UNIVERSITY OF PATRAS
M.Sc. BIOMEDICAL ENGINEERING
DEPARTMENTS OF ELECTRICAL AND COMPUTER
ENGINEERING, COMPUTER ENGINEERING & INFORMATICS,
MECHANICAL ENGINEERING & AERONAUTICS,
SCHOOL OF MEDICINE

MSc Thesis
Of the Biomedical Engineering Master of Science Student
Andreas Dimopoulos
A.M.: 1069924

‘’A novel polymeric fibrous microstructured biodegradable small caliber tubular scaffold for cardiovascular tissue engineering’’

Supervisor: Prof. Dimosthenis Mavrilas, Dept. of Mechanical Engineering & Aeronautics, University of Patras, GR

Patras 27/01/2021
Preface

This Master Thesis was written in the frame of Master program in Biomedical Engineering (BME), University of Patras, GREECE, the academic year 2020-2021.

The main theme deals with the design and biomechanical evaluation of a novel polymeric fibrous microstructured biodegradable small caliber tubular scaffold, for cardiovascular tissue engineering.

The thesis is divided into 7 parts. The part 1 refers to introductory chapters needed for the implementation of the work, part 2 presents the materials and methods used to conduct the experiments. In the 3rd and the 4th are presented the results of the research as well as their comparison with the results of reports. End in the 5th, 6th and 7th the conclusion discussion and bibliography respectively.

This work was supervised by Prof. Dimosthenis Mavrilas, Dept. of Mechanical Engineering & Aeronautics, University of Patras. I would like to special acknowledge him, for all the opportunities the patient advice, valuable help, and his special academic revision that gave me.

The other two members of the advising and examination committee are: Prof. Despina Deligianni, Dept. of Mechanical Engineering & Aeronautics and Prof. Konstantinos Moustakas, Dept. of Electrical & Computer Engineering, University of Patras.

This Master thesis could not be completed without the active advice of Dr. Dionysios Markatos, PostDoc., University of Patras, to whom I want to acknowledge my special appreciation.

In addition i would like to thank the laboratory PhD student Athina Mitropoulou for her revision under the in vitro biodegradation experiments.

I also appreciate the advice and help of Prof. Efstratios Koletsis, Cardiothoracic Surgery, Dept. of medicine & University Hospital, Patras, as well Prof. Petros Koutsoukos, Department of Chemical Engineering, University of Patras and his laboratory PhD student Giota Natsi for the support and the helpful advises.

Lastly couldn’t forget to thank my family and all of my friends, who believed and encouraged me, at every stage of my studies.
CONTENTS

Preface ................................................................................................................................. 3
Contents ................................................................................................................................. 4
Figures .................................................................................................................................. 6
Tables .................................................................................................................................. 8
ABSTRACT ............................................................................................................................. 9
ΠΕΡΙΛΗΨΗ ............................................................................................................................. 10
1. INTRODUCTION .............................................................................................................. 12
  1.1. Cardiovascular System ............................................................................................... 15
    1.1.1. The Heart .............................................................................................................. 16
    1.1.2. Blood vessels ........................................................................................................ 16
    1.1.3. Structure of Blood Vessels .................................................................................. 18
  1.2. Cardiovascular diseases ............................................................................................. 19
    1.2.1. Atherosclerosis .................................................................................................... 19
    1.2.2. Coronary heart disease ....................................................................................... 20
  1.3. Biomaterials ............................................................................................................... 20
    1.3.1. Biomaterials generations ..................................................................................... 21
      1.3.1.1. First generation ............................................................................................... 21
      1.3.1.2. Second generation ......................................................................................... 21
      1.3.1.3. Third generation ............................................................................................ 21
    1.3.2. Biomaterial categories ......................................................................................... 22
      1.3.2.1. Metallic .......................................................................................................... 22
      1.3.2.2. Ceramic .......................................................................................................... 23
      1.3.2.3. Composite ...................................................................................................... 23
      1.3.2.4. Polymers ........................................................................................................ 23
  1.4. Scaffolds ...................................................................................................................... 26
    1.4.1. Natural scaffolds .................................................................................................. 27
1.4.2. Synthetic scaffolds ................................................................. 27
1.4.2.1. Polymeric scaffolds ......................................................... 28
1.4.2.2. Biodegradable polymeric scaffolds .................................... 29
1.4.2.3. Permanent materials ....................................................... 30
1.4.3. Scaffold properties ............................................................... 30
1.4.3.1. Biocompatibility ............................................................... 31
1.4.3.2. Bioactivity ........................................................................ 31
1.4.3.3. Biodegradation ................................................................. 32
1.4.3.4. Mechanical properties ...................................................... 33
1.4.3.5. Structure ......................................................................... 33
1.4.3.6. Functional Requirements for Vascular Grafts ......................... 34
1.5. Electrospinning ..................................................................... 35
1.5.1. Application of Electrospun Scaffolds in TEVG ....................... 36
2. MATERIALS AND METHODS ......................................................... 37
   Preparation of the Polymer Solution ........................................... 37
2.1. Scaffold fabrication by electrospinning ..................................... 38
2.2. Scaffold morphology assessment ............................................ 39
2.3. Contact angle measurements ................................................ 40
2.4. In Vitro Degradation Tests ..................................................... 40
2.5. Fabrication of electrospun tubular scaffolds ............................. 41
2.6. Mechanical characterization .................................................... 42
2.6.1. Uniaxial Tests .................................................................... 42
2.6.2. Suture Retention Strength Tests .......................................... 43
2.6.3. Cyclic Tensile Tests ........................................................... 43
2.6.4. Stress Relaxation Tests ....................................................... 44
2.6.5. Radial Compliance Measurements ...................................... 45
3. RESULTS .................................................................................. 46
3.1. Electrospun scaffold morphology .......................................... 46
3.2. Wettability of the scaffold ................................................................. 48
3.3. In Vitro Degradation Results ............................................................. 49
3.4. Mechanical Tests .............................................................................. 51
   3.4.1. Uniaxial Tests ............................................................................ 51
   3.4.2. Suture Retention Strength .......................................................... 52
   3.4.3. Cyclic Tensile Tests ................................................................. 52
   3.4.4. Stress Relaxation ...................................................................... 53
   3.4.5. Radial Compliance Measurements ............................................ 54
4. SUMMARY OF THE RESULTS AND COMPARISON WITH NATURAL VESSELS .................................................................... 55
5. DISCUSSION ........................................................................................... 59
6. CONCLUSIONS ....................................................................................... 61
7. BIBLIOGRAPHY ..................................................................................... 62
8. ANNEXES ............................................................................................... 71

FIGURES

Figure 1 Human heart ................................................................................. 16
Figure 2 Coronary arteries ........................................................................... 17
Figure 3 Schematic cross-sectional view of a blood vessel structure. Obtained from [27]. ........................................................................................................... 18
Figure 4 Comparison of a normal and a atherosclerotic blood vessel .......... 20
Figure 5 Application of metallic biomaterials .............................................. 22
Figure 6 Dental implant application ......................................................... 23
Figure 7 Application of polymeric biomaterials .......................................... 24
Figure 8 Examples of scaffolds implantation .............................................. 26
Figure 9 The electrospinning experimental setup ....................................... 35
Figure 10  PCL pellets and Acetic acid ......................................................... 38
Figure 11  The electrospinning process .......................................................... 39
Figure 12  Contact angle meter ........................................................................ 40
Figure 13  In vitro accelerate degradation bioreactor .......................................... 41
Figure 14  The multilayered polymeric vessel .................................................... 41
Figure 15  Bench type miniature tensile tester ................................................... 42
Figure 16  Experimental process for suture retention strength tests ....................... 43
Figure 17  Experimental setup for cyclic tensile tests .......................................... 44
Figure 18  Experimental setup for the radial compliance measurements ............... 45
Figure 19  SEM images of the micro-nano structured electrospun scaffolds .......... 46
Figure 20  SEM image of the micro-nano structured electrospun scaffolds .......... 47
Figure 21  SEM image for the estimation of fiber diameter ................................... 47
Figure 22  SEM image for the estimation of pore size .......................................... 48
Figure 23  Contact angle estimation for PCL membranes ...................................... 49
Figure 24  Degradation rate (% remaining mass) of the electrospun PCL membranes ... 50
Figure 25  The PCL scaffold after a) 1st week and b) 28th week of insertion into the PBS ...................................................................................................................... 50
Figure 26  Stress-strain plot of the electrospun PCL membranes a) parallel and b) perpendicular to the fiber axis ................................................................. 51
Figure 27  Load-time plot of the sutured PCL electrospun scaffold ....................... 52
Figure 28  Stress-Strain cycle of the tubular polymeric vessel: a) 1-90th cycle and 2) 90-200th cycle ........................................................................................................ 52
Figure 29  Stress relaxation profile of the polymeric tubular vessel ...................... 53
Figure 30  The pressure-diameter profile of the electrospun tubular vessel .......... 54
Figure 31  Comparison of fiber diameter results with natural vessels .................. 55
Figure 32  Comparison of lumen diameter results with natural vessels ............... 55
Figure 33  Comparison of wall thickness results with natural vessels ................. 56
Figure 34  Comparison of Young modulus perpendicular to the fiber alignment results with natural vessels ................................................................. 56
Figure 35 Comparison of Young modulus parallel to the fiber alignment results with natural vessels

Figure 36 Comparison of elongation at break results with natural vessels

Figure 37 Comparison of compliance results with natural vessels

Figure 38 Comparison of suture retention results with natural vessels

TABLES

Table 1 Electrospinning parameters
Table 2 Estimation of fiber diameter
Table 3 Estimation of pore size
Table 4 Contact angle measurements for PCL membranes
Table 5 Radial compliance values at four different pressure ranges (AVG±STD)
Table 6 Biodegradation results
Table 7 Young modulus perpendicular to the fiber alignment results
Table 8 Young modulus parallel to the fiber alignment results
Table 9 Suture Retention Strength results
Table 10 Cyclic Tensile Tests results
Table 11 Radial Compliance Measurements
ABSTRACT

Increasing morbidity of cardiovascular diseases in modern society has made it crucial to develop small caliber arterial grafts, as alternatives to the gold standard autologous implants, to replace diseased coronary arteries. Synthetic small caliber grafts are still not in use due to increased risk of restenosis, lack of lumen re-endothelialization and mechanical mismatch, leading sometimes either to graft failure or to unsuccessful remodeling and pathology of the distal parts of the anastomosed healthy vascular tissues.

In this work, we aimed to synthesize small caliber polymeric (polycaprolactone) tissue-engineered vascular scaffolds that mimic the structure and biomechanics of natural vessels. Electrospinning was implemented to prepare micro structured polymeric membranes with controlled parallel fiber alignment. Consequently, we designed small caliber multilayer anisotropic biodegradable nanofibrous tubular scaffolds, giving attention to their radial compliance.

Polycaprolactone scaffold morphology and mechanical properties were assessed, quantified and compared with those of native vessels and commercial synthetic grafts. Results showed a highly hydrophobic scaffold material with a 3-layered tubular morphology, 4 mm internal diameter/0.25 ± 0.09 mm thickness, consisting of predominantly axially aligned thin (1.156 ± 0.447 um), homogeneous and continuous microfibers, with adequate (17.702 ± 5.369 um) pore size. Mechanical anisotropy was attained as a result, almost one order of magnitude difference for the elastic modulus (18±3 MPa axially/1±0.3 MPa circumferentially), similar to that of natural arterial walls. Furthermore, a desirable (physiological) radial compliance (5.04±0.82%, within the physiological pressure range) as well as cyclic stability of the tubular scaffold were achieved.
ΠΕΡΙΛΗΨΗ
Η αυξημένη καρδιοαγγειακή νοσηρότητα έχει οδηγήσει σε προσπάθειες παρασκευής αγγειακών μοσχευμάτων ως εναλλακτική των αυτόλογων μοσχευμάτων που δύναται να χρησιμοποιηθούν για την αντικατάσταση νοσούντων αγγειών.
Ο αυξημένος κίνδυνος επαναστένωσης, η απουσία επιθηλιοποίησης και η μηχανική αναντιστοιχία παραμένουν τα κύρια προβλήματα που οδηγούν σε αποτυχία της παρασκευής αγγειακών μοσχευμάτων. Προσπαθήσαμε να συνθέσουμε ένα μικρής διαμέτρου πολυμερικό αγγειακό ικρίωμα που μιμείται τη δομή και τις εμβιομηχανικές ιδιότητες των γηγενών αγγειών.
Χρησιμοποιήθηκε η ηλεκτροστατική ινοποίηση για τη δημιουργία πολυμερικών μεμβρανών με ελεγχόμενη ενδογραμμισμό των επιμέρους ινών. Ακολούθως σχεδίασαμε μικρής διαμέτρου πολυστρωματικά μοσχευμάτα αγγειακών ακρίων μοσχευμάτων δίνοντας ιδιαίτερη σημασία στην ακτινική τους ενδοτικότητα. Εκτιμήσαμε τα μορφολογικά χαρακτηριστικά και τις μηχανικές τους ιδιότητες τις οποίες και συγκρίναμε με τα αντίστοιχα των γηγενών αγγειών και των υπαρχόντων συνθετικών μοσχευμάτων.
Τα αποτελέσματα έδειξαν ένα υψηλά υδρόφοβο ικρίωμα με κυλινδρική μορφολογία τριών στρωμάτων με εσωτερικό διάμετρο 4 mm, πάχους 0.25±0.09 mm αποτελούμενο από ευθυγραμμισμένες ομοιογενείς λεπτού πάχους (1.156 ± 0.447 μμ) μικροίνες με επαρχιακό διαστάσεων πόρους (17.702 ± 5.369 μμ) που θα μπορούσαν να επιτρέψουν την κυτταρική διείσδυση in vivo και ενδεχόμενα την επιθηλιοποίηση.
Η βιοδιάσπαση του υλικού in vitro έδειξε απώλεια μάζας της τάξης του 5% εντός χρονικού διαστήματος 17-25 εβδομάδων. Επίσης αποδείχθηκε ότι τα ικριώματα/μοσχευμάτα είχαν επαρκή μηχανική ανισοτροπία (18±3 MPa κατά μήκος/1±0.3 MPa κυκλοτερω), αντίστοιχη αυτής των γηγενών αγγειών και καλύτερη αυτής των συνθετικών. Τέλος η κυκλική σταθερότητα της κυλινδρικής δομής απεδείχθη διατηρήσιμη.
Τα αποτελέσματα είναι πολύ ενθαρρυντικά σχετικά με τη χρήση της ηλεκτροστατικής ινοποίησης για την κατασκευή αγγειακών μοσχευμάτων για την αντικατάσταση αγγειών μικρής διαμέτρου. Το κύριο πλεονέκτημα στη συγκεκριμένη περίπτωση είναι η άριστη
ακτινική ενδοτικότητα που προσομοιάζει αυτή των γηγενών αγγείων. Ο συνδυασμός με υδρόφιλα πολυμερή θα μπορούσε να βελτιώσει περαιτέρω τη δυνατότητα επιθηλιοποίησης.
1. INTRODUCTION

Cardiovascular diseases (CVDs) are the main cause of death in industrialized countries and among the top three worldwide. In Europe, 3.9 million deaths annually (1.8 million in the EU alone) are owed to CVDs, while the total costs are calculated for the EU in 210 billion euros annually (2017 statistics) [1]. The increasing need for surgical therapies of cardiovascular diseases in the modern society has made it crucial, among others, to develop blood vessel substitutes especially for diseased small caliber vessels (less than 6mm).

Although autologous vessels remain the gold standard for small caliber grafts, inability to use autografts due to vessel pathologies or previous surgical interventions, can limit their clinical application. On the other hand, limited availability of homografts due to unavailable tissue donors makes sometimes implantation of living blood vessels a difficult task [2, 3].

Prosthetic grafts, such as polytetrafluoroethylene (ePTFE, a.k.a. Gore-Tex) and poly(ethylene terephthalate) (PET, a.k.a. Dacron), have been clinically used alternatively as replacements of large diameter arteries. However, restenosis, infection, thrombosis, lack of reendothelialization, compliance and other biomechanical mismatches to the anastomosed native blood vessels are common problems within these synthetic grafts [4-8]. Among others, biomechanical properties, radial compliance, suture retention strength and cyclic stability are critical properties for a prolonged graft patency. Mismatch in radial compliance (a measure of the graft diameter changes under a pressure range) between the graft and the end to end anastomosed native vessel, may cause, among other problems, local increase in shear stress disturbances that may induce intimal hyperplasia and ultimately graft failure [9].

Over the last few decades, vascular tissue engineering (VTE) has emerged as an alternative approach to overcome the limitations of the afore-mentioned vascular grafts [10]. An ideal tissue-engineered vascular scaffold should completely integrate with the surrounding vessels and function as a 3D framework for host infiltration of undifferentiated mesenchymal stem cells (MSCs) as well as differentiated cells (endothelial, smooth muscle
cells and fibroblasts), in order to fabricate the vascular extracellular matrix (ECM) in vivo. To provide an adequate 3D structure and enable full integration into the site of the damaged vessels, a biomimetic scaffold should fulfill the following requirements: a) mimic the physicochemical and biochemical properties of natural blood vessels ECM, b) be biocompatible, c) demonstrate enough porosity and porous size, to allow host cell infiltration and endovasculature formation when needed, avoiding however hemorrhage, d) present appropriate mechanical properties (e.g. matching radial compliance to the native vessels, high suture retention strength and cyclic stability), e) degrade safely in synchronization with the newly growing tissue [11, 12].

Acellular xenogeneic extracellular matrix scaffolds have been utilized for VTE applications. Different decellularization techniques, like glutaraldehyde or alternative agent tissue fixation have been used since 60ties for biological heart valves. However, tissue calcification, leading to stiffening and severe dysfunction is a common problem following tissue fixation. The lack of any regeneration ability makes the fixed tissues not suitable candidates for VTE. Non-fixed xenogeneic tissues, although used in different cardiovascular applications are not currently used for the construction of small caliber blood vessels due to a combination of factors needed to overcome all failure mechanisms [13].

Synthetic biodegradable polymeric scaffolds are an alternative for scaffold design. The freedom to utilize polymer synthesis and 2D or 3D construction makes them challenging to design a scaffold with predetermined geometry and internal structure. Hydrophobic and hydrophilic polymers can be used in different combinations to successfully achieve final geometrical, structural, biomechanical and biocompatibility characteristics of a small caliber VTE scaffold.

Many research groups have focused on the design of VTE grafts that mimic the structure of natural blood vessels by using different scaffold fabrication techniques such as molecular self-assembly, solvent-casting/particulate-leaching technique, thermally induced phase separation, and electrospinning [14]. In recent years, the interest on the electrospun scaffolds for the development of VTE has increased tremendously [15]. Although studies on this field are currently in the early stages, recent reports have
highlighted the potential use of electrospun scaffolds due to their tunable mechanical and biological properties [16-21]. Among others, electrospinning has a great potential to mimic vascular tissue ECM architecture, composed of 3-layer organized 50-500 nm diameter fibrous proteins. Electrospinning offers the ability to fine-tune mechanical properties during the fabrication process, while also controlling the necessary biocompatibility and structure of the tissue engineered grafts. The ability of electrospinning technique to combine the advantages of synthetic and natural materials makes it attractive for tissue engineering applications where a high mechanical durability, in terms of cyclic stability and radial compliance, is required. Furthermore, incorporation of natural polymers, with abundance of cell binding sites, can promote the formation of a continuous monolayer of EC in the lumen and proliferation of other cell types in the matrix of the graft’s wall. The electrospinning technique also offers precise control over the composition, dimension, and the alignment of fibers that have impact on the porosity, pore size distribution and the architecture of scaffolds. This method allows for engineering of a wide range of tunable structural and mechanical properties as required for specific applications.

The mechanical properties of electrospun scaffolds are controlled by changing various microstructural parameters such as fiber diameter, porosity and alignment. In addition to uniaxial tensile properties, the burst strength and compliance are also among the important mechanical properties for vascular grafts. However, these latter properties are rarely measured and reported. Even though electrospinning process has numerous advantages over other methods, challenges still remain that need to be overcome prior to the clinical use of electrospinning in tissue-engineered vascular grafts (TEVG). The major issues associated for the application of electrospun scaffolds, particularly for TEVG include: lack of adequate cell penetration in thick scaffolds due to small pore sizes; poor surface properties that may have negative impact on the cell viability, proliferation and growth; shortage of favorable cells; poor control over mechanical properties and degradation; and biological response of the tissues/cells towards TEVGs [10, 22]. On the one hand, robust physical and mechanical properties, including burst strength, water permeability and suture strength, are required for grafting. On the other hand, high compliance is critical, because the compliance mismatch between a vascular graft and neighboring arteries at the site of
anastomosis is a major cause of graft failure [10, 15]. Thus, selecting an appropriate polymer is one of the most essential parameters of vascular graft tissue engineering to overcome the above limitations.

Tissue-engineered vascular grafts from electrospun PET-PU [23] or polylactine/gelatin [24] have been proposed as small-diameter vessel substitutes, with satisfying biological and mechanical characteristics. Among other biocompatible biodegraded polymers, PCL has been proven ideal as a long-term implant material for the production of blood vessel scaffolds [10, 15] due to its slow degradation rate (>2.5 years) in human body. PCL chemically degrades due hydrolytic cleavage of the back-bone ester bonds and thereby converting long polymer chains into shorter water-soluble fragments. Electrospun membranes made of polycaprolactone (PCL) and its composites are widely used in biomedical applications, especially as scaffolds in tissue-engineering applications and have been shown to possess excellent biomechanical features and structural similarity with the extracellular matrix (ECM) of blood vessels [9, 10, 15].

The current Master Thesis is a part of a pilot study towards the design anisotropic biodegradable nanofibrous small caliber polymeric VTE scaffolds that mimic the structure and biomechanics of natural blood vessels, through the electrospinning method, giving emphasis to the radial compliance of the scaffolds. We used polycaprolactone (PCL), a hydrophobic highly biocompatible and biodegradable (FDA approved) aliphatic polyester as the high-strength material of a multilaminate final design that can support the creation of a polymer-cell complex in vitro with subsequent implantation in vivo [25].

1.1. Cardiovascular System

The cardiovascular system is an organ system that is responsible for supplying oxygen to the body as well as for transporting and exchanging substances in its cells. It consists of the heart, veins, arteries and capillaries. These organs constantly recycle blood in the body.
1.1.1. The Heart

The heart is the center of the circulatory system. It acts as a pump and drains oxygenated blood from the lungs to all tissues of the body through the arterial tree while in parallel recirculate the non-oxygenated blood from vein system to the lungs. It is located inside the thoracic cavity between the two lungs. The cardiac muscle is pulsed within a frequency range 60-150 beats per minute and weigh 250 to 350 grams.

![Human heart](image)

*Figure 1 Human heart*

The heart mainly consisted of three tissues: a thin connective tissue (the pericardium), the main muscular tissue (myocardium), while in its last layer we meet the epithelial cells’ monolayer (endocardium) that constitute the interface between myocardium and blood circulating inside the heart chambers. The interior of the heart consists of four muscular cavities (chambers), two atria and two ventricles and four unidirectional tissue valves. It is divided into two parts, the left and the right, which are divided by the presence of a muscular septum, the interventricular and a special membrane, the vaginal septum. Through this separation the prevention of mixing of the arterial blood with the venous is achieved.

1.1.2. Blood vessels

The circulatory system includes three types of blood vessels that carry blood to the body throughout the heart. These are the arteries, capillaries and veins.
Arteries

The arteries are responsible for transporting blood from the heart to the tissues. They are divided into three categories, the elastic, the muscular and the arterial. The wall of the arteries is thicker than that of the veins, so that it can compensate for the strong pressures they receive.

Coronary arteries

The coronary arteries protrude and branch from the base of the aorta, above the aortic valve and they spread all over the surface of the heart.

Figure 2 Coronary arteries

Each coronary artery has a trunk and many branches. The smaller branches penetrate the heart muscle from the outer surface to the inner surface of the heart cavities, transporting blood to the myocardial cells. The smaller arteries branch into even smaller vessels and finally into capillaries.
Capillary vessels

The capillaries are inserted between arteries and veins and supply almost every tissue in the body. They are the thinnest blood vessels and consist of a single layer of epithelial cells.

Veins

Veins are responsible for transporting non-oxygenated blood from the tissues to the heart. They are larger than the arteries and contain less muscle and elastic tissue.

1.1.3. Structure of Blood Vessels

The native blood vessels in the human body have complex structures with distinct features. The arterial wall is composed of three different layers: (i) Tunica intima, (ii) Tunica media and (iii) Tunica adventitia, (Figure 3). Tunica intima, the innermost of three concentric layers, consists of a continuous monolayer of endothelial cells (ECs) directly attached to the basement membrane which is mainly comprised of connective tissues. Tunica media is the middle layer comprised of dense populations of concentrically organized smooth muscle cells (SMCs) with fibers or bands of elastic tissues. Tunica adventitia is consisted of a collagenous ECM that mainly contains fibroblasts and perivascular nerve cells. An internal and an external elastic lamina separate the intima, media and adventitia from each other. Each layer serves a specific function: the collagenous adventitia functions to add rigidity while the elastic lamina provides elasticity to the vessel walls [26].

Figure 3 Schematic cross-sectional view of a blood vessel structure. Obtained from [27].
1.2. Cardiovascular diseases

Cardiovascular diseases may also arise due to rheumatic fever and infectious endocarditis. High blood pressure and myocardial infarction could indirectly affect heart function. In terms of cardiovascular diseases, atherosclerosis and coronary heart disease are the most common, as well as deep vein thrombosis and pulmonary embolism. Peripheral arterial disease and cerebrovascular disease constitute two more common CVDs.

1.2.1. Atherosclerosis

Atherosclerosis is characterized by cellular necrosis, inflammation, and complexes of lipoproteins and phospholipids that promote calcium deposition in atherosclerotic lesions leading progressively to stenosis or total vascular lumen infraction, resulting to myocardial infarction areas.

It is a pathological process that takes place in elastic arteries such as the coronary arteries and is the underlying cause of cardiovascular dysfunction, stroke and peripheral arterial disease. In the early stages, we have the damage of the endothelium of the-arterial lumen, followed by deposition of oxidized low-density lipoprotein, in the form of lipoid lines. Activated monocytes enter the endothelial region, where they are transformed into macrophages, and then into foam cells. This is followed by proliferation of the smooth muscle fibers of the sheath, and production of extracellular connective tissue, through growth factors. Atherosclerotic plaque formed consists of a central nucleus, consisting mainly of esterified cholesterol in the form of crystals, which is surrounded by a fibrous capsule. The most advanced atherosclerotic lesions are called fibrous plaques [28]. In human arteries, fibrous plaques develop into complex atherosclerotic lesions that are prone to rupture [28]. The development of atherosclerotic lesions in human arteries can be considered as a modified form of chronic inflammation. The initial key event seems to be endothelial cell damage. Areas with high shear stress present high risk of being offended of atherosclerosis. Atherosclerosis is a chronic inflammatory condition that turns into an acute clinical phenomenon through plaque rupture and thrombosis.
1.2.2. Coronary heart disease

Coronary heart disease is the most common cause of morbidity and mortality. It is often complicated by atherosclerotic plaque rupture and thrombosis, leading to a coronary episode. Created by the form of cholesterol-rich atherosclerotic plaques in the wall of the epicardial coronary arteries, resulting in narrowing of their lumen and obstruction of blood flow through them. Therefore, when blood reaches the myocardium through the coronary artery, it is not enough for the heart to function properly and then heart ischemia and pain occur. The presence of calcium deposits in the vascular wall is indicative of advanced atherosclerosis and the degree of coronary calcification adds serious prognostic significance to conventional risk factors for coronary heart disease (STD) [29].

![Comparison of a normal and a atherosclerotic blood vessel](image)

**Figure 4** Comparison of a normal and a atherosclerotic blood vessel

Risk factors that contribute to the likelihood of developing coronary heart disease are: smoking, sedentary lifestyle - lack of physical activity, stress, family history, alcohol abuse, obesity, hypertension, high blood lipids, diabetes. This disease can lead to angina, acute myocardial infarction, heart failure and sudden death. Common methods of treatment are medication, balloon angioplasty and coronary artery bypass grafting.

1.3. Biomaterials

The term biomaterials refer to materials of natural or artificial origin that meet biological systems (tissue, blood or biological fluids) for medical, therapeutic or diagnostic purposes. They have helped the quality of humans’ life as there is the possibility of replacing or treating a pathological and/or malfunctioning tissue or organ. Biomaterials are used for the design and construction of artificial implants into the human body, such as
heart valves, dental implants, intraocular lenses, joint ligament replacements, and artery or tissue implants, etc. That materials necessarily have to be with the organism that come in contact so that they are accepted by its biological system for its proper functioning. These unique features are also useful in many, other than organ implants, biomedical applications, such as building biosensors, scaffolding for tissue engineering, neural signal detectors, drug delivery systems, and bioactivators.

1.3.1. Biomaterials generations

1.3.1.1. First generation

The first generation of biomaterials in the period 1940 to 1950 included mainly metal materials, alloys and polyester polymers. Characteristic of these materials was biotolerance, their ability to be tolerated by the body, without necessarily being chemically bonded to tissues, and to meet mainly mechanical needs. In general, these materials were not toxic to the organism, but their biocompatibility with the biological environment was restricted [30].

1.3.1.2. Second generation

In the second generation of biomaterials in the period 1960 to 1990 belong the materials that are more bioactive and biocompatible with the human body, which is why they were called "pharmaceutical grade". These materials were intended to permanently repair the damage and be bioabsorbable. This category includes surgical sutures, grids, etc. However, for most prostheses full bioabsorption was not achieved and thus the patient ended up in need of replacement, usually after ten years expiration. Although these materials grow better and are non-toxic, they are not fully accepted by tissues [30].

1.3.1.3. Third generation

The third generation of biomaterials, which began to develop after 2000, aims to produce hybrid materials which must not only be biocompatible and biodegradable, but also work with the tissues in the implant site without toxicity and degeneration over time.
They are made based on tissue engineering, which relies on the cultivation of suitable cells and through the signaling biomolecules and biochemical pathways to form the material that meets the environment of the implant site. In biomaterial, which will be implanted in the area of tissue damage, or by stimulating cells in the host area to grow and repair the damage. Many adverse reactions, cellular or histochemical, cause serious implant problems. Research is therefore focused on this area and seeks to understand the chemical, physical and dynamic behavior of the interfaces of these materials in order to produce stable biomaterials [30].

1.3.2. Biomaterial categories

1.3.2.1. Metallic

The most common metal implants are made of stainless steel, cobalt, titanium and gold. They are widely used, due to their resistance to mechanical stress, in Orthopedics and Dentistry, in Maxillofacial Surgery but also in cardiovascular implants such as prosthetic valves [31].

*Figure 5 Application of metallic biomaterials*
1.3.2.2. **Ceramic**

This category includes implants made of alumina, titanium, zirconia, bioactive glasses and hydroxyapatite with basic application in Dentistry. However, their use in other fields of Biomedicine is not as extensive, compared to metal and polymeric biomaterials.

![Figure 6 Dental implant application](image)

1.3.2.3. **Composite**

They are usually a combination of metal, ceramic and polymeric materials for this and are applied in many fields of Medicine. They are ideal as prosthetic limbs as they have low density, weight and high strength [31].

1.3.2.4. **Polymers**

Polymers have been used successfully for more than 20 years in all fields and specifically in the field of Medicine. Most polymeric biomaterials are applied to a variety of biomedical applications such as facial prostheses and tracheal tubes, as pieces of kidney or liver, as well as medical adhesives, sealants and coatings that serve a variety of functions [31].

Many of the synthetic polymers are applied as permanent biomaterials, that is, as biomaterials that are required to carry out their in vivo functional mission for a long time,
even for life. The category of permanent polymers includes methyl polymethacrylate (PMMA), polyethylene (PE), polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), polyamides (nylon), polyurethanes (PU), etc.

![Figure 7 Application of polymeric biomaterials](image)

Metal and ceramic materials are not expected to be suitable for soft tissue development as opposed to polymers used as scaffolds to replace damaged tissue. Most scaffolding materials are synthetic polymers such as:

➢ Polylactic acid, Poly (D, L-lactic acid), PLA

Polylactic acid (PLA) is a biodegradable polyester and is derived from the polymerization of lactic acid. Lactic acid is present in two stereoisomeric forms D and L. L-lactic acid is produced during the metabolism of animal organisms and microorganisms. It is completely non-toxic, as it results from the breakdown of polylactans [32]. Polylactic acid (D, L) has very good properties and is used in tissue engineering, mainly for the manufacture of scaffolding.

➢ Polylactic- Polyglycolic Poly (lactic-co-glycolic acid), PLGA

It results from the copolymerization of polylactic (PLA) and polyglycolic (PGA) acids. It is a crystalline biodegradable polymer, with a high breaking point and low solubility in organic solvents. The presence of polyglycolic acid makes the polymer more hydrophilic. After implantation, the ester bonds are gradually hydrolyzed and the products are metabolized.
➢ Polyethylene glycol, (PEG)

It is also known as polyethylene oxide (PEG) or polyoxomethylene (PEG). It is the most widely used commercial polyether. Characteristic of polyethylene glycol is that it inhibits the absorption of proteins and the adhesion of cells. These properties make it valuable as a coating material, because it reduces the development of inflammation at the implant site. Hydrophilic polyethylene glycol is suitable for the preparation of hydrogels with various possible cross-linking formations for scaffolding.

➢ Hydroxyethyl Methacrylate, (pHEMA)

Hydroxyethyl Methacrylate is derived from the polymerization of hydroxyethylmethyl acrylate (HEMA), a water-soluble monomer, which can be polymerized at temperatures between -20 ºC to +10 ºC. Poly-2-hydroxyethyl methyl acrylate, because it is biocompatible and can be "molded" chemically easily, is used to make contact lenses, corneal implants.

➢ Polycaprolactone - Poly-e-caprolactone, (PCL)

Polycaprolactone (PCL) is a synthetic, linear, semi-crystalline, thermoplastic, biocompatible aliphatic polyester and is formed by polymerization, opening the ring of ε-caprolactone. It is also biodegradable and due to its slow degradation compared to other polymers, it is more suitable for long-term applications. Polycaprolactone and its degradation products, which are acid monomers and can removed from the body through normal metabolic pathways, are non-toxic, although these degradation products can lead to side effects. Polycaprolactone has a glass transition temperature close to -60 ºC, a low melting point - about 60 ºC, depending on its crystallinity - and low viscosity, factors due to its ability to be easily processed. It is used in the field of biomedicine especially in medical devices, in drug delivery systems and as a scaffolding material in Tissue Engineering, where it is often used as the main ingredient for the creation of cartilage and bone tissues. However, the main disadvantages of polycaprolactone are the lack of functional groups and hydrophobicity, which prevents it from protein absorption, cell adhesion and motility. To overcome these obstacles polycaprolactone can be mixed with a natural hydrophilic polymer such as chitosan.
1.4. Scaffolds

The scaffold is the substrate on which new tissue is created to replace, repair or regenerate tissues. It gives the appropriate shape and structure and functionally supports the construction until it acquires stability. Since the development of tissue engineering, the positioning of cells for therapeutic purposes in porous scaffolds from degradable materials is the most common and well-documented approach. This approach represents the main body of biomaterials research in tissue engineering and has led to the enormous development of different types of biomaterials and manufacturing technologies.

![Image of scaffolds implantation](image)

*Figure 8 Examples of scaffolds implantation*

Indeed, modern scaffolds are not only limited to supporting cell growth but can also be implanted with bioactive molecules that will have a specific biological function. In addition, the latest generation scaffolds are intended to be as biomimetic as possible in terms of 3D morphology, mechanical performance, surface chemical treatment and are designed according to these requirements. In order to control the scaffold in vitro, a bioreactor is used, a device or system that, strictly controls environmental and functional conditions, allowing or inducing specific behavior of living cells or tissues. Bioreactor technologies for use in tissue engineering can be used for development of functional cells and tissues for transplantation, as well as for in vitro controlled studies on the regulatory effect of biochemical and mechanical stimuli on the growth of cells and tissues. Tissue growth is affected by four main factors: a) The type of cells to be isolated and used, b) the
growth factors to be used, which help in the proper, desired differentiation of cells, c) the scaffold material and design and d) the mechanical environment in which the scaffold with the cells will be found, which can also facilitate the proper expression of the phenotype.

1.4.1. Natural scaffolds

Scaffolds derived from natural polymers can be used as biomaterials for tissue engineering applications. Natural polymers typically have more complex structures than synthetic polymers. Elastomers can be both natural and synthetic and are thus classified as a common subgroup. Natural polymers include chitosan, collagen, hyaluronic acid, gluten, heparin, etc. Great interest in these natural polymers comes from their biocompatibility, relative abundance and commercial availability, as well as their easy processing. Natural polymers have certain advantages such as bioactivity, but also the ability to bind between the receptor and cells. Of course, this bioactivity also has some disadvantages. These include a strong immunogenic reaction associated with most polymers, any complications associated with their clearance, and the possibility of disease transmission. Natural polymers can be classified into proteins (collagen, elastin, keratin, etc.), polysaccharides (chitin, cellulose, dextran, etc.) and polynucleotides (DNA, RNA). Natural polymers have many advantages, such as bioactivity, the ability to exhibit cell-binding receptors, cell sensitivity - to induce proteolytic degradation, and natural remodeling. But they also have disadvantages, such as a strong immune response, complexity and the ability to transmit the disease. Synthetic polymers on the other hand are biologically inert, have more predictable properties, have specific specifications and lack many of the disadvantages of natural polymers [33].

1.4.2. Synthetic scaffolds

Natural polymers are usually in short supply because they are expensive, suffer from batch-to-batch variants, and are susceptible to cross-contamination by unknown viruses or unwanted diseases. Synthetic polymeric biomaterials have easily controlled physicochemical properties and quality and are not immunogenic. They can also be processed with a variety of techniques and supplied in large quantities. In order to be able
to regulate the physical and mechanical properties of histomechanical scaffolds in a desired part of the human body, the molecular structure and molecular weight can be easily adjusted during the process of their synthesis [34].

### 1.4.2.1. Polymeric scaffolds

The need to address medical problems of the human body related to the insufficiency or improper functioning of tissues and organs, led to the need for tissue production. For the production of tissues it is necessary to use the scaffolds, on which the cells grow. The use of biodegradable polymers for the production of porous scaffolds has been successful in tissue development. In recent decades significant progress has been made in the development of biodegradable (hydrolytically and enzymatically degradable) polymeric materials suitable for biomedical applications. Detachable polymeric biomaterials are preferred for the development of therapeutic devices, such as temporary prostheses, 3D porous structures as scaffolds for tissue engineering, and as controlled drug delivery structures. Each of these applications requires materials with specific physical, chemical, biological and mechanical properties to provide effective treatment. The purpose is for permanent prostheses used in temporary therapy to be replaced by biodegradable devices that could help the body repair and regenerate damaged tissues. The properties of the scaffolding depend on the biomaterial that will be used for their production as well as on the characteristics of their construction. These properties affect the cell differentiation and tissue formation in the scaffold structure. Regarding the morphology of the scaffold, there is a need for 3D scaffolding, as many studies claim that the three-dimensional structures allow cell growth and organization in a space like the parent in terms of cell growth conditions in tissues in vivo.

So far, solutions for the treatment of damaged or non-existent tissues, such as mechanical medical devices or artificial prostheses. Also have disadvantages as they do not repair the damaged tissue and when used for a long time there is a possibility of damage and cause inflammation in the human body. In particular, in cases of transplantation where a healthy organ or tissue is transplanted to a patient, or tissue is transferred from a healthy organ of the patient to another diseased site, there may be incomplete tissue matching. An
additional disadvantage may be the limited number of transplants, due to the number of donors. Thus, in the field of Tissue Engineering they use an alternative method of treatment that aims to create, regenerate or even replace tissues and organs with the use of cells, biomaterials or biologically active molecules. The construction of the tissue can be done in a laboratory and it is either a fully functional tissue at the time of implantation, or a tissue that has the ability to be completed and acquire the desired properties after implantation. For their construction, three-dimensional scaffolds are used, which create the appropriate environment for guiding the new tissue, supporting its growth, spreading and differentiating the implanted cells into cells of the desired tissue and determining the possible space in which the new tissue will develop. [35-36]

1.4.2.2. Biodegradable polymeric scaffolds

The term biodegradable polymers refer to those polymers which are degradable in the biological environment, whether enzymatically or not, by the production of biocompatible or non-toxic by-products [37]. When the polymer is degraded in vivo the products in which it is metabolized are normal metabolites of the organism, or products which can be eliminated by the body, with or without further metabolic transformations, through normal physiological pathways. The basic criteria for selecting biodegradable polymers as biomaterials is that their potential degradation products should be non-toxic, and that both the rate of degradation and the mechanical properties of the material should match the desired result of the application. The mechanical behavior of biodegradable materials depends on their chemical composition, production process, storage, processing and application conditions.

Replacement of non-biodegradable polymers with biodegradable polymers was necessary due to several limitations in their use in short-term medical applications. More specifically, in several cases a second surgery was necessary to remove the implant, while in implants made from biodegradable polymers there is no additional surgery. In addition, the use of biodegradable polymers avoids shielding stress.
Biodegradable polymers can be broadly classified into natural and synthetic, based on their origin as mentioned earlier.

Synthetic biodegradable polymers are divided into polymers derived from renewable raw materials and polymers derived from petrochemical raw materials. Biodegradable polymers produced by nature or by natural processes are classified as natural polymers.

1.4.2.3. Permanent materials

Permanent materials have a wide range of medical applications. They can be used as coatings on medical implants thus improving implant biocompatibility, as membrane and porous scaffolds for tissue regeneration, in controlled drug delivery systems, in vascular grafts, in orthopedic implants and in orthopedic implants as well. The choice of polymer is based on various parameters, such as mechanical strength, ease of processing, inertia in a biological environment, abrasion, wear, biocompatibility, tissue adsorption as well as oxygen permeability. Which property will prevail depends on the application for which the polymer was to be used. The property of polymers depends on the nature of the chemical bonds that form the basis of the polymer. In general, a carbon-carbon bond is chemically and biologically quite inert. By increasing the oxidation of the carbon skeleton, the melting process is improved due to the reduced crystallinity which also favors the dissolution of these polymers in common organic solvents. Increased crystallinity can be achieved by substituting hydrogen atoms, for example substitution on hydrogen polyethylene with fluorine or methyl group. Furthermore, this process leads to polymers which due to their high crystallinity are less flexible and can only be processed at high temperatures.

1.4.3. Scaffold properties

Some of the main characteristics that the ideal scaffold must have are biocompatibility, biodegradability, suitable mechanical properties, suitable porosity and being marketable. These depend on the choice of biomaterial from which the scaffold will be produced.
1.4.3.1. Biocompatibility

The most important criterion for scaffolding selection is biocompatibility. The cells should be able to adhere to the surface of the scaffold, to function normally, but also to migrate on the surface of the scaffold and eventually through it, to multiply and thus develop the tissue [35]. Biomaterials are foreign bodies and biological responses are characterized as responses to a foreign body. The recognition of an active interface between the biomaterial and the biological system has led to important ideas that more fully shape the substance of the term biocompatibility. Interface interactions occur for both, biomaterial and tissue upon to the introduction into the biological environment, either through corrosion, chemical modification, deposition, degradation or other mechanisms. This exchange of responses leads to the conclusion that the interface is not static, but dynamic. Thus, the interface is constantly changing during its life. We therefore conclude that reactions at the material-tissue interface are a function of the tissue itself where the interface is formed. It is therefore essential that a biomaterial has the ability to perform its desired function in relation to medical treatment, without causing any adverse local or systemic effects on the recipient of the respective treatment, but to generate the most beneficial cellular or tissue response, and optimization of clinical performance of treatment. Some of the inherent properties of polymeric biomaterials that can affect biocompatibility are: material chemistry, molecular weight, solubility, shape and structure of the implant, whether hydrophilic or hydrophobic, surface energy, absorption water, degradation and the mechanism of corrosion. Due to the range of applications, there is no polymer system that could be considered as an ideal biomaterial. This is the reason for the development of a range of biodegradable materials for implants. In summary a material to be considered biocompatible must be non-carcinogenic, non-pyrogenic, non-toxic, non-allergenic, hemocompatible, non-inflammatory.

1.4.3.2. Bioactivity

By this term we refer to materials that play an active role in the body. The physical as well as the mechanical characteristics of the implants must meet the requirements of the application. The bioactive materials cause specific reactions between material and tissue
environments, while the biocompatible materials can affect the balance of the body to a limited extent. should not adversely affect the local and systemic environment such as bones, soft tissues, ionic plasma composition, as well as intracellular and extracellular fluids. It is important that biomaterials do not affect the structure of cells, tissues or organs, so by-products of the mass of the material should not be degraded by it and migrate into the body causing damage, unless it is specifically designed for such requirements. The study of the parameters that ensure the best results in the creation of these biomaterials, is today one of the most important applications of Biomechanics. These tissue scaffolds provide a mechanically stable and porous structure that allows cells to migrate into the environment and promote tissue regeneration in the body and in an artificial environment.

1.4.3.3. Biodegradation

It is essential that any polymer transplanted scaffold allows the body's cells to replace it over time. The scaffold should therefore remain stable until the development of a continuous extracellular matrix (ECM) from the implanted cells and then be gradually degraded as this matrix develops. The by-products of this biodegradation should be non-toxic, not impair the ability of implanted cells to grow and be able to escape from the body without mixing with other organs [38]. In order for a polymer to be considered biodegradable, its biodegradation time must be determined. The degree of biodegradation is measured by certified tests and is calculated from the amount of carbon resulting from the biodegradation, while the toxicity of the biodegradation products is calculated from toxicity tests using plants and animals sensitive to toxic substances. The rate of biodegradation depends directly on the geometry of the product, its surface per volume and its porosity. When synthetic polymers have only carbon atoms in their main chain, they are not biodegradable. The only synthetic polymers that can be biodegradable are condensation polymers. Synthetic condensation polymers are biodegradable at a rate that depends on the group they include in their chain, morphology, molecular weight while hydrophilic biodegradates faster than hydrophobic ones. Biodegradation is divided into three possible classifications:
i) Primary, when a change is caused in the chemical structure of the substance so that loses a special property.

ii) Environmentally acceptable, when a substance is degrade in order to remove adverse side effects.

iii) Complete, when a substance is completely degraded, resulting in conversion from organic to fully oxidized molecules such as CO$_2$, H$_2$O, ammonia, etc.

1.4.3.4. Mechanical properties

Ideally, the scaffold should have mechanical properties like the tissue that going to developed, but it should also be durable enough to be handled during implant surgery [38]. As the mechanical properties of scaffolds affect the differentiation of implanted cells [39]. They have to be maintained during the healing period as new tissue develops until the scaffold is finally degraded [40]. Also must be continuous monitoring of the mechanical properties of the scaffold throughout the time that stays in the human body.

1.4.3.5. Structure

Scaffolds should have a network of open pores to communicate with biological fluids and high porosity to ensure cell infiltration and spreading within the scaffold, as well adequate diffusion of nutrients. In addition, porosity is a necessary property to allow the diffusion of waste from the scaffold. One of the problems that occur is the degradation of the nucleus due to the lack of vascular system and waste disposal. Another scaffold property is the pores size. For any scaffold there is a critical area which depends on the type of cells used. The right combination of proper mechanical properties and proper porosity and pore size is therefore essential [34]. Various scaffolding techniques have been developed.
1.4.3.6. **Functional Requirements for Vascular Grafts**

It is crucial that TEVG anastomosed to the adjacent blood vessels sustains the load from blood pressure, and allows blood flow without leakage, immediately after implantation. Biocompatibility and bioactivity are other primary requirements for engineering vascular grafts. In addition, the mechanical properties, adhesive ligands and growth factor release should mimic the relevant ECM environment to a reasonable extent. In addition to degradation and transport kinetics of the materials used for the scaffold, it is favorable to use cost-effective and cytocompatible materials with tunable properties for a particular tissue replacement [41]. An exact replication of the native tissue is not always necessary. However, in order to create a functional construct, it is necessary that an engineered vascular graft fulfills certain basic criteria [42, 43]. For example, burst strength above 260 kPa is required for a TEVG in order to prevent rupture owing to variations in blood pressure [44].

The engineered vascular graft should be compatible with the adjacent host vessel and provide an anti-thrombotic lumen (autologous endothelium) [45]. The ability of the scaffold to provide initial mechanical function for the vascular graft is another important factor, even though the structural and mechanical characteristics of the native vessels are expected to be gradually acquired through remodeling, repair, and growth upon implantation. It is also of great importance that the implanted vascular grafts minimize intimal hyperplasia and allow for regeneration of arterial tissues. Finally, development of any tissue engineering product, including TEVG, demands that researchers consider their accordance with the requirements described by the international standards regulations for the process series needed from bench to bedside procedure.


1.5. Electrospinning

The electrospinning process is based on the stretching of a viscous polymer solution into nano-micro fibers using a high electrostatic force field. In brief, the polymeric material to be electrospun is diluted in a proper solvent (water, inorganic acids or specific organic compounds) till a certain concentration/viscosity, loaded into a syringe and is pumped at a slow flow rate by a syringe pump (Figure 9). A high DC voltage (some KV in magnitude), between the syringe metallic needle tip and a metallic surface (collector) opposed in a certain distance, is applied to the solution exhausted from the needle tip, producing strong repulsive force to the charged polymer macromolecules.

![Figure 9 The electrospinning experimental setup](image)

Under the large applied electric field, the tip of the liquid droplet makes a cone shaped film, called the Taylor cone. When the applied voltage is high enough to overcome the surface forces acting on the Taylor cone, a narrow jet of liquid generates from the cone and travels towards the collector. The opposite polarized or grounded collector electrode collects the fibers. As the liquid jet travels through the ambient towards the collector, the solvent from the fiber jet evaporates and a solid polymeric fiber is finally deposited on the collector. Depending of the collector type, fibers can be aligned in a random worm-like conformation (using a flat collector) or as nearly straight fibers aligned in preferential directions, using a drum type collector with a specific angular velocity [46, 47].

The morphology of electrospun fibers is affected by various parameters including the density, viscosity, electrical conductivity, molecular weight, surface tension, applied
voltage, flow rate, distance of the collector from the tip, and environmental parameters such as humidity and temperature [48, 49-51]. In brief, an increase in the concentration of solute increases the fiber diameter in a power law relationship, which in turn enhances the porosity. As an example, micron size fibers generate a more porous scaffold compared to nanofibers. Similar trends are observed for the effect of polymer molecular weight and viscosity: by increasing these parameters the fiber diameter, and hence the pore size increased. However, prior to fiber formation it is critical to determine the range for each of these variables for the formation of uniform, continuous and stable fibers. An increase in the electrical conductivity of solution generally decreases the fiber diameter. Contradicting results were reported for the effect if applied voltage [48]. While some researchers reported no significant difference in fiber diameter with variation of applied voltage [52], others reported an increase or decrease of size with increase in applied voltage [53]. An increase in flow rate increases the fiber diameter. However, a lower flow rate is commonly used to ensure that the solvents is fully evaporated from fibers before collection. These data demonstrate that by controlling process parameters it is possible to tune the fiber characteristics, hence the mechanical properties.

1.5.1. **Application of Electrospun Scaffolds in TEVG**

Electrospinning offers the ability to fine-tune mechanical properties during the fabrication process, while also controlling the necessary biocompatibility and structure of the tissue engineered grafts. The ability of the electrospinning technique to combine the advantages of synthetic and natural materials make it particularly attractive for TEVG where a high mechanical durability, in terms of high burst strength and compliance (axial and radial strain per unit load), is required. Incorporation of natural polymers, with abundance of cell binding sites into the fibrous polymeric materials obtained, with sufficient open microporous 3-D structure can promote the formation of a continuous monolayer of EC in the lumen and infiltration and proliferation of other vascular cell types (like fibroblasts, smooth muscle and undifferentiated mesenchymal stem host cells) into the matrix of the graft’s wall in vivo. Furthermore, the enrichment of polymeric fibrous components with biodegradable biochemical entities or factors of a sustained release
further enhance cell infiltration, attachment, growth and proliferation on the scaffold surface and bulk material [54-55]. The electrospinning technique also offers precise control over the composition, dimension, and the alignment of fibers that have greatest impact on the porosity, pore size distribution and the 3-D architecture of scaffolds. This method allows for engineering a wide range of tunable structural and mechanical properties as required for specific applications. Moreover, aligned nanofibers can be used for orienting cells in a specific direction necessary to provide the anisotropy encountered in certain organs including blood vessels.

Although electrospinning technique has been widely used in fabrication of scaffolds, with a few exceptions, most of the investigations have been limited to in vitro characterizations. Tubular scaffolds have been electrospun using a broad range of materials such as segmented polyurethane (SPU), polyethylene oxide (PEO), poly (D, L-lactide-co-glycolide) PLGA polymers, poly ε-caprolactone (PCL), collagen and elastin [56-58]. The addition of natural polymers significantly improves cellular attachment and infiltration. Zhu et al. [59] demonstrated that aligned PCL fibers coated with fibrin induce the alignment of human smooth muscle cells (hSMCs) along the direction of these fibers. Other studies, e.g. [60, 61], demonstrate the feasibility of fabricating strong and pliable electrospun scaffolds with a multilayer structure, like that of native blood vessels.

2. MATERIALS AND METHODS

2.1. Preparation of the Polymer Solution

20% w/v PCL pellets (Mn 80,000, Sigma-Aldrich, 440744) were dissolved in glacial acetic acid (≥ 99.8%, Honeywell Fluka). The solution was transferred in 20 ml cylindrical plastic vials and stirred with a laboratory roller mixer for 24 hours under 40 °C in order to
make a transparent solution. The solution was then left to cool at room temperature and used within 2 days.

![Figure 10 PCL pellets and Acetic acid](image)

**2.2. Scaffold fabrication by electrospinning**

An in-house electrospinning set-up was used in this study. A 10 ml syringe with a plastic tube extension and a 20G (inner diameter 0.6 mm) stainless steel blunt needle at the end was filled with the PCL solution and driven by a syringe pump (New Era Pump Systems Inc. NE-1000), at a feed rate of 2 ml/h. A high DC voltage of 20 kV (Spellman SL300 DC power supply unit) was applied between the metallic needle (+) and a metallic collector surface (earth) at 22cm (Table 1). A custom-designed and constructed Arduino-driven (Arduino Mega 2560 Rev3 with motor shield Rev3) rotating aluminum cylindrical drum with accurate angular velocity control and dimensions of 5 cm width and 10 cm diameter, was used as the earthed collector plate [17]. The average temperature and humidity recorded during the experimental process was 20°C-25°C and 50-60 %, respectively. After preliminary experiments, to achieve better fiber quality and peripheral alignment on the cylindrical surface, a rotational drum speed of 1280 rpm was selected for the electrospinning process.
The collector surface was covered with aluminum foil, to collect the electrospun membranous scaffolds, used to form the small caliber tubular scaffolds in a later stage. Electrospinning was performed for 2 hours.

![Electrospinning process](image11)

Figure 11 The electrospinning process

| Electrospinning parameters |  |
|---------------------------|--|
| Temperature (°C)          | 24  |
| Humidity (%)              | 55  |
| Needle size (G)           | 20  |
| Collector velocity (RPM)  | 1200 - 1280 |
| Kilovolt (kV)             | 24  |
| Distance from collector (cm) | 22  |
| Flow rate (ml/h)          | 2   |
| Experiment time (Hours)   | 2   |

2.3. Scaffold morphology assessment

The morphology of the fibrous scaffold was observed by Scanning Electron Microscopy, SEM (JEOL 6300). Rectangular-shaped specimens were gold-sputtered prior to SEM observation. Based on the SEM images, fiber alignment was assessed and the fiber diameter as well as the pore size was measured using an image processing software (Image J, version 1.51n, National Institutes of Health, USA).
2.4. Contact angle measurements

To determine wettability of the PCL scaffolds and possible alterations associated with the electrospinning procedure and fiber morphology and orientation, contact angle measurements (sessile drop method) were performed at room temperature at a contact angle meter (CAM 101, KSV instruments Ltd.) using phosphate buffer saline (PBS, pH 7.4) as the test liquid. Scaffolds were freeze-dried prior to the wettability measurements and six (6) specimens 10x10 mm were tested.

![Contact angle meter](image)

Figure 12 Contact angle meter

2.5. In Vitro Degradation Tests

Accelerated degradation test in PBS was used to assess the degradation properties of the scaffold material. To achieve that, rectangular specimens 10x10 mm from the scaffolds were freeze-dried and the initial weight (WI) was recorded. After that, they were placed in 20 mL plastic vials, each containing 10 mL of 0.1 M, pH 7.4 PBS and immersed into a stirred water bath at 37 °C for a total period of 28 weeks. Every two weeks, specimens (n=3) were taken out, washed 3 times with distilled water and vacuum freeze-dried. The PBS in the vials was renewed every two weeks.

The weight of each specimen was measured (WF) and the percentage mass loss was calculated as follow:

\[
\text{mass loss (\%)} = \left( \frac{\text{WI} - \text{WF}}{\text{WI}} \right) \times 100 \quad (1)
\]
To assess the products of degradation, an additional sample was kept into PBS for 28 weeks in order to analyze the extracted PBS by means of Raman Spectroscopy.

![Figure 13 In vitro accelerate degradation bioreactor](image)

2.6. Fabrication of electrospun tubular scaffolds

Rectangular PCL electrospun membrane strips 4x30 cm were detached from the peripheral aluminum foil of the cylindrical drum. Fiber alignment was predominately at peripheral orientation, as verified by SEM analysis.

![Figure 14 The multilayered polymeric vessel](image)

The tubular scaffolds were then fabricated through wrapping of the electrospun PCL membrane specimens, cut in a way that fiber alignment being predominately in the longitudinal direction of the tubular vessel. A multilayer design was chosen in order to mimic the native artery wall and match the mechanical properties of natural vessels’ ECM. A surgical cyanoacrylate glue (Dermabond) was utilized in order to fix the ends of the
tubular vessel after the wrapping. The final form of the fabricated vessel as well as its dimension can be seen in Figure 14.

2.7. Mechanical characterization

Assessment of mechanical properties of the multilayered fibrous polymeric small caliber scaffold is an important issue, as both, mechanical strength and matching of vascular biomechanics are key parameters (together with biological response) for an increased patency of the scaffold. Uniaxial tests were performed to assess the material anisotropy. In addition, suture retention test for the safety of anastomosis, cyclic tensile and stress relaxation testing for the detection of dynamic mechanical stability and viscoelastic performance, as well as tests for the detection of the radial compliance of the tubular scaffolds were performed.

2.7.1. Uniaxial Tests

The uniaxial mechanical properties of the electrospun fibrous membranes were determined using a bench type miniature tensile tester (MiniMat 2000, Rheometric Scientific Inc.) equipped with a 200 N load cell, at a constant rate of 10 mm/min until rupture [18]. To assess the anisotropy of the scaffolds, rectangular specimens 20x5 mm were cut along the directional axis of the fibers (the direction of the rotating drum perimeter) and perpendicularly to the fiber axis.

![Bench type miniature tensile tester](image)

*Figure 15 Bench type miniature tensile tester*

Seven (7) samples were tested for each case and the Young’s modulus was assessed. For each specimen, the greatest slope, corresponding to the linear region of the stress-strain curve at a strain range 0%-15%, was used to calculate the Young’s modulus.
2.7.2. Suture Retention Strength Tests

Suture retention strength tests were performed in 20x5 mm rectangular strips of the electrospun membranes (n=7) using the previous testing device. The average thickness of the samples was 500 ± 20 um. Test protocol was adapted from the methods described within ISO 7198 [62]. A polypropylene surgical suture (4-0, PROPYLEN, medipac®) was inserted at 2 mm distance from the end of the sample. The extension rate was set at 100mm/min which falls within the range specified by the protocol of the standard. The force required to pull the suture through the sample was recorded with time and the suture retention strength (maximum stress at rupture) was determined.

![Experimental process for suture retention strength tests](image)

**Figure 16** Experimental process for suture retention strength tests

2.7.3. Cyclic Tensile Tests

Cyclic stability and performance are very important for the elastic expansion and retraction of blood vessels in tissue engineering applications. Although vascular grafts are increasingly developed and exploited, their cyclic mechanical stability, determining their practical applications, is seldom investigated and understood. In order to mimic the effects of repetitive cyclic loading-unloading (similar to that expected in vivo), a set of cyclic loading-unloading mechanical tests was carried out to determine the cyclic mechanical stability of the tubular vessels. An effective way of measuring energy loss is to calculate materials hysteresis. Hysteresis calculations were reported as the total loop area between the loading and unloading curves divided by the area under the loading curve. The higher the amount of energy loss, the less effective the vascular graft.
Tubular specimens of 20 mm (free length between the grips) and 4 mm internal diameter were fitted in specially designed grips to maintain the tubular form. The tests (n=6) were carried out using a dynamic tensile testing machine (800 LM electromechanical testing device, Test Resources, USA). They were loaded using the sinusoidal loading procedure of the testing machine from 0 to 10% strain at a frequency of 1.25 Hz (i.e. the average heart rate of normal adults at 75 beats per minute). A preload of 0.1 N was used as a “zero state” condition, corresponding to a physiological preloading of the natural vessels. After a 90 cycles preconditioning procedure the remaining 90-200 cycles of testing were evaluated. The force/elongation of the tubes with time was recorded on-line and the dynamic stress (force/circular ring section area) and strain [(l –l0)/l0] data and the hysteresis were computed consequently. L0 was the length of the specimens after the application of 0.1 N preload, considered as the “zero state” of the testing procedure.

2.7.4. Stress Relaxation Tests

Stress relaxation tests were additionally performed after the end of the cyclic testing, for a time which is adequate for a full relaxation of the specimens, in order to evaluate the viscoelastic properties of the tubular vessels. In that frame, the tubular specimens (n=6), were stretched up to 10% strain using a ramp signal procedure (which corresponds to the linear portion of the stress-strain curves obtained from the uniaxial mechanical tests) and held there for 10 minutes. Stress/time data were computed from force/time on-line data acquisition recordings.
2.7.5. Radial Compliance Measurements

Radial compliance measurements were performed following a testing procedure based on recommendations defined in ISO 7198:2016 standard [26], which describes standardized compliance and burst test methods for vascular grafts and scaffolds. For the specific testing procedure however the pressure range limited to slightly above the physiological range. The vascular tubular grafts (n=6) were subjected to gradually increased internal pressure, and the compliance, i.e. the ability of a prosthesis to elastically expand and contract in the circumferential direction in response to pulsatile pressure, was calculated. Tests were performed in a custom made setup (Figure 18), adapted on the same dynamic tensile machine.

The internal pressure was applied into the tubular vessel through compressed air from a CO2 chamber with a pressure regulator. The internal pressure of the tubular vessel was measured at the opposite side via a cannulated T-connector, the third end of which was tampered, using an on-line connected electronic pressure gauge with an operating range of -50 to 300 mmHg (MLT1199 Transducer/Cable Kit, AD INSTRUMENTS). The diameter change, following the pressure increment, was recorded non-contact using an on-line connected laser micrometer (optoCONTROL 1200, Micro-epsilon). Pressure and diameter data were sent to a programmed data acquisition system, from which the radial compliance was calculated at four different pressure ranges according to the equation (2), as described in [26].
\[
\% \text{ compliance} = \frac{R_2 - R_1}{R_1} \times \frac{P_1 - P_2}{100 \text{mmHg}} \times 10^4 \quad (2)
\]

where \( R_2 \) and \( R_1 \) are the internal radii at the higher pressure \( P_2 \) and the lower pressure \( P_1 \) of each range respectively.

3. RESULTS

3.1. Electrospun scaffold morphology

The set of the electrospinning parameters chosen as above (voltage, feed rate, needle to collector distance, rotary collector angular velocity) was found to maintain a stable electrospinning process, leading to a smooth scaffold surface. After a period of two (2) hours, a membranous scaffold was produced covering the cylindrical surface of the drum collector. The thickness of the scaffolds was measured at different points using a high precision micrometer and found to be average \( 0.25 \pm 0.09 \) mm. Figure 19-20 shows representative SEM images of the electrospun scaffolds. It can be observed that smooth, uniform, defect-free and round-shaped fibers were produced, and a dominantly parallel alignment of the fibers achieved for the chosen collector velocity.

\[ \text{Figure 19 SEM images of the micro-nano structured electrospun scaffolds} \]
The average fiber diameter was $1.156 \pm 0.447 \text{ um}$.

### Table 2  Estimation of fiber diameter

| Sample | Diameter (um) | Sample | Diameter (um) | Sample | Size (μm) |
|--------|---------------|--------|---------------|--------|-----------|
| 1      | 16.419        | 4      | 16.366        | 7      | 13.497    |
| 2      | 23.108        | 5      | 19.830        | 8      | 27.295    |
| 3      | 11.610        | 6      | 13.487        | AVG±SDV | 17.702 ± 5.369 |
The average pore size was found to be 17.702 ± 5.369 um.

![SEM image for the estimation of pore size](image)

**Figure 22** SEM image for the estimation of pore size

| Sample | Pore size (um) | Sample | Pore size (um) | Sample | Pore size (um) |
|--------|----------------|--------|----------------|--------|----------------|
| 1      | 1.144          | 5      | 1.447          | 9      | 0.516          |
| 2      | 0.685          | 6      | 1.596          | 10     | 1.035          |
| 3      | 1.911          | 7      | 0.673          |        |                |
| 4      | 1.131          | 8      | 1.424          | AVG±SDV| 1.156 ± 0.447  |

**Table 3 Estimation of pore size**

3.2. Wettability of the scaffold

The results of the contact angle measurements (Table 4) showed that the surface of the freeze-dried PCL scaffold was very hydrophobic, having an average contact angle of 116⁰±1, n=3. That correlates well with the fact that polycaprolactone is known to be a highly hydrophobic material, a property remained unaffected after the electrospinning process.
3.3. In Vitro Degradation Results

Results of the in vitro biodegradation tests (Figure 24) showed that there was no significant weight loss till week 3 (~4%). After this period, the polymeric scaffolds showed a gradual slight increase in degradation till week 17 (~5%), after which, no further weight loss was observed till week 28. Figure 25 shows the surface morphology of the scaffold a) in the 1st week and b) after 28 weeks. It can be observed macroscopically that the degradation process occurs at the polymer surface. Use of Raman Spectroscopy on the extracted PBS solution after 28 weeks showed no presence of polymer sub-products.

| Sample | Contact angle (θ) | Sample | Contact angle (θ) |
|--------|-------------------|--------|-------------------|
| 1      | 117               | 1      | 116               |
| 2      | 116               | 2      | 117               |
| 3      | 115               | 3      | 115               |
| AVG±SDV| 116 ±1            | AVG±SDV| 116 ±1            |

Table 4 Contact angle measurements for PCL membranes
possibly due to very low concentrations of the PCL products into the solution, lower than the instrument’s sensitivity.

Figure 24 Degradation rate (% remaining mass) of the electrospun PCL membranes

The PCL scaffold after a) 1st week and b) 28th week of insertion into the PBS

The Raman Spectra (not shown here) for the sake of briefness showed not differentiation from that of a typical PBS content. The detailed biodegradation results are presented on annexes charter in table 9.
3.4. Mechanical Tests

3.4.1. Uniaxial Tests

The average Young’s modulus values (parallel with and perpendicular to the fiber axis) were calculated by analyzing the stress-strain curves. Typical stress-strain curves are depicted in Figure 26 (a,b). For both axes, an initial high-slope linear region is observed till the yield point, followed by a second, lower slope linear region, till the rupture of the polymeric scaffold. For the purpose of the study we considered the first linear region as region of interest, as the strain was into physiological values for small caliber vessels (<15%).

![Stress-strain plot of the electrospun PCL membranes a) parallel and b) perpendicular to the fiber axis](image)

*Figure 26 Stress-strain plot of the electrospun PCL membranes a) parallel and b) perpendicular to the fiber axis*

However, a possible explanation for the decrease of the slope in greater strain values can be attributed to the necking of the central region of the specimens and possible micro damages on the fibers, common in polymers at high strain levels. According to the results, a mechanical anisotropy has been attained, as the modulus in the direction parallel with the fiber axis (18±3 MPa, AVG±SDV, n=7) was found to be one magnitude higher than that in the perpendicular direction (1±0.3 MPa AVG±SDV, n=7). It must be notified that the high modulus corresponds to the axial direction of the tubular scaffold while the lower ones to the circumferential direction. The detailed uniaxial tests results are presented on annexes charter in table 7-8.
3.4.2. Suture Retention Strength

A typical load-time plot of the suture retention test is shown in Figure 27. The suture retention strength of the electrospun PCL scaffold was found to be 4.5±1 N (AVG±SDV, n=7). The detailed suture retention tests results are presented on annexes charter in table 9.

![Figure 27 Load-time plot of the sutured PCL electrospun scaffold](image)

3.4.3. Cyclic Tensile Tests

The cyclic tensile stress-strain curves of the tubular specimens are shown in Figure 28. In terms of loading and unloading regions, we can see an obvious hysteresis in the first cycle (Figure 28a). In the following stress-strain cycles, the maximum cycle stress decreases due to the afore-mentioned irreversible hysteresis.

![Figure 28 Stress-Strain cycle of the tubular polymeric vessel: a) 1-90th cycle and 2) 90-200th cycle](image)
Thus, from cycle 2 to cycle 90, there was a gradually increased overlap in the stress-strain curves. This may be attributed to transient rearrangements of the fiber architecture until a stable state, during which a greater part of the loading energy was absorbed. After cycle 90, no further loss of total strain was observed and the cyclic mechanical properties remained stable (Figure 28b), i.e. the loading and unloading regions are almost identical in each cycle, indicating that the energy stored during each cycle didn’t change. The hysteresis ratio (dissipated energy index) after 90th cycles was found to be 0.153±0.017 (AVG±SDV, n=6). The detailed cyclic tests results are presented on annexes charter in table 10.

### 3.4.4. Stress Relaxation

Figure 29 shows a typical stress relaxation behavior of the tubular vessel at 10% strain. The immediately generated stress gradually decreased with time and reached an equilibrium stress state within a few seconds.

![Stress Relaxation Profile](image)

**Figure 29** Stress relaxation profile of the polymeric tubular vessel

The stress drop percentage did not exceed 20% of the initial stress achieved ( n=6).
3.4.5. Radial Compliance Measurements

Figure 30 shows a typical pressure-diameter versus time plot. From this plot we can observe that diameter increases almost linearly under application of internal pressure into the tubular vessel. The radial compliance measurements for the pressure ranges considered are shown in Table 5. Compliance in the close physiological range 80-120 mmHg was calculated to 5.04±0.82 (AVG±SDV, n=6). The detailed uniaxial tests results are presented on annexes charter in table 7-8. The detailed uniaxial tests results are presented on annexes charter in table 11.

![Figure 30 The pressure-diameter profile of the electrospun tubular vessel](image)

| Pressure Ranges (mmHg) | Compliance (%) |
|------------------------|----------------|
| 50 - 90                | 7.01±0.94      |
| 80 - 120               | 5.04±0.82      |
| 110 - 150              | 4.80±1.06      |
| 140 - 180              | 4.61±0.96      |
4. SUMMARY OF THE RESULTS AND COMPARISON WITH NATURAL VESSELS

The results of the scaffold properties presented in the current thesis were compared with corresponding results from the literature. Fiber diameter, lumen diameter, wall thickness, tensile properties, radial compliance and suture retention strength where compared (figures 31-38) and found to be comparable with corresponding values of natural vessels.

**Fiber diameter**

![Figure 31 Comparison of fiber diameter results with natural vessels](image)

**Lumen Diameter**

![Figure 32 Comparison of lumen diameter results with natural vessels](image)
**Wall thickness**

![Graph showing wall thickness comparison](image1)

**Figure 33** Comparison of wall thickness results with natural vessels

**Young modulus perpendicular to the fiber alignment**

![Graph showing Young modulus comparison](image2)

**Figure 34** Comparison of Young modulus perpendicular to the fiber alignment results with natural vessels
**Young modulus parallel to the fiber alignment**

![Figure 35 Comparison of Young modulus parallel to the fiber alignment results with natural vessels](image)

**Elongation at break**

![Figure 36 Comparison of elongation at break results with natural vessels](image)
**Compliance**

![Graph showing comparison of compliance results with natural vessels.](image)

*Figure 37* Comparison of compliance results with natural vessels

**Suture retention**

![Graph showing comparison of suture retention results with natural vessels.](image)

*Figure 38* Comparison of suture retention results with natural vessels
5. DISCUSSION

Currently, there is a continuous search for shelf-ready small-caliber vascular prostheses with satisfactory early and late results, especially in the field of cardiovascular surgery. Aim of the current work was to synthesize small caliber polymeric (polycaprolactone) tissue-engineered vascular scaffolds, in order to mimic the structure and biomechanics of natural vessels. The use of electrospinning process has a great potential to meet the needs for proper synthesis of an appropriate scaffold for medium and small size tissue engineered vascular grafts. The produced scaffold presented a smooth, uniform, porous, and defect-free fibrous structure, while a dominantly parallel alignment of the fibers was achieved. Fiber alignment and controlled porosity has been found to have a significant effect on cellular behavior in vivo, inducing cell attachment, migration and differentiation [63]. The achieved fiber diameter of the produced scaffold is comparable to the collagen fibers found in native vessels [64], while its pore size can permit cell infiltration through it (e.g. endothelial cells diameter lies between 10-20 um). The gradual loss of weight after the degradation tests of the scaffolds, presented in figure 24, can be attributed to polycaprolactone hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages [65]; however the rate of degradation is rather slow (2–3 years). The observed weight loss is in accordance with other studies [e.g. 62]. Our scaffold seems to present a smooth and scheduled degradation in vitro, which is a perquisite for the achievement of a satisfactory functionality and longevity [10, 15]. The results from in vitro biocompatibility tests, showed a significant cell survival in contact with our scaffold material, verifying the approval of PCL as a biocompatible polymer and the freedom from toxic effects that possibly could induced due to different procedures used for the tubular scaffold construction.

The differences in structural fiber orientation resulted in analogous mechanical anisotropy of the polymeric scaffolds achieved, as shown in figures 19-20 and 26. The scaffolds were found to be stiffer in axial testing direction than the circumferential one. This is in accordance with vessel mechanical anisotropy encountered in natural arteries and veins [67-68]. The Young’s modulus found for both directions considered (parallel and perpendicular) match well with those of natural vessels (coronary arteries) as well as
autografts used for vascular graft applications, such as the gold standard saphenous vein, and internal mammary artery (IMA) [64, 68-72]. The significant difference observed between the axial and the peripheral direction is very important, as lower peripheral young modulus corresponds to higher peripheral compliance, hence important radial dilatation of the tubular scaffold, a situation not so common in polymeric tubular blood vessel substitutes in current clinical use. Moreover, suture strength of our scaffold, shown in figure 27, was found to be superior to the suture strength of saphenous vein (around 2.5 N) and IMA (around 2 N) [70, 72, 73], and matched the suture strength of synthetic grafts such as ePTFE [39]. Furthermore, the hysteresis ratio (dissipated energy index), as determined from the hysteresis loop shown in figure 28, was found to be comparable with corresponding values (close to 0.18) of native aortic wall [74].

Radial compliance of the polymeric vessels, was found to be comparable with compliance data from natural blood vessels (saphenous and umbilical cord veins) [76] and superior to that of synthetic grafts (ePTFE and Dacron) [72, 73]. It is of great interest that compliance decreases as the pressure increases (i.e. vessels become stiffer at higher pressures), a behavior also observed in natural vessels [75]. Radial compliance is critical, because the compliance mismatch between a vascular graft and neighboring arteries at the site of anastomosis is a major cause of graft failure. The main criteria for engineering a long-lasting vessel include among others, matching compliance and tensile mechanical properties [77]. A prosthetic vessel must be compliant enough to accommodate flow with high pulsatile pressure and tensile enough in order to prevent rupture. In similar studies, mechanical properties of tissue engineered vessels have been found to be comparable to that of native vessels; however, compliance mismatch was identified as the key factor that limits long-term patency of the graft [e.g. 12, 78, 79]. The fact that our polymeric tubular scaffold exhibits biomechanical matching of the radial compliance and tensile properties with natural blood vessels is a very important achievement making it and, from a biomechanical point of view, it could a potential candidate to be used alternatively as vascular implant. However, further in vitro and in vivo tests are required to assess the full tissue regeneration ability of the scaffold.
6. CONCLUSIONS

During the last two decades, many efforts have been made to develop a functional and mechanically sound tissue-engineered vascular graft, in order to replace autografts used in coronary bypass surgery. To achieve that, an ideal combination of mechanical, structural and biological properties is required, to make the graft suitable for cardiovascular applications.

In this work, small caliber polymeric grafts were fabricated through the electrospinning method, with a specific fiber morphology and fiber orientation. The structural architecture and the resulted mechanical properties of the graft were tuned in order to match these of native vessels and arteries. The Young’s modulus and suture retention strength of the fabricated grafts were in the range of the ones found in natural vessels. Radial compliance, which is a key determinant for a successful patency of the graft, coincided with the compliance of native vessels (saphenous vein, IMA, etc.) and was found to be superior to compliance of synthetic grafts (ePTFE, Dacron) in current clinical use.

Although this research illustrates the potential of electrospun vascular grafts for vascular tissue engineering applications, extensive investigation is yet to be conducted, prior to clinical usage. However, the results are very promising towards the use of electrospun vascular grafts for small-caliber vessel replacements. Electrospinning has a great potential to meet the needs for proper design of a polymeric scaffold for small caliber tissue engineered vascular grafts. Future work includes combinations of polycaprolactone with hydrophilic polymers (e.g. PVA or PGA), to promote host cell access and adhesion. The results throughout this ongoing research will offer further advancements in the field, necessary before proceeding with extended studies to assess in vitro and in vivo (animal model) cell-scaffold interactions.
7. BIBLIOGRAPHY

[1] European Heart Network. European Cardiovascular Disease Statistics. (2017).

[2] Bouten CVC, Dankers PYW, Driessen-Mol A, Pedron S, Brizard AMA, Baaijens FPT. Substrates for cardiovascular tissue engineering. Adv. Drug Deliv Rev. 2011; 63:221–41.

[3] Wise SG, Byrom MJ, Waterhouse A, Bannon PG, Ng MKC, Weiss AS. A multilayered synthetic human elastin/polycaprolactone hybrid vascular graft with tailored mechanical properties. Acta Biomater. 2011; 7:295–303.

[4] Wilson WR, Bower TC, Creager MA, Amin-Hanjani S, O'Gara PT, Lockhart PB, Darouiche RO, et al. Vascular graft infections, mycotic aneurysms, and endovascular infections a scientific statement from the American Heart Association. Circulation. 2016; 134:E412-E60.

[5] Stowell CET, Wang YD. Quickening: translational design of resorbable synthetic vascular grafts. Biomaterials. 2018; 173:71–86.

[6] Kuang HZ, Wang Y, Hu JF, Wang CS, Lu SY, Mo XM. A method for preparation of an internal layer of artificial vascular graft co-modified with salvianolic acid B and heparin. ACS Appl. Mater. Interfaces 2018; 10:19365–19372.

[7] Wise SG, Liu HJ, Kondyurin A, Byrom MJ, Bannon PG, Edwards GA, Weiss AS, Bao SS, Bilek MM. Plasma ion activated expanded polytetrafluoroethylene vascular grafts with a covalently immobilized recombinant human tropoelastin coating reducing neointimal hyperplasia, ACS Biomater Sci. Eng. 2016; 2:1286–1297.

[8] Peck M, Gebhart D, Dusserre N, McAllister TN, L'Heureux N. The Evolution of Vascular Tissue Engineering and Current State of the Art. Cells Tissues Organs. 2012; 195:144-58.
[9] Salacinski HJ, Goldner S, Giudiceandrea A, Hamilton G, Seifalian AM, et al. The mechanical behavior of vascular grafts: a review. J Biomater Appl. 2001; 15:241-278.

[10] Uyar T, Kny E. Electropun Materials for Tissue Engineering and Biomedical Applications. 1st ed. Woodhead Publishing; 2017

[11] N Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv. 2010; 28:325–47.

[12] Heydarkhan-Hagvall S, Schenke-Layland K, Dhanasopon AP, Rofail F, Smith H, Wu BM, et al. Three-dimensional electropun ECM-based hybrid scaffolds for cardiovascular tissue engineering. Biomaterials. 2008; 29:2907–14.

[13] M. Lopera Higuita & G. Griffiths. Small diameter xenogeneic extracellular matrix scaffolds for vascular applications. Tissue Engineering part B, 2020; 26(1);26:45

[14] Barnes CP, Sell SA, Boland ED, Simpson DG, Bowlin GL. Nanofiber technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev. 2007; 59:1413–33.

[15] Ashfari Mehdi. Electrospun Nanofibers. Woodhead Publishing; 2016.

[16] Wolf F, Schnoring H, Chalabi K, Mertens ME, Morgenroth A, Gesche VN, Koch S, et al. In-vivo Monitoring of Tissue-Engineered Vascular Grafts. The Thoracic and Cardiovascular Surgeon. 2015; 63

[17] Rocco KA, Maxfield MW, Best CA, Dean EW, Breuer CK. In vivo applications of electropun tissueengineeredvascular grafts: a review. Tissue Eng Part B Rev. 2014; 20:628–40.
[18] Markatos DN, Sarakinis A, Mavrilas D. Tuning Fiber Alignment to Achieve Mechanical Anisotropy on Polymeric Electrospun scaffolds for Cardiovascular Tissue Engineering. J. Material Sci Eng. 2018; 7(4):466

[19] Repanas A, Lauterboeck L, Mavrilas D, Glasmacher B. Polycaprolactone and polycaprolactone/chitosan electrospun scaffolds for tissue engineering applications. SJAMS. 2016; 4(1C): 228-232.

[20] Mitropoulou A, Markatos D, Antimisiaris S, Mavrilas D. A Novel Design of a PVA Electrospun Nanofibrous Scaffold Incorporating Liposomes as Drug Delivery Carriers for Tissue Engineering. Ann Biomed Technol Eng. 2018; 1(1):1003.

[21] Repanas A, Glasmacher B, Mavrilas D. Chitosan/Polycaprolactone blend electrospun fibers as a novel biodegradable carrier for dipyridamole. Scholars Acad J Biosci. 2016; 4(10A):827-831.

[22] Progress in vascular graft substitute P. Menua,* J.F. Stoltzb and H. Kerdjoudjc

[23] A. Hasan, G. Deeb, K. Atwi, S. Soliman and A. Hasan, "Electrospun PET-PU scaffolds for vascular tissue engineering," 2015 International Conference on Advances in Biomedical Engineering (ICABME), Beirut, 2015, pp. 217-221

[24] A. Yazdanpanah, M. Tahmasbi, G. Amoabediny, J. Nourmohammadi, F. Moztarzadeh, M. Mozafari. Fabrication and characterization of electrospun poly-L-lactide/gelatin graded tubular scaffolds: Toward a new design for performance enhancement in vascular tissue engineering, Progress in Natural Science: Materials International, Volume 25, Issue 5, 2015; Pages 405-413

[25] Ledger WJ. Current problems in antibiotic treatment in obstetrics and gynecology. Rev Infect Dis. 1985;7(Suppl 4):S679–S689

[26] Pugsley MK, Tabrizchi R. The vascular system – an overview of structure and function. J Pharmacol Toxicol Methods. 2000; 44:333–40.
[27] Uyar T, Kny E. Electrospun Materials for Tissue Engineering and Biomedical Applications. 1st ed. Woodhead Publishing. 2017

[28] Andrew D. Lucas and David R. Greaves, Atherosclerosis: role of chemokines and macrophages, Exp. Rev. Mol. Med. 2001

[29] Trion A, van der Laarse A. Vascular smooth muscle cells and calcification in atherosclerosis. Am Heart J. 2004; 147(5):808-14.

[30] Buddy D. Ratner, Allan S. Hoffman, Frederick J. Schoen, Jack E. Lemons, Introduction - Biomaterials Science: An Evolving, Multidisciplinary Endeavor, Biomaterials Science (Third Edition), Academic Press, 2012

[31] Davis, J. Overview of Biomaterials and Their Use in Medical Devices. and book of Materials for Medical Devices. ASM International; 2003

[32] Kricheldorf, H. R., Stricker, A. and Langanke, D., Polylactones, 52. Tin Carboxylates as Initiators of ε-Caprolactone. Macromol. Chem. Phys., 2001; 202: 2963-2970.

[33] Wray, L., & Kaplan, D. Biomaterials for Scaffolds: Natural Polymers. Scaffolds for Tissue Engineering, 2014

[34] O’Brien, F. J. Biomaterials & scaffolds for tissue engineering. Materials Today, 2011; 14(3), 88–95

[35] Mrunal S. Chapekar. Tissue Engineering: Challenges and Opportunities. Journal of Biomedical Material Research, 2000; 53: 617–620

[36] J. Leor, Y. Amsalem, S. Cohen. Cells, scaffolds, and molecules for myocardial tissue engineering. Pharmacology & Therapeutics. 2005; 105:151-163.
[37] Dhandayuthapani, B., Yoshida, Y., Maekawa, T., & Kumar, D. S. Polymeric Scaffolds in Tissue Engineering Application: A Review. International Journal of Polymer Science, 2011; 1–19

[38] M. W. Kessler, D. A. Grande. Tissue engineering and cartilage. Organogenesis. 2008; 1:28-32

[39] Engler, S. Sen, H. L. Sweeney, D. E. Discher. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell. 2006; 126: 677-689.

[40] A. H. Yusop, A. A. Bakir, N. A. Shaharom, M. R. A. Kadir, H. Hermawan. Porous Biodegradable Metals for Hard Tissue Scaffolds: A Review. International Journal of Biomaterials. 2012; 1-10.

[41] Ramakrishna S, Fujihara K, Teo WE, Yong T, Ma Z, Ramaseshan R. Electrospun nanofibers: Solving global issues. Mater Today. 2006; 9:40–50.

[42] Ratcliffe A. Tissue engineering of vascular grafts. Matrix Biol. 2000; 19:353–7.

[43] L’Heureux N, Dusserre N, Marini A, Garrido S, de la Fuente L, McAllister T. Technology Insight: the evolution of tissue-engineered vascular grafts - from research to clinical practice. Nat Clin Pract Cardiovasc Med. 2007; 4:389–95.

[44] Gong Z, Niklason LE. Blood Vessels Engineered from Human Cells. Trend Cardiovasc Med. 2006; 16:153–6.

[45] Stegemann JP, Kaszuba SN, Rowe SL. Review: Advances in vascular tissue engineering using protein-based Biomaterials. Tissue Eng. 2007; 13:2601–13.
[46] Markatos DN, Sarakinis A, Mavrilas D. Tuning Fiber Alignment to Achieve Mechanical Anisotropy on Polymeric Electrospun Scaffolds for Cardiovascular Tissue Engineering. J Material Sci Eng. 2018; 7(4):466.

[47] Uyar T, Kny E. Electrospun Materials for Tissue Engineering and Biomedical Applications. 1st ed. Woodhead Publishing; 2017

[48] Bhardwaj N, Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv. 2010; 28:325–47.

[49] Sill TJ, von Recum HA. Electrospinning: Applications in drug delivery and tissue engineering. Biomaterials. 2008; 29:1989–2006.

[50] Soliman S, Sant S, Nichol JW, Khabiry M, Traversa E, Khademhosseini A. Controlling the porosity of fibrous scaffolds by modulating the fiber diameter and packing density. J Biomed Mater Res A. 2011; 96A:566–74.

[51] Gaumer J, Prasad A, Lee D, Lannutti J. Structure-function relationships and source-to-ground distance in electrospun polycaprolactone. Acta Biomater. 2009; 5:1552–61.

[52] Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced by electrospinning. Nanotechnology. 1996; 7:216–23.

[53] Meechaisue C, Dubin R, Supaphol P, Hoven VP, Kohn J. Electrospun mat of tyrosine-derived polycarbonate. 2012, 1033-1056

[54] Mitropoulou A, Markatos D, Antimisiaris S, Mavrila D. A Novel Design of a PVA Electrospun Nanofibrous Scaffold Incorporating Liposomes as Drug Delivery Carriers for Tissue Engineering. Ann Biomed Technol Eng. 2018; 1(1):1003.
[55] Repanas A, Glasmacher B, Mavrilas D. Chitosan/Polycaprolactone blend electrospun fibers as a novel biodegradable carrier for dipyridamole. Scholars Acad J Biosci. 2016; 4(10A):827-831

[56] Ekaputra AK, Prestwich GD, Cool SM, Hutmacher DW. Combining electrospun scaffolds with electrosprayed hydrogels leads to three-dimensional cellularization of hybrid constructs. Biomacromolecules. 2008; 9:2097–103.

[57] Kidoaki S, Kwon IK, Matsuda T. Mesoscopic spatial designs of nano- and microfiber meshes for tissue-engineering matrix and scaffold based on newly devised multilayering and mixing electrospinning techniques. Biomaterials. 2005; 26:37–46.

[58] Stitzel J, Liu J, Lee SJ, Komura M, Berry J, Soker S, et al. Controlled fabrication of a biological vascular substitute. Biomaterials. 2006; 27:1088–94.

[59] Zhu YB, Cao Y, Pan J, Liu YX. Macro-Alignment of Electrospun Fibers For Vascular Tissue Engineering. J Biomed Mater Res B Appl Biomater. 2010; 92B: 508–16.

[60] Ju YM, Choi JS, Atala A, Yoo JJ, Lee SJ. Bilayered scaffold for engineering cellularized blood vessels. Biomaterials. 2010; 31:4313–21.

[61] Vaz CM, van Tuijl S, Bouten CVC, Baaijens FPT. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. Acta Biomater. 2005; 1:575–82.

[62] ISO 7198-2016. Cardiovascular implants: tubular vascular prostheses. 2016

[63] Denchai A, Tartarini D, Mele E. Cellular Response to Surface Morphology: Electrospinning and Computational Modeling. Front Bioeng Biotechnol. 2018;6:155.
[64] Hao-Yang Mi, Yongchao Jiang, Xin Jing, Eduardo Enriquez, Heng Li, Qian Li, Lih-Sheng Turng. Fabrication of triple-layered vascular grafts composed of silk fibers, polyacrylamide hydrogel, and polyurethane nanofibers with biomimetic mechanical properties. Mater Sci Eng: C. 2019; 98: 241-249.

[65] Heimowska A, Morawska M, Bocho-Janiszewska M, Anita. Biodegradation of poly(ε-caprolactone) in natural water environments. Polish Journal of Chemical Technology. 2017; 19(1):120-126.

[66] Claes, E., Estudio mecánico de las arterias coronarias humanas y sus sustitutos vasculares, tesis doctoral, in Escuela técnica superior de ingenieros de caminos, canales y puertos. 2010, Doctoral Thesis. Universidad Politécnica de Madrid: Madrid, Spain. p.310.

[67] Donovan, D.L., et al., Material and structural characterization of human saphenous vein. Journal of Vascular Surgery. 1990. 12(5): 531-537.

[68] Jenkins, T. L., & Little, D. (2019). Synthetic scaffolds for musculoskeletal tissue engineering: cellular responses to fiber parameters. NPJ Regenerative medicine 2019; 4:15.

[69] Lorenzo Soletti, Yi Hong, Jianjun Guan, John J. Stankus, Mohammed S. El-Kurdi, William R. Wagner, David A. Vorp. A bilayered elastomeric scaffold for tissue engineering of small diameter vascular grafts. Acta Biomaterialia. 2010; 6(1): 110-122.

[70] Wise SG, Byrom MJ, Waterhouse A, Bannon PG, Martin K.C. Ng, Weiss AS. A multilayered synthetic human elastin/polycaprolactone hybrid vascular graft with tailored mechanical properties, Acta Biomaterialia. 2011; 7(1): 295-303.

[71] Hao-Yang Mi, Xin Jing, Emily Yu, Xiaofeng Wang, Qian Li, Lih-Sheng Turng. Manipulating the structure and mechanical properties of thermoplastic polyurethane/polycaprolactone hybrid small diameter vascular scaffolds fabricated via electrospinning using an assembled rotating collector. J Mech Behav Biomed Mater. Volume 2018; 78: 433-441.
[71] Johnson J, Ohst D, Groehl T, Hetterscheidt S, Jones M. Development of Novel, Biodegradable, Small-Diameter Electrospun Vascular Grafts. J Tissue Sci Eng. 2015; 6:151.

[73] Gkizas SI, Apostolakis E, Pagoulatou E, Mavrilas D, Papachristou DJ, Koletsis E, et al. Aldosterone receptor inhibition alters the viscoelastic biomechanical behavior of the aortic wall. Experimental Biology and Medicine 2010; 235(3):311–316.

[74] Stephen P. Glasser, Donna K. Arnett, Gary E. McVeigh, Stanley M. Finkelstein, Alan J. Bank, Dennis J. Morgan, Jay N. Cohn, Vascular Compliance and Cardiovascular Disease: A Risk Factor or a Marker? American Journal of Hypertension. 1997; 10(10): 1175–1189.

[75] Johnson R, Ding Y, Nagiah N, Monnet E, Tan W. Coaxially-structured fibres with tailored material properties for vascular graft implant, Mater. Sci. Eng. C. 2019; 97: 1-11

[76] Stephen P. Glasser, Donna K. Arnett, Gary E. McVeigh, Stanley M. Finkelstein, Alan J. Bank, Dennis J. Morgan, Jay N. Cohn, Vascular Compliance and Cardiovascular Disease: A Risk Factor or a Marker? American Journal of Hypertension. 1997; 10(10): 1175–1189.

[77] Dimitrievska S, Niklason, LE. Historical perspective and future direction of blood vessel developments. Cold Spring Harb. Perspect. Med 2018; 8:025742

[78] Catto V, Fare S, Freddi G, Tanzi MC. Vascular Tissue Engineering: Recent Advances in Small Diameter Blood Vessel Regeneration. Int Sch Res Notices 2009; 2014

[79] Stewart SF, Lyman DJ. Effects of a vascular graft/natural artery compliance mismatch on pulsatile flow. J Biomech. 1992;25(3):297–310
8. ANNEXES

**Table 6 Biodegradation results**

| weeks | 100-weight% | stdev         |
|-------|-------------|---------------|
| 0     | 100         | 0.005773      |
| 2     | 100         | 0.011547005   |
| 3     | 96.39344262 | 0.015275252   |
| 5     | 96.31147541 | 0.015821926   |
| 10    | 95.90163934 | 0.0080829     |
| 17    | 95.08196721 | 0.0028284     |
| 22    | 94.75409836 | 0.010148892   |
| 28    | 94.73409836 | 0.010448892   |
Table 7 *Young modulus perpendicular to the fiber alignment results*

| sample | Young modulus (Mpa) |
|--------|---------------------|
| 1      | 1.175               |
| 2      | 0.7                 |
| 3      | 0.86                |
| 4      | 0.89                |
| 5      | 1.48                |
| 6      | 1.21                |
| 7      | 1.3                 |

Average 1.087857

Standard deviation 0.277727
Table 8 *Young modulus parallel to the fiber alignment results*

| Sample | Young modulus (Mpa) |
|--------|---------------------|
| 1      | 19.019              |
| 2      | 16.436              |
| 3      | 22.042              |
| 4      | 17.349              |
| 5      | 16.005              |
| 6      | 21.706              |
| 7      | 15.006              |
| Average | 18.22329          |
| Standard deviation | 2.786142 |

| sample | Retention (N) |
|--------|--------------|
| 1      | 4.56         |
| 2      | 5.5          |
| 3      | 4.106        |
| 4      | 4.9          |
| 5      | 3.37         |
| 6      | 4.02         |
| 7      | 5.6          |

Average 4.579429

Standard deviation 0.815677
## Table 10 Cyclic Tensile Tests results

|    | Area inside the hysteresis loop | Area under the loading curve | Results  |
|----|----------------------------------|------------------------------|----------|
| 1  | 0.371483903                      | 3.056407448                  | 0.121542664 |
| 2  | 0.439230883                      | 2.750957972                  | 0.159664701 |
| 3  | 0.406572116                      | 2.638547272                  | 0.154089381 |
| 4  | 0.353012376                      | 1.991138694                  | 0.177291706 |
| 5  | 0.312206333                      | 2.073998433                  | 0.150533543 |
| 6  | 0.386529525                      | 2.626836079                  | 0.14714642 |
| 7  | 0.248340141                      | 1.551845578                  | 0.1600289  |

**average** 0.152899617

**stdev** 0.016906304
| sample | 50-90mmHg | 80-120 mmHg | 110-150 mmHg | 140-180 mmHg | 170-210 mmHg |
|--------|-----------|-------------|--------------|--------------|--------------|
| 1      | 6.33383   | 5.544779    | 4.923912     | 4.375584     | 3.884177     |
| 2      | 6.185821  | 4.755687    | 3.914558     | 3.437023     | 3.135805     |
| 3      | 8.549417  | 6.409871    | 6.797882     | 5.595548     | 3.971766     |
| 4      | 7.117568  | 4.138278    | 3.934835     | 3.831331     | 3.9796       |
| 5      | 6.880121  | 4.953268    | 4.663777     | 4.989929     | 4.405263     |
| 6      | 7.103425  | 4.456939    | 5.089544     | 5.031132     | 6.391433     |
| Average| 7.028364  | 5.043137    | 4.887418     | 4.543425     | 4.294674     |
| Standard deviation | 0.841597 | 0.821305 | 1.057917 | 0.812842 | 1.106676 |
