stretch (-) experimental responses circumferential (σ_{11}) and longitudinal (σ_{22}) directions comparing the results from 2.5% and 1:2 ratio protocol and 30% and 1:1 ratio loading sets performed in a homograft sample (H5).....	62
Figure 58: Young's modulus E (GPa) plotted against density ρ (Mg/m^3). The heavy envelopes enclose data for a given class of material. Dacron and Gore-Tex belong to the polymers field while Vectra and the homograft to the elastomers.....	67
Figure 59: Workflow of the study and design of new bypass prosthesis.....	68
Figure 60: A continuum body at reference and deformed configurations is represented in a global $x_1x_2x_3$ reference. The infinitesimal deformation of each reference (dx') and deformed (dx) configurations is represented in the figure [94]	71
Figure 61: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a homograft (H5) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step.....	73
Figure 62: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Gore-Tex (G4) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step.....	73
Figure 63: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Vectra (V14) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step.....	74
Figure 64: Stress (MPa) vs strain (-) curves for brittle polymer (A), plastic (B) and highly elastic or elastomeric (C) behaviours [95]	75

LIST OF TABLES

Table 1: Elastic modulus of components of an artery and a woven Dacron graft [19]	13
Table 2: Advantages, disadvantages and healing process of synthetic vascular grafts in clinical use [24].....	16
Table 3: Rupture limits of the woven Dacron specimens [29]	17
Table 4: Comparison of compliance given as per cent diameter change per mmHg x 10 ² [20, 23]	18
Table 5: Advantages, disadvantages and healing process of composite, biosynthetic and totally engineered vascular grafts under research [24]	23
Table 6: Brief description of the morphological parameters assessed in this study, determined via image analysis of the micro-CT images	28
Table 7: Notation to indicate the level of significance.....	29
Table 8: Average and standard deviation of the thickness of Dacron, Gore-Tex, Vectra and Homografts samples measures with Matlab (ML) and DataViewer (DV) software.....	47
Table 9: Maximum force (N) recorded in each loading cell for each Dacron sample and average and standard deviation	50
Table 10: Maximum force (N) recorded in each loading cell for each Gore-Tex sample and average and standard deviation.....	51
Table 11: Maximum force (N) recorded in each loading cell for each Vectra sample and average and standard deviation	52
Table 12: Maximum force (N) recorded in each loading cell during the low strain protocol for each Homograft not treated sample and average and standard deviation.....	54
Table 13: Maximum force recorded in each loading cell during the high strain protocol for each Homograft not treated sample in Newton (N) and average and standard deviation	54
Table 14: Conclusions extracted from the visual inspection of the samples.....	64
Table 15: Conclusions extracted from the morphological analysis of the samples.....	64
Table 16: Conclusions extracted from the mechanical analysis of the samples.....	67
Table 17: Conversion formulas relating the Cauchy stress (σ) and the first Piola-Kirchhoff stress (P)	72
Table 18: Modulus of elasticity (GPa) and deformation to failure or ductility (%) for different types of polymers [95]	75

1 INTRODUCTION

According with the World Health Organization cardiovascular diseases (CVDs) are the number one cause of death globally [1]. Around 17.9 million people died from CVDs in 2016, this represents a 31% of all the deaths occurred that year. Despite most CVDs can be prevented with behaviours that decrease the occurrence risk (no smoking or drinking, performing physical activity, having a balanced diet...), their lethal effect rises the number of deaths every year.

For changing this tendency, apart from stimulating a preventive behaviour, proper surgical actions must be present. If the obstruction in the blood vessel is rapidly detected the blood clot can be removed performing a surgical catheterisation. However, in the case of Peripheral Arterial Occlusive Disease, where the clot is obstructing the vessel for a long time, the blood vessel tends to calcify and infrainguinal arterial bypass surgery must be performed [2].

Bypasses are made using autologous vessels or prosthetic vascular conduits. The limited availability of healthy autologous blood vessels has led to the use of synthetic polymeric material, especially for long occlusions [3]. The mechanical properties of the prosthetic grafts are however quite different from the autologous grafts. While the mechanical strength often provides an appropriate behaviour, the existing compliance and biocompatibility mismatch might still cause several complications.

The main mid to long-term failure of existing synthetic grafts is caused by unfavourable healing processes, known as incomplete endothelialisation and myointimal hyperplasia (IH) [4]. The reaction between the graft and the body not only can cause the artificial blood vessel to collapse, but can also, depending on the composition of the synthetic material, lead to carcinogenic effects. Elastic polymers have been used with the aim of matching the elastic behaviour of their host arteries, since lack of this property may contribute to IH. Too elastic materials might remove all the pulsatility of the blood stream, while if it is too rigid a high systolic and low diastolic pressure can be observed. This behaviour is similar to the one detected in arteries affected with atherosclerosis.

The structure and the manufacturing process of the synthetic vascular grafts may also affect their mechanical behaviour. High porosity reduces graft compliance by causing fibrovascular infiltration, whereas low porosity discourages the development of an endothelial lining and hence effective antithrombogenicity. Therefore, consideration of structural and mechanical properties is necessary in graft development [5, 6]. The manufacturing process influences also the mechanical properties, such as the dilatation or contraction of the graft when inserted in the body, which would impact in the patency level meaning the long-term percentage of occlusion.

The aim of this master thesis is to define a cluster of experiments with which the structural and mechanical properties of different graft materials in comparison with the native artery can be defined.

2 STATE OF THE ART

In what follows, the state-of-the-art related to the structure and mechanical properties of the native artery will be described to establish the parameters a synthetic graft must match. Then, the existing materials used to manufacture synthetic vascular grafts will be detailed, because together with their manufacturing method, they will define their structural and mechanical properties. Also, the existing experiments to determine the mechanical properties of synthetic grafts will be described. Finally, an insight of coatings, designs and materials for developing novel synthetic grafts will be depicted.

2.1 Native tissue

The heterogeneous structure of an artery imparts unique mechanical properties, which up until now still make it difficult to mimic its behaviour using synthetic grafts. These properties are non-linearity, anisotropy, viscoelasticity and compliance.

2.1.1 Structure

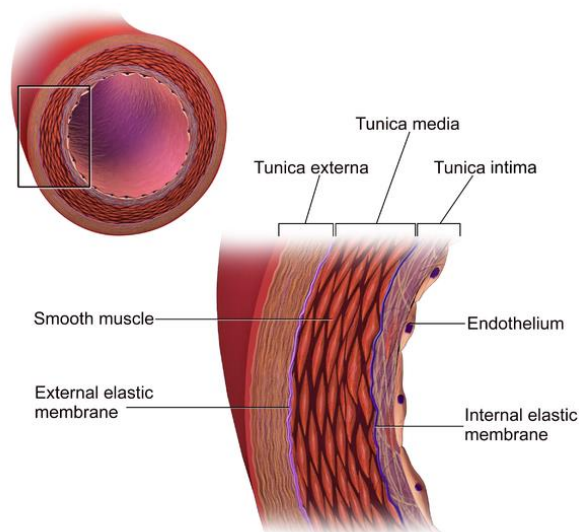


Figure 1: Structure of an artery wall [9]

The arterial wall is composed of three major layers, tunica intima, tunica media and tunica adventitia (Figure 1). The tunica externa or tunica adventitia is composed of longitudinally oriented collagen fibres of large diameter and elastic tissue. The tunica media, which mainly defines the mechanical behaviour of the artery, is the thickest one. It is composed of circumferentially arranged elastin fibres, smooth muscle cells and collagen fibres. Finally, the innermost layer, tunica intima, is in direct contact with the blood flow and its elastic tissue allows the artery to bend and adequately transmit the pressure wave through the vessel [9].

Elastin and collagen are the main structural components of an artery. Their elastic modulus significantly differs with 0.6-1 MPa for the elastin and around 1 GPa for the collagen [10, 11]. This will induce anisotropy which is translated into a non-linear stress response. This behaviour will be described in detail here below.

2.1.2 Mechanical properties

To define the mechanical properties of a native blood vessel, experimental tests and computational models have been performed obtaining interesting data about the blood vessel behaviour, as they can be its anisotropy, non-linearity, viscoelasticity or compliance. Special attention will be paid to this last property because it is the most difficult to replicate in synthetic materials.

2.1.2.1 Stress response

An artery shows a low stress response at low pressures contributed entirely by elastin fibres and shows a steep increase in elastic modulus as the pressure is increased due to the collagen fibres. The mid-region of a stress-strain curve involves successive transition of load from elastin to collagen; this property is known as non-linearity and is key to preserve the elastic stability in arteries. In Figure 2, the behaviour of an artery under different stress levels is depicted. The transition between the actuation of both structural components can be easily observed.

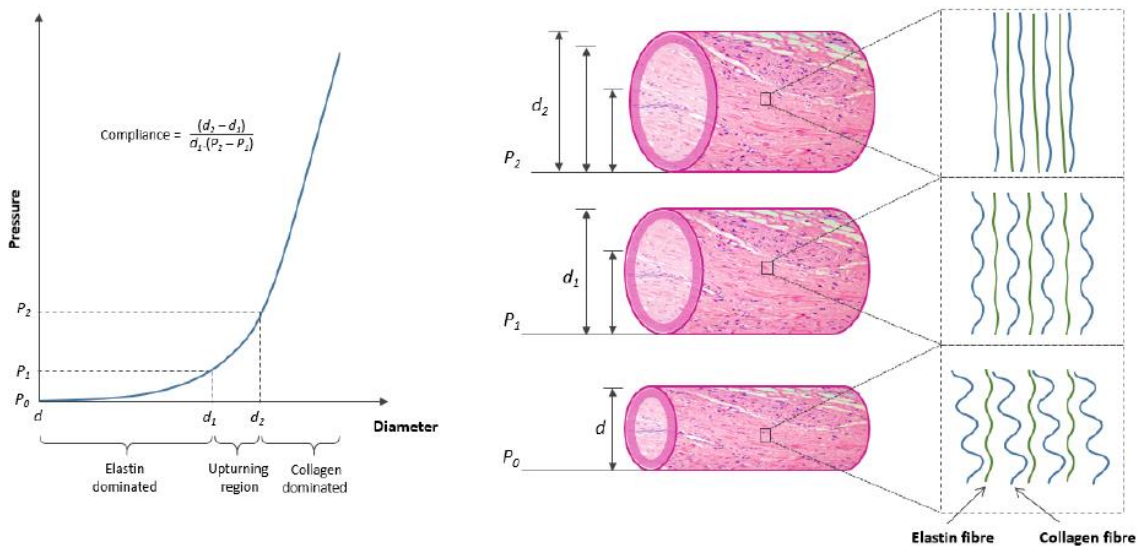


Figure 2: Elastin and collagen effect in pressure-diameter relation of an artery. In the right, the graft shows the relation between the pressure of the blood flow and the variation that it provokes in the diameter, information needed to compute the compliance with the depicted formula. In the left the collages (blue) and elastin (green) fibres under different levels of pressure can be observed [7]

At the same time, the geometrical arrangement between the structural components of an artery gives to the vessel an anisotropic character, which is difficult or even not possible to replicate with a homogenous structure as the one that will be presented in synthetic implants [7]. The circumferential orientation of collagen fibres and their unequal orthogonality in unstressed state, gives the arterial tissue interesting properties as, for instance, a circumferential stiffness 40% higher compared to the axial one [14].

In uniaxial extension tests a load is applied in one direction. Uniaxial extension tests of soft tissue do not result in a strain path that coincide with the strain domain in a physiological state as the performance in the circumferential and axial directions is not combined. In modelling, the data obtained through this procedure is not sufficient for the determination of multidimensional

material models that aim to predict the material behaviour in physiological loading states. However, the advantage of this kind of testing is its simplicity.

In Figure 3, a schematic plot indicating the experimental paths for uniaxial extension of two arterial strips with orientations orthogonal to each other is depicted. x_1 denotes the circumferential and x_2 the axial direction of an artery, while convex contours represent states of constant energy of a hypothetical strain-energy function. The grey area represents the physiological loading conditions of an artery [49].

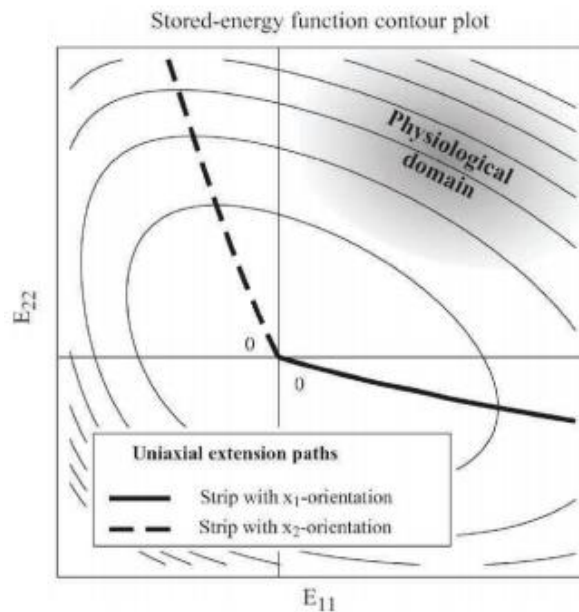


Figure 3: Schematic plot indicating the Green Lagrange strains E_{11} and E_{22} in the (x_1, x_2) plane of an artery [49]

In biaxial tests two loads are applied independently in two different directions. The physiological loading of most soft biological tissues is biaxial (e.g. arteries, skin...). The combination of the directions in which apply the two loads can be planar, extension-shear or extension-inflection as shown in Figure 4.

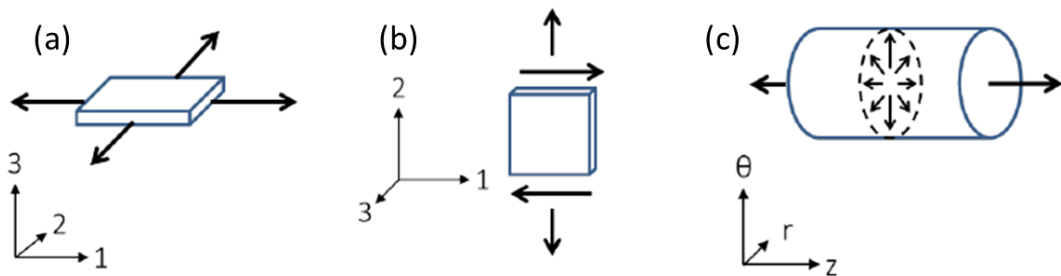


Figure 4: Different combinations of strengths in biaxial testing (a) planar extension, (b) extension-shear, (c) extension-inflection [50]

A recent study, proposed a standardized method for planar biaxial testing of soft biological tissues that combines mechanical testing, images analysis and finite element model (FEM) for determining the stress response of tissues such as arteries [50]. Triaxial testing and tests for non-standardized geometries also exist, but those are out of the scope of the tissue aimed to study, i.e. arteries, because their physiological behaviour is most likely under biaxial stress.

There are many arterial models that try to recreate the native tissue stress response. Most of them consider the arterial wall as a homogeneous single layer. However, the structure of an artery is much more complex. Increased insight into the mechanical properties and geometrical dimensions of the individual vascular tissues is needed to understand their functional behaviour. With that aim, Holzapfel G.A. and Ogden R.W. [82] developed a study in which they compared experimental and computational stress-stretch curves after circumferential and longitudinal testing of the different arterial layers. The results of this study are presented in Figure 5, media and intima show a higher stiffness in the circumferential direction while adventitia shows higher stiffness in the longitudinal direction.

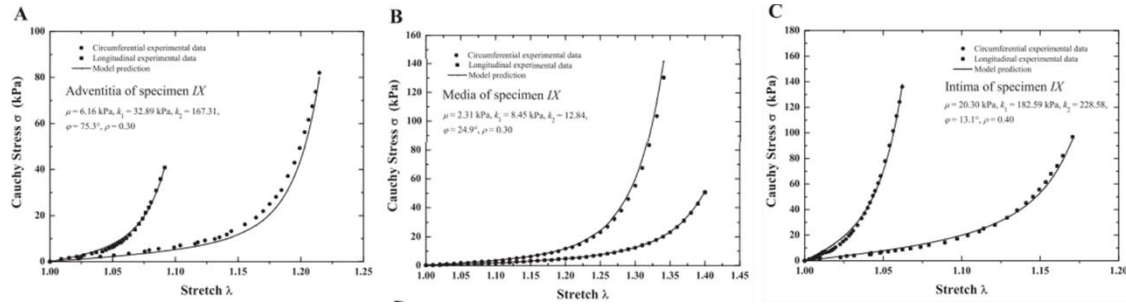


Figure 5: Circumferential and longitudinal stress-stretch responses of samples from layer of a human left anterior descending coronary artery. A: adventitia. B: media. C: intima. The experimental data is represented with dots and compared with numerical results represented with continuous lines [82]

2.1.2.2 Viscoelasticity and fatigue

The viscoelasticity of an artery impacts in the delay of response to cyclic infraction-deflation stresses; this is known as hysteresis when changes in pressure and volume occur. The area enclosed by the hysteresis loop represents the energy lost in each cycle. This energy dissipation attenuates pressure pulses and propagates them along the arteries as waves of circumferential distention. Additionally, the viscoelastic property has been suggested to improve the fatigue life of arteries by reducing dynamic stresses and strains in the wall [8].

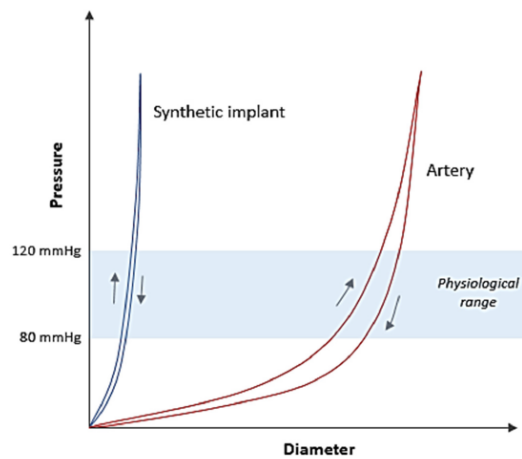


Figure 6: Comparison of pressure-diameter curves between an artery (red) and a synthetic implant (blue) [7]

The frequency dependency of the dynamic mechanical properties of a saphenous vein and internal mammary artery grafts has been experimentally studied [53]. For this study, rectangular longitudinal strips of the mentioned blood vessels from 14 patients were tested under cyclic

uniaxial tensile loading in the frequency range of 0.1-20 Hz at 37°C in wet conditions. The storage modulus, the hysteresis ratio and the collagen phase modulus were computed as a function of frequency.

For each tissue strip, the Fourier mathematical periodic functions were used to obtain the chart in Figure 7. The following mechanical parameters can be obtained from this chart: the storage modulus the slope of the straight line from zero to the maximum stress, the collagen phase modulus the slope of the linear part of the loading phase and the hysteresis ratio after the integration of the Fourier mathematical functions separately for loading and unloading phase as $h = (\text{loading} - \text{unloading area}) / (\text{loading area})$. This experiment demonstrated different characteristics of an artery such as non-linearity or the incremental elastic modulus. This information allows to determine the fatigue limit of an artery under a certain stress frequency.

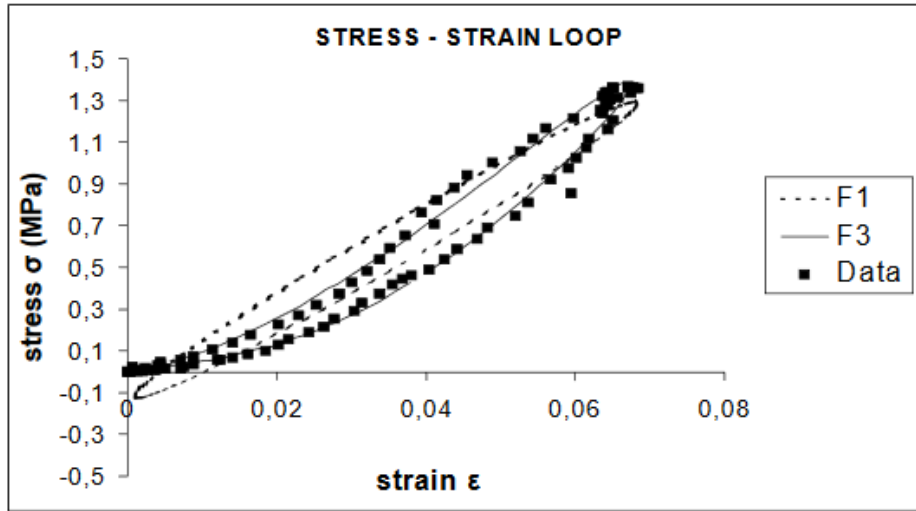


Figure 7: Stress-strain loading-unloading loop diagram of one tissue strip of the internal mammary artery. F1 and F3 are the mathematical curves obtained after Fourier analysis and synthesis of the periodic data using the first and the third Fourier functions [53]

2.1.2.3 Compliance

Compliance is defined as the radial extensibility of an artery in the physiological pressure range (80-120 mmHg) and it is measured in ml/mmHg. This characteristic is closely related to the structure and material properties. The size of the blood vessel, the thickness of the layers thereof and the distribution of the elastin and collagen fibres can result in values of different magnitudes.

The compliance is relatively controlled by the proportion of collagen and elastin fibres existing in the artery. In the ascending aorta, for example, the elastin content is about 41% of dry weight while the descending aorta presents only 30%. This matches with the decreasing compliance while moving downstream an aortic vessel [12, 13].

As depicted in Figure 2, compliance can be calculated considering the transition between the actuation of the elastin and the collagen fibres under different pressure levels as the percentage increase in diameter to a given pressure:

$$C = \frac{(d2 - d1)}{d1 \times (P2 - P1)} \quad (2.1)$$

Below, several methodologies to measure compliance will be explained. It will be shown how all the methods used to estimate volume compliance are implicitly or explicitly based on Windkessel models.

The first experiment described concerns the measure of the pulmonary compliance of a pig artery in vivo [33]. It considers two measurement techniques, the first one consists of the measure of the compliance in pulsatile flow conditions and the other in steady flow conditions.

For the pulsatile flow conditions, a flow probe was placed around the pulmonary artery (PA), a second pressure transducer-tipped catheter was inserted into the left atrial appendage (top left chamber of the heart) to monitor left atrial pressure and a pressure-volume catheter was inserted via direct puncture into the right ventricle to monitor intraventricular pressures. A schematic of the mounting is presented in Figure 8. The flow in the PA and the pressure in the left atrial and right ventricular and the right ventricle were recorded at baseline and during dobutamine continuous infusion ($15 \mu\text{gr} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) to increase cardiac output and pulmonary pressures.

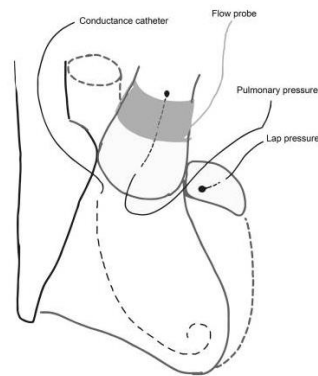


Figure 8: Pulsatile Flow setting [33]

For the steady flow conditions, the PA was encircled using umbilical tape with a tourniquet to constrict the PA. A multi-perforated cannula was inserted into the right atrium, and a straight cannula was inserted into the PA through the right ventricular outflow. The cannulas were connected to a continuous centrifugal flow pump via two silicon tubes, inflow and outflow. A schematic of the mounting is presented in Figure 9. The exact pump flow rate was recorded via a flow probe that was adapted around the arterial tubing. To perform the measures the flow was abruptly interrupted with a clamp to obtain the pressure decrease in the PA.

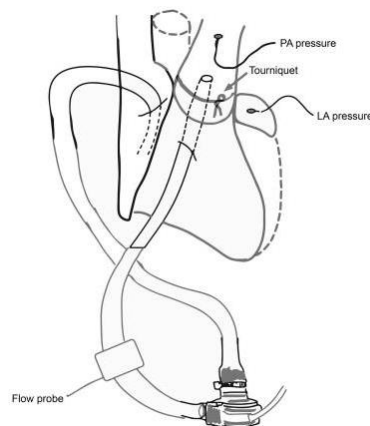


Figure 9: Steady Flow setting [33]

There are four main methods to calculate the compliance that have been used in several experiments. In the case of pulsatile flow conditions compliance have been calculated with all of them and compared:

1. The ratio of stroke volume (in mL) and pulse pressure (in mmHg). Where the stroke volume was calculated using the integral of the positive values of the PA flow meter.
2. Diastolic pressure decay time method. During diastole, the pressure drops mono-exponentially in a Windkessel system according to the following equation:

$$P = P_{\text{diast}} * \exp(-t/RC) \quad (2.2)$$

Where R is the resistance and it is measured as: (mean pulmonary pressure – left atrial pressure)/(mean flow).

3. The area method [34]:

$$C = \text{Stroke volume} / (K * (P_{\text{diast}} - P_{\text{end diast}})) \text{ with } K = (A_s + A_d) / A_d \quad (2.3)$$

with $P_{\text{end diast}}$ defined as the pressure at the end of the diastole in mmHg, A_s the systolic area and A_d the diastolic area.

4. The pulse pressure method [35, 36]. This method iteratively adapts the value of C in a two-element Windkessel model [51] until the measured and predicted pulse pressures are identical, considering a calculated value of R.

Another type of experiment was performed in a young adult's peripheral artery, also in vivo [37]. In this experiment the compliance was determined through the pressure waveforms present in the artery. Radial artery waveforms were obtained using a calibrated tonometer device and characterized waveform morphology to determine large artery (C1, capacitive compliance) and small artery (C2, oscillatory compliance) compliance. Blood pressure (BP) was measured using random zero sphygmomanometers, a device for measuring the blood pressure where the observer does not know where the zero pressure is in the device [52].

The diastolic pressure at time t relative to the aortic valve closure was calculated using the following algorithm:

$$P(t) = A_1 * \exp(-A_2 * t) + A_3 * \exp(-A_4 * t) * \cos(A_5 * t + A_6) \quad (2.4)$$

The first part of the equation represents the overall exponential decay in pressure during diastole and the second represents an exponentially decaying envelop of the sinusoidal oscillations occurring in early diastole, as a result of reflecting waves and resonances within the arterial system. The algorithm determined the best set of A_i values for matching the diastolic portion of the averaged beat to this equation. The C1 and C2 values were then calculated from the A_2 , A_4 , and A_5 found with the best fit algorithm, in accordance with the modified Windkessel circuit analysis. The reproducibility of this test is quite high since the differences between two studies taken one week apart were small as report the results: $C1 = 0.015 \pm 0.38 \text{ mL/mm Hg}$; $C2 = 0.007 \pm 0.014 \text{ mL/mm Hg}$ [38].

One of the first experiment performed ex vivo to describe the relationship in terms of relative change in volume and pressure was proposed by Bramwell and Hill [59]. The setup of such experiment is depicted in Figure 10. With sensors located in X and Y the difference of pressure (ΔP) was measured. Since the time interval in which the pressure was applied, as well as the length of the artery and the density of the mercury, were known, the increment in volume (ΔV)

could be computed. Hence, the pulse wave velocity (PWV) that then was implemented in a model to obtain the compliance, was calculated as follows:

$$PWV^2 = \frac{\Delta P * V}{\Delta V} * \rho \quad (2.5)$$

The reason why mercury was used to perform the experiment was the difficulty to find arteries of considerable length, and the fact that the velocity of the pulse wave was inversely proportional to the square root of the density of the fluid in the vessel. Thus, in the case of replacing blood by mercury, the velocity in an isolated artery has to be multiplied by 3.58 approximately to obtain the approximate blood velocity.

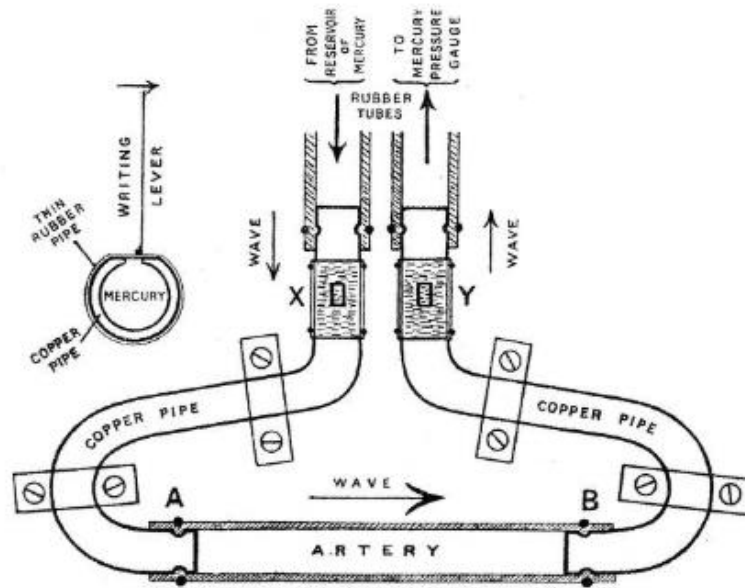


Figure 10: Setup to measure pressure increase in an artery. A and B are the clamping points between the artery and the copper tubes through which the mercury is pumped. X and Y are pressure sensors. The copper pipes are clamped to rubber tubes in their ends. The pressure wave is set up by hitting or squeezing the left-hand rubber pipe at some distant spot [59]

A more recent study has analysed 22 ex vivo porcine aortas on a tensile tester [60]. Regional and directional compliance, failure stress and failure strain were recorded. Aortic strips with a width of 15 mm were mounted in a customized tensile tester using surgical clamps as shown in Figure 11. The force-dependent distension was measured optically.

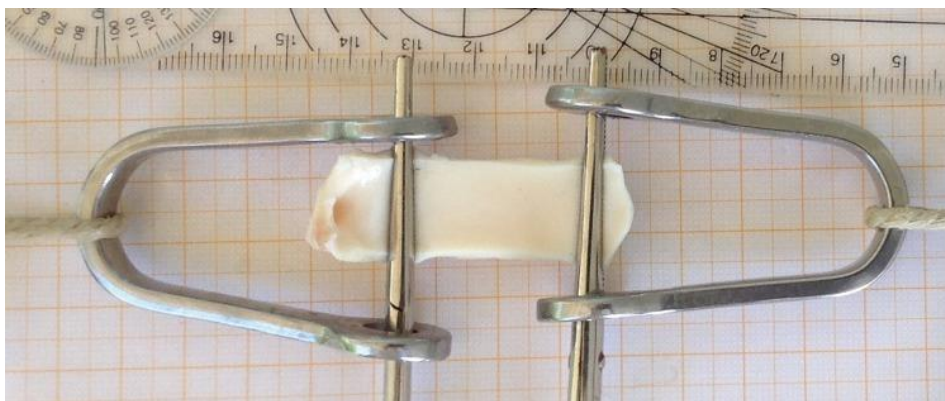


Figure 11: Customized tensile tester using surgical clamps attached to an artery strip of 15 mm [60]

To sum up, when trying to measure the compliance of a native artery stroke volume and pressure must be defined. These values can be obtained using experimental methods, in vivo or ex vivo, but for the compliance only analytical models are present to obtain its value. However, there are substantial differences between all the experiments presented that affect the results obtained. Moreover, none of these methods have been used to compare the behaviour of the native tissue with the synthetic grafts used to performed bypasses in case of occlusion of such native tissue.

2.1.2.4 Computational models

To predict the native tissue behaviour without the need of using invasive techniques, computational models have been developed, powered by the technological advances of the past decades. Mechanical and fluid-dynamical models have been built. The first group is mainly focused on reproducing the cardiovascular system with a structural approach, while the second is based on studying how the blood flow evolves according to different constraints.

2.1.2.4.1 Mechanical models

In the year 2001 Cardoso E. *et al.* [62] made a simplified mechanical model of the cardiovascular system based on Clancy's model (1991) [63]. Sometime after, the same authors developed a complete model based on LéFevre's model (1999) [64], where muscular strength and speed parameters were included. After simulation, graphics of the ventricular pressure waves as function of the time, ventricular volume and the cycle pressure-volume were obtained. In 2003, Díaz Zuccarini [65] presented a model of the left ventricle of mammals. He started from the muscular contraction mechanisms and developed models already present in previous publications (*Integrating a Bond-Graph based model of chemo-mechanical coupling with Huxley's sliding filament theory* and *un modèle Bond-Graph simple de la mécano-chimie du myocarde*). His final aim was to develop a theoretical model to illustrate mechano-chemical transduction replicating the physiological conditions of the valve to cardiac studies and clinical training.

A couple of years after, in 2008, an article was published in which they depicted the influence of the arterial pressure in a model for cholesterol accumulation and intimal growth [66]. This model qualitatively predicted the formation of the injury in the external walls of the carotid artery bifurcation and how its geometry evolved as function of the time. Also, in 2008, E. Claes, C. *et al.* performed a study in which the objective was to analyse the anastomosis, or clinical union, where a coronary artery is substituted by another vessel (homograft or synthetic prosthesis) [67]. They studied the stress distribution in the anastomosis area and its effect on the integrity of the whole construction. The mechanical behaviour of the vessels involved was characterised and a numerical model was presented to reproduce how a termino-terminal bypass works. The results demonstrated that the diameter, the thickness and the flexibility of the synthetic graft are more important than the same data for the native artery to predict the behaviour of the construction. In the same year, a simulation of the cardiovascular system and the blood obstruction in the native veins was presented [68]. To study the native blood vessel occlusion as well as other pathologies, the cardiovascular system was modelled thanks to a simplified electrical analogy.

In the work developed by José Di Paolo *et al.* [69], a numerical simulation of the bloodstream in coronary arteries with stenosis to determine the curves of the shear stresses is proposed. After the analysis it could be claimed that under different ranges of pressure, the morphology of the curves does not present significant discrepancies. The mean value of the blood flow and the

effective percentage of occlusion were more important than the variation of the pulse of the flow to predict the behaviour of the set.

2.1.2.4.2 Fluid-dynamical models

One of the eldest studies about the behaviour of the blood flow, is the one performed by F. Pythoud *et al.* in 1996 [70]. Their work was based in the study of the friction effects and the non-linearity in the blood flow in an arterial blood vessel. Another relevant study to be mentioned, is the one performed by K.H. Parker and D.G. Gibson in 2006 [71]. In the study, the laws of behaviour of the cardiovascular fluids were generally described, as well as the equations to model it and its characteristics. This publication has been useful to understand the behaviour of the bloodstream before modelling it.

In 2009, Jan Vimmr *et al.* published a numerical study of the non-Newtonian effects of the blood flow in complete coronary and femoral bypasses in 3D [72]. Also, in this same year a numerical simulation of the Newtonian and non-Newtonian blood flows in a bypass for biomedical applications was performed in 2D and 3D for stable and instable flows [73]. The equations used in the model were those for the incompressible fluid of Navier-Stokes, the concept of artificial compressibility with three steps of the method Runge-Kutta and the method of finite volumes.

A recent study performed in 2014 proposed a mathematical model of the lower limb arterial network that predicted the flow in a bypass [2]. It used a 1D model adapted to study long wave lengths in arterial networks and a 0D model, which is like an electrical circuit, to characterise peripheral networks. After simulation, the following parameters were obtained: diastolic diameter, length of the arterial segments, wall rigidity of each segment, concomitant mean pressure and velocity in the deep femoral and popliteal segments, pressure waveform in the same segments and velocity profile both in the native tissue and the synthetic graft. These data must be compared with the post-operative in-vivo conditions to validate the model.

2.2 Clinically applied synthetic vascular grafts

The definition of an ideal synthetic vascular graft requires to fulfil the following properties: biocompatible, non-thrombogenic, compliant, fatigue resistant, flexible yet robust, readily available and easy to manufacture. Among these, the two first attributes are mainly contributed by material selection while the others are influenced by the manufacturing method used and structural design of the graft [7].

The two most-often used vascular graft materials in clinical practice are Dacron (i.e. Polyethylene terephthalate, PET) and Gore-Tex (i.e. expanded Polytetrafluoroethylene, ePTFE). Different combinations of polyester-polyurethanes have been also used in medical interventions as substitutes of vascular native tissue [45]. The structure of these materials and the existing techniques used for their mechanical characterization will be described in this section.

2.2.1 Structure

2.2.1.1 Dacron

PET was first introduced in 1939. DuPont further developed it and patented Dacron fibre in 1950 used for suits manufacture and was called Dacron in the United States and Terylene in Britain [16, 40]. Vascular grafts made from Dacron were first implanted by Julian in 1957 and DeBakey in 1959 [16]. The main manufacturing processes used to fabricate Dacron grafts are weaving or knitting. A woven graft is manufactured by interlacing two sets of yarns (warp and weft) oriented

at 90° to each other. In Figure 12, different types of weave designs are presented. Knitted grafts have a looped filament construction in which a continuous interconnecting chain of yarn loops spirals around the graft circumference. The different structures of knitted Dacron can be observed in Figure 13.

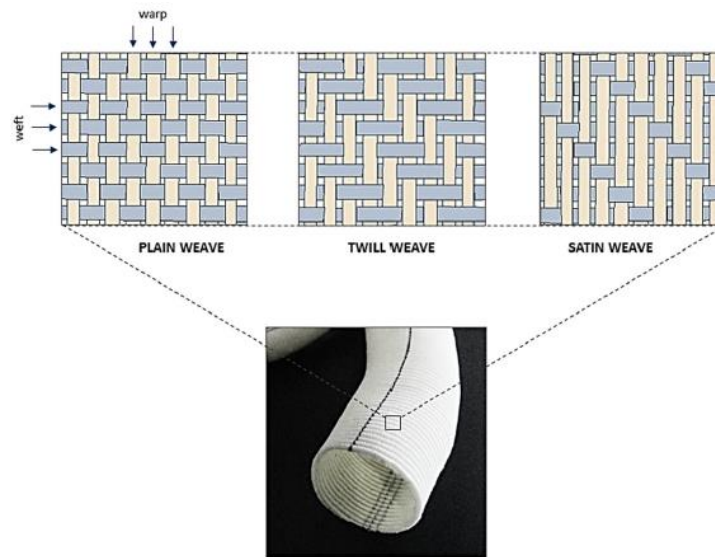


Figure 12: Structural design patterns of a woven Dacron graft [7]

Whereby the elastic behaviour of the PET fibres corresponds to the collagen fibres in an artery, no graft component performs the role of the elastin fibres. Moreover, the orthogonal arrangement of both yarn components results in low axial elongation and poor radial compliance properties in these grafts [18]. The axial elasticity is often achieved through graft crimping, which also helps to maintain the tubular shape of the graft during bending. Nevertheless, optimum compliance is still unachieved which is a problem once demonstrated its clinical importance.

Table 1 shows the comparison between the mechanical properties of native artery and Dacron.

Table 1: Elastic modulus of components of an artery and a woven Dacron graft [19]

Structure	Component	Elastic Modulus (MPa)
Artery	Elastin	0.6-1
	Collagen	1000
	Smooth Muscle	0.1
Woven graft	Dacron Polyester	800-900

The woven Dacron prostheses have better stability with minimal bleeding and lower permeability compared with knitted structure. However, woven structures present a reduced compliance and biostability due to their relatively low porosity, greater infection risk and tendency to fray at the edges.

The knitted Dacron prostheses are softer, more flexible, more compliant and have better handling characteristics than woven structures. They also have greater porosity and tissue ingrowth, but they dilate over time and have an increased risk of infection [17]. This opens another line of investigation concerning the fatigue resistance adequacy.

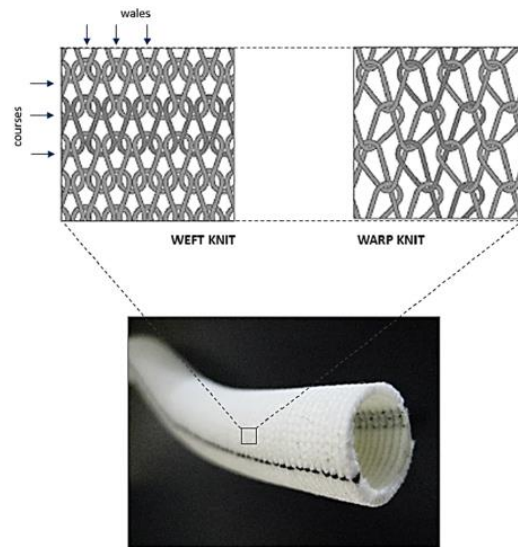


Figure 13: The structural design patterns of a knitted Dacron graft [7]

2.2.1.2 Gore-Tex

PTFE was patented by DuPont in 1937 as Teflon. Its medical use began with its application in artificial heart valves in the early 1960s. In 1969, Gore patented expanded ePTFE (Gore-tex). This biostable polymer is manufactured from PTFE through a process of heating, stretching and finally extruding that produces a microporous material more supportive of firm tissue adhesion.

The graft consists of a node-fibril structure in which solid nodes connect through fine fibrils, with an average internodal distance of 30 μm for a standard graft. Most of the times, an external wrap is performed to reduce the dilatation after implantation. An enhanced tissue ingrowth with complete endothelialisation occurred in ePTFE grafts with a 60 μm or 90 μm internodal distance, but the 90 μm internodal graft demonstrated focal areas of neointimal desquamation at late periods which is translated as the shedding of the outermost membrane or layer of a tissue, in this case it is referred to the graft [24]. This is translated as a higher patency rate in high porosity ePTFE grafts, meaning with patency the percentage of openness of a blood vessel.

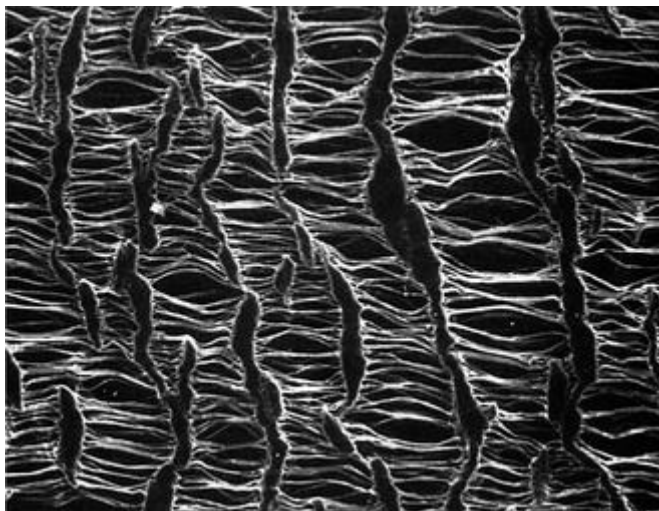


Figure 14: Electron microscopy of ePTFE node-fibril structure [22]

In Figure 14, a scanning electron microscopy image (original magnification x500) of a node-fibril structure in an ePTFE graft can be observed. The solid islands of nodes linked by fibrils can be distinguished. This node structure occupies about 20% of the total volume of the expanded polymer and results in a higher ratio of pores but with smaller dimensions.

2.2.1.3 Polyurethanes

For many years, the use of polyurethane (PU) for the fabrication of vascular grafts has been attractive because its elastomeric mechanical performance, which is higher than Dacron's and Gore-Tex's, mimics the compliance required in a synthetic blood conduit. They were originally developed in Germany in the 1930s and first commercialized by DuPont in 1962. For biomedical applications they have been available since the 1960s (Boretos and Pierce 1967) [24]. PU are composed of three monomers: a diisocyanate (hard domain), a chain extender, and a diol (soft domain). At physiologic temperatures, the soft domains provide flexibility, whereas the hard domains impart strength [22]. The reason why polyurethane is usually avoided in vascular grafts manufacturing is its tendency to chemical deteriorate due to its hydrolytic susceptibility that can derivate in carcinogenic effects due to the content of 2,4-toluene diamine [4].

The three main commercial examples of PU-based synthetic grafts are: Corvita (1992), Pulse-Tec (2000) and Thoratec (2005) [43]. The first two are an old type of endoprosthesis, which are not used anymore, while the last one is actually used to perform arterio-venous fistula. The first generation of polyester–polyurethane vascular grafts was hydrolytically unstable (Corvita). Then, it was demonstrated that a polyether-based polyurethane (Pulse-Tec), relatively insensitive to hydrolysis, underwent biodegradation in vivo due to an oxidative process [41].

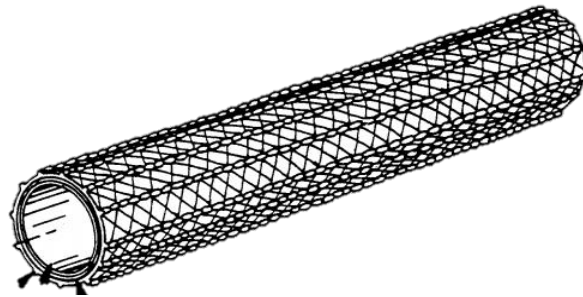


Figure 15: Corvita vascular graft structure, fibrillar polyurethane [45]

One of the latest attempts to create a self-sealing vascular access for immediate use is a three-layered polyetherurethaneurea with non-porous layer under the luminal surface (Vectra, made by Thoralon trademark of Thoratec laboratories corporation). It gave similar patency rates compared to PTFE grafts [42]. This type of synthetic polyurethane-based graft, which can be observed in Figure 16, is currently being used to perform arterio-venous fistula in order to obtain a vessel with high flow but low pressure to optimize the dialysis.



Figure 16: Vectra vascular access graft

According to their microstructure, polyurethanes can be fibrillar (Figure 15), like it was for Corvita, or foamy (Figure 17). Both tend to lack communicating spaces for potential capillary

ingrowth. In the case of the fibrillar PU, the blood surface of the graft is covered, before implantation, by a fibrin layer that is thinner than on knitted Dacron. The outer surface is typically encapsulated by scar formation containing foreign-body giant cell (FBGC), which is a collection of macrophages generated in response to the present of a foreign body, that is to say the synthetic graft. In the microporous foamy PU with an average pore size of 15 μm , little capillary ingrowth could be accomplished, whereas with increasing pore size (up to 157 μm), undesired inflammatory FBGC reaction could be diminished [24].

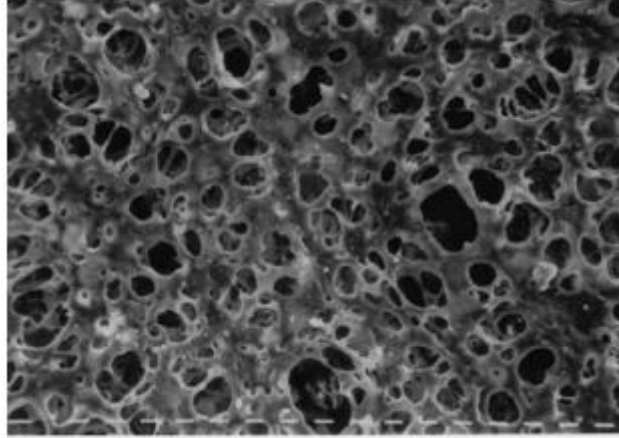


Figure 17: Scanning electron microscopy of native MyoLink, compliant poly(carbonate-urea)urethane [46]

As a summary, in Table 2 the advantages and disadvantages, as well as the healing process, of every clinical used synthetic vascular graft are presented.

Table 2: Advantages, disadvantages and healing process of synthetic vascular grafts in clinical use [24]

	Woven Dacron	Knitted Dacron	Low-porosity Gore-Tex	High-porosity Gore-Tex	Fibrillar PU	Foamy PU
Advantages	Good stability, low permeability and low bleeding.	High porosity, good tissue ingrowth and radial distensibility.	Biostability, no dilation over time.	Biostability, good cell Ingrowth.	Compliance, good hemo- and biocompatibility, low thrombogenicity.	
Disadvantages	Low compliance and tissue incorporation, low porosity, fraying at edges, infection risk.	Dilation over time, infection risk.	Stitch bleeding, limited incorporation, infection risk, perigraft seroma formation.	Late neointimal desquamation in 90 μm IND, infection risk.	Biodegradation in first generation, infection risk, carcinogenic.	
Healing process	Inner fibrinous capsule, outer collagenous capsule, scarce endothelial islands.	Fibrin luminal coverage, very sporadic endothelium, transanastomotic endothelialisation in animals.	Luminal fibrin and platelet carpet, connective tissue capsule with foreign body giant cells, no transmural tissue ingrowth.	Macrophages and polymorphonuclear invasion, capillary sprouting, fibroblast migration, certain angiogenesis, thicker neointima, endothelialisation in animals.	Thin inner fibrin layer, outside foreign body cells, limited ingrowth.	Good ingrowth with bigger pores.

2.2.2 Mechanical properties

2.2.2.1 Stress response

To study the anisotropic behaviour of the presented clinically used synthetic grafts, different tests have been performed. For the mechanical characterization of woven Dacron, uniaxial and ring tensile tests have been performed under different orientations [29]. The results of the tensile tests were used to design and validate a numerical model that predicted the graft's mechanical response under generalized loading such as those that occur under physiological conditions. The testing orientations selected for the uniaxial testing were longitudinal (0°), circumferential (90°) and bias (45°). The results obtained are presented in Figure 18. The rupture limits for the stretching according with the directions in which the test was performed are presented in Table 3.

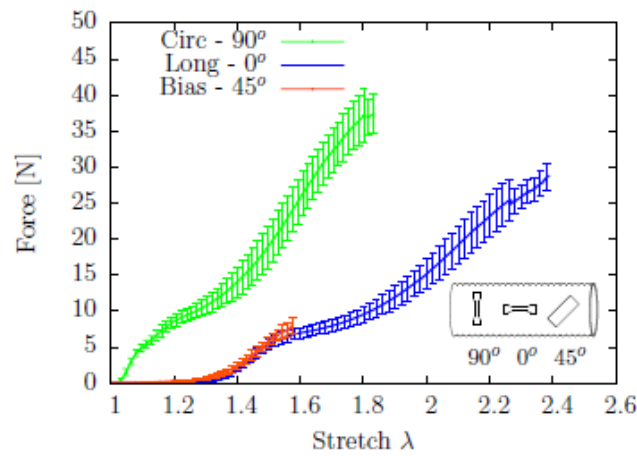


Figure 18: Experimental data force vs stretch for the three directions analysed. In the y axis the force applied is represented in Newton and the stretch in the x axis which is the increase of length divided by the initial length [29]

Table 3: Rupture limits of the woven Dacron specimens [29]

Limit	Circumferential	Longitudinal	Bias
λ_{rupt}	1.836 ± 0.080	2.390 ± 0.067	1.592 ± 0.050
$F_{rupt} [N]$	37.250 ± 4.484	28.853 ± 2.566	7.755 ± 1.669

It can be observed that the circumferential response was about twice as rigid as the longitudinal one as illustrated by the slopes. These differences can be explained due to the different filaments that compose the threads and their number in the fabric according to each direction (King et al., 2013) and, in addition, to the longitudinal corrugation that displaces the curve due to its stretching.

According to the results reported by García-Herrera *et al.* (2012, 2013) for the aortic tension in the circumferential direction, a rupture Cauchy stress of about 2 MPa was obtained, resulting in 35 times smaller than that of the Dacron (72-74 MPa for Dacron). In this relation, the graft would exhibit a high stiffness for damping the pressure wave and it would eventually modify the functioning of the cardiovascular cycle. A corrugated wall in the longitudinal direction could introduce flexibility, which would smooth the effect of the graft stiffness and would prevent the collapse.

2.2.2.2 Fatigue and viscoelasticity

As depicted in Figure 6, the woven Dacron (synthetic implant) exhibits a lower elasticity and higher linear stress response at similar levels of strain than a native artery. The experimental characterization of the static hyperelastic behaviour, the relaxation, the dynamic modulus and the loss factor of woven Dacron for aortic prosthesis has been performed to further study this behaviour [54]. Uniaxial tensile and relaxation and dynamic stiffness tests were performed to 45 x 10.5 mm strips of the material. As a result, an 11% difference of the reduced relaxation (after infinite time) between axial and circumferential directions has been observed and a very large increase in stiffness was obtained in the case of harmonic loading with respect to the static loading.

2.2.2.3 Compliance

Currently, for the synthetic grafts on the market, there is still a large graft-artery compliance mismatch, and this leads to late graft failure, as known from clinical observation, especially with synthetic grafts [30]. Around the suture line, it appears that intimal thickening is initiated by surgical injury and promoted by mismatch in the graft-to-artery compliance and by low wall shear stress [31,32]. A possible contribution to the occurrence of this phenomenon is thought to be tissue remodelling in response to mechanical injury due to mismatch in compliance [31]. Therefore, consideration of different graft-artery compliance and the determination of the intra-vascular stresses are important for further understanding of the disease process and proposing new solutions or improvements in the grafts.

Table 4: Comparison of compliance given as per cent diameter change per mmHg x 10² [20, 23]

Structure	Compliance (Dynamic Diameter %/mmHg)
Artery	7.4 e-2
Vein	2.7 e-2
Dacron Woven	1.9 e-2
Dacron Knitted	2.3 e-2
ePTFE	1.63 e-2
Segmented Design	9.8 e-2

For determining the compliance of vascular grafts, different studies and models have been developed. In a recent study [55], a test bench for determining the hydrodynamic compliance of different types of PET multifilament yarns has been developed. From the table above (Table 4) the only material tested in this set-up was the woven Dacron, also for low-twisted Viscosuisse and intermingled Diolen compliance values were obtained.

Cyclic tests have been applied to prosthesis tubes by using both mechanical testing machine and a test bench specially developed for simulating mechanical aging induced by water pressure on the prosthetic wall. This instrument allows the injection of pulsatile water adjustable in frequency and pressure in vascular grafts previously sealed by a rubber bladder while simultaneously monitoring the prosthesis wall distension by a high-resolution displacement transducer linked to a data acquisition software. The schematic of the device that generates the pulsatile hydraulic flow through prosthetic conduits is presented in Figure 19.

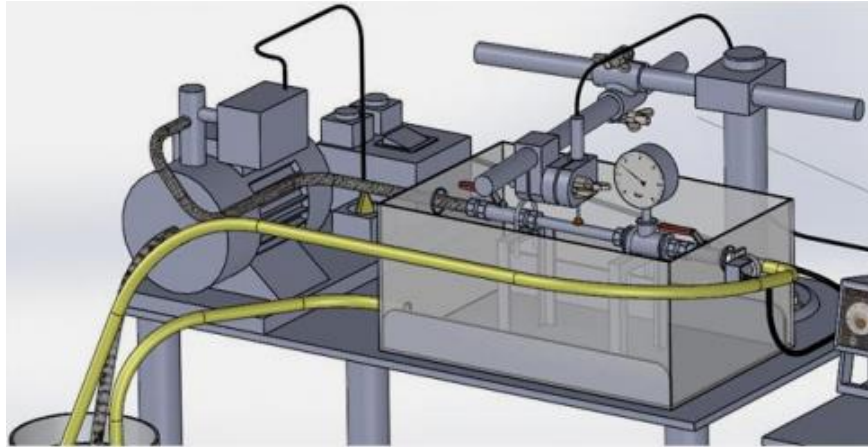


Figure 19: Schematic of the test bench used to generate the pulsatile hydraulic flow through the prosthetic conduit and wall distension measurement [55]

After the testing, compliance curves were obtained, so it was possible to identify the high-distensibility graft structures whose cyclic dilatation could be maintained almost stable throughout fatigue test. In Figure 20 the results of the test for different synthetic graft materials is shown. The fatigue inflicted on the textile graft structures was responsible for the increase of their extensibility and consequently of their compliance. Low-twisted Viscosuisse (C3) and Intermingled Diolen (C2) presented similar initial compliances ($3.3 \cdot 10^{-5}$ - $3.8 \cdot 10^{-5}$ mmHg⁻¹), but Intermingled Diolen (C2) was stabilized at $4.8 \cdot 10^{-5}$ mmHg⁻¹ and Low-twisted Viscosuisse (C3) raised to $8.8 \cdot 10^{-5}$ mmHg⁻¹. For the woven Dacron (C1) the initial compliance already has good values $7.6 \cdot 10^{-5}$ mmHg⁻¹ and remains relatively stable to $8.8 \cdot 10^{-5}$ mmHg⁻¹ at the end of the fatigue cycle. The values presented in Table 4 differs from the experimental data due to the increase in the pressure range applied in this last one (1350-2025 mmHg) being above the physiological values over two orders of magnitude.

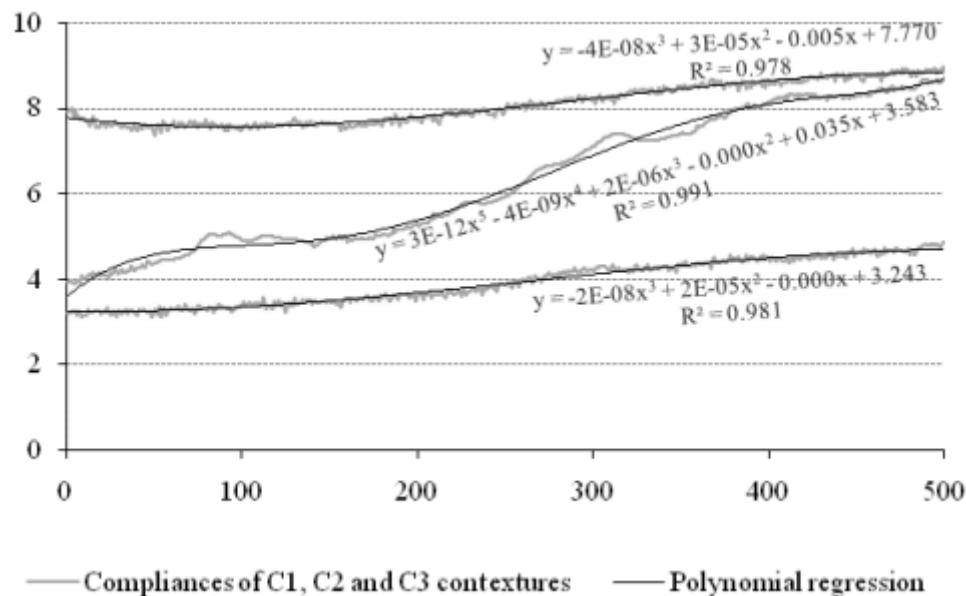


Figure 20: Dynamic compliance curves tested in 1.8-2.7 bar pressure range of textured Dacron (C1), Low-twisted Viscosuisse (C3) and Intermingled Diolen (C2) up to bottom presented [55]

2.2.2.4 Computational models

Some of the models used to predict fluid-dynamic properties through vascular synthetic grafts have already been presented in the section of computational models for the native tissue, because most of the times the blood flow model is valid for both type of vessels.

For the characterisation of the mechanical properties of the synthetic grafts many models could be found in the literature to obtain information about its permeability, rigidity, anisotropy, stress response, etc. For example, a study developed in 1998 proposed a computer model to predict fabric permeability properties taking into account variables such as yarn diameter, weave type, fabric count and filament number [74]. This study included some experimental testing for the validation of the model, consisting of pumping water through the fabric and measuring the external humidity with a sensor located some millimetres below the sample. A more recent study used uniaxial tensile tests to adjust the parameters of a hyperelastic anisotropic constitutive model applied to predict through numerical simulation the mechanical response of woven Dacron in a ring tensile test [29]. This model was capable of representing adequately the nonlinear elastic region and it captured the progressive increase of the rigidity and the anisotropy due to the stretching of the Dacron. Image correlation and finite elements modelling have been also used together to determine the deformation gradient in some materials.

Computational modelling is out of the scope of the present Master Thesis, but it is important to know the existing techniques that have been used to try to characterise both structural and mechanical properties of the materials of interest to have an input before developing an experimental template.

2.3 Novel grafts under research

2.3.1 Coatings for the vascular grafts

The high porosity of the knitted Dacron graft necessitates preclotting as a means of preventing transmural blood extravasation. Gelatin (Vascutek, Renfrewshire, Scotland), collagen (Boston Scientific, Oakland, NJ), and albumin (Bard Cardiovascular, Billerica, Mass) are currently used to seal knitted Dacron graft pores. The gelatin and collagen in the Vascutek and Boston Scientific grafts are cross-linked by low concentrations of formaldehyde, a method that results in a weak linkage that allows the gelatin or collagen to be degraded in the body in <2 weeks [25,26]. Bard uses glutaraldehyde to cross-link albumin, and the albumin is absorbed in 2 months [27].

Carbon coating is used in Gore-Tex to increase the surface electronegativity so as to diminish thrombus formation [28].

2.3.2 Composite, biosynthetic and totally engineered vascular grafts

Several attempts have been made to improve the mechanical and physiological behaviour of the current clinically used synthetic vascular grafts. The combination of different composite materials and native tissue is one of these lines of investigation.

One of the main causes of the compliance mismatch is produced due to the joint between the native blood vessel and the synthetic bypass to conduct the blood stream. This union is known as anastomosis and one of the solutions to reduce both compliance mismatch and suppressing IH was the interposition of autologous vein segment between the native tissue and the synthetic graft has performed (Bastounis et al. 1999) [24]. But this correction is not enough to solve the presented problem.

Design of alternatives to PET and PTFE has concentrated on achieving a compliant structure. This have been approached in several ways including porous materials [86] and double tubular grafts with a more compliant inner lining and a less compliant outer layer [87]. As these later grafts are distended, they become progressively less compliant as the outer sleeve properties become more prominent. The Department of Vascular Surgery, Royal Free Hospital Hampstead (NHS Trust, London, UK) has produced and characterised porous compliant poly(carbonate)polyurethane (CPU) grafts (Myolink™) [88], which compliance compared with native tissue and other clinically applied grafts is presented in Figure 21, observing a higher compliance compared with Dacron and ePTFE (Gore-Tex). However, this new graft is already under investigation and cannot be found in clinical use.

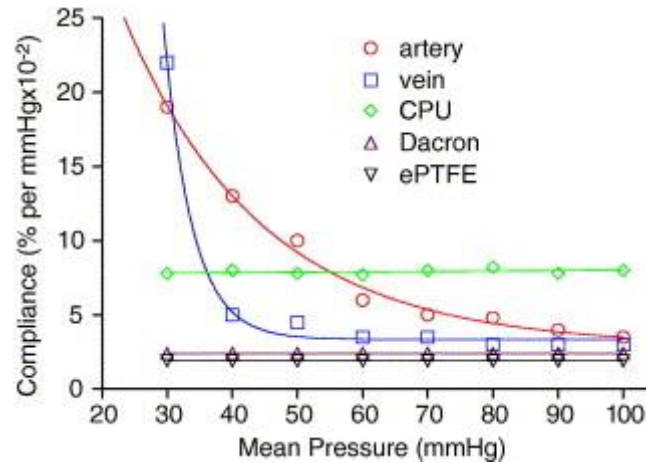


Figure 21: Variation of compliance vs. mean pressure for different conduits. The pulse pressure was 60 mmHg [88]

In a recent study [21], an innovative knitted stent-graft design was reported, which closely mimics the natural artery mechanical behaviour. The design consists of concatenating two different segments with low and high modulus. The low modulus is knitted polyurethane, this section tends to remain contracted. The high modulus is knitted polyester and maintains the straight configuration. The structure described is shown in Figure 22.

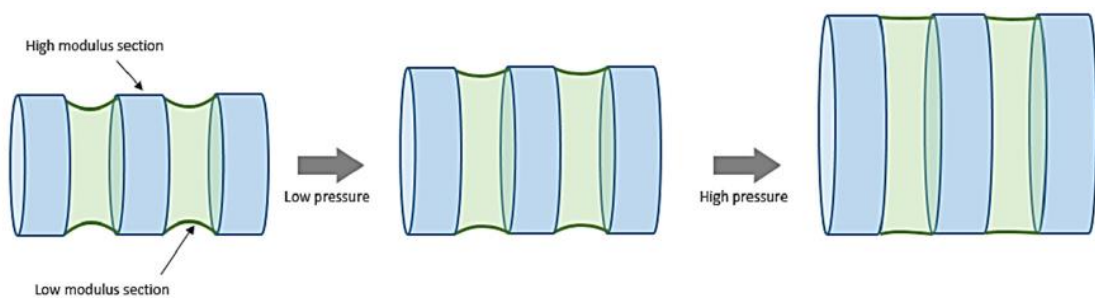


Figure 22: Segmented innovative graft design to improve the compliance [21]

With low internal pressure the low modulus segments controls the stress response until the circumference is of the same size of the high modulus segments. At high pressure both segments increase the stress response, replicating the behaviour of an artery. Values of the compliance of this new design can be observed in the Table 4. However, the in vivo performance of this design is still unavailable to demonstrate its clinical performance.

Biosynthetic vascular grafts consist of the ingrowth of viable biological components into an artificial vascular graft scaffold biocompatible both with the biological material and the native

blood vessel in which it will be allocated. According with some studies, in inflammatory diseases biosynthetic grafts have promising patency, postoperative clinical findings and lower rates of complications requiring reintervention in mid-term than other PTFE based grafts [61].

Electrospinning is a novel manufacturing technique used to produce vascular grafts scaffolds. This technique is capable to produce fibres to the scale of fibrin and collagen, which is a beneficial property for improving cell growth [40].

In the construction of tissue-engineered vascular grafts (TEVG), three major components must be addressed: a scaffold to provide the initial graft shape and strength, an adhesive matrix and endothelial cells [47].

For the scaffold, four major approaches can be identified: permanent synthetic support, natural acellular tissues, a biodegradable scaffold and non-scaffold technology. The manufacturing process of TEVG using a polymer-based scaffold is shown in Figure 23.

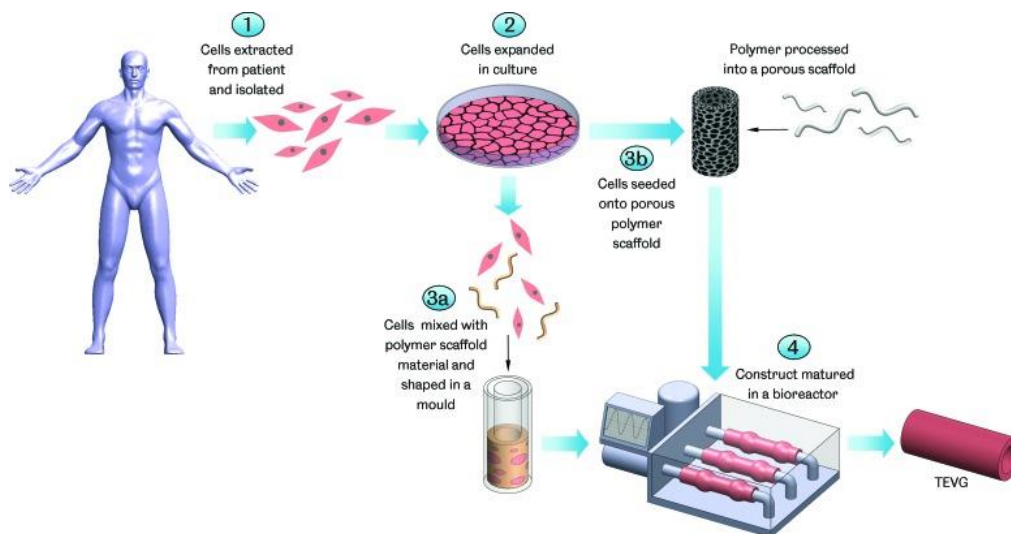


Figure 23: Scaffold-based tissue-engineered vascular graft manufacturing process [48]

Some pioneering methods used in tissue engineering that does not utilise a scaffold or supporting matrix are depicted in Figure 24. These methods are sheet-based tissue engineering, assembly of microtissues and cell printing. The first one consists of a 2D cell sheet already cultured and then shaped around a mandrel, forming a tube matured into a vascular graft. The microtissue aggregation consists of cell aggregates placed in a mould and combined. Finally, bioprinting is a process in which cells and supporting material are deposited layer-by-layer building up a 3D construct.

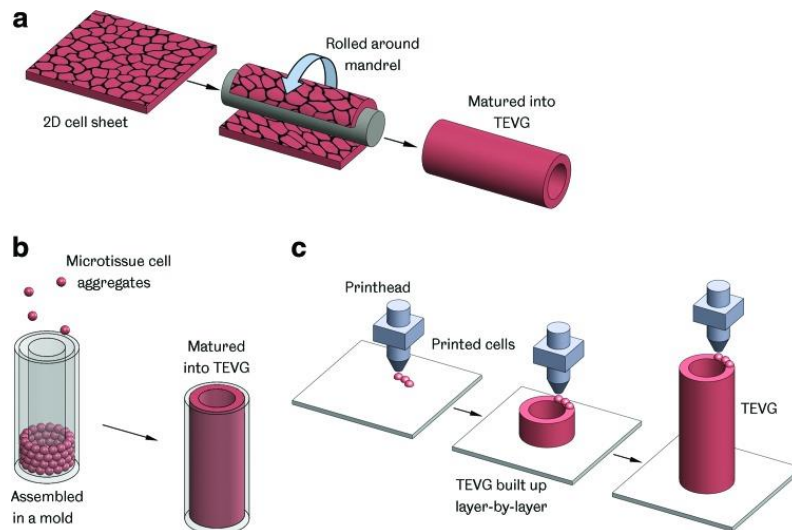


Figure 24: Pioneering manufacturing process for TEVG, (a) sheet-based tissue engineering, (b) microtissue aggregation, (c) bioprinting [48]

Totally engineered blood vessels seems the solution for covering the mechanical and biological mismatch between synthetic vascular grafts and native tissue. However, the understanding of this technique to create the ideal graft and to perform direct graft integration must be improved. A detailed characterisation of the biomechanical properties with data from simulation work and in vivo trials are needed to provide that information.

A comparison between the three exposed techniques is made in Table 5.

Table 5: Advantages, disadvantages and healing process of composite, biosynthetic and totally engineered vascular grafts under research [24]

	Composite grafts	Biosynthetic grafts	Totally engineered blood vessels
Advantages	Low compliance mismatch and intimal hyperplasia, good patency in femoro-distal bypasses, easy redo surgery.	Antithrombogenic, good patency.	Responsiveness, non-thrombogenicity, self-repair, growth, metabolically active, potentially cost-effective.
Disadvantages	Technically demanding, prolonged surgery time.	No emergency use, cell amplification problems, cell culture contamination risk.	Demanding fabrication, time- and cost consuming, bioreactor cell laboratory, specialized centres only.
Healing process	Low intimal hyperplasia.	Self-renewing functional endothelium.	Complete integration.

3 PROBLEM STATEMENT, AIM AND OBJECTIVES

Peripheral bypasses are performed by applying a graft material to substitute a blood vessel that has been occluded, or partially or completely calcified. Sometimes the blood vessel is not replaced, and the bypass is performed to guarantee blood flow through two points of the circulatory system. For the bypasses, native tissues could be used as graft material; they are known as autografts when they are obtained from the same patient, or as homografts when the tissue comes from a donor. Xenografts, i.e. grafts made out of animal tissue, are less used due to some problems of biocompatibility. Because of the lack of availability of native graft tissues, synthetic grafts are broadly used. Dacron is the most popular clinically applied graft type, but also Gore-Tex is used to perform bypasses in smaller arterial vessels. Although these synthetic grafts temporally solve the problem of occlusion, on the long-term it has been found that the mismatch in compliance between the synthetic grafts and the native blood vessels still leads to complications that require periodic interventions and graft change.

In the literature, several clinical evaluations have been done comparing the compliance and the patency rate, i.e. percentage of section of the vessel opened, of native tissue and synthetic grafts [56, 57, 58]. However, those studies only compare the clinical patency and compliance of synthetic grafts and native blood vessels, by in vivo measurements or by using computational modeling, but they do not accurately characterize the properties of the grafts, neither mechanical nor structural. Most of the studies in which the mechanical properties of synthetic grafts and native blood vessels are assessed, consist of performing experiments to acquire data that can be introduced in a computational or analytical model to analyse their stress response or their fluid-dynamic behaviour. These experiments, however, often do not recreate the physiological conditions appropriately, e.g. they assume linear or isotropic behaviour of the vessel/graft. Moreover, the comparison of the properties of the grafts with the native tissue is often not consistent as they use different test set-ups to obtain the mechanical properties.

In terms of internal structure, although microscopic images of the native tissues and the different graft materials have been presented in the literature, no 3D characterization of these materials has been performed. This information, however, could be crucial to better understand the behaviour of the synthetic graft materials in all directions, and the differences compared to the native tissue in terms of internal architecture.

Therefore, the general aim of this study is to define a cluster of experiments to characterise both the mechanical and structural properties of native tissues and synthetic grafts, and to consistently compare these results. Homografts will be taken as reference materials and they will be compared to the currently clinically applied synthetic grafts in order to evaluate the weaknesses and strengths of the grafts. Based on this, possible reasons for the unsatisfactory behaviour of the synthetic grafts will be defined, which would then serve as input for the design and manufacturing of optimized synthetic graft materials.

The general aim of this work can thus be translated in two main objectives.

1. Characterization of the mechanical properties and 3D internal structure of both native tissue and different clinically applied synthetic grafts, under the same testing conditions, reproducing the physiological environment.
2. Deciphering the mismatch between the structural and mechanical properties of native tissue and synthetic grafts and providing recommendations for design and manufacturing of graft materials with improved (biomimetic) structural and mechanical properties.

4 STRUCTURAL CHARACTERISATION

For the structural characterisation, microfocus X-ray computed tomography (microCT) has been used. Computer tomography is a non-destructive inspection technique based on differences in absorption/attenuation of X-Rays through the material. This attenuation depends on the atomic number, density and thickness of the material and on the energy of the X-Ray beam. This technique has been used to analyse the morphometric properties of the different synthetic grafts (porosity, specific surface area, composition, etc.) and to visualise the microstructure of the native tissue.

4.1 Materials and methods

4.1.1 Synthetic grafts and native tissue

Both native tissue and synthetic vascular grafts have been assessed. The native tissue corresponds to a femoral artery of 4.5 mm of diameter and 5 cm of length (homograft from the tissue bank, kindly provided by Prof. Valérie Lacroix, St. Luc, Woluwe, UCLouvain). Among the synthetic grafts, three different types of polymer-based bypasses have been used: Dacron, Gore-Tex and Vectra. These materials have been already presented in the literature overview. As it was pointed out, Dacron is based on PET, Gore-Tex on ePTFE and Vectra is a composed polyurethane which is constituted by three-layered polyetherurethaneurea.

While Dacron and Gore-Tex are currently clinically used as a bypass conduit, Vectra is used to perform arteriovenous fistulas in dialysis access, meaning clinical purification of blood as substitute for the normal function of the kidney. Even if the purpose of this last material differs from the main application that was intended to study here, i.e. repairing artery blockage, its mechanical behavior could provide insights for the design of a new material that replicates more accurately the behavior of an artery.

4.1.2 Sample preparation

The femoral artery has been stored at -80°C during a month. Then, it was gently thawed in a 4°C refrigerator and then stained using a Hafnium-substituted Wells Dawson polyoxometalate (Hf-POM) solution (35 mg/ml PBS) for 20 days.

The Dacron, Gore-Tex and Vectra samples with an initial length of 40 cm were cut in sections of 7 cm length and then opened longitudinally to obtain 6 samples cut in squares of 1x1 cm. The upper left corner of the sample, observing from the luminal side, were also cut to indicate its orientation.

For Dacron and Gore-Tex, full samples of 6 mm of diameter and 2 cm of length, for the first one, and 3 mm of diameter and 2 cm of length, for the second, were also scanned (cylindrical shape), so no specific preparation was done. These samples, however, were already manipulated, but they provide a global view of the structural properties in its cylindrical shape.

4.1.3 Micro-CT image acquisition

All samples were imaged using a Phoenix NanoTom M (GE Measurement and Control Solutions, Germany). A diamond-coated tungsten target was applied. The system was operated at a voltage of 50 kV and a current of 250 μ A. The exposure time was 500 ms, and 2400 images were acquired over 360° using the fast scan mode (frame averaging = 1; image skip = 0). These settings allowed limitation of the scanning time to 20 minutes. The synthetic grafts were examined at 3

μm isotropic voxel size, while the homograft was scanned at 4 μm voxel size. During reconstruction (Datos|x, GE Measurement and Control Solutions), a beam hardening correction of 7 and a Gaussian filter of 3 was applied.

4.1.4 MicroCT image analysis

In the frame of this study, the software used were CT-An, DataViewer and CTvox (Bruker microCT, Kontich, Belgium). 3D visual inspection, as well as measurements of the graft thickness, were performed with the software DataViewer. This program displays reconstructed results as slice-by-slice orthogonal projections. With CTAn, the main morphometric parameters of the materials were calculated (Table 6). 3D rendering of the datasets was obtained thanks to CTvox software.

Table 6: Brief description of the morphological parameters assessed in this study, determined via image analysis of the micro-CT images

Parameter	Description
Specific surface ($1/\mu\text{m}$)	Object surface divided by the object volume within the volume of interest (VOI).
Volume fraction (%)	Object volume divided by the total volume within VOI.
Structure thickness (μm)	Average object thickness measured as the mean diameter of consecutive spheres that fill the structure.
Structure separation (μm)	Average pore thickness measured as the mean diameter of consecutive spheres that fill the pore space.
Total porosity (%)	Volume of all open plus closed pores as a percent of the total VOI volume. Total porosity (%) = $100 - \sum_{i=0}^n \text{volume fraction}_i$ (%), being i every different structure composing the graft.

Qualitative inspection of the samples was performed using DataViewer and CTvox. These programs provide valuable information about the orientation, distribution and spacing between the fibres, which are indicators of the anisotropy. In CTvox, 3D rendering is performed, so the different materials composing the whole graft could be visualized in 3D. For the morphological analysis with CTAn, the following image analysis protocol was used:

1. Draw a VOI around the graft, eliminating the sample holder.
2. Image filtering (Gaussian filter) to non-binarised image for obtaining a smoother image, with less noise.
3. Obtain the full-graft binarized image, based on manual global thresholding.
4. Apply operations to fill the space occupied by the full graft (despeckling and closing).
5. 3D image analysis to obtain fundamental parameters (volume and specific surface area of the graft).
6. Make this filled-up graft the new VOI.
7. Reload the grey-scale images.
8. Use manual global segmentation for the material of interest (wire and sheet for Vectra or foam for Vectra, Gore-Tex and Dacron).

9. Apply operations to take into account only the material being studied (despeckling and closing).
10. 3D image analysis of the studied material (volume of the material of interest, structure and pore thickness distribution).
11. Repeat steps 6-9 for every material of interest in the graft.

4.1.5 Statistical analysis

For statistical comparison of the morphological parameters of the different synthetic grafts, a one-way analysis of variance (ANOVA) was used. The value of significance or p-value selected as a reference for the analysis was 0.05. The statistical software used to perform the analysis was GraphPad (Prism 8.1.1, California, United States). For all graphs, the notations in Table 7 are used to indicate the level of significance.

Table 7: Notation to indicate the level of significance

ns	P>0.05
*	P≤0.05
**	P≤0.01
***	P≤0.001
****	P≤0.0001

4.2 Results and discussion

4.2.1 Visual inspection

In this section, both three orthogonal views and 3D images will be presented and discussed for each material.

4.2.1.1 Native tissue

The structural components of a femoral artery are 68% H₂O, 44.5% collagen, 24.5% elastin and 1.89 Collagen/Elastin ratio [80]. It is composed of three main layers: intima, media and adventitia. These three layers can be clearly observed in Figure 25. Figure 25 A shows a 3D view of the stained femoral artery homograft, while the Figure 25 B presents the three orthogonal views of such tissue.

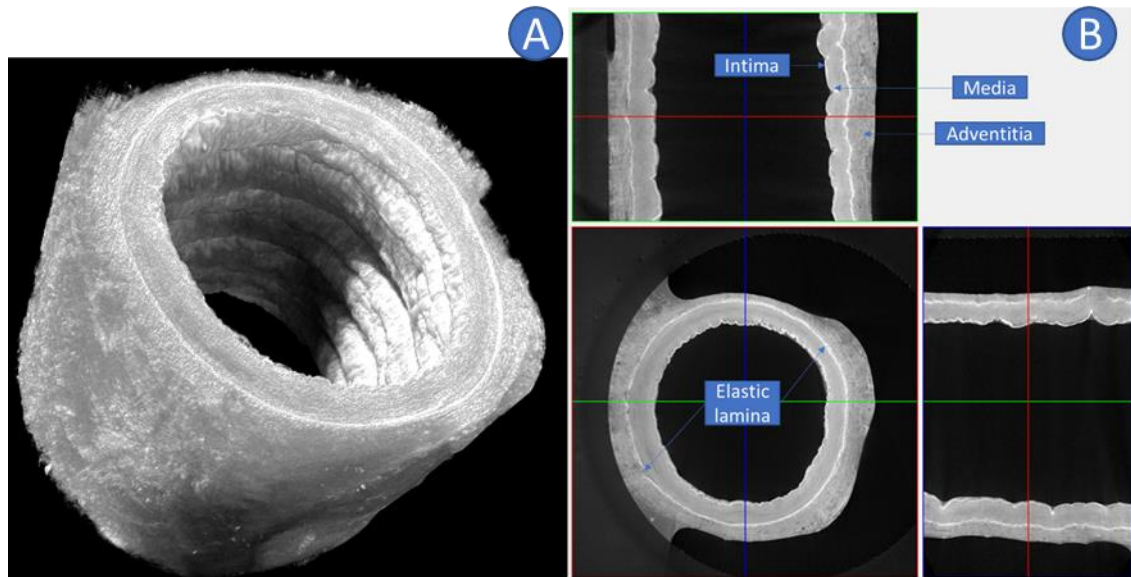


Figure 25: A: 3D view of 6mm diameter 5 cm length homograft. B: three orthogonal views of the homograft cross-section indicating the different layers, from inside to outside intima, media, elastic lamina and adventitia

The three layers are separated by an external elastic lamina, which can be distinguished as a bright line in the images. Figure 26 represents the histogram of one image of the femoral artery scanned dataset. The different exposure peaks represent different materials. The radiation absorbed for each material is related with its density so the denser the material, the less x-rays will go through it. That means that less x-ray fraction will arrive to the detector reporting a lower exposure in the histogram. As the density of elastin and collagen, main components of the artery, is similar, the exposure level for each of them will hold similar values.

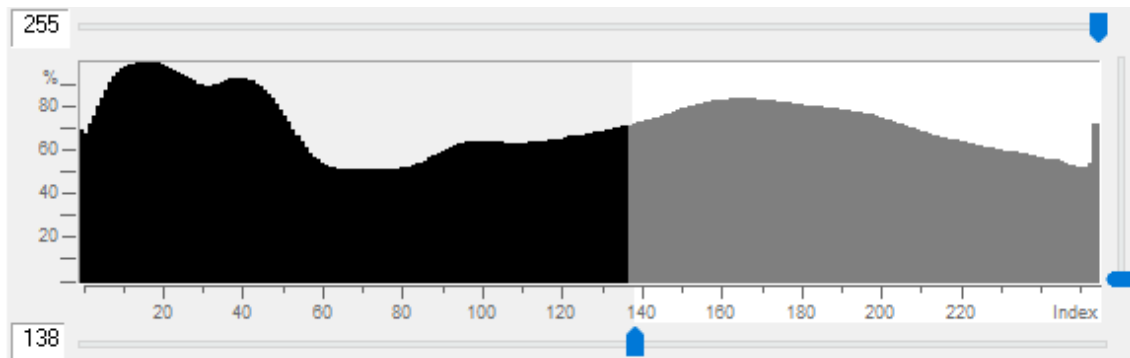


Figure 26: Femoral artery histogram. The x-axis represents the amount of exposure, and the y-axis represents the incidence of pixels for each exposure level

The inner layer, the tunica intima, is very hard to distinguish in the images. One reason for this is that this layer is very thin (only some tens of microns thick), and this approaches the spatial image resolution of the microCT images. The intermediate layer is the tunica media and it is composed of smooth muscle cells. It provides the contraction force to impulse the bloodstream through the body. In the 3D view, the wavy surface of this layer (together with the intima) can be observed. This waviness favours the longitudinal resistance of the blood vessels. In the intermediate layer, the distribution of the fibres can be observed adopting concentric circumferences along the surface. Finally, the outer layer, the adventitia, is also distinguished.

It should be mentioned that, probably because of starting degradation of the tissue, irregularities of the surface were noticed. In this layer, the main component is collagen, but also elastin is present. Due to the similar density of these two components they cannot be easily distinguished. However, the disordered distribution of the fibres in the outer layer can be noticed. These will be aligned after applying mechanical stresses to reinforce the tissue in the direction where the stress is applied.

4.2.1.2 Dacron

Dacron is based on a knitted ultrathin polyester and coated with a highly purified form of cross-linked bovine collagen to minimise intraoperative bleeding and to eliminate the need for preclotting at the time of the implant. This treatment confers the material a water permeability $< 5 \text{ ml/cm}^2/\text{min}$ at 120 mmHg. Figure 27 shows a 3D visualisation of one of the 1x1 cm prepared samples and the three orthogonal views of this one, respectively. In both images, the waviness, present to provide elasticity in the longitudinal direction, can be observed. This waviness resembles well the native structure (see Figure 25 A). In the Figure 27 A, it can be observed that the fibres are arranged forming longitudinal braids. This configuration will provide, along with the wavy structure, a high resistance and elasticity in this direction.

In Figure 27 B, it can be observed in the above view that the fibers form two layers, and in each layer they are sticking together forming clumps. The outside layer is thicker than the inside layer. In the first one, the clumps are larger and a higher porosity can be noticed, while the inside layer is more dense and with clumps of smaller size. This distribution is justified for preventing the blood leakage to the outside and tissue ingrowth around the external surface.

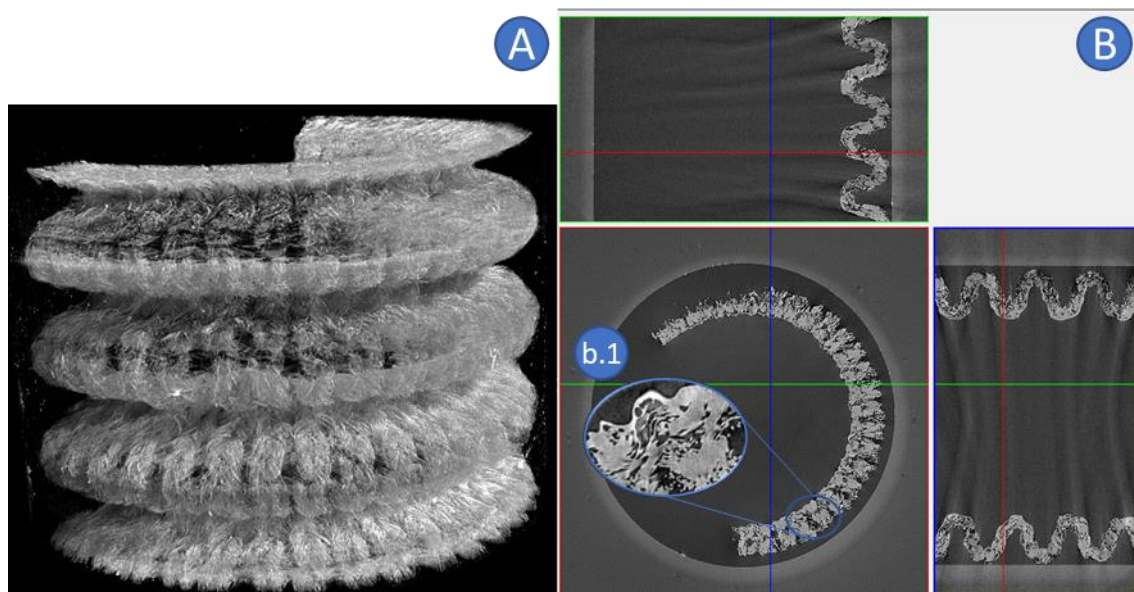


Figure 27: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Dacron graft. B: three orthogonal views of the Dacron sample cross-section. In b.1 a detail of the fibres is presented

These images were taken from a sample in as-produced state; they were not prewetted, nor dried. A sample that was prewetted and dried was also scanned (Figure 28). In these images, the agglutination of fibres is not as dense as in Figure 27, and just one layer is observed. Additionally, individual fibres can be distinguished. This can be an effect of the dissolution of the collagen coating. When the Dacron fibres were wetted the collagen provoke the agglutination, but after

drying the collagen remains in the water and the fibres, without the coating, present higher separation between each other.

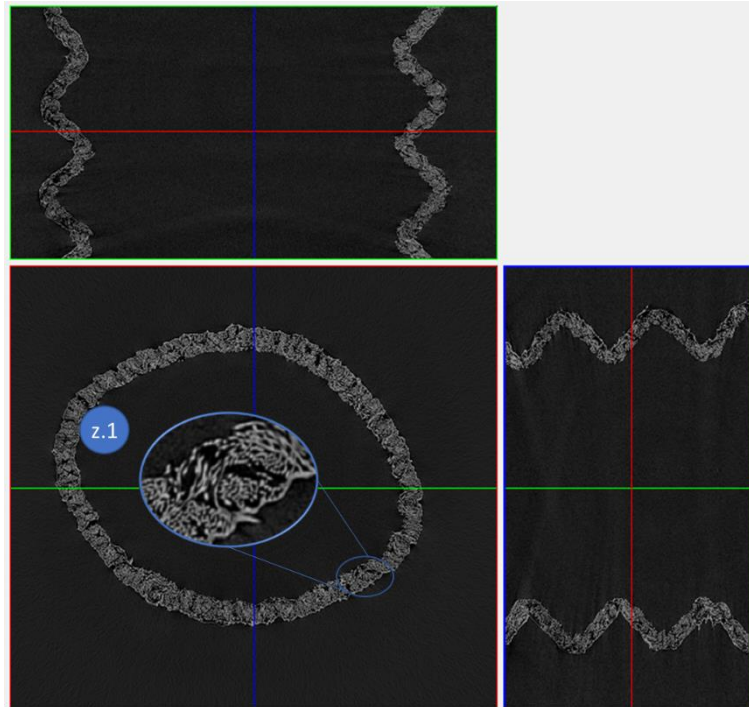


Figure 28: Three orthogonal views of a 6mm diameter and 2 cm length Dacron graft. In z.1 a zoomed image of the graft is presented wall in which the dispersion between the fibres can be observed

4.2.1.3 Gore-Tex

Gore-Tex is composed of reinforced expanded PTFE and a luminal surface bonded with fractionated heparin of porcine origin. This layer is conceived to avoid clot adhesion and ensure impermeability. However, it is not an alternative to intraoperative or postoperative anticoagulation regimens. The microstructure of Gore-Tex can be observed in Figure 29. For Gore-Tex, the structure seems to be denser than for Dacron. The structure is based on fine fibrils connected with nodes. This could confer the material a higher stiffness than for Dacron, but a lower elasticity. In Figure 29 B, it can be observed that the fibrils present a tendency of radial orientation, which is justified for the blood pressure that it has to stand applied in this direction. Examining the 3D view (Figure 29 A), these fibrils appear to form longitudinal yarns that will favour the mechanical resistance in such direction, though the transversal strength will be lower since in this direction the privileged orientation is not recorded.

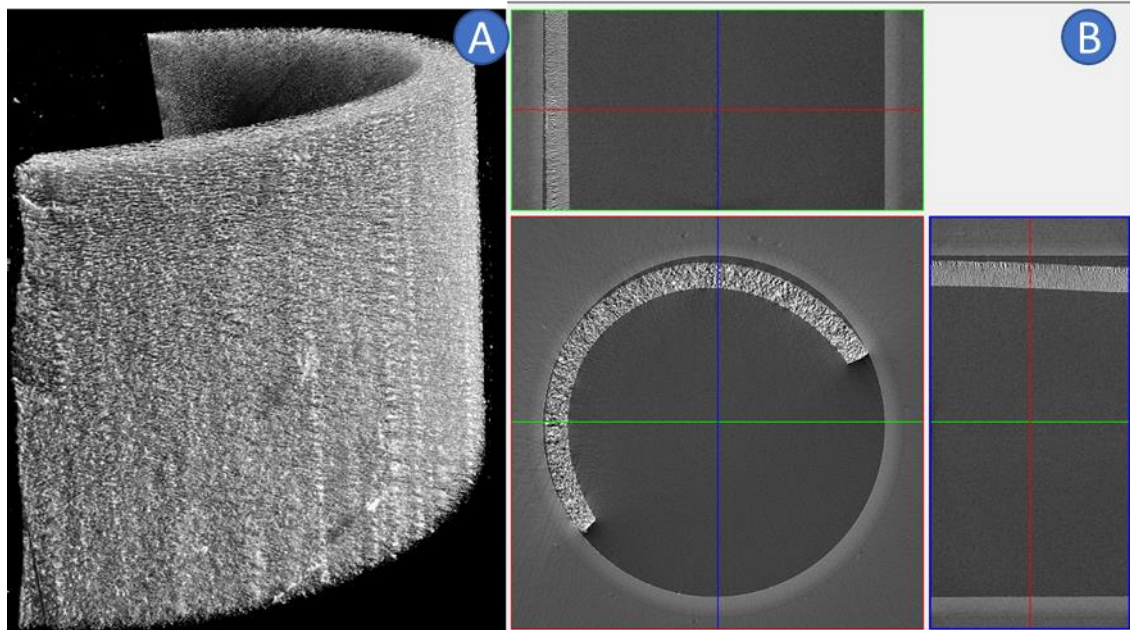


Figure 29: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Gore-Tex graft. B: three orthogonal views of the Gore-Tex sample cross-section

4.2.1.4 Vectra

Vectra is a proprietary blend of segmented polyetherurethaneure and a siloxane containing surface-modifying additive (SMA). The 3D representation of a typical sample and the three orthogonal views are presented in Figure 30. Three self-bonded microporous layers can be distinguished: an inner layer (foam), a self-sealing middle layer (sheet) and an outer layer (foam). In addition, inside this last layer, a wire coiling the surface is noticed. The sheet and the wire are presented in orange in Figure 30 A, and the foams in grey.

The inner layer is the one containing an SMA rich environment, practically indistinguishable in the images. This component is designed to minimise the platelet adhesion and reduce the risk of occlusion. The middle layer prevents any communicating pathway between the inner and the outer layer. This component offers to the graft its strength, flexibility and self-sealing properties. The microporous outer layer is designed to promote tissue ingrowth for enhanced graft anchoring, and thus has a relatively high porosity. Indeed, the pores for the foam structure in Vectra seem to be larger than for Dacron and Gore-Tex. In Figure 30 B, it can be observed that the outer foam layer consists of concentric layers of foam along the surface. This distribution leads to think that the reaction forces to the same elongation applied both in longitudinal and transversal directions would be similar if the wire inside the structure would not exist.

The aim of the wire is to reinforce the microporous structure for kink resistance with monofilament polyester fibres. 4-5 wraps/cm have been counted in the studied samples, and each reinforcement monofilament is wound circumferentially in graduated concentrations along the length of the graft. This structure not only will confer radial and longitudinal resistance, but also a higher flexibility in this last direction.

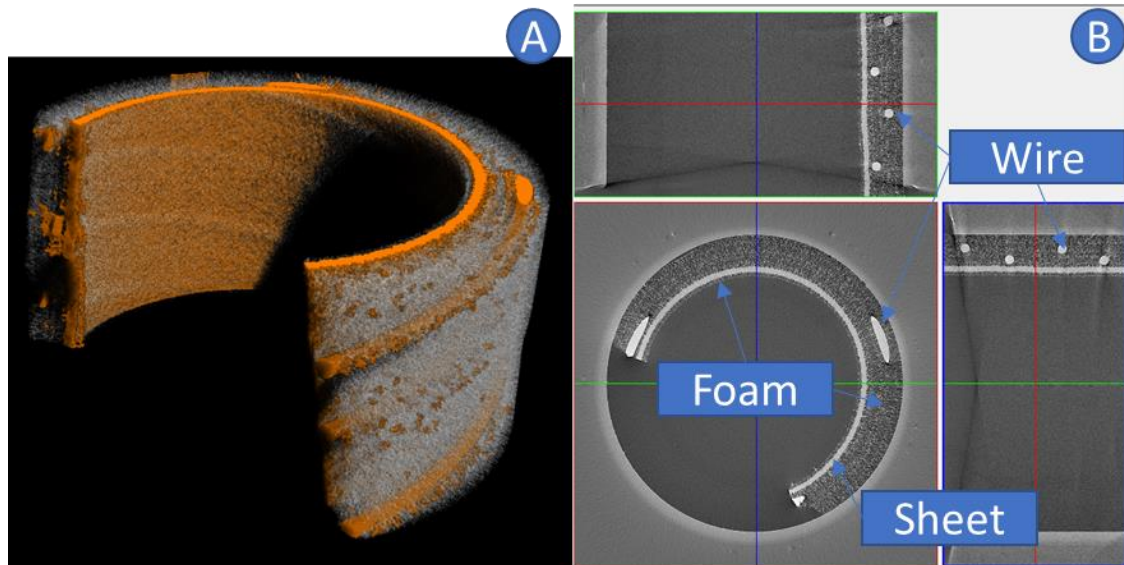


Figure 30: A: 3D view of a 1x1 cm sample extracted from a 6 mm diameter Vectra graft. In orange the wire and the self-sealing middle layer are presented and the foamy structure in grey. B: Three orthogonal views of the Vectra sample cross-section indicating the different layers and structures composing the graft

4.2.2 3D morphological analysis

4.2.2.1 Full graft

4.2.2.1.1 Specific surface area

The full graft specific surface area was calculated as the object surface divided by the object volume. This property is of a high interest, since the specific surface area is an indicator of the surface available for start the endothelial lining, that is to say, the available surface for the endothelial cells to start fixing to the synthetic graft structure. As it was mentioned in the state-of-the-art, a proper endothelial cell ingrowth might avoid complications such as thrombogenicity.

Figure 31 presents the average and standard deviation of the specific surface for each synthetic graft. It can be seen that the tendency of the endothelial cells to fix to the structure will be higher in Dacron followed by Gore-Tex and by Vectra.

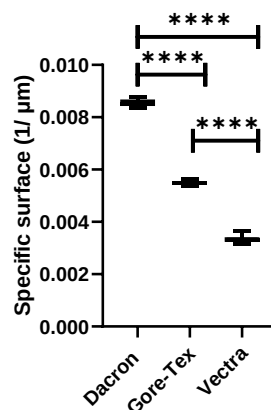


Figure 31: Box plot of the specific surface (1/μm) for Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p < 0.0001$)

4.2.2.1.2 Overall porosity

The relevance of the porosity in a synthetic graft has been already presented. It has a high influence on properties such as the arteriogenesis and low-term patency because it determines the endothelial cells ingrowth. Due to the complex structure of the arterial wall, its study has been based in many times on the use of computational models. To gather the data necessary to compare the native tissue porosity with the results obtained for the synthetic grafts, some results of different models have been used: a constant porosity over the arterial wall of 43% and an average permeability of $5 \cdot 10^{-17} \text{ m}^2$ [85].

Figure 32 presents the average and standard deviation of the porosity in % for the three synthetic grafts. It can be observed that Vectra reports a significantly higher porosity than Dacron and Gore-Tex. From the visual inspection, the high separation between the structure in the foam of Vectra graft has been already noticed, so as the higher volume covered by this component from the full graft volume. Hence, it can be claimed that this component has the highest influence in the high porosity reported. However, hereafter a detailed morphological study of the foam will be performed to obtain more insight about that particular structure. Also, Dacron and Gore-Tex show a statistical difference in porosity, with Gore-Tex being the least porous graft material.

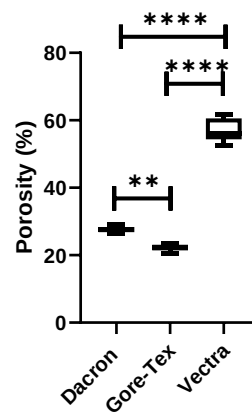


Figure 32: Box plot of the porosity (%) for Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (** $p < 0.01$, **** $p < 0.0001$)

4.2.2.2 Foam

4.2.2.2.1 Volume fraction

Several attempts have been made to model and analyse the mechanical behaviour of foams. Some of the more popular work was carried out by Gibson and Ashby [84]. They accepted bending as the essential structural deformation mechanism and used dimensional analysis to obtain the relation between the Young modulus (E_f) and the volume fraction of the foam (V_f). They stated that $E_f \propto V_f^2$, which means that the stiffness of the foam is strongly dependent on the volume fraction of the foam.

Figure 33 shows the average and standard deviation of the foam volume fraction for the three synthetic grafts studied. It should be mentioned that, whilst Dacron and Gore-Tex are completely composed of the foam, Vectra combines the foam structure with a self-sealing layer and a wire coiling the surface, both last two embedded in the foam. This remark is important because, despite the lower foam volume fraction of the Vectra foam structure, the stiffness might be

higher compared with the other materials because of the presence of the other structures. The volume fraction for each graft was significant different.

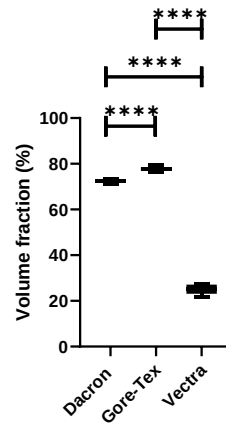


Figure 33: Box plot of the volume fraction (%) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p < 0.0001$)

4.2.2.2.2 Average structure thickness

The average structure thickness will be understood as the mean thickness of the material present between the pores of the foam. This parameter must be studied together with the pore thickness and the volume fraction to have an accurate characterisation of the foam. A structure with a high structure thickness and small pore thickness will be stiffer than one that presents a higher structure thickness but also higher pore thickness. So, conclusions about the expected mechanical properties can be settled once both parameters have been studied. The average and standard deviation from all the samples analysed for each material is presented in Figure 34. The average structure thickness for all the graft types was significant different between them.

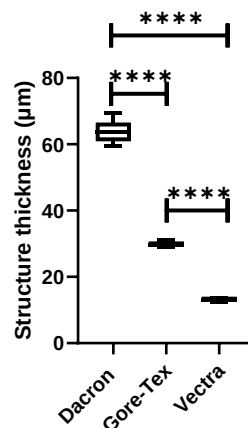


Figure 34: Box plot of the structure thickness (μm) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p < 0.0001$)

4.2.2.2.3 Average structure separation

The average structure separation represents the mean pore thickness or the open space between the structure. Its size determines the endothelial cells ingrowth and it also has an influence in the foreign-body macrophages reaction. The average and standard deviation of all the samples studied for each material is presented in Figure 35. Polyurethanes with a small pore thickness, around 15 μm , will present a little capillarity ingrowth while with higher values, around 157 μm , the foreign-body macrophages reaction is avoided. Vectra shows a value closer to the smallest value, providing a moderate cell ingrowth. Gore-Tex presents the lowest value while Dacron, even though it presents the highest structure thickness, also presents the highest pore thickness.

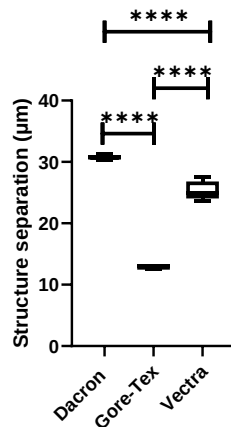


Figure 35: Box plot of the structure separation (μm) of the foam of Dacron, Gore-Tex and Vectra. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively (**** $p < 0.0001$)

To conclude the morphological analysis, a schematic representation of each graft material is presented in Figure 36. It shows the appearance of the foamy structure of each material, observing that the one that is expected to be stiffer is Gore-Tex since, there, a compromise between structure and pore thickness is reached.

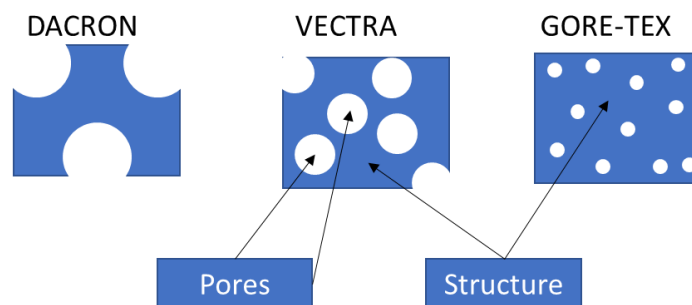


Figure 36: Schematic of the foam structure of the materials aim of study. In blue the structure is represented while the pores are presented in white

5 MECHANICAL CHARACTERISATION

To recreate the physiological conditions that a native artery would experience as accurate as possible, a mechanical test in which both sample environment and loading regime are adequate must be chosen. In this study, planar biaxial displacement testing was chosen. The testing device was specially designed for testing biological tissues and biomaterials.

5.1 Materials and methods

5.1.1 Synthetic grafts and native tissue

For the synthetic grafts, the same samples, prepared in a square shape, as the ones used for the structural analysis were used (see 4.1.1). For the human native tissue, only limited material was available. From one homograft (preserved at -80°C), three small pieces were cut with the same dimensions as the synthetic grafts (squares of $1\times 1\text{ cm}$). This homograft was a different one than the one used in the structural characterisation, because the staining solution might modify the mechanical properties of the material.

5.1.2 Sample preparation

The particularity of the femoral artery homograft used for mechanical testing is that due to operational complications, the sample was out of the freezer since extraction for 2 days. Then, it was frozen at -80°C for 2 weeks. These circumstances should be considered when analyzing the results obtained. Subsequently, the sample was thawed gently in a refrigerator at 4°C . Once it was thawed, it was cut longitudinally and then in squares of $1\times 1\text{ cm}$ obtaining 3 subsamples.

The synthetic grafts followed the preparation explained in the structural characterization section and were scanned in that shape before mechanical testing. There were 6 samples of each synthetic material (Dacron, Gore-Tex and Vectra) cut in $1\times 1\text{ cm}$ squares. Each of these samples were sunk into physiological water ($\text{NaCl } 0,9\%$) before and during mechanical testing to recreate the physiological conditions.

5.1.2.1 Thickness measurement

The thickness of the samples was measured following two different procedures. The first one, was thanks to the DataViewer software that allows direct measurement over the cross-section of the microCT images. Since the samples vary their thickness along the same image, the mean of six consecutive measurements was performed.

The second one consisted of placing the sample between two metal plates of known thickness. This allowed exerting the same pressure on every sample, but the bending tendency of the sample to return to its cylindrical shape (i.e. residual stresses) avoided both plates to be parallels. A picture was taken so through image analysis the thickness of the sample could be determined. An example of a picture is shown in Figure 37. The image analysis is performed in Matlab (Matlab R2018b, MathWorks Inc., USA).

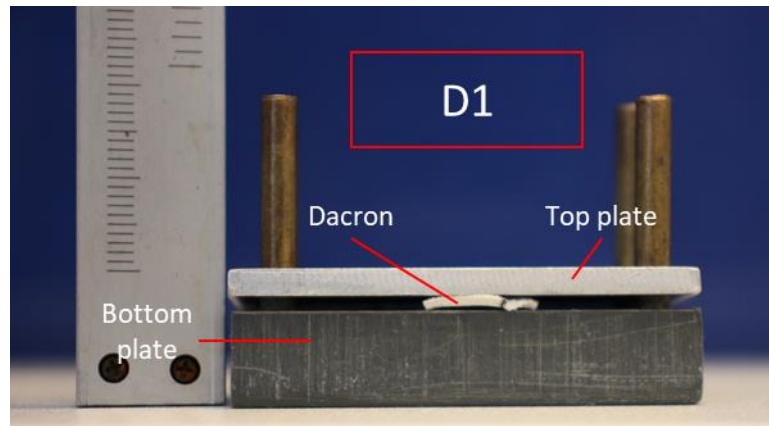


Figure 37: A Dacron sample between two plates of known thickness

5.1.2.2 Speckles for strain analysis

Graphite powder was applied with a wetted (physiological water) brush to distribute speckles on the luminal side of the graft, since mechanical loads are higher in this side under physiological conditions. It is important to uniformly distribute de graphite on the sample to obtain an accurate strain analysis after image processing. In Figure 38, a sample of Dacron mounted in the testing device can be observed. The shiny spots in the centre of the sample corresponds to the graphite speckles.

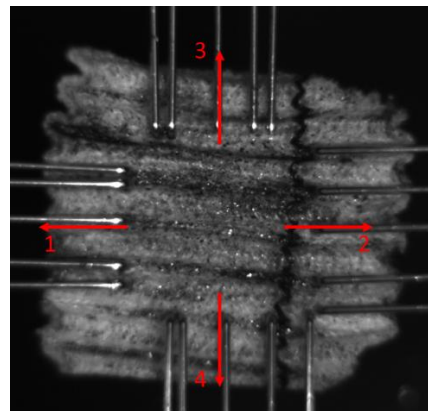


Figure 38: Dacron sample mounted in the biaxial tester with graphite speckles applied. Numbers 1,2,3 and 4 are the notation for the different loading cells. 1 and 2 will be considered x axis, the equivalent circumferential direction in the graft, and 3 and 4 y axis, the longitudinal direction

5.1.3 Planar biaxial tester

All the samples were tested in a biaxial testing machine specifically developed for mechanical testing of natural and artificial elastic tissues (Messphysik-Zwick/Roell, Fürstenfeld Austria). The samples were expected to be square shaped with an optimal size of 1x1 cm approximately.

5.1.3.1 Hardware

The planar biaxial tester imposes force or displacement along two perpendicular directions, using four independent actuators and load cells. Figure 39 shows the biaxial tester set-up. The clamping system used in this project was rakes to ensure the integrity of the sample. Each rake is composed by 5 high-quality stainless-steel hooks of 0.3 mm of diameter, 3 mm of depth and

1.25 mm space between each rake. Rake-based systems transfer a limited amount of lateral forces onto the sample and since the shear deformation is low at the load range selected it was the best option of clamping system for the material tested in this study. Also, they are easier to mount in a more equidistant distribution and to align with the materials axes. Although the applied load is still discontinuous, using rakes results in a more uniform load distribution. For better replicating physiological conditions the samples were tested completely sunk in a bath of physiological water (0.9% NaCl) at a room temperature of 20°C.

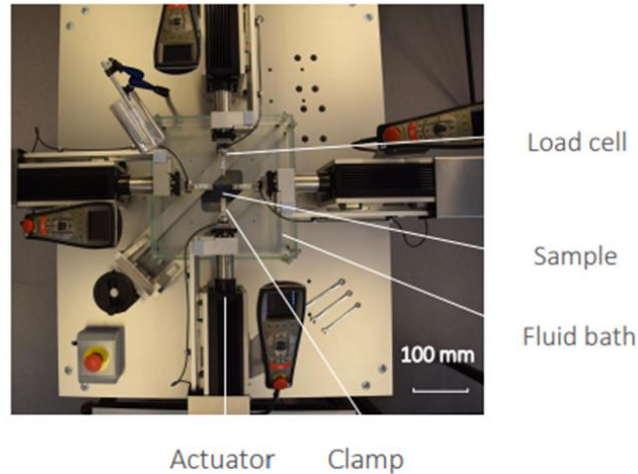


Figure 39: Biaxial tester set-up

The testing machine possesses four high-resolution linear drives (travel 69 mm) which can be position or force-controlled completely independently of each other. For the current study, they were controlled using strain ranges due to the lower distortion of the measures, tracked from previous studies, that this technique implies. Force measurement is via four load cells (two load cells each in the X- and Y-directions) with a load measuring range between 0.8 and 200 N with a relative accuracy of load signal of <0.5% of nominal load, below 0.8 N the accuracy of the load signal decreases to 1-2%.

The camera (Manta G-917 B, Allied Vision Technologies GmbH, Stadtroda, Germany) can be observed in Figure 40. It is an industrially used monochrome charged-coupled device (CCD) camera type that incorporates the high-quality Type 1 (15.972 mm diagonal) Sony ICX814 CCD sensor with EXview HAD II technology distinguished by excellent image quality and high resolution. At full resolution, this camera runs 10.1 frames per second. With a smaller region of interest, higher frame rates are possible.



Figure 40: Manta G-917 B camera, Allied Vision Technologies GmbH, Stadtroda, Germany

To conduct 2D-DIC analysis, one camera was placed above the mounted sample, perpendicular to it. The distance between the camera lens and the sample was approximately 30 cm. This led to a field of view (FOV) of 26.4mm by 21.14mm.

5.1.3.2 Software

The testXpert II operates the planar biaxial tester. The software allows the user to implement the experimental protocol and to select among displacement or force-control, preload, preload speed, initial position of the rakes or sampling rates. Also, the load level, the relative load ratio between the two perpendicular load directions and the testing speed are controlled through the program. The software generates, after each experiment, the reaction forces of the load cells and the displacement of the rakes from de reference.

The Vic-Snap 8 software enables the camera configuration. The user interface shows a view of live images of the mounted sample. Once the test is started in the testXpert software, the camera starts capturing images with a speed of around 20 images/s. The focus of the cameras is manually defined. Also, the aperture of the cameras and the light incidence must be selected so the image does not present overexposure spots.

5.1.4 Strain map calculation

5.1.4.1 Digital Image Correlation (DIC)

DIC software is an optical method to determine the displacements and strains of the sample through image correlation. To guarantee the goodness of the results, in this study, incremental correlation was used. Incremental correlation consists of comparing each image corresponding to a certain deformation state with the previously taken image state; this last one acting as a reference. The level of correlation between images depends on the uniformity and quantity of speckles applied and it should be enough to perform the calculations.

A schematic representation of the 2D-DIC principle is presented in Figure 41. When calculating the displacement of point P, a subset area is defined in the neighbourhood around that point. This step is necessary for better distinguishing the grey value of a pixel from other pixels. The matching between subsets is achieved, minimising a correlation function C [76]. The correlation function used in DIC analysis is based on the normalised squared difference and can be checked below [75]:

$$C = \sum_{i,j=-M/2}^{M/2} \left[\frac{f(x_i, y_i)}{\bar{f}} - \frac{g(x'_i, y'_i)}{\bar{g}} \right]^2, \quad (5.1)$$

$$\bar{f} = \sqrt{\sum_{i,j=-M/2}^{M/2} [f(x_i, y_i)]^2}, \quad (5.2)$$

$$\bar{g} = \sqrt{\sum_{i,j=-M/2}^{M/2} [g(x_i, y_i)]^2}, \quad (5.3)$$

with (x_i, y_i) and (x'_i, y'_i) as the pixel coordinates of a point Q in the reference image subset and point Q' in the deformed image subset subsequently taken, respectively. The reference image is indicated by f, the deformed image is indicated by g. M is the length of a subset side in pixels.

In general, the defined subset will not maintain its squared shape during the deformation. Hence, points in the subset have to be mapped by means of shape functions ξ and η , based on their coordinates in the reference subset (see Figure 41):

$$x'_i = x_i + \xi(x_i, y_i), \quad (5.4)$$

$$y'_i = y_i + \eta(x_i, y_i). \quad (5.5)$$

The shape functions are defined as follows, in order to be able to represent rotation, translation, shear and strain:

$$\xi(x_i, y_i) = u + u_x(x_i - x_0) + u_y(y_i - y_0), \quad (5.6)$$

$$\eta(x_i, y_i) = v + v_x(x_i - x_0) + v_y(y_i - y_0), \quad (5.7)$$

with x_0 and y_0 the coordinates of the subset centre in its reference state, u and v the translation components in the horizontal and vertical direction of point P , respectively and u_x, v_x, u_y, v_y the first-order displacement gradients in the horizontal and vertical direction of point P , respectively [75].

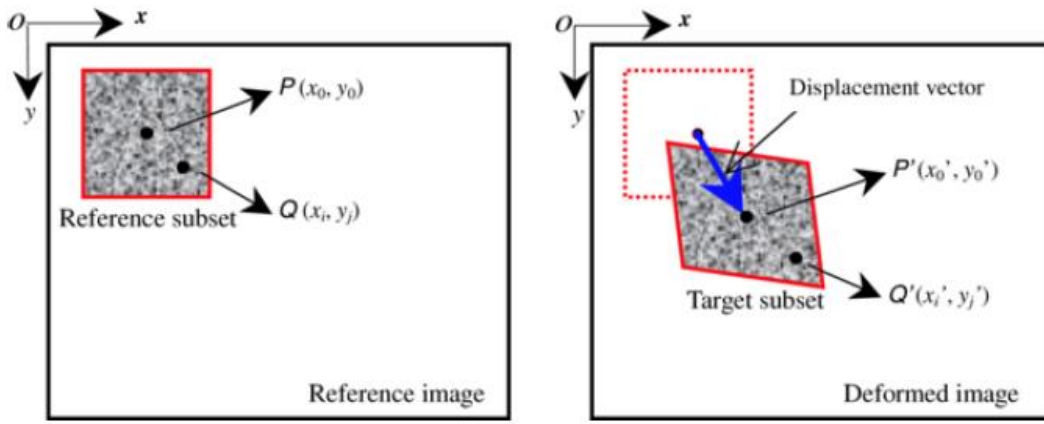


Figure 41: Schematic representation of the 2D-DIC principle. The left image presents the undeformed situation and, in the right, the deformed one. The red square indicates the subset size of point P [75]

A subset should contain the largest speckle region; however, a compromise must be found regarding the size of the area selected. A large subset provides a lower error, but a larger subset size comes with loss of spatial resolution [77]. When processing the results, a subset size is recommended regarding the σ value of the reference image, which is the uncertainty information of the results expressed in pixels and must be close to zero. A good subset size is between 20 and 80 pixels.

The matching algorithm will not be applied for every pixel of the image. In practice, the next pixel for which the strain will be defined is located at a certain distance from the previous pixel. This distance is defined by the step size. For the current analysis, the default value (7 pixels) was used, as suggested by the DIC software.

The matching algorithm generates a cloud of (x, y) -data, each with their corresponding displacement vector (u, v) . Based on this cloud of (x, y) -data and their respective displacement vectors, the strain is calculated. More insight about the mechanics of a continuum body will be presented in Appendix A.

The strain tensor selected is the Green-Lagrangian strain tensor E . It is based on the deformation gradient F and can be calculated as follows [85]:

$$E = \frac{1}{2} (F^T \cdot F - I), \quad (5.8)$$

where I is the identity matrix.

This measure is commonly used for materials undergoing large strains such as elastomers. Since the measurement is performed in 2D the terms of the tensor can be presented as:

$$e_{xx} = \frac{\partial u}{\partial x} + \frac{\left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial x}\right)^2}{2}, \quad (5.9)$$

$$e_{yy} = \frac{\partial v}{\partial y} + \frac{\left(\frac{\partial u}{\partial y}\right)^2 + \left(\frac{\partial v}{\partial y}\right)^2}{2}, \quad (5.10)$$

$$e_{xy} = \frac{\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} + \frac{\partial u \partial u}{\partial x \partial y} + \frac{\partial v \partial v}{\partial x \partial y}}{2}. \quad (5.11)$$

5.1.5 Stress calculation

The stress calculation was performed using a Matlab code created and kindly provided by dr. ir. Heleen Fehervary (Soft Tissue Biomechanics group, Biomechanics Section, Dept. of Mechanical Engineering, KU Leuven). Some adaptations of the code performed by Paulien Vandemaele (KU Leuven) were also extremely useful for this study.

The code calculates the experimental Cauchy and first Piola-Kirchhoff stresses and then plots the results for the loading cycle selected. It also allows comparing the experimental results with the ones obtained using a material model and optimises the data for such model. However, the material model parameter fitting was out of the scope of this project, and only the experimental stresses will be presented and discussed for every material mechanically tested.

The Cauchy stress S or true stress is defined for the material in deformed state as the reaction force (RF) tracked in the load cell (deformed configuration) scaled by the cross-sectional area. However, it is not accurate for large deformations or experimental measures. Therefore, other definitions are used, such as the first Piola-Kirchhoff P stress, which is defined in the reference configuration. In the program, these stresses are calculated as follows (and in Appendix A):

$$P = \frac{RF}{A_0}, \quad (5.12)$$

$$S = \frac{1}{J} \cdot P \cdot F^T, \quad (5.13)$$

where A_0 is the area in the reference state, J the jacobian, which is calculated as the determinant of the deformation gradient (F) matrix and RF the reaction forces, that will be calculated in longitudinal and circumferential directions as the mean of the forces applied in each direction.

Before this calculation was performed, some further operations were necessary to filter and prepare the data of interest. The steps were:

1. Prepare the excel exported from testXpert II selecting the loading cycle of interest.

2. Determine the offset of the camera and adjust the csv extracted from the DIC, so the delay between the moment in which the images were taken and the respective moment in the mechanical test are synchronised.
3. Sort images into loading step cycles.
4. Prepare data to extract the information from the VIC-2D output (strain maps).
5. Calculate stresses.
6. Plot reaction force and stress-stretch graphs.

5.1.6 Experimental protocol

The experimental protocol used in this study is displacement-controlled, meaning that the actuators of the biaxial tester stretch the sample until a predefined strain percentage. This percentage is calculated from the preload state position, giving the length increase. The sum of the displacement from the preload position in both sides of the sample in the same axis is equal to such length. The predefined strain can differ between x- and y-axis, changing the strain ratio between directions. The strain levels are based on the reaction forces reported from the load cells. These forces are based on the forces that are exerted on a femoral artery under physiological conditions.

The femoral artery is subjected to two types of stress under physiological conditions: a dynamic pressure due to the inflation-deflation and a shear stress due to the blood flow. The dynamic pressure is mainly directed in the circumferential direction whilst the shear stress in the longitudinal one. The circumferential stress is between three and four orders of magnitude larger than the shear stress [78]. Thus, the wall shear stress is neglected and the focus to create the experimental protocol will be the blood pressure.

Blood pressure decreases as the distance from the left ventricle increases. Systolic pressure is the maximum pressure achieved during ventricular contraction and diastolic pressure is the lowest pressure that remains in the arteries before the next ventricular contraction occurs. Figure 42 depicts the pressure levels for each vessel. The femoral artery, as one of the biggest vessels of the body, registers a systolic pressure of 100 mmHg and 70 mmHg of diastolic pressure, approximately.

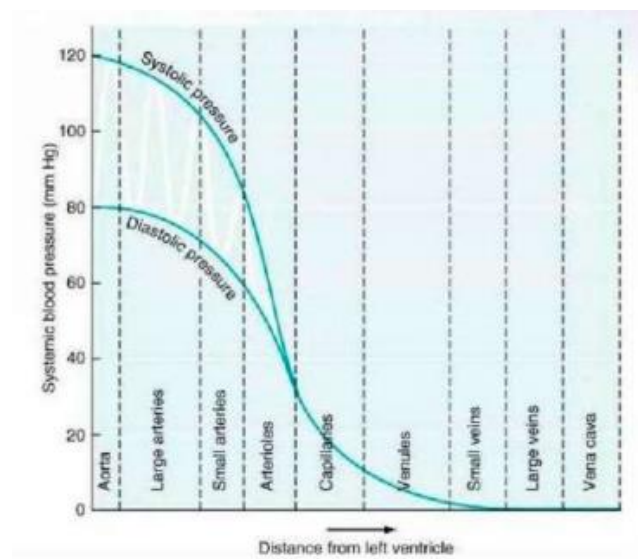


Figure 42: Systemic blood pressure (mmHg) registered in the main arteries concerning their distance from the left ventricle. Both systolic (maximum) and diastolic (minimum) pressures are presented [79]

With the knowledge of the radius r and the thickness t of the vessel, the circumferential stresses during both systole and diastole can be estimated via Laplace's law, valid for thin-walled tubes:

$$2LrP = 2tL\sigma_{circ} \rightarrow \sigma_{circ} = \frac{Pr}{t} \quad (5.14)$$

The femoral artery's wall thickness is $t=1$ mm and the radius $r=4.5$ mm, data obtained after experimental measurement in DataViewer. The maximal systolic pressure, P , as it was mentioned before, is 100 mmHg. So, the σ_{circ} of the femoral artery is:

$$\sigma_{circ} = \frac{100 \text{ mmHg} * 133.322 \frac{\text{Pa}}{\text{mmHg}} * 4.5 \text{ mm}}{1 \text{ mm}} = 60 \text{ kPa} \quad (5.15)$$

For calculating the force supported by the vessel, the concept of the stress as a force F distributed over a surface A is applied. In this case, the surface is equal to the product of the width W and the thickness t of the tissue. The force is normal to the surface and is calculated as follows:

$$\sigma = \frac{F}{A} \rightarrow \sigma = \frac{F}{Wt} \quad F = \sigma Wt \quad (5.16)$$

Solving the equation above, considering W the width of the sample 10 mm, it is obtained that the maximal physiological force stand by the femoral artery is 0.6 N. Different strain percentages have been applied on Dacron trial samples until this value is read in the load cell of the biaxial tester. With this strain ratio as a reference, the experimental protocol has been defined as described below.

5.1.6.1 Preload

As it was presented in the state of the art, when the artery has a long blockage the best option is to insert a bypass to carry the blood from an artery with good blood inflow to the affected artery below the block [2]. The connection between the synthetic graft and the blood vessel is called anastomosis. First, a proximal anastomosis is performed to fix the synthetic graft to the common femoral artery, when the synthetic graft is presented under blood pressure to the distal vessel. Then, a cut of the graft at the desired length to perform the end-to-side anastomosis is performed and both sides connected.

The synthetic graft does not suffer a high stretch during the presented operation. However, the blood pressure and the approximation to the distal blood vessel subject the synthetic graft to some load. Besides, applying a preload avoids sagging, meaning sinking downwards under the sample own weight due to lack of strength that keep it stretched. To avoid damage of the tissue and based on previous experiments, the preload was settled to 0.01 N at a deformation speed of 0.5 mm/s.

5.1.6.2 Testing protocol

All the materials tested in this study were subject to a displacement-controlled experimental protocol. As a result, they were stretched in x - and y -direction and the displacement was measured continuously.

After preloading the sample, several test sequences have been performed. Each test sequence was performed at a different strain level. For every strain level, three sets of five cycles were

conducted: the first four for sample preconditioning and the fifth one as the cycle of most interest on which the main discussion will be based. Each set was performed with a different strain ratio in the x- and y-directions: $\varepsilon_x/\varepsilon_y = 1, 0.5$ and 2 . Even if the strain levels were the same for every material, the reaction forces registered varied from one to another, depending on the resistance opposed by the material to the deformation.

Preliminary experiments on Dacron showed that the material records some damage for strains higher than 10%. For ensuring the behaviour under physiological conditions, the strain levels defined for synthetic materials were: 2.5%, 5% and 7.5%. These same levels were applied to native tissue, including a cycle of very high strain, 30%, to prove the forces that the femoral artery can endure. The testing speed was set to 2.5%/s, meaning that each second the sample registered a 2.5% of elongation starting from the preload position in both x- and y-direction. After each cycle, the sample was taken back to the preload position.

5.1.7 Statistical analysis

For statistical comparison of the maximum forces for the different synthetic grafts and the native tissue, a one-way analysis of variance (ANOVA) was used. To evaluate the difference between the different thickness measuring techniques and those forces in the different directions within each material, a two-way ANOVA was performed respectively.

5.2 Results and discussion

5.2.1 Thickness measurements

As described before, the thickness of each sample was measured via two different procedures, using Matlab and DataViewer. The average and standard deviation for each measuring procedure are presented in Table 8. For the homograft, it was only possible to present the results calculated with Matlab due to lack of microCT images of the mechanically tested samples. For performing the measure with Matlab, it was necessary to locate the sample between two plates and take a photo to finally processing it.

Table 8: Average and standard deviation of the thickness of Dacron, Gore-Tex, Vectra and Homografts samples measures with Matlab (ML) and DataViewer (DV) software

	Dacron		Gore-Tex		Vectra		Homograft	
Software	ML	DV	ML	DV	ML	DV	ML	DV
Average (mm)	0.98	0.65	1.14	0.50	1.06	0.99	1.00	-
Standard deviation (mm)	0.16	0.09	0.13	0.02	0.18	0.03	0.71	-

Significant differences were found for both measuring techniques (Figure 43). Two factors could influence these differences: the thickness and the residual stresses. The thicker the sample is, the closer the measures performed with each method will be. Also, the higher the residual stresses are, the more different the measures performed with each method will be. The materials ordered from the thickest to the thinnest are Vectra, Dacron and Gore-Tex, and through visual inspection of the inclination of the plate, the highest inclination to the lowest is presented in Vectra, Gore-Tex and Dacron. This last one has one further particularity, namely its waviness. Thus, the evaluation of the influence of such parameters is not evident.

Vectra does not present significant difference between each measure, so in this case the thickness of the material has a higher influence than the residual stresses. In Dacron, a significant difference between the measures was found, and it was hypothesised that, the waviness influences that result. It can be also observed that the variance in the measures performed in DataViewer for Dacron is the widest, again because of the waviness that constitutes a more irregular surface. Finally, Gore-Tex presents a significant difference between both methods due to the thin graft wall, but also the residual stresses observed in the material.

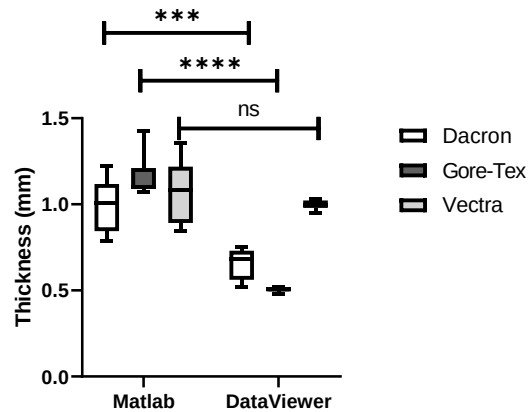


Figure 43: Box plot comparing the measure methods used to get the graft wall thickness (mm), Matlab and DataViewer. Vectra does not report a significant difference (ns) between each method, Dacron reflects a significant difference (***) $p < 0.001$, so does Gore-Tex (**** $p < 0.0001$)

5.2.2 Force and displacement

Following the protocol described in section 5.1.6, the three synthetic grafts and the homografts were tested with the biaxial tester machine. In what follows, the low strain protocol and high strain protocol will be presented. The low strain protocol was applied to all the samples, while the high strain protocol was applied only on the homografts, and this, immediately after the end of the low strain protocol.

From analysis of the force and displacement graphs, some interesting properties of the materials, such as the anisotropy, viscoelastic behaviour or damage, could be observed. Moreover, the maximum forces registered for each sample have been statistically compared.

5.2.2.1 Standard forces

5.2.2.1.1 Dacron

Figure 44 shows the forces for one biaxially tested Dacron sample. The read-out of the loading cells M1, M2, M3 and M4 follows the structure presented in Figure 38. Considering M1 and M2 as the x-axis, and M3 and M4 as the y-axis, it was found that the forces in x-direction are around 76% higher than in the y-direction. It has been considered that the reaction forces (RF) in each axis were the mean of the highest forces registered in the loading cells of this same axis, and assumed that the forces in each axis were not significant different: $RF_x = (F_{max1} + F_{max2})/2$ and $RF_y = (F_{max3} + F_{max4})/2$. These results confirm the anisotropic behaviour of Dacron, already introduced in the visual inspection. The waviness or folds observed in Dacron provide a higher elasticity in this direction, allowing a higher displacement for equal forces. However, in the x-direction the stiffness was higher, with forces above the physiological conditions (>0.6 N) from the 5% strain loading set.

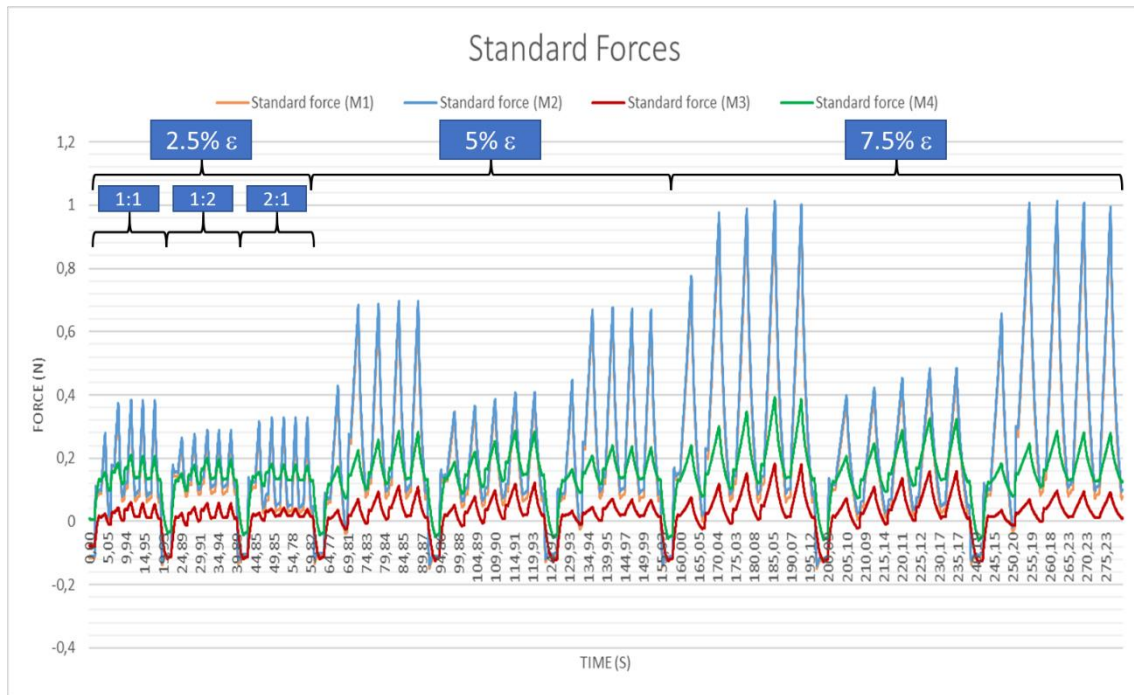


Figure 44: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Dacron (D1) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step

It can be also noticed that in the first cycle of each loading set, the force is lower. This behaviour might have two different explanations. The first one is due to the viscoelastic behaviour of the material, where the deformation does not only depend on the elasticity but also in the speed of deformation. The relaxing time that the sample needs to return to its resting state is higher than the time the machine waits to start a new cycle, so to arrive to the same preload level a higher force is necessary since the fibres are trying to return the resting state mentioned. This observation will be also present in the displacement graphs. The second possibility is that the sample has suffered damage, i.e. fibres re-orientation, etc. To evaluate the cause of this behaviour, tomographic images of the sample after mechanical testing must be analysed. Or, perform two consecutive loading sets at the same strain to see if the results are equivalent.

The difference forces recorded between M3 and M4, but even more evident between M1 and M2, could be caused by a different mounting conditions in each side of the sample or due to the inherent material properties. Proving which of these causes is the one that affects the response is not evident, because every sample preparation and mounting implies some asymmetry. However, the influence of the mounting can be evaluated with a uniaxial tensile test, to remove the influence of the forces in the perpendicular direction, in which the clamping system is carefully symmetrically set.

Table 9 presents the maximum standard forces from each loading cell of the biaxial testing set-up for the six Dacron samples. The average and the standard deviation for each force is presented determining the different range of forces registered in the x- and y-axis, respectively.

Table 9: Maximum force (N) recorded in each loading cell for each Dacron sample and average and standard deviation

	$F_{\max 1}$	$F_{\max 2}$	$F_{\max 3}$	$F_{\max 4}$
D1	0.98	1.01	0.18	0.39
D2	0.87	0.89	0.11	0.25
D3	0.83	0.88	0.13	0.23
D4	1.12	1.17	0.15	0.14
D5	1.34	1.36	0.66	0.99
D6	1.23	1.17	0.13	0.40
Average (N)	1.05	1.07	0.18	0.33
Standard deviation (N)	0.20	0.19	0.21	0.31

5.2.2.1.2 Gore-Tex

Figure 45 shows a typical force versus time graph for one Gore-Tex sample. It can be seen that Gore-Tex presents a higher anisotropy than Dacron. In the case of Gore-Tex, the forces registered in the x-direction, loading cells M1 and M2, are around 84% higher than in y-direction. As for the Dacron, the highest stiffness is recorded in the x-direction.

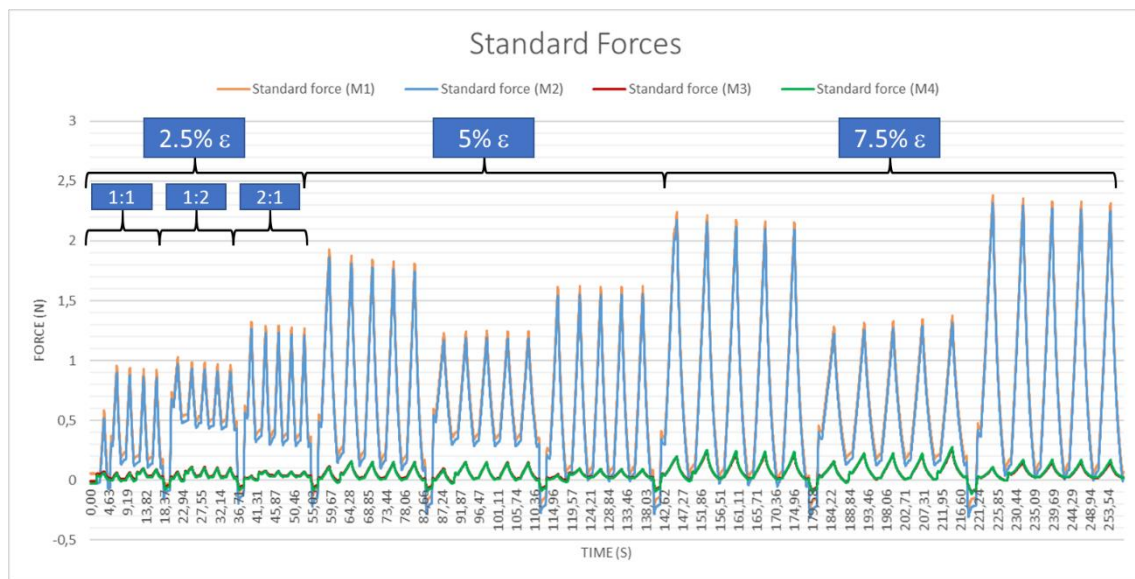


Figure 45: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Gore-Tex (G4) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step

While in the y-direction the forces resemble the values observed for Dacron in the x-direction these are much higher than Dacron's exceeding the physiological conditions even in the lowest strain loading steps. The denser structure observed in Gore-Tex might be the main reason of this increase of stiffness. Moreover, the force values recorded for each loading set rise to the same values in each cycle. This is an indication of the higher elasticity of Gore-Tex compared to Dacron, promoted by the node-fibril structure, the elastic behaviour is more marked than the viscous.

Table 10 presents the maximum force registered for every Gore-Tex sample as well as the average and standard deviation for each force. It is clear to assert the existence of a high anisotropy degree already noticed from the force-time graph.

Table 10: Maximum force (N) recorded in each loading cell for each Gore-Tex sample and average and standard deviation

	F_{max 1}	F_{max 2}	F_{max 3}	F_{max 4}
G1	2.69	2.56	0.72	0.97
G2	2.51	2.44	0.30	0.47
G3	3.00	3.03	0.39	0.59
G4	2.38	2.31	0.26	0.28
G5	2.71	2.62	0.31	0.42
G6	2.80	2.84	0.30	0.47
Average (N)	2.68	2.62	0.36	0.49
Standard deviation (N)	0.22	0.26	0.17	0.24

5.2.2.1.3 Vectra

It should be reminded that Vectra is currently used to create surgical arterio-venous fistulas, i.e. connections between an artery and a vein, to be used in dialysis for patients with a serious kidney disease. Despite its use is not the target of this study, the material it is made of, i.e. polyurethane, is of interest to be studied to verify whether this kind of material could be used for arterial bypasses or not.

Figure 46 shows the force-time graph for a Vectra sample. Again, in Vectra, as in Dacron and Gore-Tex, higher forces can be observed in x-direction compared with the ones registered in y-direction. However, the degree of anisotropy is much lower presenting M1 and M2 forces 28% higher than the ones recorded for M3 and M4 loading cells. That can be explained due to the more uniform structure of the Vectra foam but mainly because of the self-sealing layer, which provides the same stiffness in each direction. Nevertheless, the wire coiling inside Vectra's foam porosity introduces a higher elasticity in the y-direction, allowing deformation at lower forces. This elasticity can be observed in each cycle of a loading set, where the force applied is the same. The stiffness of the material is similar to the one observed in Dacron, without exceeding the physiological forces until the 5% strain loading step.

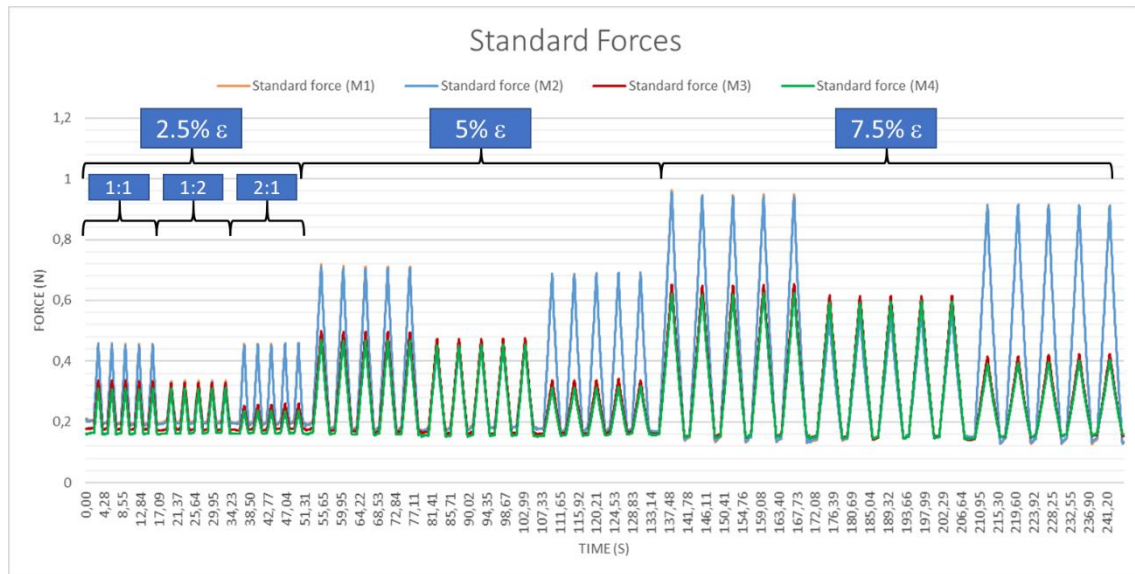


Figure 46: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Vectra (V14) sample. Low strain protocol was applied with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step

Table 11 presents the maximum forces registered for every loading cell for all the Vectra samples as well as the mean and standard deviation of every force. Similar values of maximum forces in the x-axis are recorded than in Dacron. However, in the y-direction the maximum forces are much higher than the ones registered in both Dacron and Gore-Tex. This higher stiffness registered in the y-direction can be both because of the wire and the self-sealing middle layer present in Vectra.

Table 11: Maximum force (N) recorded in each loading cell for each Vectra sample and average and standard deviation

	$F_{\max 1}$	$F_{\max 2}$	$F_{\max 3}$	$F_{\max 4}$
V12	1.45	1.21	1.04	1.03
V13	1.26	1.24	0.88	0.87
V14	0.96	0.95	0.65	0.62
V15	0.78	0.78	0.58	0.59
V16	1.12	1.11	0.72	0.74
V17	0.89	0.87	0.69	0.71
Average (N)	1.05	1.01	0.75	0.75
Standard deviation (N)	0.25	0.19	0.17	0.16

5.2.2.1.4 Homograft

The protocol applied to the homograft combines the low strain protocol with a high strain protocol, i.e. a loading step of 30% strain with the same 3 loading sets with ratios 1:1, 1:2 and 2:1. With the low strain protocol, the aim was to compare the obtained results with the ones recorded for the synthetic materials, and with the high strain protocol the target was to study the structure close to failure conditions after mechanical testing. This last part was, however, not possible to implement in the present study.

As presented in the literature [82], the stress-stretch graphs obtained for the different layers composing the artery show that, while media and intima reported higher Cauchy stresses at lower stretch in circumferential direction, adventitia presented the same behaviour but in longitudinal direction. The combination of all these singular results will be present when analysing the full homograft results after mechanical testing.

Figure 47 presents the force-time graph of the low strain protocol of a homograft. It can be observed that, in this case, the forces recorded in the y-direction were higher than the ones obtained in x-direction, and this differs from what was obtained for the synthetic grafts. The forces recorded in the y-direction are 68% higher than in x-direction. Scanning the samples after mechanical testing it could be assessed if the y-direction coincides with the axial one and the x-direction with the circumferential. It can be also mentioned, that the forces recorded in the native tissue are much lower than in the synthetic grafts, never exceeding the physiological force normally exerted in a femoral artery during the application of the low strain protocol.

The viscoelastic behaviour, especially present in Dacron, can be also observed in the homograft since the force of the first cycles of a loading set is smaller than in the end of that loading set. Again, for being sure whether this behaviour is caused by the viscoelastic behaviour of the material or due to damage or fibres orientation, the same loading protocol should be applied two consecutive times and it should be assessed whether the results are the same.

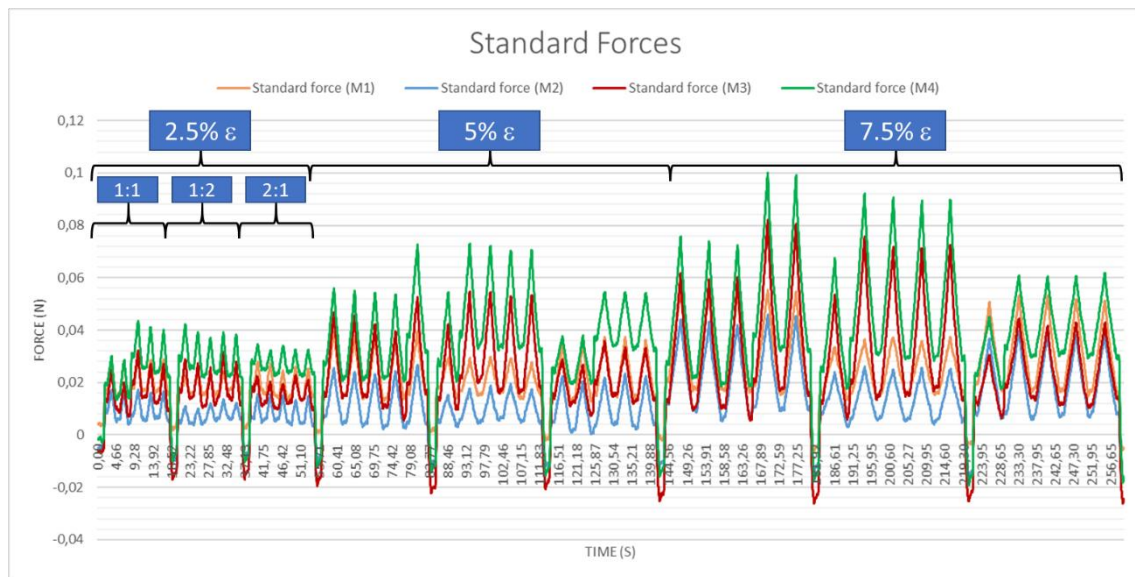


Figure 47: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Homograft (H5) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step

Table 12 shows the maximum forces recorded from each loading cell during the low strain protocol for each homograft sample. It can be observed that the forces in x-direction are much lower than in the synthetic grafts, but in the y-direction the forces have similar values than the ones obtained for Dacron.

Table 12: Maximum force (N) recorded in each loading cell during the low strain protocol for each Homograft not treated sample and average and standard deviation

	$F_{\max 1}$	$F_{\max 2}$	$F_{\max 3}$	$F_{\max 4}$
H4-	0.07	0.08	0.10	0.54
H5-	0.05	0.08	0.10	0.33
H6-	0.05	0.05	0.10	0.13
Average (N)	0.05	0.07	0.10	0.29
Standard deviation (N)	0.01	0.02	0.002	0.21

Figure 48 presents the results of the high strain protocol. The same tendency as in the low strain protocol can be observed, i.e. higher force in the y-direction, in this case 74% higher. So, it can be confirmed that the anisotropy rises at increasing strain levels. The viscoelastic behaviour can be also observed, but one particularity should be mentioned: during a loading set, there is a rise in the force for the first cycles but then a decrease. It could be explained due to the damage produced by the rakes, tearing the material.

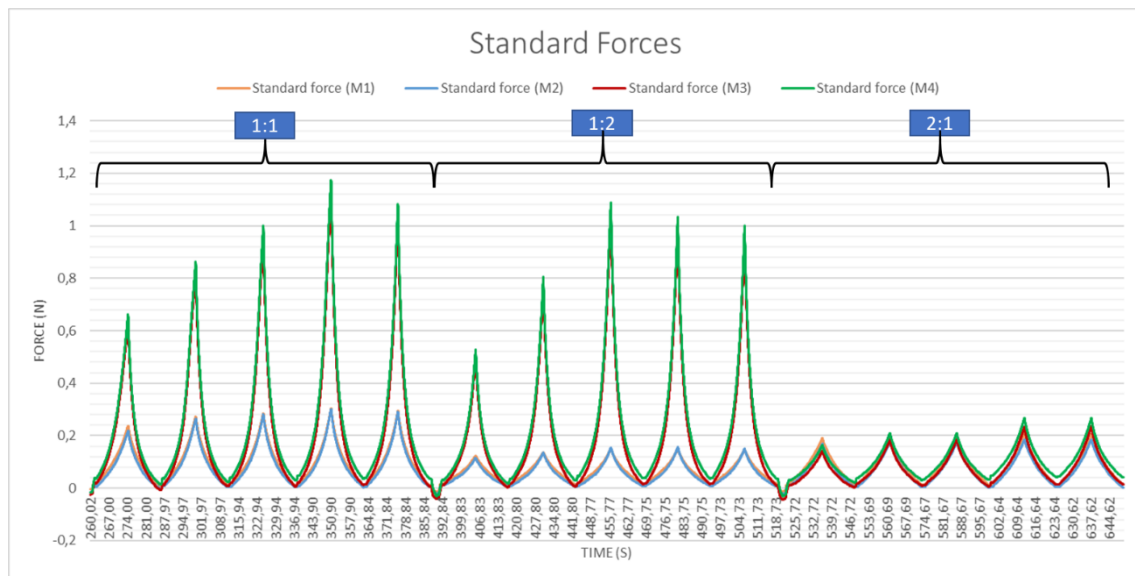


Figure 48: Force (N) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Homograft (H5) sample. High strain protocol is presented with 30% loading step and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1

Table 13 presents the maximum forces recorded from the loading cells for all the homograft samples during the high strain protocol. Also, the average and standard deviation for each force is mentioned. It can be observed that the maximum forces in the y-direction in this strain protocol are higher than the ones obtained in the x-direction.

Table 13: Maximum force recorded in each loading cell during the high strain protocol for each Homograft not treated sample in Newton (N) and average and standard deviation

	$F_{\max 1}$	$F_{\max 2}$	$F_{\max 3}$	$F_{\max 4}$
H4+	0.46	0.56	1.66	1.72
H5+	0.30	0.30	1.13	1.17

	$F_{\max 1}$	$F_{\max 2}$	$F_{\max 3}$	$F_{\max 4}$
H6+	0.33	0.38	1.60	1.66
Average (N)	0.36	0.40	1.44	1.50
Standard deviation (N)	0.09	0.13	0.29	0.30

5.2.2.1.5 Comparison of the grafts and the native tissue

Figure 49 (left) presents the results for the maximum force in load cell 1, located in the x axis. All the materials were significant different with $p < 0.0001$, except for Dacron and Vectra. They presented a similar mean value of the maximum force in load cell 1.

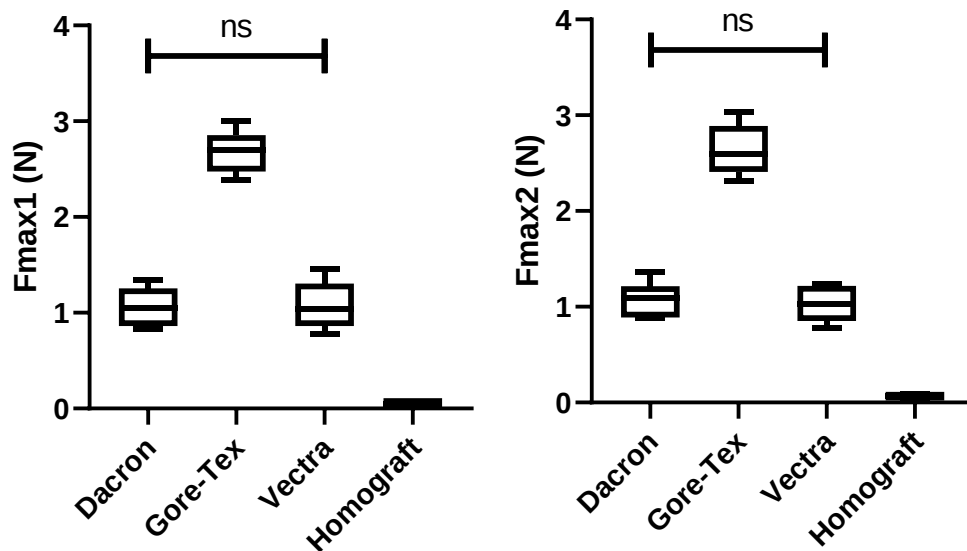


Figure 49: Box plot of the maximum force read in the load cell number 1 (left) and number 2 (right) for Dacron, Gore-Tex, Vectra and the homograft. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively. Both, $F_{\max 1}$ (left) and $F_{\max 2}$ (right), resulted significantly different ($p < 0.0001$) for all the materials except for Dacron and Vectra where not significant different (ns) was found

Figure 49 (right) presents the results of the maximum force recorded in the load cell 2, located in the x-axis, for the four different materials. As for $F_{\max 1}$, all materials resulted to be significant different except for Dacron and Vectra. Hence, both Dacron and Vectra, despite their different microstructures, present similar mechanical properties in the circumferential direction.

Figure 50 shows the maximum force recorded in the load cell 3 and 4 respectively. Regarding $F_{\max 3}$, Dacron and Gore-Tex are not significantly different compared to the native tissue, and they are also not significantly different compared to each other.

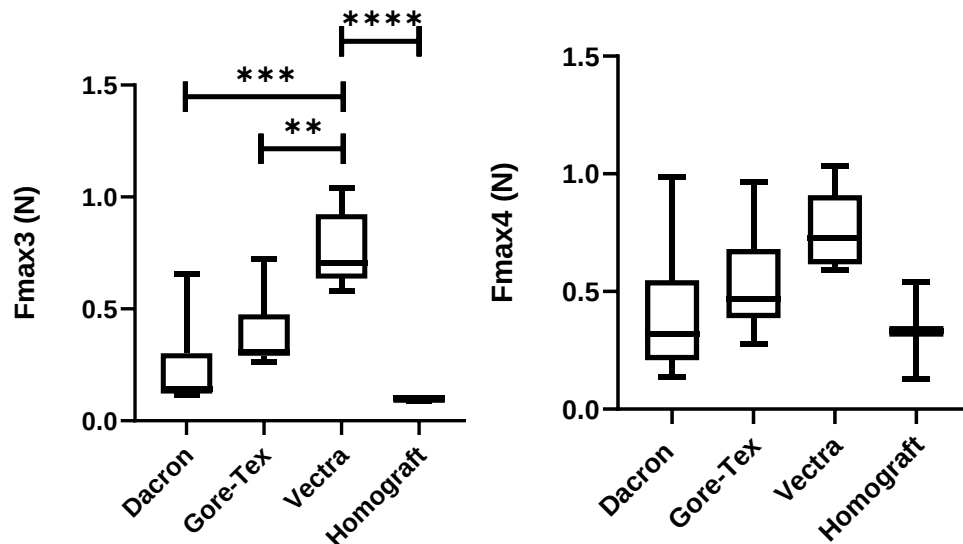


Figure 50: Box plot of the maximum force read in the load cell number 3 (left) and number 4 (right) for Dacron, Gore-Tex, Vectra and the homograft. The mean and the variance can be observed as the horizontal line in the boxes and the segments up and down the box, respectively. Fmax3 (left) did not registered a significance difference between Dacron and the homograft, while Fmax4 (right) did not found a significant difference between any of the materials

Finally, the last force to be analysed is the one measured in the load cell number 4 and the results are presented in Figure 50 (right). The mean values of the maximum force recorded in load cell 4 are not significant different for all types of samples. Once the behaviour of the materials in the longitudinal direction has been also studied, it can be established that those that better replicate the homograft's properties are Gore-Tex and Dacron, but there is a gap to cover regarding the circumferential direction.

The mean of the reaction forces recorded in each load cell was used to calculate the stresses in the materials. To evaluate the stresses both in x- and y- directions, the mean between F1-F2 and F3-F4, respectively, has been calculated. To confirm this assumption, the significance between the different forces in the materials has been evaluated following a two-way ANOVA (Figure 51).

In Dacron, all forces were significant different except for F1-F2 and F3-F4. For Gore-Tex, the same results were observed. In Vectra, the only forces that were significant different, were F1 and F3. Finally, for the homografts, all forces resulted to be not significantly different. Thus, the calculation of the stresses in each direction as the mean between the forces recorded in the load cells located in the same axis can be considered as a good solution. Ideally, this comparison would have been made between the mean force recorded in each load cell and not in the maximum force recorded.

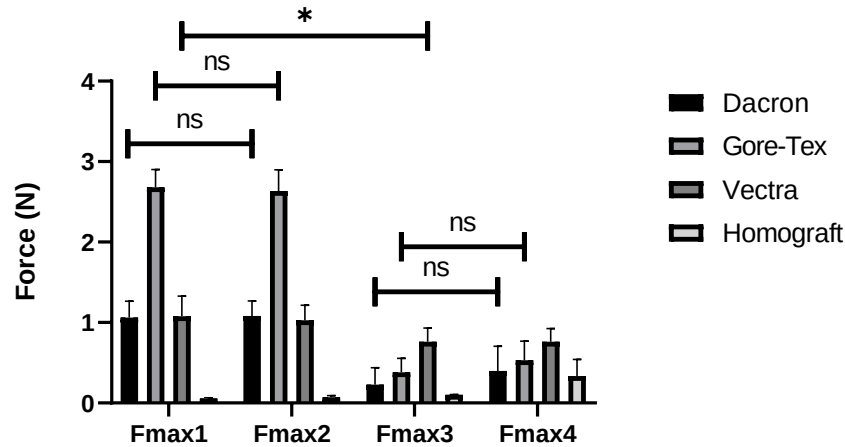


Figure 51: Box plot comparing the maximum forces recorded in each load cell for each material. In Dacron and Gore-Tex forces are significant different except between Fmax1-Fmax2 and Fmax3-Fmax4. In Vectra all were not significant different except between Fmax1-Fmax2 and in the homograft all forces were not significant different between each other

5.2.2.2 Standard travels

The tests performed were displacement-controlled, taking as reference the preload state. Hence, when observing the results of the standard travels, recorded in each direction two displacements need to be taken into account, the one necessary to arrive to the preload state and the one needed to arrive to the strain level defined in the protocol.

Similar results as the presented in the standard forces (see previous section) were recorded for the displacements with some particularities. Figure 52 shows the standard travel for a Dacron sample, for the standard travel graphs of the rest of materials see Appendix B. In the case of Dacron, despite the higher force applied in x-direction compared to y-direction, the higher displacement is registered in x-axis. This behaviour reinforces the observation that Dacron is stiffer in the circumferential direction. The waviness of the material favours the displacement in the y-direction applying low forces. Also, during clinical practice, the material suffers a slight stretch and the material should not be damage during this practice, since the ease of stretching guaranties the material integrity during the anastomosis.

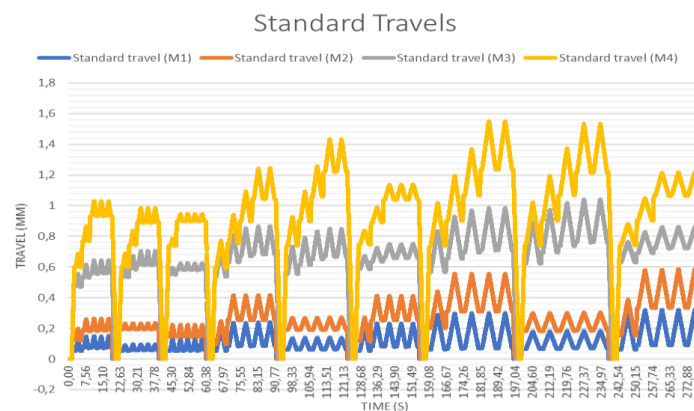


Figure 52: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Dacron (D1) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step

The discussion between viscoelastic behaviour or fibres damage is retaken when observing the displacement graphs. Again, for Dacron and Gore-Tex, it can be observed that the first cycles in a loading set present a lower travel than the one registered for the last cycles of the sets. The waviness present in Dacron in y-direction might justify the higher displacement necessary to arrive to the preload state than the one registered in x-direction.

To summarise, Dacron presents the highest displacement, followed by Gore-Tex, then the homograft and finally Vectra. Indeed, for Vectra, following the manufacturer instructions, a deformation higher than 10% might produce damage in the material due to the microporous layers of the graft. This structure justifies the observed results, in which Vectra presents a similar travel in all directions. Regarding the travel graphs, the homograft reports the same tendency than the one observed in Dacron and Gore-Tex: i.e. higher displacement in y- than in x-direction. However, the forces presented the opposite behaviour. Finally, it can be asserted that the homograft is not only stiffer than the synthetic grafts, but also it presents a more elastic behaviour, which means that higher displacement was recorded where higher forces were applied independently from the time the deformation last, in contrast with the viscoelastic behaviour present in Dacron and Gore-Tex.

5.2.3 Stress

Since the deformations in the low strain protocol are small, the Cauchy stress is a sensible way of describing the action of surface forces, given that there is not a significant difference between the deformed configuration and the sample reference shape, undeformed. As the biaxial tester used rakes as clamping system the shear terms in the stress matrix have been neglected. Besides, it should be mentioned that there is a slight underestimation of the stretch, since the displacement of the rakes was used for calculation of the displacement instead of the real displacement of the sample.

As the aim of this study was to evaluate different materials under physiological conditions, the loading set selected to obtain the stress response must work with reaction forces lower than the physiological limit estimated for a femoral artery, <0.6 N. After analysing the force- time graphs obtained for all the materials, it has been detected that the only loading set that works under those conditions for all is the one with 2.5% strain and 1:2 ratio. The constraining material is the Gore-tex which reported noticeable higher forces than the rest of materials, especially in the circumferential direction. That is why, despite the ratio selected, in which higher strain was applied in the longitudinal direction, forces were kept under the physiological limit in all directions.

Figure 53 presents the experimental Cauchy stress calculated for all the materials. Ideally, those graphs would present, for each material, the fifth cycle of the selected loading set for all the samples tested. However, it was just possible to evaluate one sample per material.

The graphs present the circumferential stress in the upper left side of the square and the longitudinal in the lower right, index 11 and 22 respectively in a stress matrix. The circumferential stresses showed more irregularities, at some points they even dropped back to lower stretch values. This behaviour can be due to fibres orientation or damage, but also can be a response to the Poisson compression effect [83], since the strain in the longitudinal direction is double the circumferential. Assessing the structure after mechanical testing for possible damage, would reveal which effect is causing the present behaviour. Other remark to make is that, in this direction, the stress lines for every cycle are more grouped than in the longitudinal

one. This shows that the preconditioning effect did not have the same influence in the circumferential direction as in the longitudinal, except for Vectra.

In the longitudinal direction, the stress curves are smoother for every material. For Dacron, Gore-Tex and the homograft, a higher dispersion of the curves is observed compared to Vectra, while in Vectra every cycle presents similar response. Hence, for the first group, arriving to a preconditioned state takes more cycles than for Vectra. The last remark to be made is the offset that can be observed in each curve due to the preload.

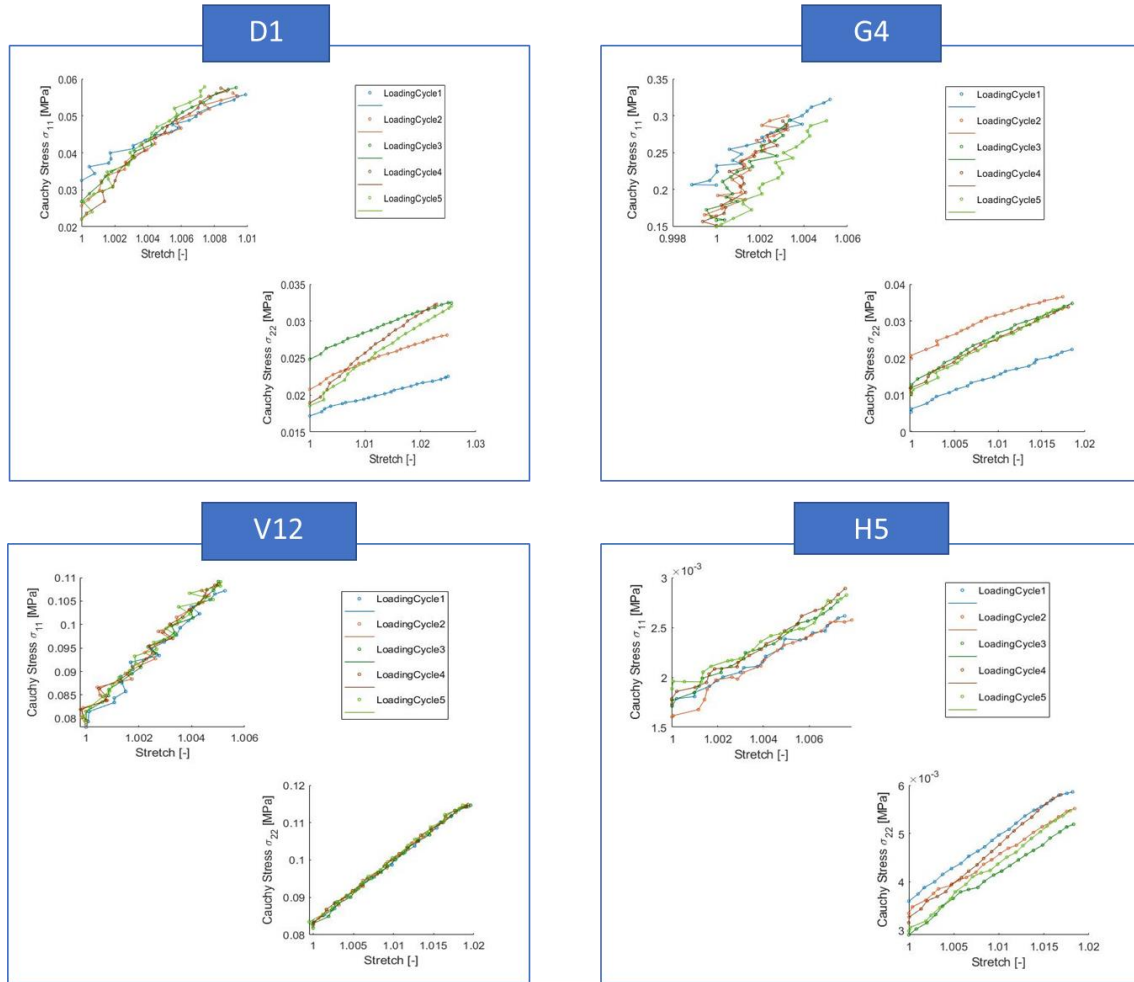


Figure 53: Cauchy stress (MPa) vs stretch (-) experimental responses in circumferential (σ_{11}) and longitudinal (σ_{22}) directions for a low strain protocol 2.5% strain, 1:2 ratio and 5 cycles (Loadingcycle1-4 for preconditioning) for synthetic grafts and native tissue. One significant sample of each of the materials have been selected. D1: Dacron.

G4: Gore-Tex. V12: Vectra. H5: homograft

Figure 54 represents the comparison between the stress-strain curves obtained for Dacron with the computational tool and the response of PET to uniaxial tensile test [90]. In this figure, the behaviour of the PET film used for the product of PET/whey bi-layered and the same material after washing with protease-based detergents during 2 hours at 50°C is presented. The aim of using that kind of detergents is to break down the protein chains. The interest of these findings for the present study is to evaluate the variation of the mechanical properties of PET after washing, reporting a reduction in the elongation at break and a stiffer behaviour. In the visual inspection

of the Dacron, it was observed that after washing its fibres appeared more separated among each other. The stress-stretch graph obtained with Matlab for Dacron represents approximately the start of the full stress-strain curve of PET, since low strain is being applied in the sample. It can be asserted that, with that load level, the material has not exceeded the elastic limit, so the possible damage discussed in the forces section might be, in case of being present, an issue of the fibres orientation.

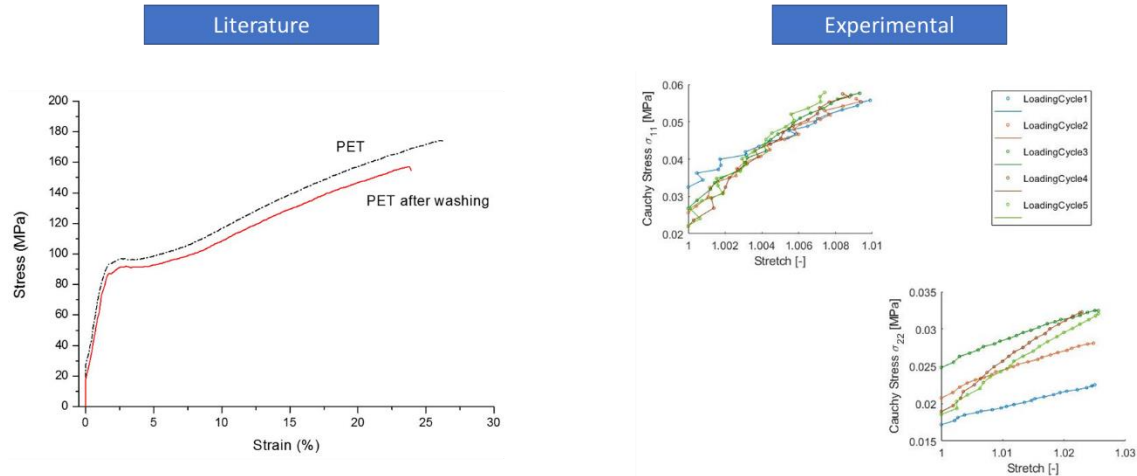


Figure 54: In the right representative stress/strain curves of PET films before (black) and after (red) coating with whey and whey removal by washing with Alcalase at 50°C for 2 h [90]. In the left, Dacron's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions

For Gore-Tex, the literature was again checked to discuss the obtained results in this study. In Figure 55, ePTFE stress-strain curves of a uniaxial tensile test of layers of different thickness, both in x- and y-directions, are presented [91]. The x- and y-directions of those layers correspond to the circumferential and longitudinal directions, respectively, in this study. It can be observed that the tendency of the curves is similar, both in the current study and the researched data. The slope in the circumferential direction is higher and in the longitudinal direction it is smoother.

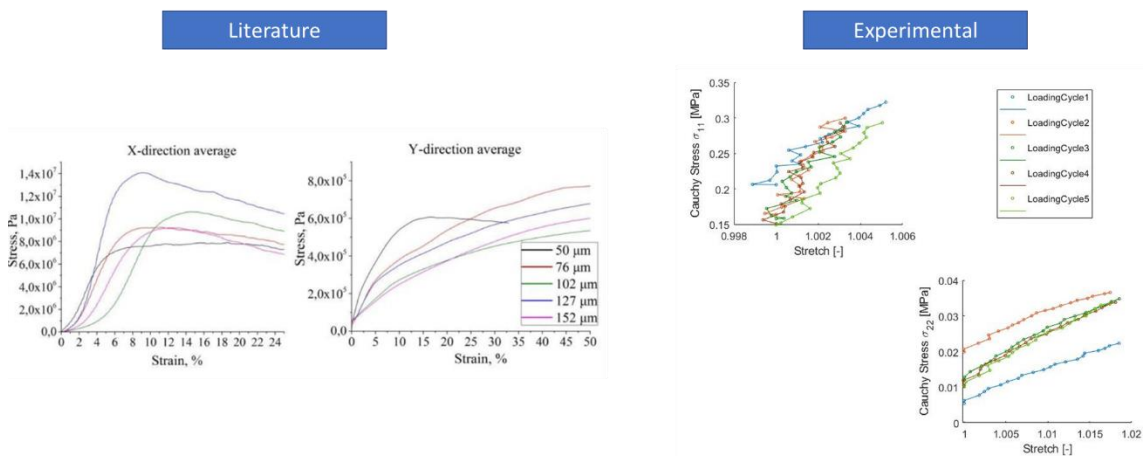


Figure 55: In the right representative stress/strain curves of ePTFE layers of different thickness (see legend) after uniaxial tensile test both in x and y direction are presented [91]. In the left, Gore-Tex's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions

Figure 56 compares the results obtained in tensile test of cellulose nanofibrils (CNF) modified polyurethane foams (PUF) [92] with the results obtained in this study for the Vectra, which composition is PU-based. The reference graphs show that the elastic limit of the PU modified foams is exceeded over 0.2 MPa. Vectra's sensibility to high deformations has been already stated and the results recorded from literature verify these observations. So, the use of this material for peripheral bypasses might be risky, even if it is reinforced with stiffer materials as the wire and the self-sealing layer.

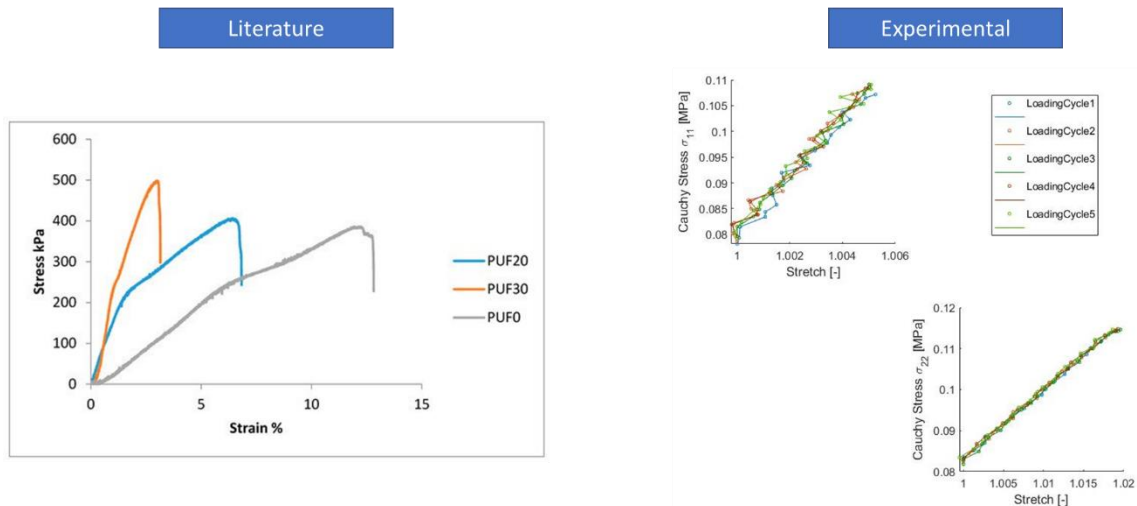


Figure 56: In the right, representative stress/strain curves of cellulose nanofibrils (CNF) modified polyurethane foams (PUF) of different densities PUF0 0.059, PUF20 0.05 and PUF 0.051 all in gr/cm^3 [92]. In the left, Vectra's stress-strain curves after applying the low strain protocol in both circumferential and longitudinal directions

To evaluate the homograft response under high strain, the 1st Piola-Kirchhoff stresses applying the low strain protocol presented before and a high strain protocol of 30% strain and 1:1 ratio are compared for the samples. The results are presented in Figure 57. The 1st Piola-Kirchhoff stress is selected in this case, since this stress is a better solution for representing the higher deformation level recorded after applying the high strain protocol.

Also, for the high strain protocol a higher dispersion in the longitudinal direction can be observed in the lines of each loading cycle. The most interesting remark to make, after comparing the low and high strain protocol's response, is the shape of the graphs. The high strain protocol resembles the shape observed in Figure 5, where the stress response of the different layers of an artery was presented. However, at low strains, the graph presents a concave shape, which can represent the very start of the high strain curves. The curves obtained from the high strain protocol represent an elastomeric behaviour of the homograft since the slope of the curve rises at higher deformation level. A description of elastomers' and polymers' main properties is presented in Appendix C for having further insight about their behaviour.

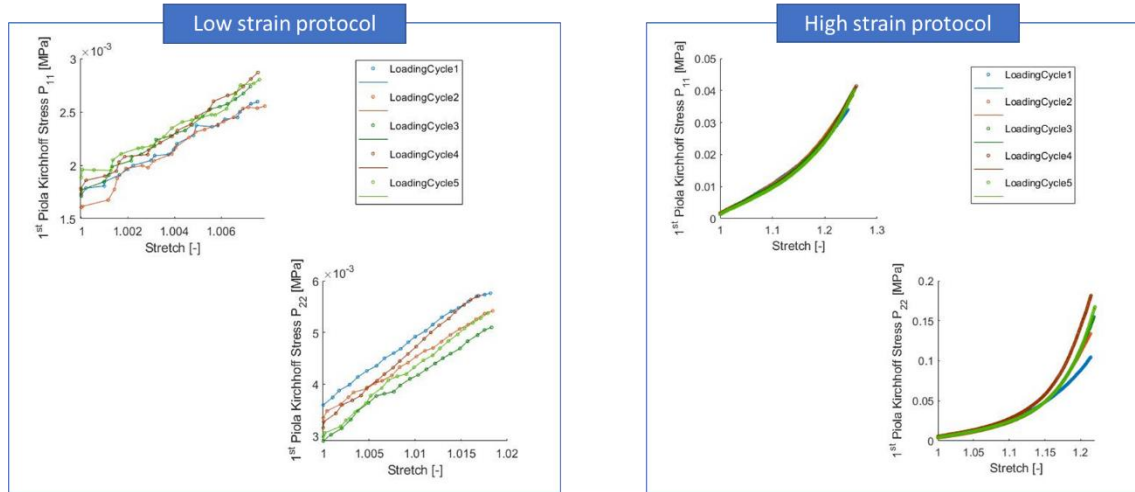


Figure 57: 1st Piola-Kirchhoff stress (MPa) vs stretch (-) experimental responses circumferential (σ_{11}) and longitudinal (σ_{22}) directions comparing the results from 2.5% and 1:2 ratio protocol and 30% and 1:1 ratio loading sets performed in a homograft sample (H5)

6 CONCLUSION

Two main objectives were defined in the frame of this Master Thesis. The first one consisted on a deep characterisation of the structural and mechanical properties of different synthetic grafts clinically applied. Hence, the second one was, based on literature and experimental results, deciphering the mismatch between the properties of the synthetic grafts and the native tissue. With the results obtained from this comparative study, some of the gaps between the existing synthetic grafts and the native tissue properties could be detected and form the basis for the design of novel synthetic grafts.

As a general conclusion, it can be stated that this study clearly determines the basis of a standardised methodology to study the structural and mechanical properties of synthetic grafts and native tissue. As it was presented in the literature, there are a wide range of testing protocols, and because of this, it is very hard to be able to compare the results. Additionally, no 3D structural characterisation has been done so far. Hence, it is of a high interest to standardise the characterisation protocols to obtain unified and comparable results. Despite the temporal and operational limitations, a wide range of properties have been defined and discussed within this thesis, successfully determining the main discrepancies between synthetic grafts and native tissue. In the following sections specific conclusions for both the structural and mechanical characterisation will be presented and a proposal for the workflow to be followed in the study and design of new bypass prosthesis is provided.

6.1 Structural characterization

In the structural characterisation, a visual inspection of the microCT images was developed and after the 3D morphological analysis of those images it was possible to compare structural components of the grafts. The conclusions extracted from each part of the study are presented below.

Visual inspection:

- Regarding the homograft images the different layers were clearly differentiated. More advanced image processing or sample treatment will, however, be necessary to be able to study the fibrous structure of the native tissue.
- Dacron is fibre-based with braided fibres orientated in the longitudinal direction. This will give resistance to the structure, but the waviness provides elasticity.
- Gore-Tex presents a structure composed by fibres united with nodes, orientated in the longitudinal direction, and with higher resistance in this direction, like Dacron.
- Vectra is based on three different components: foam, wire and self-sealing layer. The foam is thinner than the one observed in Gore-Tex, and the pores are larger. That means more space for endothelial tissue ingrowth but less stiffness, which is overcome with the wire structure coating the inner surface and the self-sealing layer.

As a summary, Table 14 gathers the conclusions extracted from the visual inspection of the samples. The type, orientation and distribution of the microstructure has a great influence in the mechanical properties.

CONCLUSION

Table 14: Conclusions extracted from the visual inspection of the samples

Material	Type	Microstructure		Conclusions
		Orientation	Pore size	
Homograft	3 layers separated by an elastic lamina; corrugated lumen.	-	-	More advanced image processing or sample treatment to distinguish components.
Dacron	Braided fibres and corrugated surface (waviness).	Longitudinal	High	-Higher fracture resistance and elasticity in longitudinal direction. -As-produced sample denser microstructure than wetted then dried samples which seems more rigid and porous → collagen layer dissolution.
Gore-Tex	Node-fibril.	Longitudinal	Low	-Denser microstructure than the other grafts → stiffer. -Higher fracture resistance and elasticity in longitudinal direction.
Vectra	Foam, sheet and wire.	-Foam: concentric circumferences. -Sheet: embedded in the foam. -Wire: embedded in the foam coiling the structure.	-Foam: high. -Wire and sheet: low.	-Foam porous structure will favour the tissue ingrowth. -Wire: kink resistance and elasticity. -Sheet: uniform distribution of stresses.

Morphological analysis:

- The specific surface area of Dacron and Gore-Tex is higher than of Vectra. This means that there is more surface for endothelial tissue ingrowth. After statistical analysis, the specific surface area resulted significant different for all materials. Those materials with a higher specific surface will register higher speeds of endothelial cells fixation.
- Vectra possess the highest porosity, being significantly different compared with the one of Dacron and Gore-Tex. Dacron and Gore-Tex have a lower porosity, but again among each other this parameter is significant different.
- Regarding the foam, the volume fraction is significant different between all the materials. For Vectra the foam volume fraction was far below the values registered for Dacron and Gore-Tex. Following the study of Gibson and Ashby, Vectra's stiffness is supposed to be lower than the one of Dacron and Gore-Tex, but the wire and self-sealing layer provides a higher stiffness than the one expected from Vectra's foam due to their elastomeric-based composition.
- The structure thickness for all the materials was significant different between all the materials, being higher for Dacron, then Gore-Tex and then Vectra. It can be assumed that those materials with higher structure thickness will report a higher stiffness, but regarding the foamy nature of the materials studied, the pore thickness has a meaningful effect in the mechanical properties and it has to be also evaluated to come to a definitive judgement. Hence, Gore-Tex appears as the stiffer material, since it reaches the most optimal compromise with a structure thickness between the one of Dacron and Gore-Tex and with the smallest pore thickness.

Table 15 presents the conclusions extracted from the morphological analysis.

Table 15: Conclusions extracted from the morphological analysis of the samples

Component	Property	Conclusions
Full graft	Specific surface	Higher surface higher endothelial cells fixation (Dacron>Gore-Tex>Vectra).

Component	Property	Conclusions
Foam	Porosity	Higher porosity higher tissue ingrowth lower resistance to deformation (Vectra>Dacron>Gore-Tex).
	Volume fraction	-Stiffness grows with the volume fraction squared (Gore-Tex>Dacron>Vectra).
	Structure thickness	-Compromise between structure and pore thickness to define stiffness level.
	Pore thickness	-Evaluating just the foam Vectra presents a low stiffness but the effect of the wire and the sheet is being neglected → mechanical testing needed.

6.2 Mechanical characterisation

To assess the mechanical properties of synthetic grafts and homografts, planar biaxial tensile testing was performed. Both force and displacement were tracked over the duration of the experiment, and images of the sample were taken during all this process. The speckles applied on the sample surface during the testing allowed to perform DIC to obtain the strain map of the samples. Finally, this information was processed to obtain the strain-stretch curves. The conclusions extracted from the mechanical characterisation are:

- The variability of the thickness measured via optical measurements is higher than when obtained from microCT images. The waviness present in Dacron provides a high variability, even in the measures performed on the microCT images. The optical measures are influenced both by the thickness of the graft and the residual stresses. A higher thickness reports more accurate measures, and high residual stresses infer distortion in the measures. Due to these influences, the measures obtained from the microCT images were more accurate than the ones obtained with optical measurements.
- All the materials present anisotropic behaviour, being of a similar magnitude for Dacron, Gore-Tex and the homograft, but very low for Vectra (20% approximately). However, the stiffer direction is different for the synthetic grafts and the native tissue. For the synthetic grafts, it is the circumferential direction, while for the native tissue the stiffer direction is the longitudinal. It should be evaluated from microCT images taken from the native tissue after mechanical testing, though, whether the orientation of the sample during the testing is congruent with the one adopted by the synthetic grafts. Considering an affirmative answer, it can be assumed that the grafts are design with this different configuration compared with the native tissue to ensure the pulsatile blood flow. In light of a higher tension, the graft wall will transfer a higher energy to the flow during the diastole when returning to the relax state. Also, it can happen that the elastic limit in this direction is exceeded earlier due to the high stress standed by the material, so this will provoke an inverse behaviour reducing the blood flow velocity and favouring the thrombus formation.
- Regarding the standard travel of the rakes, it has been reported that under the same strain required in both, x- and y-directions, it is in y-axis or longitudinal direction that the highest displacement was observed for Dacron and Gore-Tex. This is an interesting response since, for these same materials, the forces were higher in the x-direction under the same strain condition. The orientation of the fibres and fibrils in this favourable direction could be the cause of this behaviour. For Dacron, this behaviour is emphasised by presence of the waviness over its surface. The fact that, after each cycle in a same loading set, the displacement is higher, invites to think that the material presents viscoelastic properties or damage. To discard the second assumption, some further tests

are necessary and will be described in the future prospective. Homografts and Vectra report a coherent behaviour; i.e. the direction in which the highest force is applied is the one which presents the highest displacement.

- Some similarities between the synthetic materials, Dacron and Gore-Tex, and the homograft have been already highlighted. After statistical analysis of the maximum forces recorded in every load cell, it could be seen that in y-direction the maximum forces do not present significant differences between Dacron, Gore-Tex and the homograft. In the load cell number 3, in y-direction, there are differences between the mean maximum force reported by all the Vectra samples and the other materials. Regarding the x-direction, all the forces are significant different ($p < 0.0001$) except for Dacron and Vectra, being able to assert that in this circumferential direction, their mechanical behaviour might be similar.
- To assess whether the mean of the reaction forces located in the same axis could be considered to calculate the stress in such direction, the significance of the difference between the maximum forces in each load cell was statistically evaluated. Due to the fact that for any material a significant difference was found between the forces of the load cells located in the same axis, it has sense to consider the mean of the reaction force of those load cells for the calculation of the stress in that particular direction.
- From stress-stretch graphs, some of the behaviours and conclusions already extracted are reinforced. The load set examined must work under the physiological conditions, that is to say under a maximum force of 0.6 N for a femoral artery. The loading set that meets this specification is that of 2.5% strain and 1:2 ratio, double of strain imposed in the longitudinal direction. Despite this higher strain imposed in the longitudinal direction, the highest tensions for Dacron and Gore-Tex appear in the circumferential direction. This higher solicitation in the circumferential direction might lead to an earlier damage, provoking the thrombogenic effect already mentioned.
- Due to the fact that the strain imposed in the circumferential direction is lower, a Poisson contraction effect is recorded, observing that some points of the strain-stretch curve return to lower stretch values in this direction.
- The stresses observed in Vectra are similar in both directions. Due to the recommendation of the manufacturer of the graft, Vectra reports damage exceeded the 10% strain. This is a limiting factor of its use in peripheral bypasses. It would be a good practise to implement the data recorded for this graft in a mathematical model that models the blood stream to predict its behaviour in the cardiovascular system for a bypass application. Like that, it will be possible to assess the potency of the graft or directly discard the use of this material in the peripheral bypasses although its interesting mechanical properties.
- An elastomeric behaviour has been observed in the homograft stress-strain curve under the high strain protocol, since the slope of the curve rises at higher deformations.
- Finally, after observing the Ashby graphs [89] (see Figure 58) and locating the artery on it [93], and confirming with the stress-stretch graph of the homograft at a high strain of 30%, it can be confirmed that the type of material that better reproduces the native tissue behaviour is elastomer-based. Hence, it is suggested that the line of study for finding a new bypass prosthesis, this family of materials should be considered. More insight about these families of materials could be found in Appendix C.

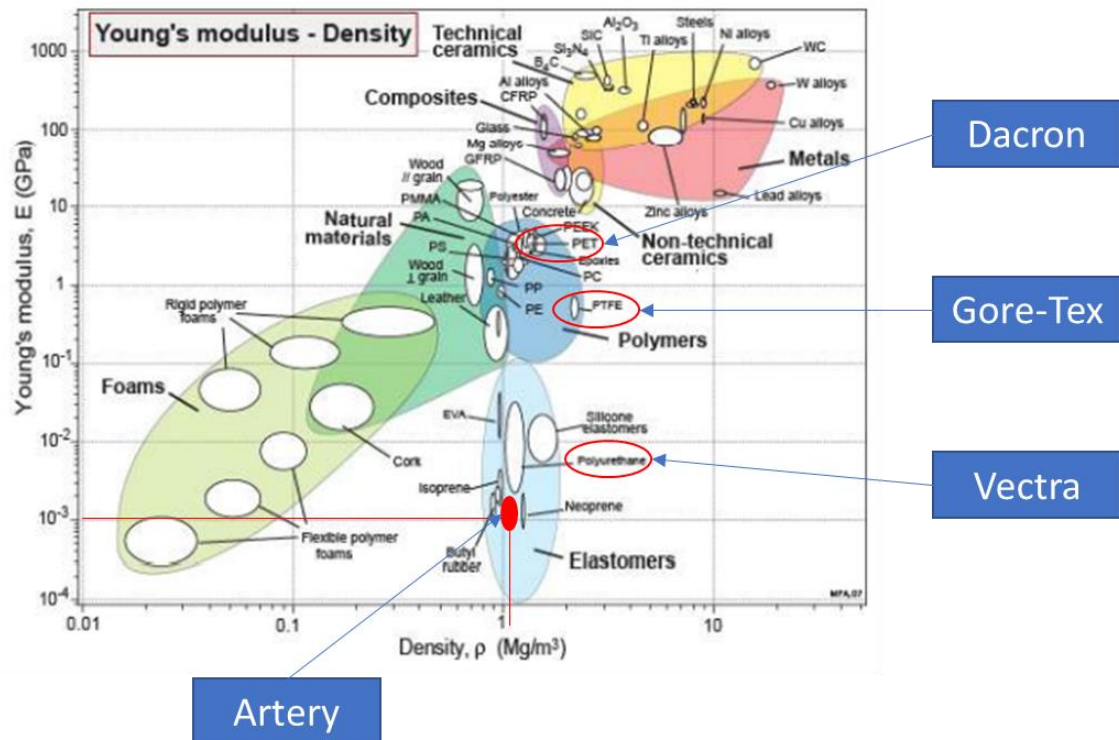


Figure 58: Young's modulus E (GPa) plotted against density ρ (Mg/m³). The heavy envelopes enclose data for a given class of material. Dacron and Gore-Tex belong to the polymers field while Vectra and the homograft to the elastomers

To sum up, the conclusions extracted from the mechanical characterisation are presented in Table 16.

Table 16: Conclusions extracted from the mechanical analysis of the samples

Property	Conclusions
Thickness	-DataViewer is more accurate measuring toll than Matlab. -Variance of the results grows with the residual stresses and is reduced with material's higher thicknesses. -High variance with DataViewer when measuring Dacron because of the folds.
Forces	-Circumferential: much higher in Gore-Tex. Maximum force similar between Dacron and Vectra → similar mechanical behaviour in this direction. -Longitudinal: Higher in Vectra. Maximum force similar between Dacron, Gore-Tex and Homograft → similar mechanical behaviour in this direction. -Anisotropy: Gore-Tex>Dacron>Homograft>Vectra. Opposite direction of anisotropy between the homograft and the synthetic grafts → different testing direction or intended design to increase blood pulsatility in synthetic grafts. -Rising force in every cycle → viscoelasticity or damage. Observed in Dacron, Gore-Tex and the homograft. Vectra elastic behaviour. -Not significance difference between maximum forces of the same loading direction → The mean of these forces can be calculated to determine the reaction forces.
Displacement	-Rising displacement in every cycle → viscoelasticity or damage. Observed in Dacron, Gore-Tex and the homograft. Vectra elastic behaviour. -Low deformation resistance in longitudinal direction in Dacron and Gore-Tex. Higher displacement when forces were lower.
Stress	-Poisson contraction in circumferential direction due to higher strain in longitudinal direction. -Higher stresses in circumferential direction in Dacron and Gore-Tex even if the strain was double in the longitudinal direction. -Circumferential direction acquire preconditioning faster. -Similar stresses in both directions recorded in Vectra. -Homograft in high strain protocol showed an elastic behaviour → curve slope grew with higher deformations.

6.3 Work methodology

The need of defining a standardised protocol to characterise the properties of the synthetic grafts used in peripheral bypasses has been presented along this study. Once these properties are characterised, their behaviour in the cardiovascular system should be predicted with a mathematical model. Prof. Valérie Lacroix and her team have already developed and validated a model suitable for this aim [2]. Finally, regarding the results of the model, the material can be optimised to reach an optimal behaviour once inserted in the human body. The workflow proposed is depicted in Figure 59 and it can be applied as a design methodology for new materials for bypass prosthesis.

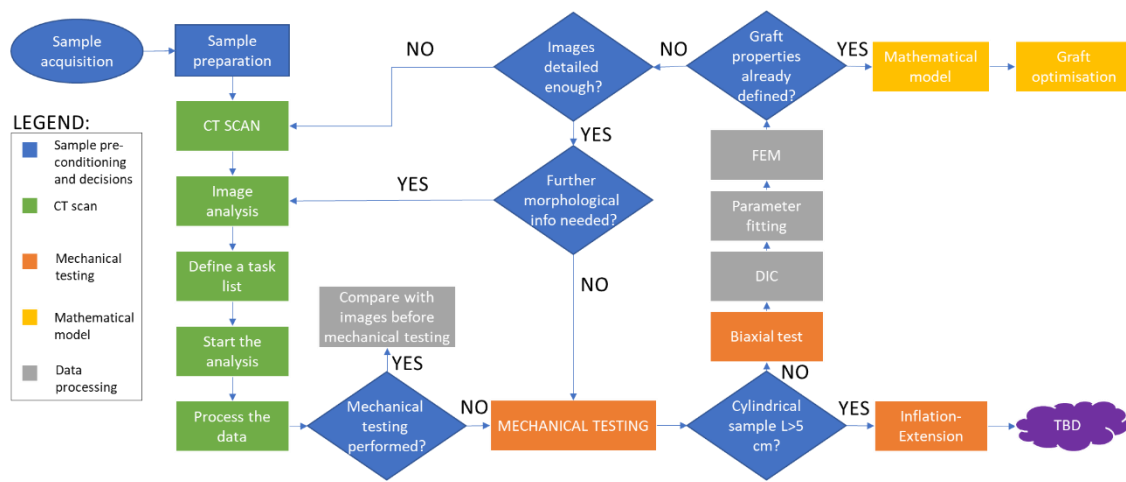


Figure 59: Workflow of the study and design of new bypass prosthesis

7 FUTURE PROSPECTIVE

The work performed in this thesis presents a roadmap to evaluate the structural and mechanical properties of synthetic grafts used to perform peripheral bypasses, as well as native tissue. Even if the obtained results already give a detailed view of the properties of such materials, further studies of some of those properties, and others, is necessary to further characterize the materials. Also, some processes of the used methodology must be improved to really be used in a standard manner.

Concerning the structural analysis, some proposals to improve the results obtained are:

- Scanning the samples after mechanical testing using microCT and performing visual inspection and morphological analysis to evaluate the changes compared with the ones taken before mechanical testing. Following this process every time after mechanical testing the damage in the sample, if present, can be evaluated. This practice will be also useful to clear doubts appeared after mechanical testing. One example of that occurred in this study, is the need to scan the homograft after mechanical testing to confirm that the mounting in the biaxial tester was analogous to the one in which the synthetic grafts were mounted.
- Calculating the full graft thickness using based on 3D analysis of the microCT data. The images analysis software calculates this parameter evaluating the 3D structure of the material, so it is the most accurate way to obtain this parameter. It can be statistically compared with the results obtained from optical measurements and 2D microCT images to have a quantitative measure of its accuracy.
- Applying advanced image processing and treatment techniques to the samples to be able to visualize the inner structure of the homograft in detail. In the results, the different layers composing the homograft were clearly distinguished, but not the structure inside them. With images that clearly represents the inner structure, it will be possible to perform morphological analysis in which the different layers and their microstructure could be evaluated in detail.

There are also some points to be improve in the mechanical characterization performed in this study, but also new techniques and measurements to obtain further information of the samples.

- Evaluating if the behaviour observed in the mechanical testing in which the displacement between consecutive cycles of one loading set was risen, happened because of the viscoelastic nature of the materials or because the material presented damage. To do so, a new planar biaxial test of the sample is proposed. In it, the same strain level should be applied and two consecutive loading sets of 1:1 1:2 2:1, and again, 1:1 1:2 2:1 strain ratios should be performed to compare the results obtained after each loading set. If those are the same, it can be confirmed that there is viscoelastic behaviour, otherwise there is damage and the type of damage should be evaluated. Some of the possible damage are fibres re-orientation or elastic limit overtaking.
- Perform creep test to evaluate the viscoelasticity of the materials.
- Presenting in the stress-stretch graphs only the fifth cycle of the same loading set, evaluated for all the samples for each material. It is interesting to present the four first cycles to evaluate how the preconditioning affects the sample, but for a mechanical analysis, only the fifth one must be considered to perform a consistent assessment.
- Evaluating the materials response to an inflation-extension test, in which a flow of changing pressure is irrigated inside a circumferential graft of a minimum length of 5

cm. This test will give insight about the material compliance, a property which interest has been presented along this study. It has been observed that after irrigating water for some time inside the different grafts tested in this study with a syringe, Dacron was the only one that presented leakage. However, it might be interesting to also evaluate this material in the extension-inflation set-up to evaluate the level of this leakage and the mechanical response of all the materials studied in this thesis.

- It was found that the residual stresses present in the samples once they were planarly prepared affected not only the optical measure of the thickness, but also in the mounting in the biaxial tester. It would be interesting to determine the opening angle of the grafts once they are prepared in 1x1 cm squares to evaluate the influence of the diameter on the residual stresses and, also, the influence of these stresses in the testing itself.

Going further in the study, once the properties of the material are fully defined, it will be interesting to evaluate the response of the graft in the cardiovascular system. Specially for novel grafts, it is a risky practice, even if the structural and mechanical properties mimic precisely the behaviour of the native tissue, to directly being implemented in the human body. To eliminate that risk, mathematical models have been created to assess the response of the graft after being inserted in the human body. One of those models have been developed and validated by Prof. Valérie Lacroix and her team. In the frame of this study, it will be of a high interest to evaluate Vectra with the model to determine if it could be really used in peripheral bypasses or, although its beneficial properties, they are not appropriate.

Regarding the design of an optimised synthetic prosthesis to perform peripheral bypasses, the average stiffness and density of an artery has been evaluated and located in an Ashby graph [89] together with the synthetic grafts tested in this study, as it can be observed in Figure 58. Before this classification, the elastomeric response of the artery has been already observed in the stress-stretch curves. This result leads to state that, in the design of new materials for peripheral bypasses, the field to which these materials must belong must be the elastomers or at least somewhere in the boundary of elastomers and polymers, after seeing with Dacron and Gore-Tex the acceptably adequate response under physiological conditions that they reported.

APPENDIX A: Continuum Mechanics

The aim of this appendix is to provide insight into some important continuum mechanics principals and concepts that were presented in chapter 5 to describe the stress calculation from the strain maps. The information given in this appendix is not exhaustive and it is orientated to a better understanding of this study. The interested reader is referred to [94].

A.1. Deformation

Consider a body in the 3D space characterised by the global reference $x_1x_2x_3$, as presented in Figure 60. The reference or undeformed configuration is the condition of the body in 3D space before loads have been applied to it, vector x' is defined in the reference configuration. The deformed configuration is the location and shape of the body after loads have been applied to it, vector x is defined in the deformed configuration. The difference between x and x' will define the displacement vector, u .

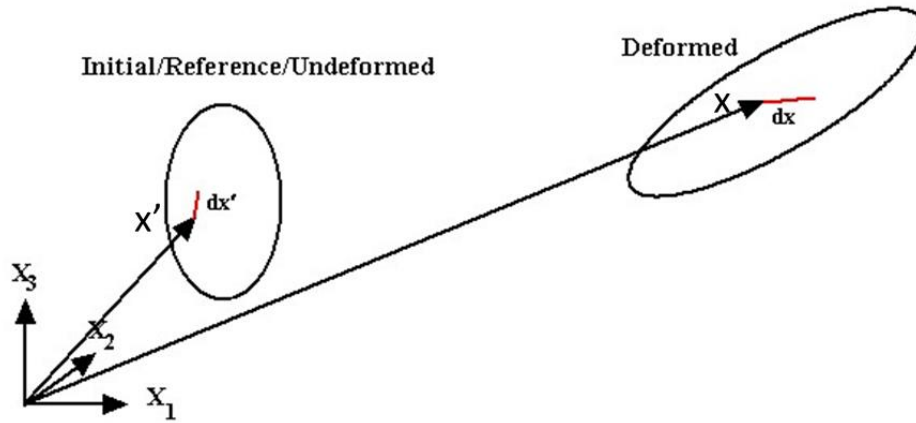


Figure 60: A continuum body at reference and deformed configurations is represented in a global $x_1x_2x_3$ reference.

The infinitesimal deformation of each reference (dx') and deformed (dx) configurations is represented in the figure

[94]

Knowing how a point displaces from the reference to a deformed configuration, the next step is to define how it is stretched and rotates as it performs this displacement. In red in Figure 60, infinitesimal material vectors are presented dx' for the reference and dx for the deformed configuration. Since the length of dx' can change when going to the deformed configuration, it is interesting to find the relation between dx' and dx . It can be done through the chain rule as:

$$dx_i = \frac{\partial x_i}{\partial x'_j} dx'_j \quad (\text{A.1})$$

The above equation gives a relationship between a material vector in the undeformed and deformed configuration. The mapping itself between those two positions is defined as the deformation gradient tensor:

$$F_{ij} = \frac{\partial x_i}{\partial x'_j} = \frac{\partial x'_i}{\partial x'_j} + \frac{\partial u_i}{\partial x'_j} = \delta_{ij} + \frac{\partial u_i}{\partial x'_j} \quad (\text{A.2})$$

Where δ_{ij} is the kronocker delta that is equivalent to the identity matrix.

F is a second order tensor, since it has two independent indices and it is not symmetric. The determinant of the F matrix is called its jacobian and its interest will be presented when defining the stress tensors.

A.2. Strain

Considering the case of small deformations, the strain can be defined as the ratio between the change in length and the initial length. The equation that defines small strain is A.3. For large deformations, the gradient tensor cannot be used as a strain measure directly because it can contain rigid body modes like translation and rotation. The equation that defines large strain is A.4, and it is also known as the Green-Lagrange strain.

$$\varepsilon_{ij} = \frac{1}{2} \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) \quad (\text{A.3})$$

$$E_{ij} = \frac{1}{2} \left(\frac{\partial u_i}{\partial x'_j} + \frac{\partial u_j}{\partial x'_i} + \frac{\partial u_k}{\partial x'_i} \frac{\partial u_k}{\partial x'_j} \right) \quad (\text{A.4})$$

A.3. Stress

Stress occurs when forces are applied to a body that is constrained. It can be defined as a measure of the internal force intensity developed within a body in response to external forces. The importance of determining the stresses of a body is crucial to describe a force balance or to assess material failure. The simplest way of defining stress is in the deformed state as the force scaled by the cross-section area, and this stress is known as the Cauchy stress tensor (σ). It is a symmetric stress tensor and allows to calculate the force per unit area ($\mathbf{t}$) for a given direction ($\mathbf{n}$) as:

$$\mathbf{t} = \mathbf{n} \cdot \sigma \quad (\text{A.5})$$

With $\mathbf{t} = \frac{d\mathbf{f}}{dA}$ the ratio of the current force and the current cross-section area, and $\mathbf{n}$ the normal to dA , expressed in the current (deformed) configuration.

Cauchy's stress tensor is not practical to use for large deformation analysis or experimental measures. Therefore, an alternative definition of stress must be defined. One of those alternatives is the 1st Piola-Kirchhoff stress tensor ($\mathbf{P}$). It is an asymmetric tensor, defined in the reference configuration and its definition is similar to the one for the Cauchy stress tensor:

$$\mathbf{t}_0 = \mathbf{n}_0 \cdot \mathbf{P} \quad (\text{A.6})$$

With $\mathbf{t}_0 = \frac{d\mathbf{f}}{dA_0}$ the ratio of the current force and the current cross-section area, and $\mathbf{n}_0$ the normal to dA_0 , expressed in the reference or original configuration.

Table 17 shows the conversion formulas between the Cauchy stress and the first Piola-Kirchhoff stress. Note that here the deformation gradient (F) and its jacobian (J) are used to obtain the results.

Table 17: Conversion formulas relating the Cauchy stress (σ) and the first Piola-Kirchhoff stress ($\mathbf{P}$)

From	To		
	σ	$\mathbf{P}$	
	σ	$J\mathbf{F}^{-1}\sigma$	
	$\mathbf{P}$	$J^{-1}\mathbf{F}\mathbf{P}$	$\mathbf{P}$

APPENDIX B: Standard Travels

Section 5.2.2.2. recorded the results and discussion about the standard travels graphs obtained for the different materials studied. In that section the results obtained for Dacron were already presented. This appendix gathers the standard travel grafts for one representative sample of each homograft, Gore-Tex and Vectra.

Homograft

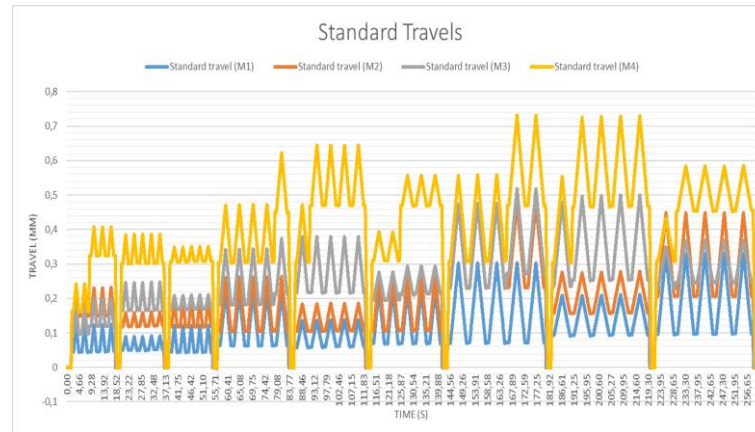


Figure 61: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a homograft (H5) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step

Gore-Tex

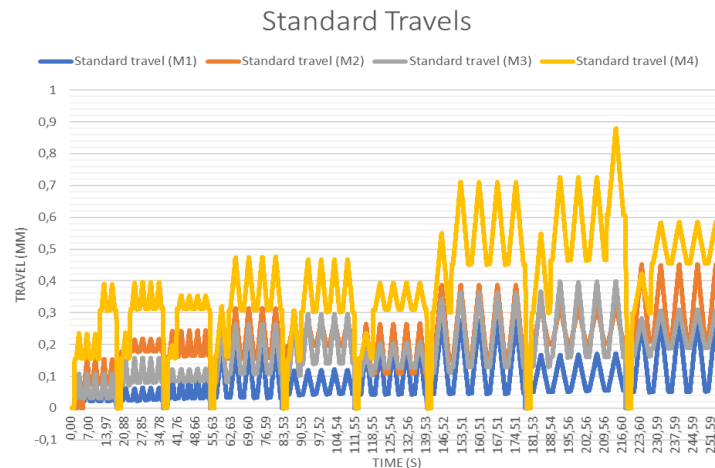


Figure 62: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Gore-Tex (G4) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\varepsilon_1:\varepsilon_3$) 1:1, 1:2 and 2:1 for each step

Vectra

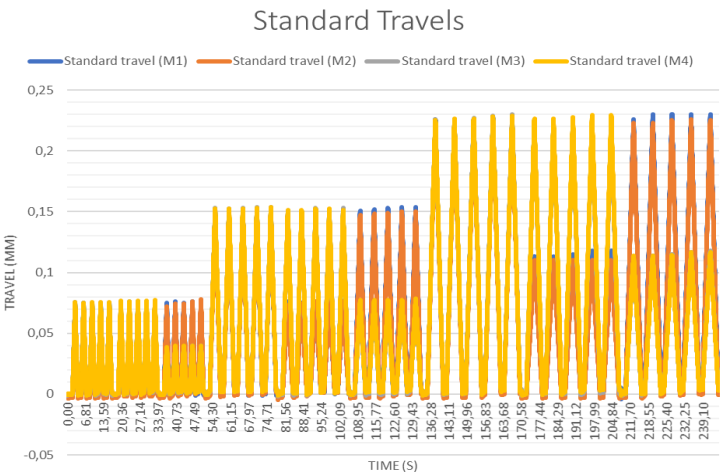


Figure 63: Displacement (mm) vs time (s) registered in each loading cell of the biaxial tester set-up M1, M2, M3 and M4 for a Vectra (V14) sample. Low strain protocol is presented with 3 different loading steps 2.5, 5 and 7.5 % of strain and 3 loading sets of ratios ($\epsilon_1:\epsilon_3$) 1:1, 1:2 and 2:1 for each step

APPENDIX C: Polymers and Elastomers Overview

The goal of this appendix is to provide an overview of two classes of materials from which the arterial tissue takes some of its properties. Material selection is a key step in the design of new objects. So, the insight presented in this section will help the reader to better understand some of the behaviours observed in the materials tested in this study but also to start thinking about the properties and components that a new synthetic graft might present. The focus will be set in the mechanical properties of these groups of materials. Since this is not an exhaustive explanation the interested reader is referred to [95], [96] and [97].

C.1. Polymers

For many polymeric materials, simple stress-strain tensile tests are employed for the characterisation of their main mechanical parameters (modulus of elasticity and yield and tensile strengths). These family of materials is generally highly sensitive to the rate of deformation (strain rate), the temperature and the chemical nature of the environment (the presence of water, oxygen, organic solvents, etc).

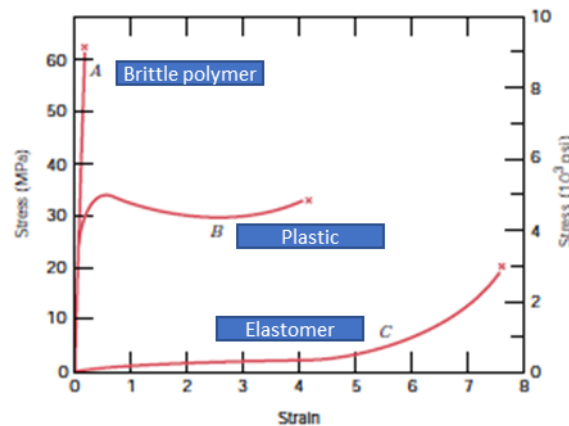


Figure 64: Stress (MPa) vs strain (-) curves for brittle polymer (A), plastic (B) and highly elastic or elastomeric (C) behaviours [95]

Three typically different types of stress-strain behaviour that are found for polymeric materials are presented in Figure 64. The brittle polymer fractures while deforming elastically. The plastic material stress-strain curve is similar to that found for many metallic materials. Initial elastic deformation followed by yielding and a region of plastic deformation. Finally, the class that mimics the best the arterial behaviour, the elastomers, present a stress-strain curve rubberlike, meaning that it presents large recoverable strains produced at low stress levels.

Table 18 presents the modulus of elasticity and the ductility, understood as the percent of elongation at break, of different polymer-based materials. The arterial modulus of elasticity has a value around 1 MPa [93], so it can be noticed that this family of materials is way stiffer. This behaviour has been already recorded in the results of the present study.

Table 18: Modulus of elasticity (GPa) and deformation to failure or ductility (%) for different types of polymers [95]

	Modulus of elasticity (GPa)	Ductility (%)
Nylon 6.6.	1,58 – 3,80	15 - 300
Polycarbonate (PC)	2,38	110 - 150

	Modulus of elasticity (GPa)	Ductility (%)
Polyester (PET)	2,76 – 4,14	30 -300
Polytetrafluoroethylene (PTFE)	0,40 – 0,55	200 - 400
Polyethylene low density (LDPE)	0,17 -0,28	100 - 650
Polymethyl Methacrylate (PMMA)	2,24 – 3,24	2,0 – 5,5

The mechanical characteristics of polymers are much more sensitive to temperature changes within the vicinity of room temperature. Taking the polymethyl methacrylate (Plexiglas) to describe the effect of increasing the temperature the following features are worth to mention: elastic modulus decreases, there is a reduction of the tensile strength and there is an enhancement of the ductility, the material is considerable deformable at 50-60°C. It has, also, to be mentioned that, in general, decreasing the deformation rate has the same influence on the stress-strain characteristics as increasing the temperature, that is, the material becomes softer and more ductile.

Finally, a particular characteristic exhibit by the polymers is viscoelasticity. They present an intermediate range of properties between an elastic behaviour and a viscous liquid. The viscoelasticity infers a time-dependent behaviour on the material properties, that is, while under a constant stress, a viscous body strains at a constant rate, when the load is removed the material remains in deformed state. To evaluate the influence of this property dynamic tests are applied as creep test, where at a continuous temperature the stretch of the material can be recorded, and it can be observed how the inner stresses relax with loads maintained over time.

C.2. Elastomers

According with the ISO 1382:1996 an elastomer is a high molar mass material which when deformed at room temperature reverts quickly to nearly original size and form when the load causing the deformation has been removed. These materials are strongly influenced by the type of loading, e.g. whether it is static or dynamic.

Polyurethanes (PUs) are the most well-known polymers used to make foams. Ester- based PUs are used to produce elastomers, flexible foams and coatings [96]. Their structure is highly influenced by intermolecular forces and there may be crystalline regions between flexible chains, that increase their stiffness. In a loading cycle, as the strain is increased, intermolecular interactions in the PU structure become more favourably and thus the stress required for any particular strain increases. The stress-strain curves of some polyurethane foams have been already presented in Figure 56 (right).

The values of the tensile strength of rubbers and thermoplastic elastomers are taken as satisfactory for mechanical design in a range of 7-15 MPa, and excellent when they exceed the 15 MPa [97]. The values of elongation at break vary in the range of 300-1000%. This material family also presents viscoelastic behaviour, but that property is less marked than it is for polymers.

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