TECHNICAL UNIVERSITY OF LIBEREC
Faculty of Textile Engineering

Production of vascular grafts for tissue engineering

Master Thesis

Study programme: N0723A270002 Textile Engineering
Study branch:

Author: **Ulhas Balasaheb Sangave**
Thesis Supervisors: Ing. Petra Harciníková
Department of Nonwovens and Nanofibrous materials

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Master Thesis Assignment Form

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1. Yalcin, I., Horakova, J., Mikes, P., Sadikoglu, T. G., Domin, R., & Lukas, D. (2016). Design of Polycaprolactone Vascular Grafts. Journal of Industrial Textiles, 45(5), 813–833. https://doi.org/10.1177/1528083714540701
2. Yalcinkaya, F. (2019). Preparation of various nanofiber layers using wire electrospinning system. Arabian Journal of Chemistry, 12(8), 5162–5172. https://doi.org/https://doi.org/10.1016/j.arabjc.2016.12.012
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4. Kharazi, A. Z., Atari, M., Vatankhah, E., & Javanmard, S. H. (2018). A nanofibrous bilayered scaffold for tissue engineering of small-diameter blood vessels. Polymers for Advanced Technologies, 29(12), 3151–3158. https://doi.org/10.1002/pat.4437

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Ulhas Balasaheb Sangave

ABSTRACT

The world's population growing faster. As population digit going high diseases in human kinds also exploring faster. In those diseases, cardiovascular disease is the biggest among those. This disease mostly resulting in death. To get rid of this problem, many types of solutions are present in the world. The production of synthetic vascular graft is one of the solutions to this problem.

In this dissertation, the synthetic graft production, required material for it, testing of the graft explained. There are different ways to replace or recover diseased organs, over those techniques this thesis chooses the best technique.

Implementing those methods to produce synthetic graft and development possibilities are considered. The results and discussion in this diploma thesis will help in further study and expanding the scope for tissue engineering.

Key words - Tissue engineering, Vascular graft, Polymer, Polyurethane, Electrospinning, Cells, Fibre diameter, Biocompatibility.

Abstrakt

Světová populace roste rychleji. Vzhledem k tomu, že počet obyvatelstva stoupá, stále častěji se objevují choroby u lidí. Nejčastější z nich jsou kardiovaskulární onemocnění. Tato nemoc má většinou za následek smrt. Abychom se tohoto problému zbavili, existuje na světě mnoho typů řešení. Výroba syntetického cévního štěpu je jedním z nich.

Tato diplomová práce je studií výroby syntetického štěpu, popisuje vyžadovaný materiál a vysvětluje testování štěpu. Existují různé způsoby jak nahradit nebo obnovit nemocné orgány a pro tyto práce volí nejlepší techniku.

V této práci je uvažováno o implementaci těchto metod k výrobě syntetických štěpů a o možnostech vývoje. Výsledky a diskuse v této diplomové práci pomohou při dalším studiu a rozšíření záběru tkáňového inženýrství.

Klíčová slova - tkáňové inženýrství, cévní štěp, polymer, polyuretan, elektrospinning, buňky, průměr vláken, biokompatibilita.

CONTENTS

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1 INTRODUCTION

In the current scenario, tissue engineering was used to less extent and proceeding gradually in medical applications. Also, it has a limited number of medical trials. In world Technology growing faster and faster but die rate of peoples also increasing due to diseases. This is happening due to a lack of organ donors or due to substitutes, we are not getting. In the field of regenerative medicine tissue engineering is creating a great image by replacing damaged or diseased organs by constructing artificial tissue or organ planted in vitro and then transplanted in vivo. Tissue engineering depends basically on cell, scaffold, and growth factors. Besides this tissue engineering has many factors such as the construction of scaffold, cell source, cell seeding, and cultural environment, matrix preparation, mechanical characteristics of the cell, scaffold construct, and animal model. The source of cell effects more vigorously on tissue engineering. there are three types of cell resources found in tissue engineering as autologous cells (from a patient), allogenic cells (other than patient cells), and xenogeneic cells (derived from animal cells). Recent reports show that xenogeneic cells are considered as least safe. Autologous cells have a problem during harvesting from aged and ill people. Where allogenic cells best fit for skin tissue but Allogenic cells had lack donors and xenogeneic cells are not safe to use because of the virus [1].

If we talk about worldwide deaths of human being due to different diseases. in those diseases, cardiovascular disease will be in large portion. Coronary heart disease and peripheral arterial disease are the main reasons behind the atherosclerotic narrowing of supplying arteries [2].

Artificial vascular grafts in big diameter vessels showed better and comparable properties with native blood vessels, which is positive for tissue engineering. Large diameter grafts have shown better results than small diameter. Because small diameter synthetic grafts continue to display a variety of deficiencies that have restricted their effects. These shortcomings include low patency rates (< 6 mm in diameter) for small diameter vessels, a lack of growth capacity that needs repeated treatments for the pediatric population, and vulnerability to infection. At the stage of microvasculature, ischemic disorders also occur. Currently, small diameter grafts fail due to acute grafting, Graft thrombogenicity, anastomotic intimal hyperplasia, development of aneurysms, inflammation, atherosclerotic disease progression. With the vast number of patients requiring replacement grafts and the need for an alternative small-diameter graft, these criteria are immense and have motivated scientists to look for new materials [3,4].

In natural polymers, collagen and elastin-based biomaterials are used due to their excellent mechanical properties, wherein synthetic polymers should be biocompatible, non-immunogenic, and degradable, in synthetic mostly polyglycolic acid (PGA), polycaprolactone (PCL), poly-L-lactide (PLLA), and their mixtures are used as biomaterials. The native artery is an extremely complex multilayered tissue consisting of multiple different proteins and cell types of the extracellular matrix (ECM). An artery is composed of three

distinct layers called the tunica intima, tunica media, and tunica adventitia to withstand the high flow rate, high pressure, and pulsating nature of blood flow [3,4].

Looking at the situation this study was conducted on synthetic vascular graft to deal with the current scenario. This study focused on the production of vascular grafts by using suitable polymer in the electrospinning process. Other polymers mainly show a slow degradation rate and thrombosis occurring after implementation. Over the failure of other polymers, this thesis going to experiment on polyurethane. Polyurethane under coming a biocompatible material, it's been used in the medical sector for over 50 years, it can spin by different techniques, its physical and chemical properties can be tailored according to needs. Polyurethane shows excellent resistance to oxidation and hydrolysis. Looking over the advantages of polyurethane, it is a dominant candidate over other biomaterials [3].

2 THEORETICAL PART

The theoretical part of this thesis contains a detailed description of vascular graft and its production, polymers using to produce a graft, electrospinning process, and lastly standard testing methods to test the product.

2.1 Tissue engineering

The birth of tissue engineering occurred in the "Boston" city of the United States. In this city, one of the fine orthopedic doctors "W.T Green did several experiments to create new fresh cartilage in the year 1970. But he failed in those experiments, but he gave a statement that we can create a new tissue by using biocompatible material with a perfect scaffold in which we are planting biocompatible materials [5].

Both tissue engineering and cell therapy field has its strength to work in the surgical field. Tissue engineering is an updated version of cell therapy. Tissue engineering has objectives like – replace the damaged or broken tissues, enhance the work of tissue and it should continuously maintain the functions of tissue [6].

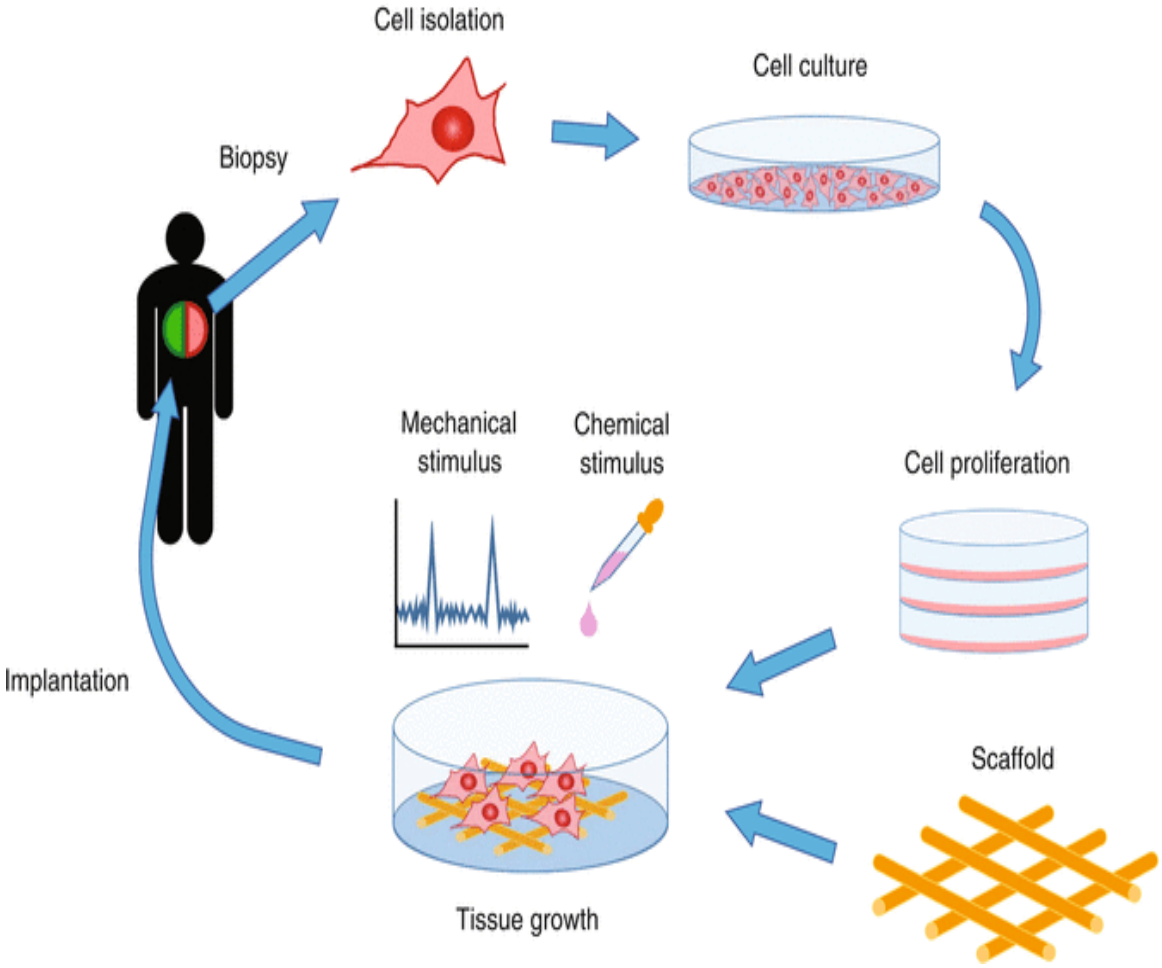


***Figure 1**: Process of Tissue Engineering [7].*

Every human being on earth's surface is suffering from different kinds of injuries, in his life. That injured part of the human body can repair or replace with the help of tissue engineering. But for transplantation we required a donor, to donate an organ, unfortunately, we are lack donations. In this situation, tissue engineering plays a great role in human lives. we have 206 bones in our body and an uncountable but limited number of tissues and muscles. Tissue regeneration means that our damaged body parts or tissues or cells are replaced or repaired by a new one. When our body parts burn highly then that part becomes unrecoverable or can't heal naturally, like this situation tissue engineering promises damaged part regeneration [8,9].

In past days tissue engineering researches have highly occurred with the regeneration of pancreas, bone, cartilage and vasculature, dental implants, vasculature, and other organs [8,9].

2.2 Scaffolds in Tissue Engineering

Tissue is made up of cells, which are the basic building blocks. Extracellular matrix (ECM) is a type of support structure that is made and secreted by groups of cells. This matrix, or scaffold, serves as a relay station for various signaling molecules in addition to supporting the cells. Where scaffold artificial structures are made by natural or synthetic materials. On the scaffold, tissue grows to mimic biological processes outside of the body and is used to replace the diseased or damaged tissue inside the body [6].

Regenerative medicine we can classify into two parts based on scaffold uses as follows -

a. Cell therapy – scaffolds are not used as regenerative medicine.

b. Tissue engineering – scaffolds are used as regenerative medicine.

In the human body to make a three-dimensional distinct structure of cells of organ or tissue, cell required support, that support is named as – scaffold or artificial extracellular matrix. The object of the scaffold is mimic as an extracellular matrix (ECM), It should be providing proliferation (population of cells increasing), differentiation of cells, and biosynthesis of cells [10].

2.2.1 Structure and composition of extra cellular matrix

All cells' physical maintenance is handled by the extracellular matrix (ECM). The idea that the ECM plays a passive role in cellular activity, on the other hand, has been debunked. It is now recognized that it is involved in a variety of cellular processes such as cell proliferation, differentiation, and migration. The important components in |ECM structure and composition are proteins like collagen and fibronectin. to matrix metalloproteinases (MMPs) [11].

Composition of ECM - The ECM is made up of various matrix proteins, with matrix proteins serving as the ECM's main component. Depending on their function, the proteins that make up the ECM can be classified as structural or non-structural (also known as glycoproteins). Collagens and elastin are examples of structural proteins, while fibronectin,

laminin, and tenascin are examples of non-structural proteins. Integrins, growth factors (GFs), and a group of MMPs are also essential components of the ECM[11].

Structure of ECM - The ECM's various components is structured into a recognizable three-dimensional (3D) structure that may be divided into two distinct forms: the base membrane and the interstitial matrix. Although these two forms differ in several ways, the underlying structural outline is the same [11].

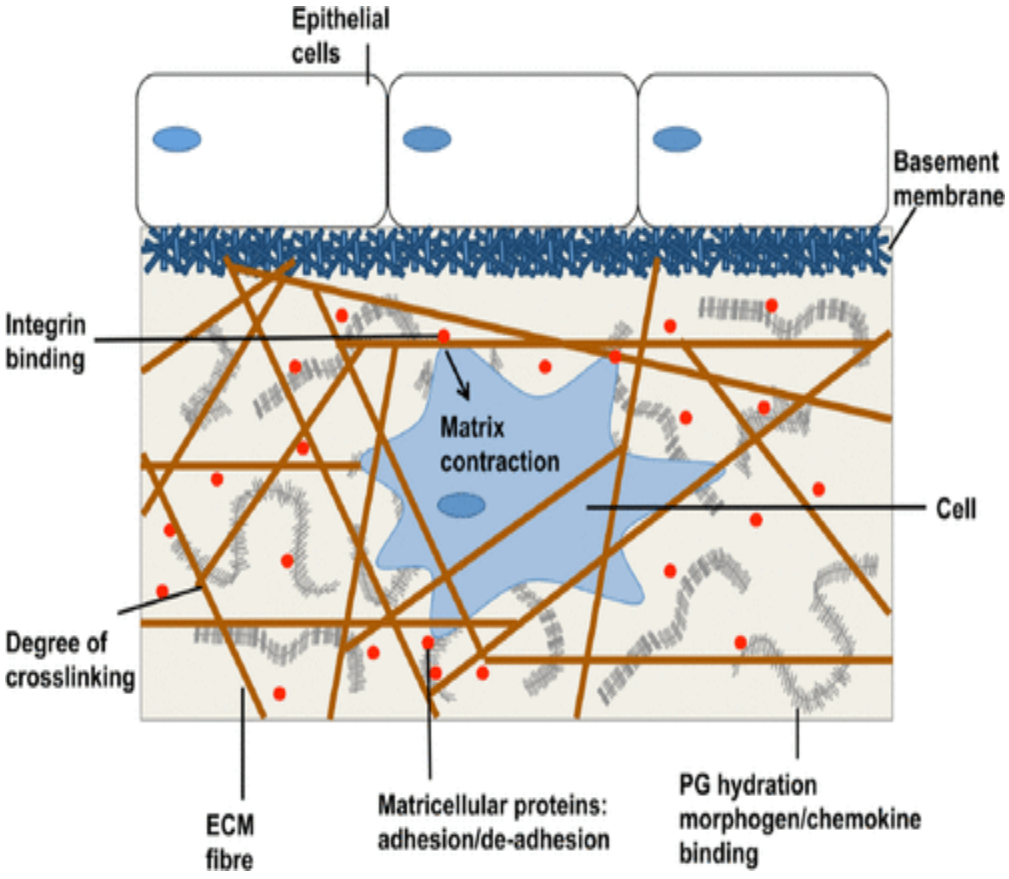


Figure 2: Structure of ECM[11].

2.2.2 Scaffold properties

In tissue regeneration and repair, a scaffold plays an important role. A scaffold can be described as a three-dimensional platform necessary to finish actions ranging from cell-biomaterial interaction, cell adhesion to a controllable rate of biodegradation that matches cell proliferation [12].

Scaffolds are generally from Biomaterials; biomaterial should be acceptable by cell components for engineered tissues. Biomaterials of the scaffold should enhance mechanical properties like strength and structural stability. The scaffold should help to tissue to do its regular activities [12,13].

Both vascular development and waste movement are made feasible by micropores. This is critical for the cells' survival inside the scaffold. The ideal pore size is between 100 and 500 micrometers. In this range, porosity cells grow properly maintained way. If it exceeds this range cell growth will not happen. affect Furthermore, the scaffold should have high porosity, a large surface area, and good mechanical strength[10].

Scaffolds can be produced by different methods like freeze-drying, porogen leaching, delicate processes like a solid-free prototype, and electrospinning [10].

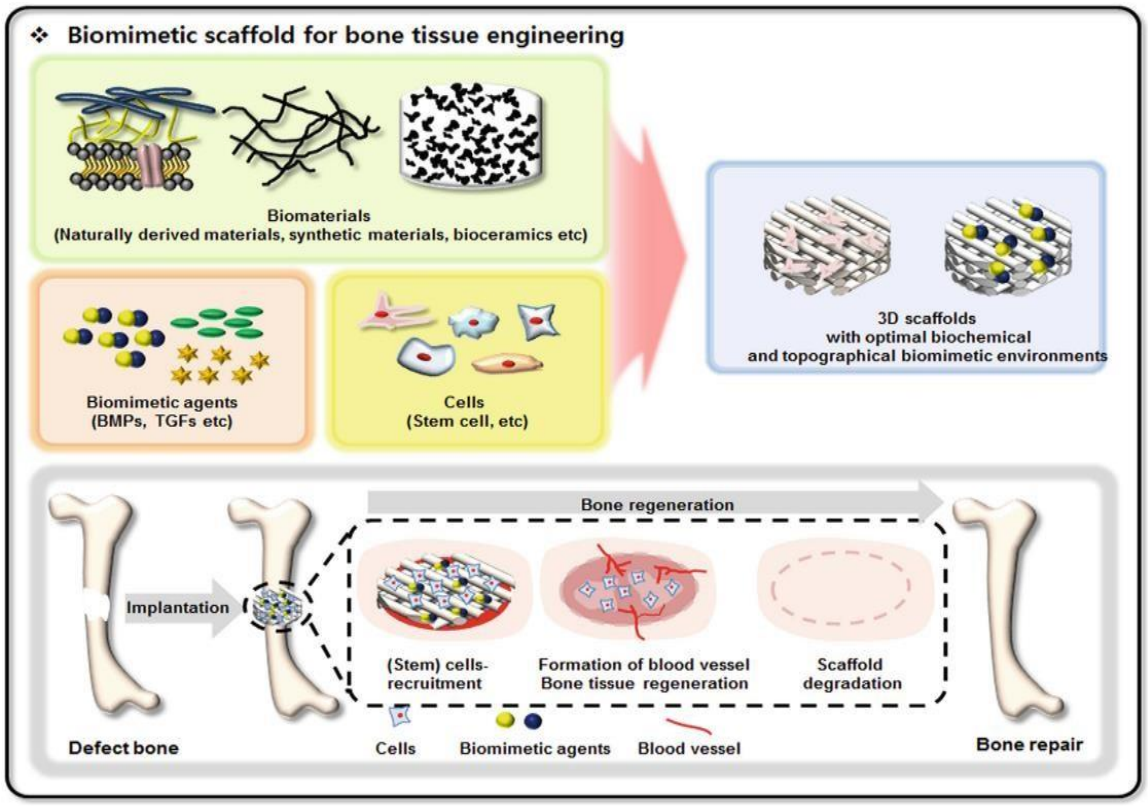


***Figure 3**: Scaffold approaches for tissue engineering [14].*

2.2.2.1 Properties for vascular graft

The creation of graft requires several parameters and properties into consideration. The synthetic vessel should comply with native blood vessels. The produced graft should show the properties like Non-destructive to blood cells and enzymes, low thrombogenicity and non-immunogenicity, do not alter plasma proteins or trigger blood electrolyte depletion, remodeling capability, and other technical advances that minimize the time and cost of implantable grafts, making it even more patient-friendly. The vascular graft should be high patency, compliance, consistency in properties values and sterility, resistance for kinking, stretchy and circumferential strength to face up to blood pressure with simple handling, and suturability are alternative fascinating features of it [12].

The overall tissue-engineered vascular graft should show good mechanical and biological properties. The biology of cell-scaffold interaction plays a crucial role in the efficient development of tissue-engineered conduits. In biologically properties scaffolds should be thromboresistance. Where thromboresistance comes from intact endothelial cell lining in the scaffold lumen [12].

2.3 Blood vessel

Tissue engineering defined has an idea about copying or mimicking the extracellular matrix, structure, and composition of blood vessels. Our thesis focusing on mimicking the original vascular graft. We should understand the working and structure of original blood vessels.

2.3.1 Function and structure of blood vessel

If we measure the length of a blood vessel in a single human body it will be around 60,000 miles, which is 3 times more than the earth circle. The heart pumps blood, through the veins and arteries network supplies to the whole body. Through those networks of blood vessels, oxygen and other nutrients are provided to all cells of the body. Blood is a simply leaving fluid which carries oxygen and other substance which prevents sickness and plays other vital roles in human body system [15].

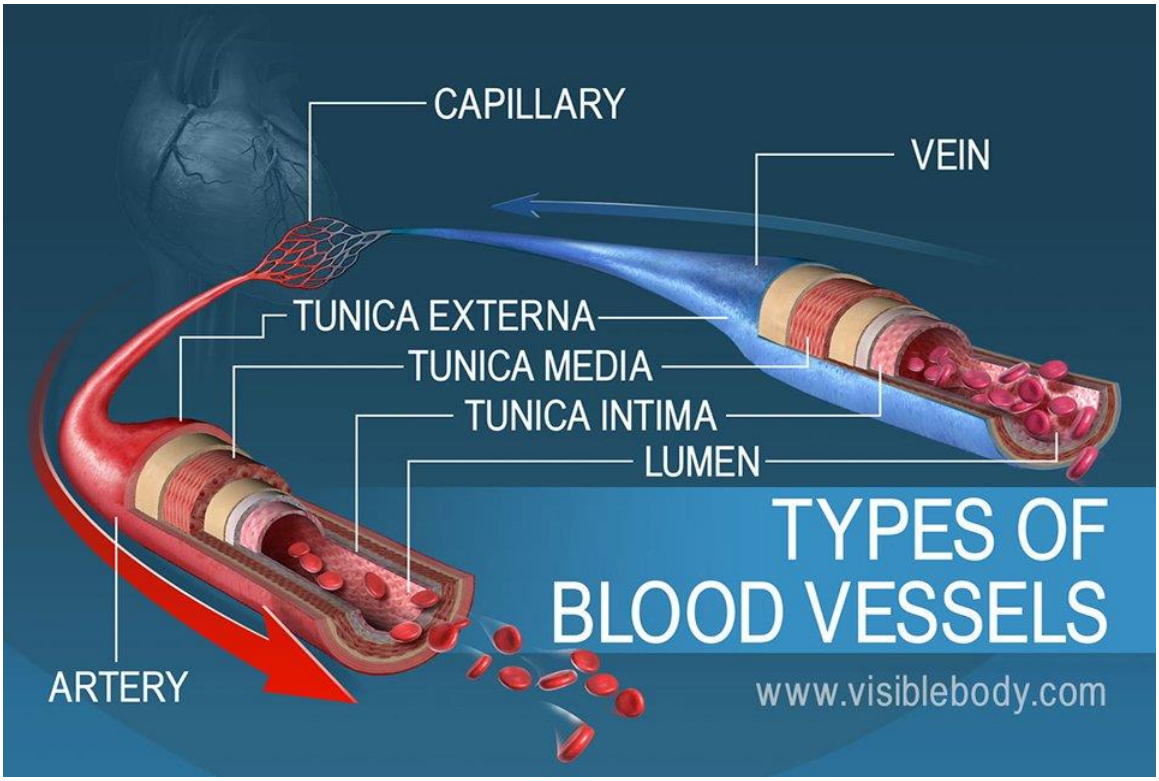


Figure 4: *Blood vessels [15].*

Blood is made from 55% plasma and 45% other elements. Where other elements contain, platelets, white and red blood cells. Blood is also named as fluid connective tissue, not only fluid. Blood doing multi-task in the human body, it controls the temperature of the body, transportation of hormones and nutrients, platelets are clot by the blood during blood vessel damage, brings unnecessary waste of the body to the kidney and liver. In the blood, red blood cells transfer oxygen to organs and white blood cells improve the immune system of the body [15].

Blood vessels are composed of different layers; those layers should sustain the force exerted by blood flow. Blood flow applies pressures and pulsating nature on the layer of blood vessels. Three layers are present in blood vessels named tunica intima, tunica media, tunica adventitia [13].

The inner surface of the blood vessel is composed of the Tunica intima. Tunica intima is made of endothelial cells. This layer is in contact with the flow of blood. Which prevents thrombogenesis of blood flow [13].

The central part of a blood vessel is made from Tunica media. Tunica media is composed of smooth muscle cells and elastic fibers. It separates the layers of tunica intima and adventitia. It provides elasticity to the blood vessel. Elastic fiber and smooth blood vessels are separated

by inter lamella matrix made of collagen, proteoglycans, and glycoproteins. The outer side of a blood vessel is a tunica adventitia, which protects the blood vessel from the outer environment. Tunica adventitia is made of fibroblast and collagen fibers [13].

2.4 Vascular Tissue engineering

Vascular tissue engineering applies engineering and life science to construct a vascular graft, which shows biological and mechanical properties similar to native blood vessels. Design and production of the tissue-engineered graft must do by considering cell adhesion, proliferation, differentiation, and choice of cell types [16].

Both small and big diameter blood vessels require properties that are discussed in scaffold properties points. The rate of degradation of the engineered scaffold should match to regeneration rate of native blood vessel cells to create a functional substitute. The key point for tissue engineering is the maintenance of structural and mechanical integrity of the neovascular tissue overall through the regeneration process. Vascular tissue engineering uses natural or synthetic polymers, whereas both have their advantages and disadvantages. Where natural polymer shows low mechanical properties but they show excellent biological properties and Synthetic polymers maintain good physical and mechanical properties but lag in biological properties. Recent developments going on to reduce those disadvantages and develop a blood vessel that will replace native vessels in the future. Small and big diameter vascular tissue engineering is done in different ways as shown in Figure3. By decellularized matrices or scaffolds formed by using natural or synthetic polymers followed by using different production technologies [16].

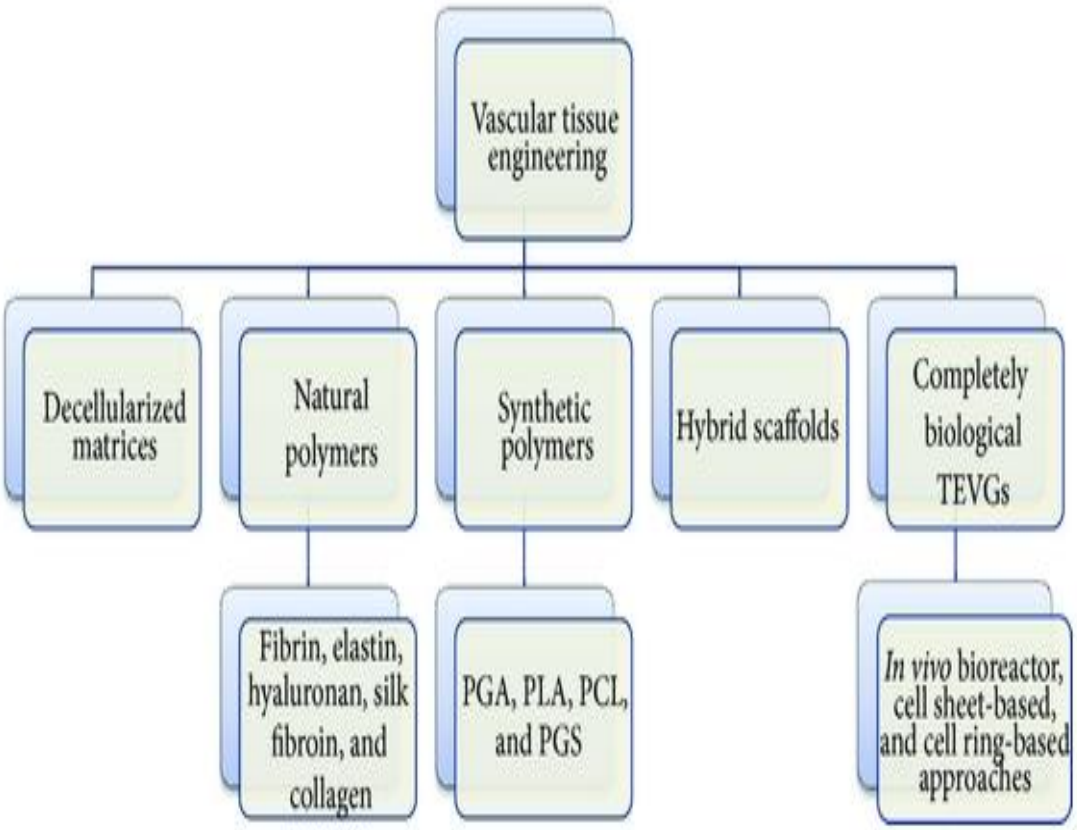


Figure 5: *Vascular tissue engineering [16].*

Vascular structures have been engineered by in vivo stimulation of angiogenesis, by endothelial cell implantation, or by decellularized organ reendothelialization. Moreover, microfabrication technologies will make important promises for the future of in vivo vascular tissue engineering. Vivo stimulation of angiogenesis- To form a new capillary or new graft, endothelial cells should break the base layer of the existing blood vessel. To stimulate endothelial cells different types of growth factors are used. Implantable engineered microvasculature- In an embedded matrix or scaffold, vascular self-assembly often occurs with freely integrated endothelial cells [17].

Decellularization methods have been utilized to replicate the normal vascular organization of a particular organ. Decellularization regimes have been developed, typically using mild detergent solutions, DNAase applications, and extensive washing, that maintain the organ's extracellular matrix scaffold, including microvascular basement membranes [17].

2.4.1 Textile Structures as vascular implants

From 1952 vascular implants by using textile started which can replace replaced diseased aortic vessels. Vascular implants are manufactured by different textile technologies, where technologies involve weaving, knitting, braiding, and electrospinning. Each of these processes has its benefits and drawback.

2.4.1.1 Weaving

By interlacing, two sets of yarns with each other with an orientation at 90° vascular grafts are produced. These grafts fabric contain different types of weave structure like plain weave, satin, twill weave, etc. weaved blood vessel shows smooth surface, ease of handling, water permeability, bursting strength. But weaved grafts lack compliance which can result in fatal [18].

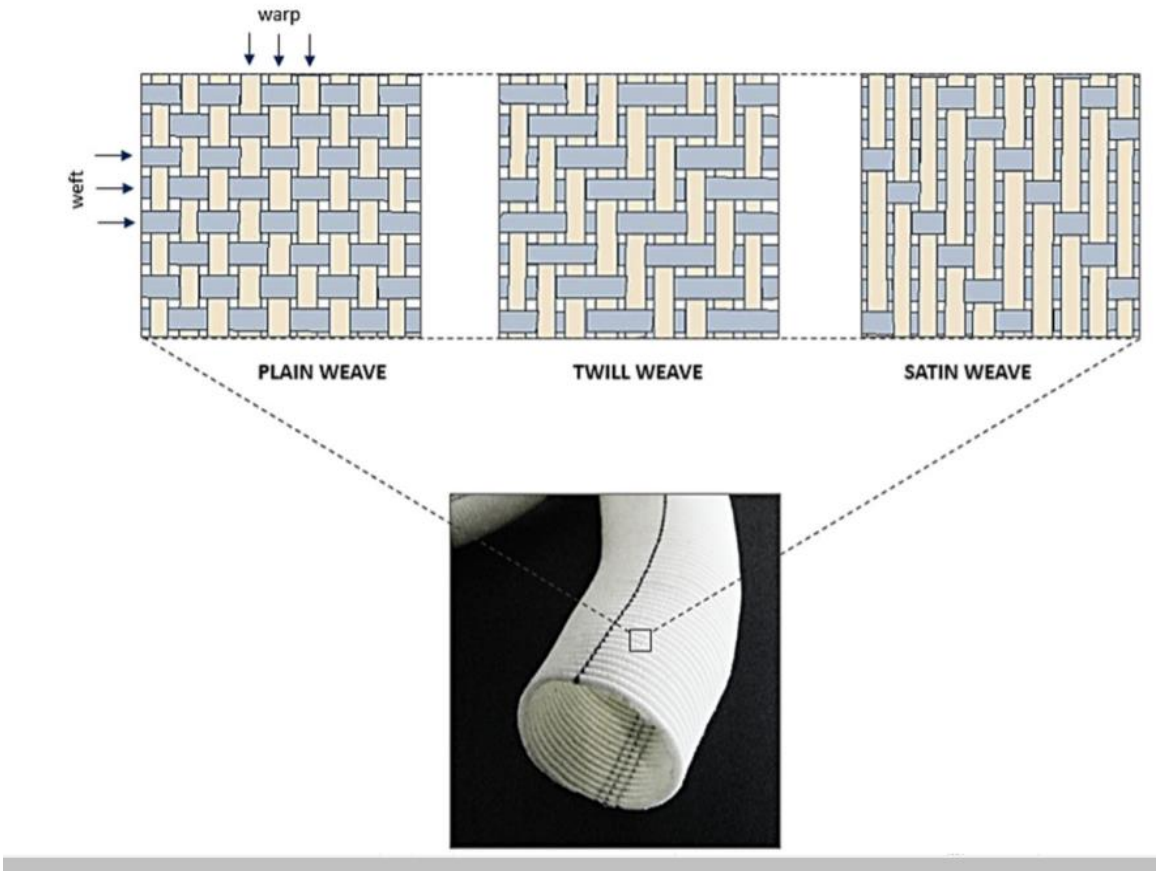


Figure 6: Woven graft structural design [18].

2.4.1.2 Knitting

Looped filament constructions are used to produce a vascular graft. Knitted grafts show more compliance, flexibility, softer and easy to handle than the woven graft. Warp and weft knitting designs are used to construct grafts mostly. Where warp knitted structures show more stability than weft knitting due to less stretchability. Different studies show Dacron, spandex, and polyurethane filaments are used in knitted grafts. Like woven graft knitted graft is also required to improve elastic behavior concerning native blood vessels [18].

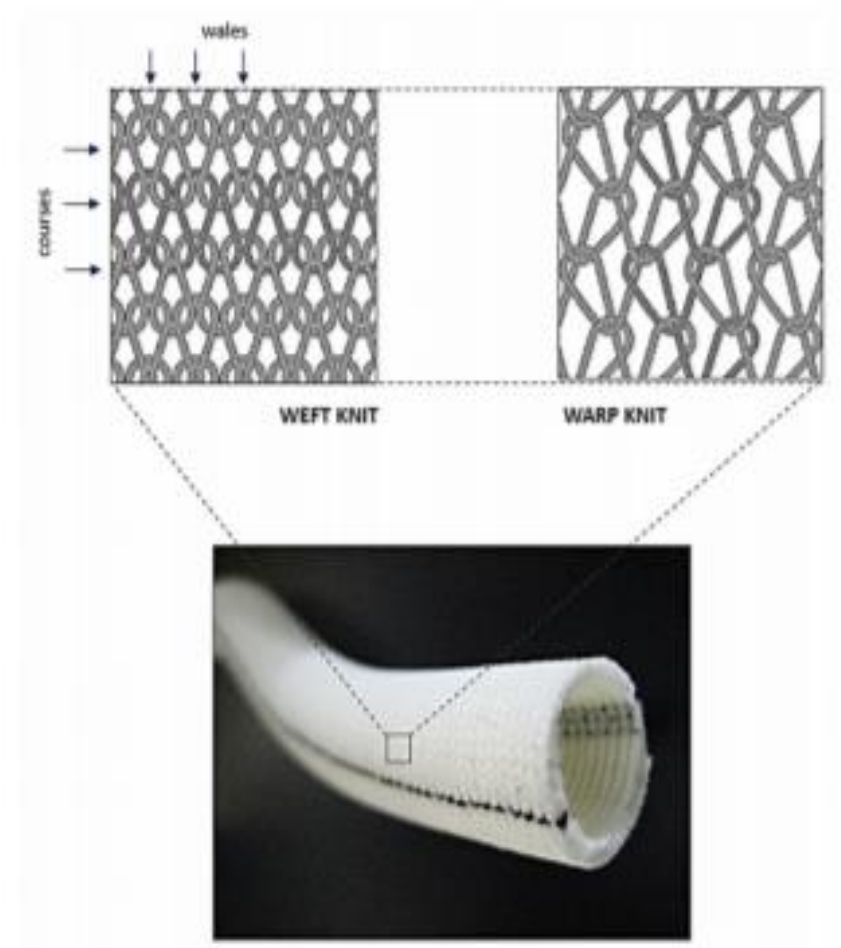


Figure 7:Knitted vascular graft [18].

2.4.1.3 Expanded polytetrafluoroethylene (ePTFE)

Currently, mainly polyethylene terephthalate (PET), polyurethane (PU), expanded polytetrafluoroethylene polymers are used to manufacture artificial vascular grafts. In 1937 PTFE was patented by Dupont by the name -Teflon. Ideally, due to its inert nature, it is used as an electrical insulator and in medical applications. Later on, Gore patented expanded PTFE as Gore-tex in the 1960s, ePTFE material used in vascular graft manufacturing[19].

ePTFE produced from the heating, stretching, and extruding process results in a highly porous material and more supportive to tissue adhesion. ePTFE graft showed a 5year primary patency rate at 91% to95% in aortic substitute. The 3- and 5-year patency rates for femoropopliteal bypass grafting are just 61 % and 45%, respectively, while the 5- and 10-

year combined patency rates for autogenous vein grafts are 77 % and 50 %, respectively. But ePTFE failed in small diameter vascular graft due to biocompatibility and occurrence of thrombogenesis [19].

2.5 Polymers in Tissue engineering

Plenty of polymers are present in the world. Every polymer has its areas of application. Polymers used in tissue engineering are called biomaterials. Selection of biomaterial depends on the following properties of polymer-

- Molecular weight
- Chemistry of material
- Hydrophobicity/hydrophilicity
- Solubility
- Structure
- Lubricity
- Degradability
- Surface energy
- Erosion mechanism [16,20].

Natural polymers have an advantage concerning biological performance due to they do not activate chronic implementation or toxicity. But natural polymers have weak mechanical properties and can transmit the infection. Whereas synthetic polymers show tailorable properties as compared with natural polymers. Synthetic scaffolds show higher flexibility and controlled reproducibility than natural scaffolds. polymeric scaffolds give optimum characteristics like strength, porosity, rate of degradation, microstructure, size, and shape Also, synthetic polymers can produce it in a large amount[16,20].

As we know tissue-engineered vascular grafts should meet the properties of native blood vessels, properties like strength, compliances, and deformability. However, strength and compliance can achieve in tissue engineering, but deformability is key property during the selection of biomaterial. The selection of a scaffold depends on the degradation rate during the Vivo remodeling process. The degradation rate of the scaffold at which it degrades must match to regeneration time of tissue, to develop a functional tissue [16,20].

2.5.1 Degradability of polymers

Classification of polymers is going on the source of origin, depending on the structure and depending on the application. Depends on the origin of polymer they come in three parts as natural, modified natural and synthetic polymers. Polymers have areas of application as – rubber, plastic, fibers, paints, and coatings[21,22].

Both degradable and nondegradable polymers have their importance. There are plenty of natural and synthetic polymers which could degrade over time. Degradation may occur by biodegradation or by an enzymatic process. Polymers are organic or inorganic. Most of the polymers contain carbon and hydrogen atoms. Polymer is generally defined as; component made of the number of monomers. Polymers chain may be linear, branched, or maybe cross-linked polymer. Linear and branched polymers - can be dissolved in appreciating solvents, molecules can be separated from each other or they can melt by heat[21,22].

When Polymer is in the influence of processing conditions or due to one or more environmental factors, losses its chemical and mechanical properties then it's called polymer degradation. This loss occurs due to polymer chain breakage or polymer fragmentation. The degradability of polymers is going in two ways chemically and mechanically. Polymers that contain a "c-c" straight bond in their backbone oppose degradation. ***Figure 5*** shows the process of degradation in polymers[21,22].

Polymers degrade in different ways like thermal, mechanical, hydrolytic, chemical, biological, photolytic, ultrasonic, pollution contact, radiolytic, and sludge activation. Where the rate and amount of degradation of the polymer depend on the type of polymer, molecular weight, the morphology of polymer, and conditions to which polymers are subjected. In many of the applications of the polymer life span of polymer plays an important role. For increment in the life span of polymer, stabilizers are added to extend his life [21,22].

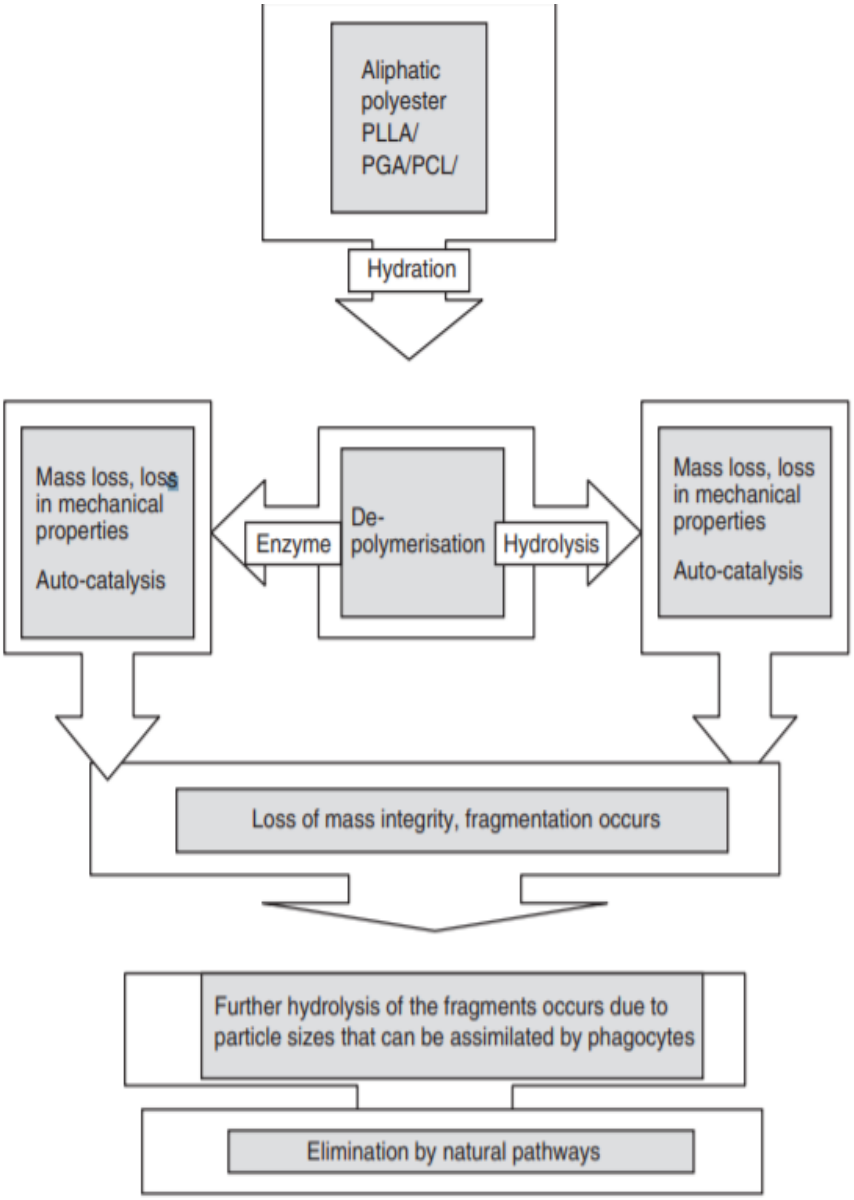


Figure 8: *Sequence in degradation of aliphatic polyester [21].*

2.5.2 Polycaprolactone (PCL)

Alpha-caprolactone is the monomer for polycaprolactone, PCL is a biodegradable polyester. We can look at ***Figure 4*** shows a Sami crystalline structure with a glass transaction temperature of -60 degrees. This polymer has a slow rate of degradation. Electrospun PCL

vascular grafts have remarkable patency because of their optimal hemocompatibility and mechanical properties. But the number of cells and capillaries in the electro-spun PCL vascular graft walls decreased in the long term (12 and 18 months), meanwhile, calcification

Density (ρ/g cm^{-3})	1.11
Av. mol. weight (M_n/g mol^{-1})	530–630 000
Solubility	Highly soluble in benzene, chloroform, DCM, and toluene at room temperature. Slightly soluble in acetone, 2-butanone, DMF and acetonitrile. Insoluble in water, alcohols, diethyl ether.
Melting point (T_m) and glass transition (T_g)	$T_m = 65°C$; $T_{g,} = -65$ °C to $-61°C$
Crystallinity(%)	67
Modulus (MPa)	190
Elongation at break (%)	>500
Tensile stress at break or max (MPa)	14
Water permeability at 25°C (g/m^2/day)	177
Surface tension (g) in mN/m	51

***Figure 9**: Properties of PCL [23].*

was observed, due to the slow degradation, lower compliance, and dense fibrous structure. PCL has a melting point of 60 degrees. PCL undergoes hydrolytic degradation with the enzymatic process [21].

The elasticity of PCL closely correlates to native blood vessel values and has a high extension Pre-breakage rate, but PCL tensile strength is less evident. An electrospun tubular PCL scaffold revealed a 4,000 mm Hg burst strain, which is very similar to natural tissue. Small

diameter vascular constructs based on PCL have been reported to have good suture retention value and compliance with blood vessel physiological conditions. The slow rate of degradation of PC limits vascular regeneration in vascular tissue engineering. Due to the slow rate of degradation capillary walls decreased over time (12-18 months). This result limits the PCL application in vascular tissue engineering [12].

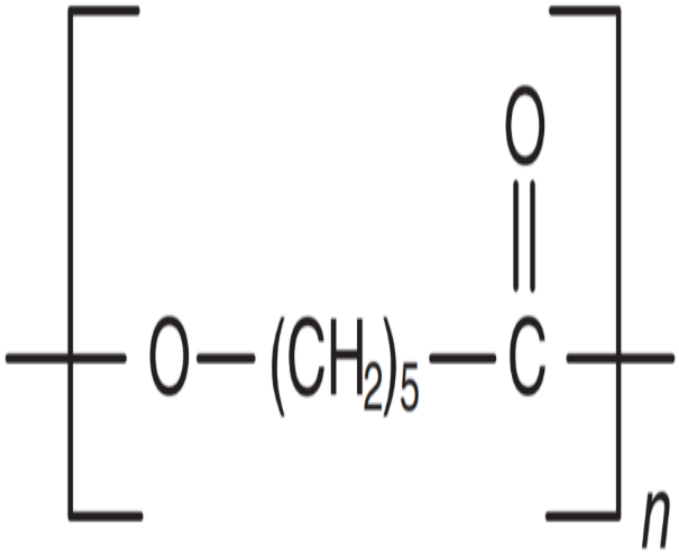


Figure 10 : *Polycaprolactone [21].*

2.5.3 Polyurethane

PU integrated from poly (alpha-caprolactone) diol,1-4-butadiisocynate, and L-lysine ethyl ester dihydrochloride. The repeating unit of polyurethane is urethane moiety. polyurethane is derivative of carbamic acids. This acid is present in the form of esters. Generalize structure of urethane we can see in Figure 7 and Figure 8 [24].

The structure of the polyurethane 'R' group and substitute amide group creates different urethane. polyurethane consists of a repeating urethane group with other moieties like ester, ether, and aromatic. Polyurethane is not formed exclusively by only carbon atoms. It is composed of heteroatoms, carbon, oxygen, and nitrogen [24].

Polyurethanes are recognized biocompatible materials. Polyurethane was used in the biomedical field for the last 50 years due to its versatile properties like toughness, durability, biocompatibility, and most important tailored degradation rate [24].

Polyurethane was the dominant candidate for medical application. Because PU can fabricate by conventional methods as well as new methods. Conventional like salt latching, gas foaming, and electrospinning and Advances like fused deposition and bioprinting. PU constructs can be tailored as per the physical and chemical properties of the soft tissue engineering application [24].

From the medical point of view, polyurethane showed better resistance towards macromolecular oxidation, hydrolysis, and calcification. Due to high elasticity, toughness, resistance to tear, oxidation, and humidity polyurethane elastomers are used as elastomers. Polyether derivatives of polyurethane are inexpensive concerning production [25].

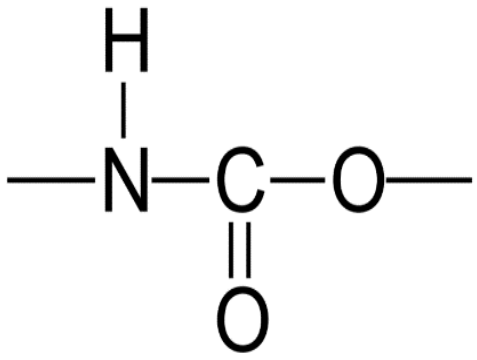


Figure 11:*Monomer of polyurethane [26].*

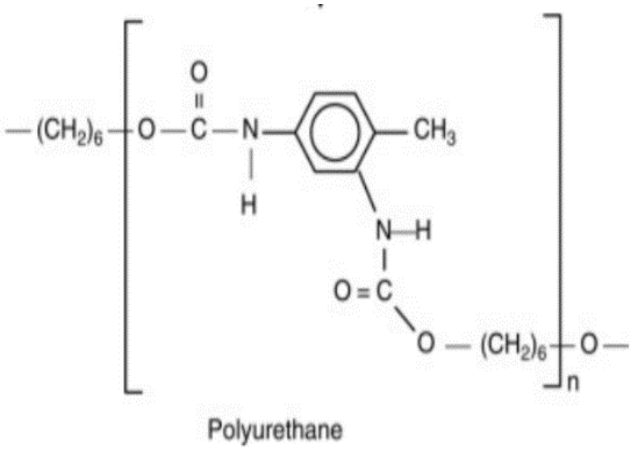


Figure 12: *Structure of polyurethane [24].*

The degradability of the polyurethane can be adjusted by inserting chemical linkage into the copolymeric structure of Polyurethane. Where looking at this versatility of polyurethane it has a high potential ability to be used in tissue engineering. polyurethane has a bright future in the tissue engineering field [25].

Application within the biomedical discipline started in the 1950s with the paintings of Pangman. Pangman used polyester-urethane foam as a breast prosthesis coating, in pangman experiment material rapidly degrades in vivo. Another polyester-urethane foam was generated by ostamer in 1959. Ostamer used PU foam as a bone gap filler. This experiment was successful than pangman development [26].

In 1959 B. F. Goodrich from the USA has used a PU-based "estane" name product, this product was used for the preparation of heart valves and aortic grafts. Nowadays PU was extensively used in tissue engineering for different purposes [26].

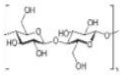
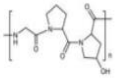
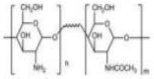
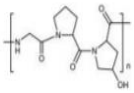
Looking at the dominancy of polyurethane, this thesis selected polyurethane polymer for the production of the electrospun vascular graft.

2.6 Production of blood vessel

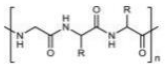
For the production of a blood vessel, the Thesis should follow the basic steps. Steps from material selection, fabrication techniques, surface modification, mechanical properties, and bioactivity of material [27].

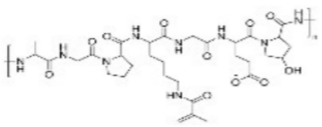
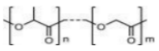
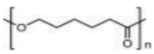
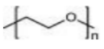
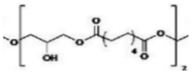
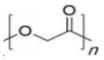
2.6.1 Material selection

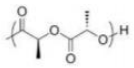
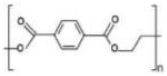
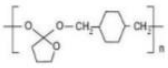
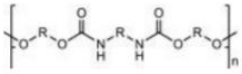
Different synthetic and natural polymers are used to produce a tissue-engineered vascular graft. polymers that show good biocompatibility are used as biopolymers for graft production. Following are the polymers used in tissue engineering –

Natural Polymers	Chemical Structures	Main Advantages & Applications
Cellulose		Mechanical properties,[34] shape-memory, self-rolling[26]
Collagen		Cell adhesion,[35] elastic modulus,[31] hydrogel[7]
Chitosan		Cell encapsulatin,[36] surface modification[37]
Gelatin		Cell growth,[38] cell proliferation,[30] hydrogel,[39] ECM[40]

DOI: 10.1039/C9TB018

Silk		Biocompatibility,[41] compliance,[42] hydrogel[43]

Synthetic Polymers	Structural Units	Main Advantages & Applications
Gelatin methacrylate (GelMA)		Hydrogel,[44] cell encapsulation[45]
Polyacrylamide (PAM)		Hydrogel[43]
Poly (D,L-lactic acid-co-glycolic acid) (PLGA)		Tissue engineering and biocompatibility,[46] cell affinity[47]
Poly (ε-caprolactone) (PCL)		Tissue engineering[13,48,49]
Poly (ethylene glycol) (PEG)		Surface modification,[50] biocompatibility[51]
Poly (glycerol sebacate) (PGS)		Elastomer[52,53]
Polyglycolic acid (PGA)		Thermoplastic polymer[54]
Polylactic acid (PLA)		Mechanical properties,[55] tissue engineering[56]

Poly (L-lactic acid) (PLLA)		Mechanical properties,[55] tissue engineering[56]
Polyethylene terephthalate (PET)		Biofabrication[8]
Polyorthoester (POE)		Mechanical properties[57]
Polytetrafluoroethylene (PTFE)		Clinical reference,[58] surface modification[5,59,60]
Polyurethane (PU)		Elastomer, elastic modulus[38,43]
Polyvinyl alcohol (PVA)		Hydrogel[61]
Thermoplastic polyurethane (TPU)		Tissue engineering[33]

Natural polymers had limitations like they are costly nowadays, limited stock, batch to batch variation, cross contaminations. In natural polymer - Collagen was used most of the time in tissue engineering. Collagen fabricated into grafts via electrospinning and implanted into arterial vessels. The vessel showed good biocompatibility, and the grafts have effectively shown growth, migration, and proliferation. To improve degradation time and mechanical properties researchers went with a synthetic polymer. Synthetic polymers are available at different rates of degradation which makes them versatile. In biodegradable synthetic polymers - polyglycolic acid (PGA), polycaprolactone (PCL), polylactic acid (PLA), poly (glycerol sebacate) (PGS), copolymers of poly (lactic-co-glycolic acid) 30 (PLGA) and, thermoplastic polyurethane (TPU). wherein the non-degradable polymer includes polymers like polytetrafluoroethylene (ePTFE) and polyethylene terephthalate (PET) [27].

2.6.2 Fabrication technique

To get the required properties in vascular graft we can use the following fabrication techniques for the production of the vascular graft. From these techniques, we can achieve require mechanical, chemical, and biological properties of the vascular graft.

1. Extrusion followed by expansion
2. Electrospinning
3. Braiding
4. 3D printing
5. Gas foaming

6. Thermal induced phase separation

7. Synthesis of hydrogel

8. Combination s of these methods [27].

2.7 Electrospinning

Due to morphological resemblance to the extracellular matrix, nanofibrous scaffolds are currently under study in connection with a variety of tissue engineering applications. Nanofibrous scaffolds accelerate the regeneration of repaired tissue. Cell proliferation and cell adhesion it's better in nanofibrous structure. Nanofibers are mainly known for their size (diameter). 50nm to 1μm is the range of nanofiber diameter. Nanofiber shows specific properties with excellency Nanofibers can be produced: electrospinning, centrifugal spinning, fiber drawing, isolation of phases, and self-assembly. However, due to its simplicity and possible upscaling of output, electrospinning is the most commonly used technique. Also due to the simplicity of construction and ease of adjustment of the setup and parameters, needle electrospinning is used for the conduct of laboratory experiments. Where needless used for high production usually[28].

William Gilbert was the first person who invented the electrostatic attraction of liquid in the year 1600. After this first patent of electrospinning was came in 1900 by John Francis Cooley. Before this patent, the process of manufacturing nanofibers was described by Charles Vernon boys [29].

Vernon boys constructed one apparatus with a little dish, which is connected with an electrical machine, which will supply power to the dish. Around 22 patents on electrospinning are done in a period between 1933 to 1944 by Anton Formhals. Formhals designed a machine that had a fiber emitter with a saw tooth. circular saw dips in a spinnable liquid, on wetted tooth's electrical charges are concentrated which will cause for generation of fibers, those created fly off fibers collected by a rotating disc. Between 1964 to 1969 mathematical modeling of electrospinning was done by Geoffery Ingram Taylor. after 1995 there are plenty of publications that have come mostly by Reneker. Reneker did too much advertising for the electrospinning process, with the advertising plenty of publications come into the studies [29].

2.7.1 Principle of Electrospinning

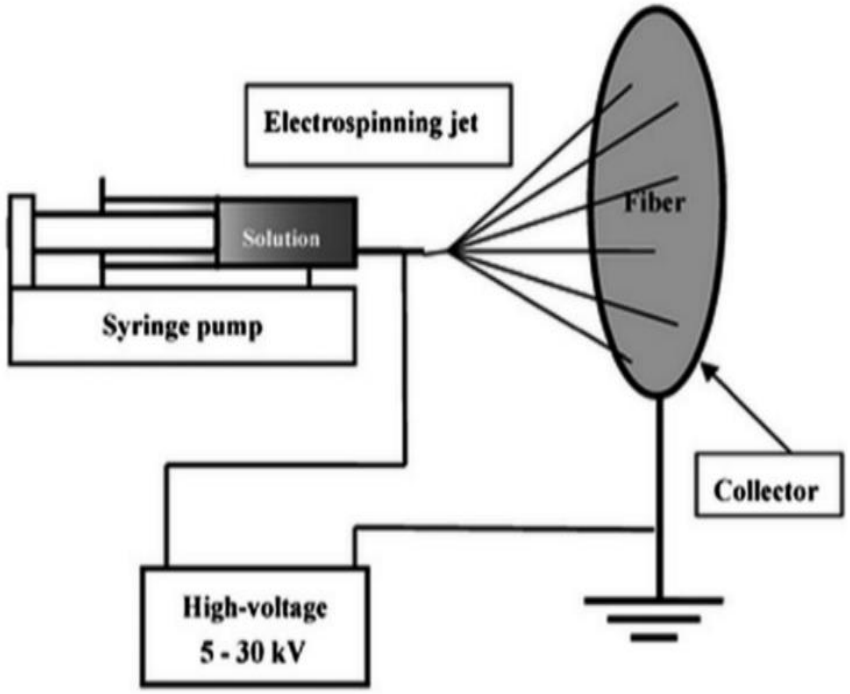


Figure 13: *Electrospinning process [29].*

Electrospinning apparatus mainly contains 3 components – firstly syringe field with the polymer solution, solution in syringe must carry the electrical charges and should have enough viscosity to spinout the fibers. The syringe output rate decides the critical properties of the fiber. The second Component is a high voltage, supply to give enough electrostatic force to the polymer solution and to stretch out the polymer solution [29].

This stretched polymer solution is collected by a third component called a ground plate. applied voltage around 5-30 kV. by increasing supply voltage, we can produce high electrified polymer solution droplets. Current induced in a solution with the help of supplying high voltage, that solution passed through the needle to form a Tylor cone. After the formation of a cone, the cone started to deforming and producing nanofibers from the solution. As we increase the supply voltage it elongates fibers. Up to a certain point of voltage, it will elongate the fiber. The basic principle we can see in given Figure 10 [30].

Until now different types of polymers are used for the production of vascular graft on electrospinning. in those PLGA, elastin, polyethylene dioxide, PPLA, gelatin/PCL, ST-gelatin, collagen, and poly (ethylene oxide) types of polymer used. Oxide morphology control of synthetic polymers is easier than natural polymers in the electrospinning process. The shape and structure of polymeric materials are affected by the chemical structure, spatial order of the chain, and molecular weight of the polymer. Where polymeric solution

properties depend on the solvent type, surface tension, concentration, electrical conductivity, solvent volatility, solvent dielectric constant, and soluble temperature. To produce a nanofibrous scaffold some process parameters should be considered as follow

- Applied voltage
- Spinning distance
- Feeding rate
- The geometry of the capillary tube
- Collector shape
- Environmental factors [31].

Polymeric electrospun nanofibers	Cultured cell	Graft
Collagen	Endothelial α-SMA positive cells	Artery
Collagen type I	canine jugular α-SMA positive cells	Venous
PCL/collagen	Endothelial cells	Artery
PGA	Bovine aorta α-SMA positive cells	Artery
Chitosan-PCL (CS/PCL)	Human umbilical vein endothelial cell (HUVECs)	Artificial blood vessel

***Figure 14**:Electrospun nanofibers for the fabrication of engineered vascular grafts [31].*

In recent decades Electrospun scaffolds are used for vascular tissue engineering. Weinenberg and Bell first researchers who developed multilayer vascular grafts by using collagen and Dacron mesh. They prepare grafts similar to the artery to absorb physiological pressure. Like this Hirai and his colleagues produced tabular vascular tissue by using collagen. Hirai wrapped produced graft in Dacron mesh which is implanted in canine posterior vena cava for up to 2 years. They observed that tissue becomes dense with time[31].

Tillman et al shown from their experiment that an electrospun scaffold with vascular cells is a good alternative for a vascular graft and reconstruction. Tillman produced a PCL/collagen scaffold. The produced scaffold can maintain its structural integrity and biomechanical endurance which we can compare with native blood vessels[31].

Niklason and his colleagues used biodegradable PGA to form a bovine aorta which shown better-ruptured strength as compared to a native blood vessel. Also, this vessel is shown an appreciative contractile response to drug stimulation[31].

In another study by Fengyi et al, a heparinized three-dimensional nanofibrous vascular scaffold was developed to prevent thrombosis using chitosan and poly-caprolactone. The results of platelet adhesion assay and activated partial thromboplastin. Time confirmed that the Anti-thrombogenic properties of these scaffolds increased with their heparinization. Therefore, the use of chitosan /PCL heparinized scaffolds could create a method for the production of artificial blood vessel arrays [31].

To prepare tubular vascular graft needle spinning electrode and rotating mandrel collector type of electrospinning machine was used. From a proper graft on electrospinning, the Electrospinning process requires a perfect solution to spin it out into a graft. The polymer should dissolve in the solvent and the formed solution should be viscous. To get a proper solution we have to keep stirring for over 24 hours on a magnetic stirrer[4].

Orientation of fiber can be achieved by varying the rotational speed of the mandrel. Fiber orientation will improve the mechanical properties and cell organization. temperature and relative humidity are maintained continuously throughout the process. The reciprocating flow of the needle is formed by a sliding holder. Step motor giving the power to sliding holder. The length of the slide depends on us, how much long graft we wanted we can set sliding length [4].

2.7.2 Types of machines used for electrospinning

The conventional electrospinning technique uses a needle as a spinneret, due to that, it is named needle electrospinning. Conventional Electrospinning is a versatile technique but it had some drawbacks, it had a minimum limited amount of production rate and use of toxic solvents concern to the environment. Due to this drawback's electrospinning started to come in different designs [30].

2.7.2.1 Melt electrospinning

The polymers cannot dissolve in any solvents, for those polymers this design was created in an electrospinning machine. Lar rondo and Manley, applied electrostatic force to a molten polymer to spin it out. The whole setup of the machine is similar to conventional, polymer heater is added to it. It has a complicated configuration due to the heater plantation in the machine. It comes with many advantages like /removal of toxic solvents, high throughput rate, ease of fabricating polymeric fiber blends. Over this, it has limitations like the

requirement of a high-temperature melting system, electric discharge problem associated with the melt, low conductivity of the melt [30].

2.7.2.2 MultiJet electrospinning

To increase the production multi needles are used. In MultiJet electrospinning comes with different configurations concerning many needles, needle design, and needle gauge. The main problem with MultiJet is a jet deviation which creates instability in the process. Instability includes dripping of polymer solution and difficulty in the collection on collector [30].

2.7.2.3 Electroblowing / gas jet electrospinning

This technique uses two forces simultaneously to produce nanofiber, It uses electric force and air-blowing shear force. Same design as conventional electrospinning with an additional air blower. This design is mainly used for a highly viscous solution where electric force has less power to overcome surface tension [30].

2.7.2.4 Centrifugal electrospinning

This type of electrospinning uses centrifugal and electrical force combine to produce nanofiber. This design provides a higher orientation of polymer chains and giver higher production with less electrical energy. But this design is expensive as compared with others. The main drawback is that produced fibers may be non-continuous [30].

2.7.2.5 Needleless electrospinning

Above mentioned all types of electrospinning are based on needle electrospinning. To overcome limitations of needle electrospinning. Mainly needleless electrospinning provides solutions to problems like needle clogging, fibers with similar average diameter than the needle, easy to scale up, mainly its setup configuration is easy and simple. It provides a higher production rate and reduces problems due to needle-in needle spinning (like needle whole blocking due to polymer). Needleless electrospinning design came in an industry where fabrication of nanofibrous is done directly from the open liquid surface. In 1979 firstly needleless electrospinning was raised. In needleless electrospinning different designs are

present, which include employment of bubble, conical wire assisted by the act of gravity, metal plate, splashing spinneret, rotary cone, bowl edge, toothed wheel, linear flume, and shear added spinneret. In needle spinning, polymeric jet formation occurred due to the capillary action but in needleless polymeric jet, formation occurred on a free liquid surface [32].

2.7.2.6 Nanospider

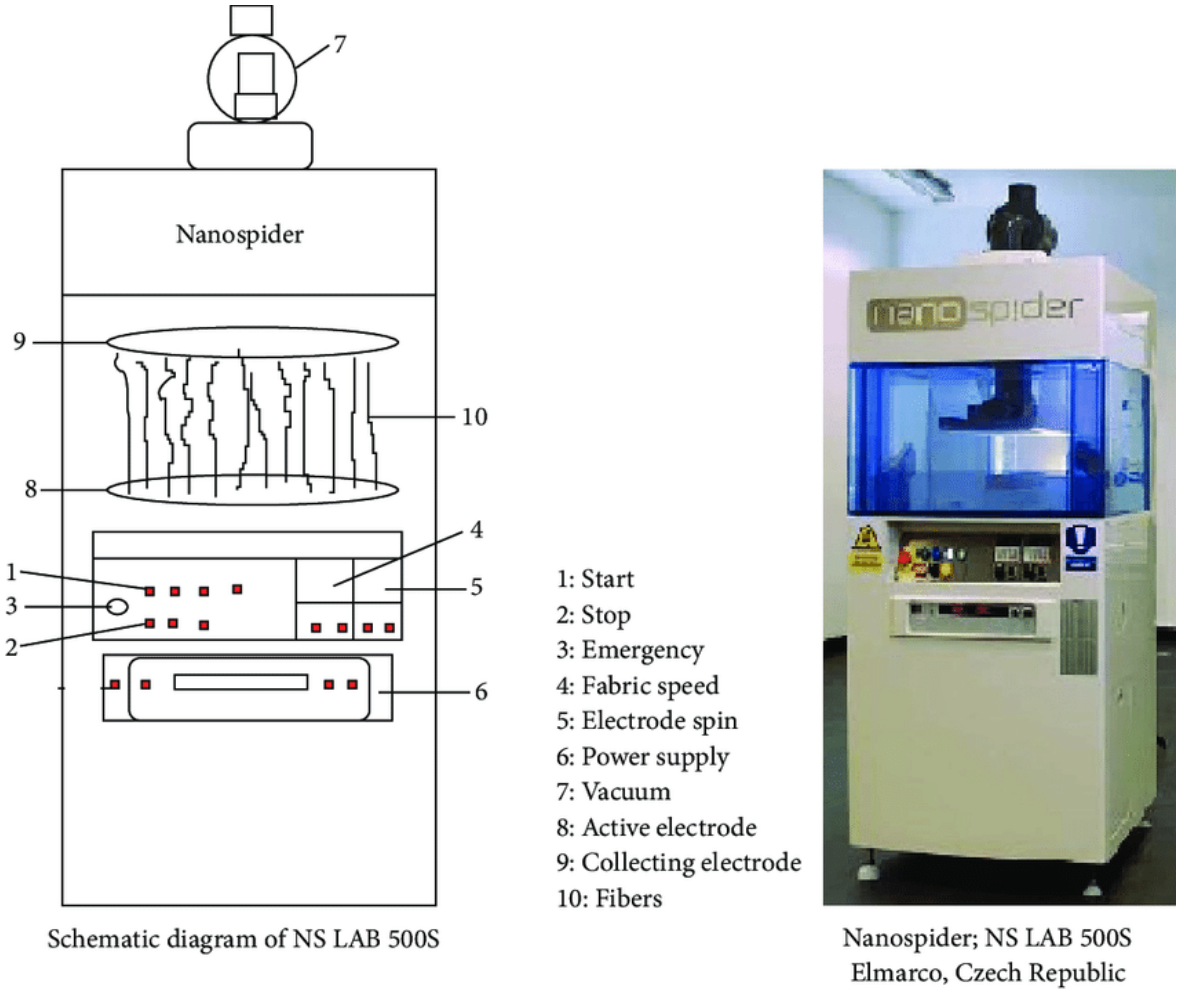


Figure 15: *Nano spider electrospinning [33].*

In 2005 needleless electrospinning was patented by Jirsak et al. which is now sold under the name "Nanospider" by Elmarco in the Czech Republic.

Two types of spinnerets in needless electrospinning – rotating and stationary. Nano spider uses a rotating spinneret. In pharmaceutical and biomedical applications needleless electrospinning use. Needleless uses different type of spinnerets as - (i) cylinder (ii)disc(iii)ball(iv)wire/rod spinnerets [32].

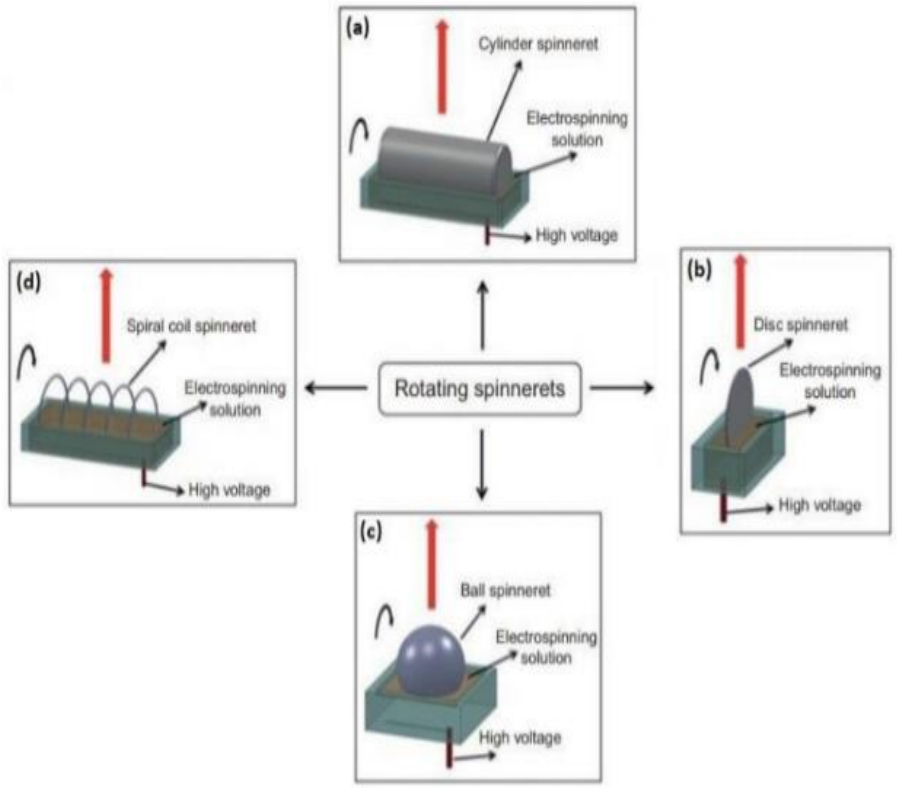


Figure 3. Schematic diagram depicting the rotating spinnerets: (**a**) cylinder spinneret, (**b**) disc spinneret, (**c**) ball spinneret and (**d**) wire/coil spinneret in needleless electrospinning (electrospinning direction

Figure 16: *Spinnerets used in needleless electrospinning [32].*

Nano spider electrospinning setups mostly use rotating spinnerets, where the direction of electrospinning is upwards. Where the cylinder type of spinneret is used in nano spider. Spinneret is partially immersed in the polymeric solution. Over the surface of the spinneret, spikes are formed [32].

Process flow of nano spider – (I) spinneret is emerged in polymeric solution, as spinneret starts to rotate a thin layer of polymer is deposited on the surface of the spinneret. (II) rotation of roller will cause to generate agitation in the solution layer which will result in conical spikes. (III) applied high voltage creates Taylors cone by deforming conical spike (IV) polymer solution stretched out from Taylor's cone to form a fiber [32].

2.7.3 Parameters in electrospinning

Properties of nanofibers are dependent on parameters of electrospinning which we are keeping during the production of graft. electrospinning parameters are mainly two types - solution parameter and process parameter. Where solution parameters - solution viscosity, conductivity, surface tension, and molecular weight of the solution. Process parameters exist with applied current voltage, tip to collector distance, feeding flow rate. Process parameters are quite important for us because the morphology and diameter of fibers depend on process parameters. For achieving aimed diameter and morphology ambient condition also helps. ambient conditions like surrounding temperature and humidity [34].

Current induced in a solution with the help of supplying high voltage. As we increase the supply voltage it elongates fibers. Up to a certain point of voltage, it will elongate the fiber, if exceed the value beds will form in the fiber. for that purpose, the supply voltage should be precise [34].

A flow rate of solution also affects the fiber characteristic, as flow rate increases mean droplets are not dry, so the diameter of fiber will be high, which will cause bed formation in fiber. For correct results flow rate should be minimum. If we talk about the distance between the spinning electrode and collector, it affects the morphology of fiber. Because these distances show an effect on the time of deposition of fiber on collector and evaporation. A small distance will not give enough time for evaporation which results in a higher diameter. More distance will result in the elongated fiber [34].

Solution concentration and viscosity of solution- if the concentration and viscosity of the solution are low then beads formation will occur in nanofiber. Whereas higher concentration and high viscosity produce uniform fiber. The conductivity of the solution affects Tylor cone formation. if conductivity less Tylor cone will not form, we can see the effect of conductivity on the Taylors cone [34].

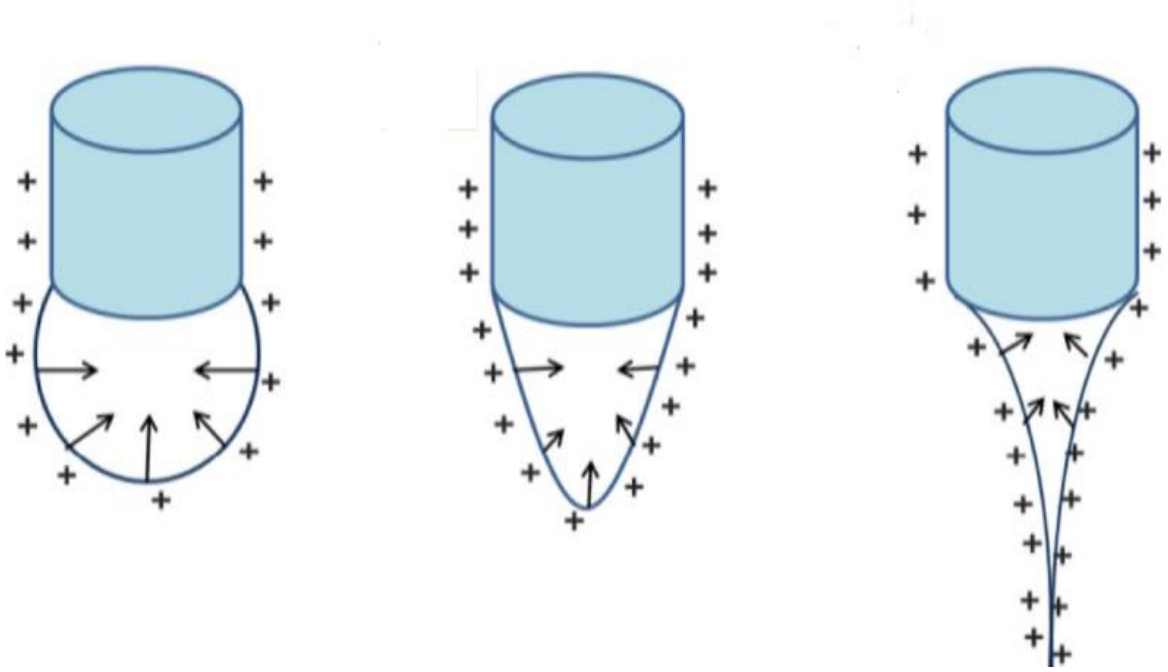


Figure 17: *Formation of Tylor cone according to the conductivity of a solution.[34].*

Whereas selection of solvent depends on the boiling point of solvent and surface tension of solvent. A lower boiling point will cause higher evaporation and a higher boiling point will cause less epuration. The process of solidifying fibers depends on the humidity and temperature provided in the surrounding. If we increase temperature and humidity fiber surface becomes porous. The high temperature will cause more evaporation of the solvent and high humidity will cause condensing of water vapor [34].

During the production of fibers, we should look out mainly on

- Diameter of fiber
- Continuity
- Surface (should be defect-free)

The diameter should be equal throughout the length. The surface shouldn't contain any beads and pores in it which will affect the fiber properties. Continuity of fiber is important to collect the fiber on the collector [34].

Technical parameters by nano spider elmarco are as follow-

Spinning voltage – 0-100kV

Substrate speed – 5-5000mm/min

Spinning distance – 120-240mm

Working temperature – 20-30°C

Working humidity: 20 - 40% RH

Process airflow: 30 - 250 m3/hod [35].

2.8 Testing Methodology

Testing of the manufactured product is as important as it is we are producing it. After the production of the material, we have to carry testing under certain conditions. For checking its physical, chemical, and biological properties, we should do testing. For the thesis part, we do the following testing of a vascular graft.

2.8.1 Fiber diameter

Physical properties and application of nonwoven products are dependent on fiber properties like fiber diameter, pore size, and fiber orientation. Electrospinning produces nanofiber which had a diameter range in nanometers, ranging from 50nm to 1μm.

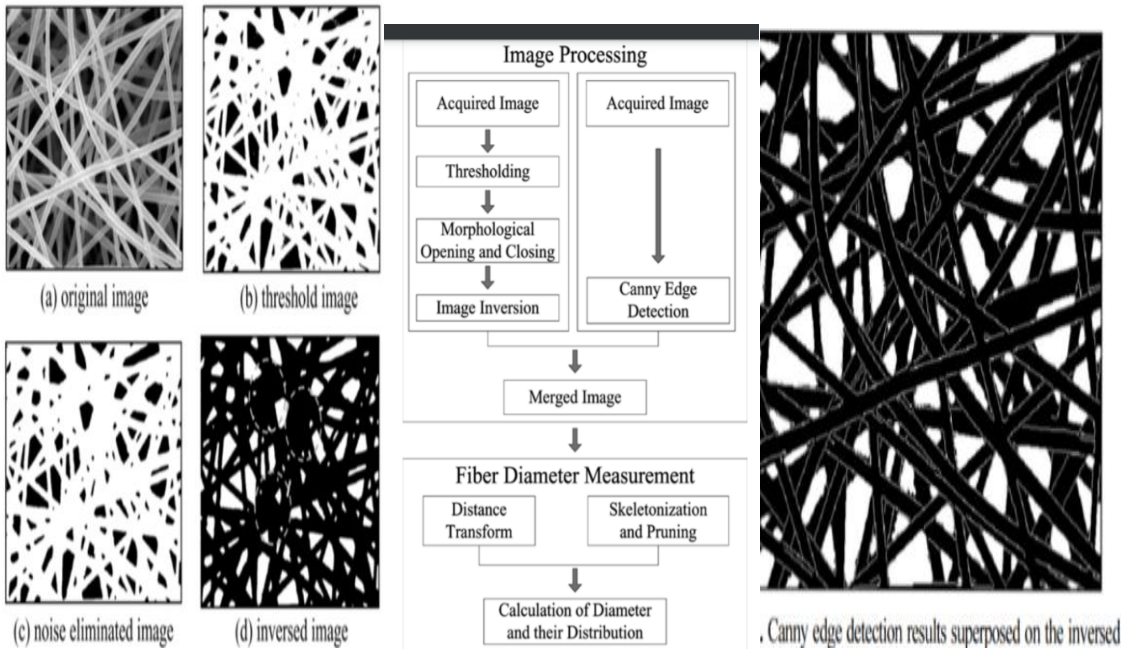


Figure 18: *Image processing analysis method on scanning electron microscope [36].*

To calculate the precise diameter of fibers scanning electron microscopy (SEM) technique was used. For the measurement of the diameter, we have to take a large number of images from different locations of the sample. which is time-consuming and difficult to obtain results. Although et al Eun Ho Shin researcher found that, we can find the diameter of nanofiber by image analysis processing method from scanning electron microscope image [36,37].

This method can calculate the diameter of fiber from a thick web by splitting the web layer into multiple layers from the image. This method can find accurate results. The scanning electron microscope method can be done in two phases- firstly image acquisitions and second image analysis. Image acquisition – hear simply we are taking pictures of the web from many angles to cover the better surface. Where image analysis process has two steps, firstly the boundaries of each fiber are detected from a binary image. The binary image is formed due to the thresholding of the image. Somehow at the crossing fiber places, boundaries of fiber are difficult to find for that reason, we have to use canny edge detection. In the second step center line is drawn by using skeletonization. The distance from the centerline to the boundary is calculated by using distance transform [36,37].

2.8.2 Porosity

The porosity of the membrane is defined as - present volume of voids in the membrane to the total volume of the membrane [37].

Porosity provides to graft to transfer ions and fluid through the graft matrix and provides a scaffold for the ingrowth of cells in tissue. Fabric porosity and graft porosity had a similarity. To test the fabric porosity, we use air and in graft we use fluid. A high porous vascular graft has the drawback that during the implantation blood leakage occurs through the graft wall. Scaffold requires optimum porosity level where scaffold can work properly without any effect on the biological performance of scaffold [38].

The porosity of the material can find out by mercury intrusion porosimeter (MIP), X-ray computed tomography, and image analysis method. Mercury intrusion porosimeter was quite commonly used. Mercury intrusion porosimeter uses the Washburn equation to find out porosity[39].

External pressure is applied to force the mercury fluid to go throughout the pores of the product. As the pressure goes up mercury bubble will form on the surface, after some time of extent, at a single pressure point formed mercury bubble will break down, by putting that pressure into in Washburn equation we can calculate pore size [39].

2.8.3 Strength & Elasticity

Essential physical properties for an ideal vascular graft should contain the following points

- Flexible
- Resistance to kinking and squashing
- Stretchable
- Range of size which will match native blood vessel
- Tensile strength minimum to withstand at cut and suture stitch.
- Circumferential strength withstands blood pressure [40].

The strength and elasticity of vascular grafts provide help to flow blood without any disturbance and withstand blood pressure. The ideal vascular graft should show good mechanical strength, elasticity, suture retention strength, and j shaped mechanical response concerning physiology. Suture retention of the graft is also we should consider from the point of implantation in the human body mechanical properties of the graft depends on the passive and active components of the graft. Passive components are elastin and collagen fiber and active are smooth muscle cells. Measurement of physical properties can be done by a universal strength tester. Under the universal strength tester, the stress-strain curve is a measure. Samples are clamped in two jaws of the tester and stretched by force at a constant rate. During the test from force and elongation, we can draw the stress-strain curve [41].
Figure 18, shows the required range of mechanical properties of the ideal vascular graft.

D Graft Configuration	Inner Diameter (mm)	Wall Thickness (μm)	Suture Retention Strength (gF)	Burst Pressure (mmHg)	Circumferential UTS (kPa)	Circumferential Strain to Failure	Death (POD)
Longitudinal Fibrin	0.6	151 ± 50	4 ± 1	115 ± 45 (n=2), > 200 (n=3)	1019 ± 329	2.80 ± 0.83	2 ± 1
Multidirectional Fibrin	0.6	218 ± 85	5 ± 1	> 200	677 ± 263	3.68 ± 1.53	9 ± 2 **
Symmetric Multidirectional Fibrin	0.6	340 ± 3 **	9 ± 2	> 200	330 ± 44	5.37 ± 0.12	3 ± 1
Multidirectional Fibrin + PCL Sheath	0.6	268 ± 85	21 ± 9 *	> 200	1637 ± 64	8.98 ± 3.95 *	N/A
PCL Sheath (heat treated)	1.04 ± 0.17	30-50	37 ± 4 **	> 200	6742 ± 1461 ****	9.07 ± 2.63 *	N/A
Mouse Native Abdominal Aorta	0.25 ± 0.04	123 ± 31	10 ± 4	N/A[a]	552 ± 319	5.98 ± 1.35	N/A
Human Saphenous Vein	3.95 ± 0.26	510 ± 30	196	2134	2610 ± 670	1.55 ± 0.06	N/A
Human Carotid Artery	7.10 ± 0.62	730 ± 70	200	3000	1022 ± 427	1.53 ± 0.27	N/A

Figure 19:Properties of the vascular graft [42].

The human saphenous vein has an inner diameter of around 4 mm and wall thickness up to 510 μm, where burst pressure 2100 mmHg which can with stand human blood pressure, circumferential strength is up to 2600 kPa with allowance ± 670 kPa [42].

2.8.4 Biocompatibility

Biocompatibility is made from different words like bio-functionality, bio-inertia, bioactivity, and biostability. Biocompatibility of synthetic implants means surrounding tissue or human body accepts that implant without any undesirable immunity response, allergic infection, and chronicle or inflammatory infection.

Factors influencing biocompatibility are as follow

- How it interacts with the surrounding
- Period of application of implements
- Suitability of host tissue with the implant
- The adaptability of implants to the host tissues mechanical properties
- Function of implant

- Size and shape
- Material [43].

Biocompatibility of material tested by different test methods as follow:

- ***MTT Test***

The quality of material being toxic to the tissue or human body and its evaluation is called cytotoxicity. Its also called cell viability in biology. Cytotoxicity can be qualitative and quantitative analysis[44].

Quantitative cytotoxicity includes 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) Cytotoxicity Test and Colony Formation Cytotoxicity test. Where qualitative test includes – direct contact, agar diffusion assay, and MEM elution assay [44].

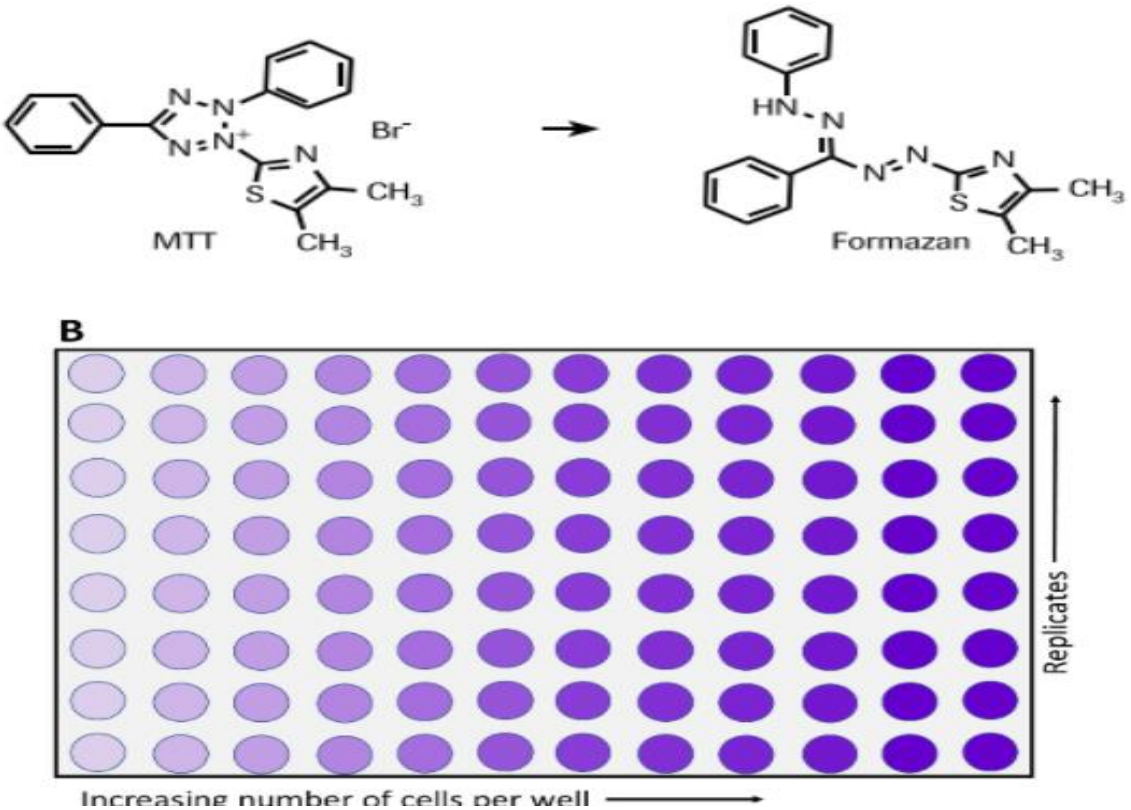


Figure 20:*MTT Test [45].*

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From the above-mentioned assays, the MTT test best fits for evaluating the cell viability and studies on cell proliferation. MTT test is a colorimetric test, where yellow tetrazolium salt is

reduced and becomes purple formazan crystals by metabolically active cells. The viable cells contain oxidoreductase enzymes which reduce MTT to formazan. After that soluble formazan crystals are dissolved in a soluble solution. That solution creates color which is quantified by using a multi-well spectrometer. depending on a shed of the color cell viability decide. darker the color, cell viability higher [45].

- ***Fluorescence microscopy***

  Fluorescence microscopy is widely used in the biological and medical fields. Fluorescence microscopy gives high sensitivity and high specificity. To find out low concentration other substances. Fluorescence microscopy finds particles below the light microscopy resolution and histochemistry which we cannot see by conventional microscopy[45].

  Fluorescence microscopy is used for studies on leaving tissue for a long time. Mostly used in vivo study of the concentration of inorganic ions and measurement of pH and calcium ion concentration. By using fluorescence esters, we can test cell viability. 6-carboxyfluorescein diacetate is nonfluorescent which can pass through the cell membrane. Inside the cell ester will hydrolyze, which will create a free anion, and if the membrane is intact, accumulated in the cell[45].

  Microscopy uses relatively short wavelength light (blue or ultraviolet), which is illuminated on the specimen. A barrier filter was used to examine the specimen, filter absorbs short-wavelength light which is used for illumination and transmits the fluorescence. lastly, we can see bright against a dark background [45].

- ***Cell counting kit (CCK8)***

  Objective - In cell proliferation and cytotoxicity experiments, the Cell Counting Kit-8 (CCK-8) enables sensitive colorimetric tests to determine cell viability. Advantages of this test kit are - It's easy to use, Colorimetric test that is non-radioactive, and Outperforms assays from previous generations (e.g. MTT, MTS, or XTT) [46].

  The working principle of CCK8 - WST-8, a highly water-soluble tetrazolium salt developed by Dojindo, is reduced in cells by dehydrogenase activities to produce a yellow-colored formazan dye that is soluble in a tissue culture medium. The amount of formazan dye produced by dehydrogenase activities in cells is directly related to the number of live cells [46].

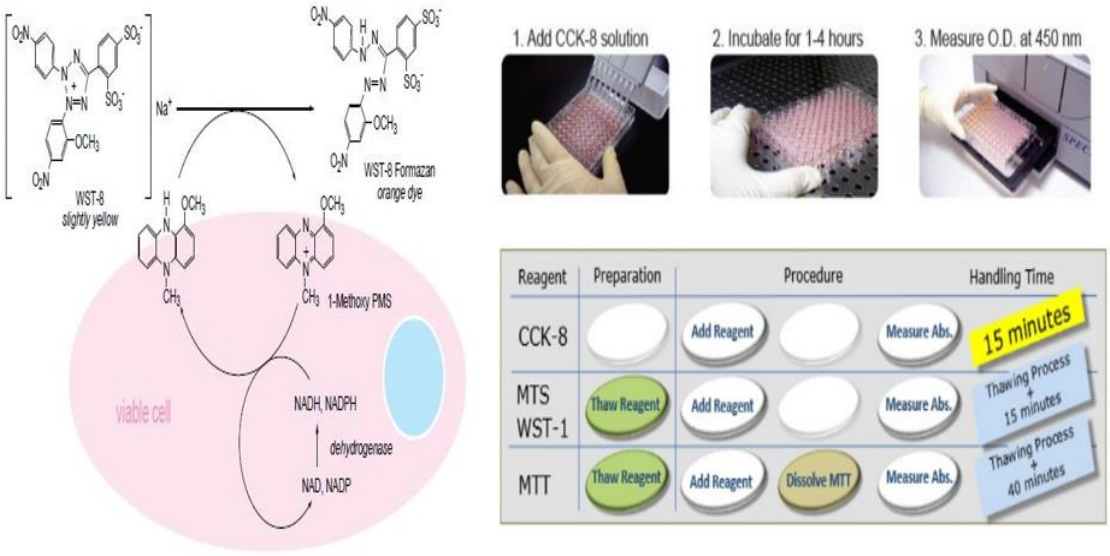


Figure 21:Working principle of CCK8 [46].

2.8.5 Characterization of wettability

Surface wettability is one of the most important factors to consider when designing membranes for a variety of purposes, including liquid separation, fluid management, corrosion protection, anti-fouling surfaces, self-cleaning materials, and protective fabrics. The contact angle derived from Young's equation is the most generally used method for determining a solid surface's wettability [47].

Based on the premise that a chemically homogenous surface is completely smooth, the static contact angle is calculated from the surface energy of the related solid and liquid. When the total surface energy of the solid surface is low, the wettability is low, and the contact angle is large. Polypropylene (PP), polyvinylidene fluoride (PVDF), polyurethane (PU), and polytetrafluoroethylene (PTFE) are common petroleum-based synthetic hydrophobic materials used in electrospun membranes for wettability manipulation. As a result, the development of electrospun membranes made from biodegradable materials is crucial. In textile research, the well-known sessile drop method for determining contact angle is commonly used [47].

The balance of interfacial energy at the solid-liquid (γ_{sL}), solid-vapor (γ_{sv}), and liquid-vapor (γ_{Lv}) interfaces completely determine the spreading of a liquid on a smooth surface, and hence the wetting behavior. The well-known Young's equation describes the relationship between these quantities and the (static) contact angle (ɵ) of a droplet resting on top of the surface (“Young angle”).

$$\cos(\theta) = {(\gamma_{sv} - \gamma_{sL})}/{\gamma_{Lv}}$$

For a better result, 30 readings should be taken on both sides nanofibrous layer [47].

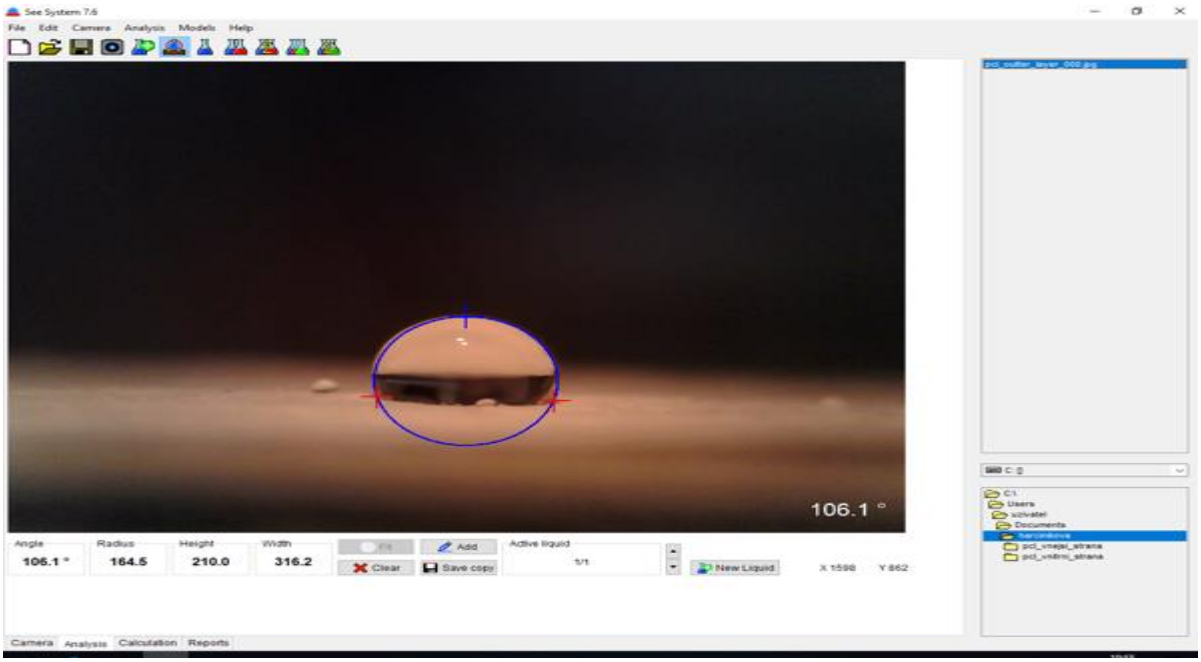


Figure 22:Measurement of contact angle.

3. EXPERIMENTAL PART

The experimental part of this thesis is devoted to the production of synthetic vascular grafts by electrospinning production technology. Where for the production of vascular graft this thesis selected different materials. Over the studies above discussed, polycaprolactone has limitations overuse in vascular graft as compared with polyurethane. Looking at the versatility polyurethane was chosen. In this thesis Biocompatible, Biodegradable, and non-biodegradable polyurethanes are used as a polymer for electrospinning. Also, needle electrospinning had drawbacks and limitations during the production of the vascular graft. To overcome those drawbacks this thesis going with needleless electrospinning technology. This thesis producing a 6 mm diameter synthetic vascular graft by using needleless electrospinning technology. The beginning thesis will look at the optimization of parameters on electrospinning using Nanospider NS 1WS500U. A sufficient amount of samples thesis needs to be prepared for material characterization and mechanical properties. Some of the properties test in the planer and tubular forms has to be done.

3.1 Material and Method

In the material and method section, this thesis studied all kinds of possible materials which can be used to produce a synthetic vascular graft.

3.1.1 Material

Different types of PU are available in markets according to properties concerning biocompatibility and biodegradability. The biodegradation rate of PU depends on the amount and type of di-isocyanates used in PU. So, for this thesis, I am going to select some degradable and non-degradable PU. Bilayer electrospun nanofiber architectures were created to mimic the morphological and mechanical characteristics of a native blood vessel scaffold. The bilayer scaffold shows mechanical properties comparable to native vessels and the combination of the two layers allowed for better cell integration and development of the cell. The inner layer had a high cellular density due to the disparity in porosity between the two layers [48].

Polyurethane is composed of two segments – Hard and Soft segments, where hard segments are made from a diisocyanate, and soft segments are composed of Polyol.

Both of the segments provide different features in Polyurethane. Toughness, strength, modulus property governed by hard segment. Soft segment causes water absorption, softness, and degradability in Polyurethane [48].

The identification of degradation products is important, but the ultimate toxicity of the degradation products is also important. If the biomaterial degrades, either naturally or as a result of biological activity, components may leach into the surrounding tissues and cause an inflammatory reaction if they are not easily metabolized by natural pathways. Because of the ester groups in the soft segment, polyester urethanes are susceptible to hydrolytic degradation, while polyether urethanes are susceptible to oxidative degradation [48].

Aromatic polyurethane shows in some studies toxicity, where the aliphatic type of polyurethane shown bio-compatible properties.

Juan V et al provided different types of polyurethane according to its biostability and biodegradability. Looking over the study did by "Juan V" this thesis chosen polyurethane composition [48].

Following polyurethanes are selected according to degradability and biostability-

Biodegradable polyurethane –

1) 4,4′-methylene bis (cyclohexyl isocyanate) (HMDI) + Poly(caprolactone) (PCL) + ethylene glycol.
2) 4,4′-methylene bis (cyclohexyl isocyanate) (HMDI) +Poly (lactic acid) (PLA)+ ethylene glycol
3) 1,6- hexamethylene diisocyanate (HDI) + Poly(caprolactone) (PCL) + ethylene glycol [48,50,65,66].

Non -Biodegradable / Biostable polyurethane –

1) 4,4′-methylene bis (cyclohexyl isocyanate) (HMDI)+ Poly(tetramethylene oxide) (PTMO)+ ethylene glycol.
2) 4,4′-methylene bis(cyclohexyl isocyanate) (HMDI)+ Poly(ethylene oxide) (PEO)+ ethylene glycol
3) 1,6- hexamethylene diisocyanate (HDI) + Poly(tetramethylene oxide) (PTMO)+ ethylene glycol[48,50,67].

This type of PU is available in market. Leading name of provider is sigma Aldrich company.

The properties of Polyurethane depend on different types of monomers, which are used during the manufacturing stage. Polyether forms of polyol and various aromatic diisocyanates were first used to create biostable polyurethanes. The chemical composition of polyols, as well as their molecular weight and concentration, have a significant impact on polyurethane activity. They can be used in a variety of quantities, but usually, 50-75 percent of the polyol is used [48]. Biostable and biodegradable polyurethanes are made from a variety of monomers, which are as follow-

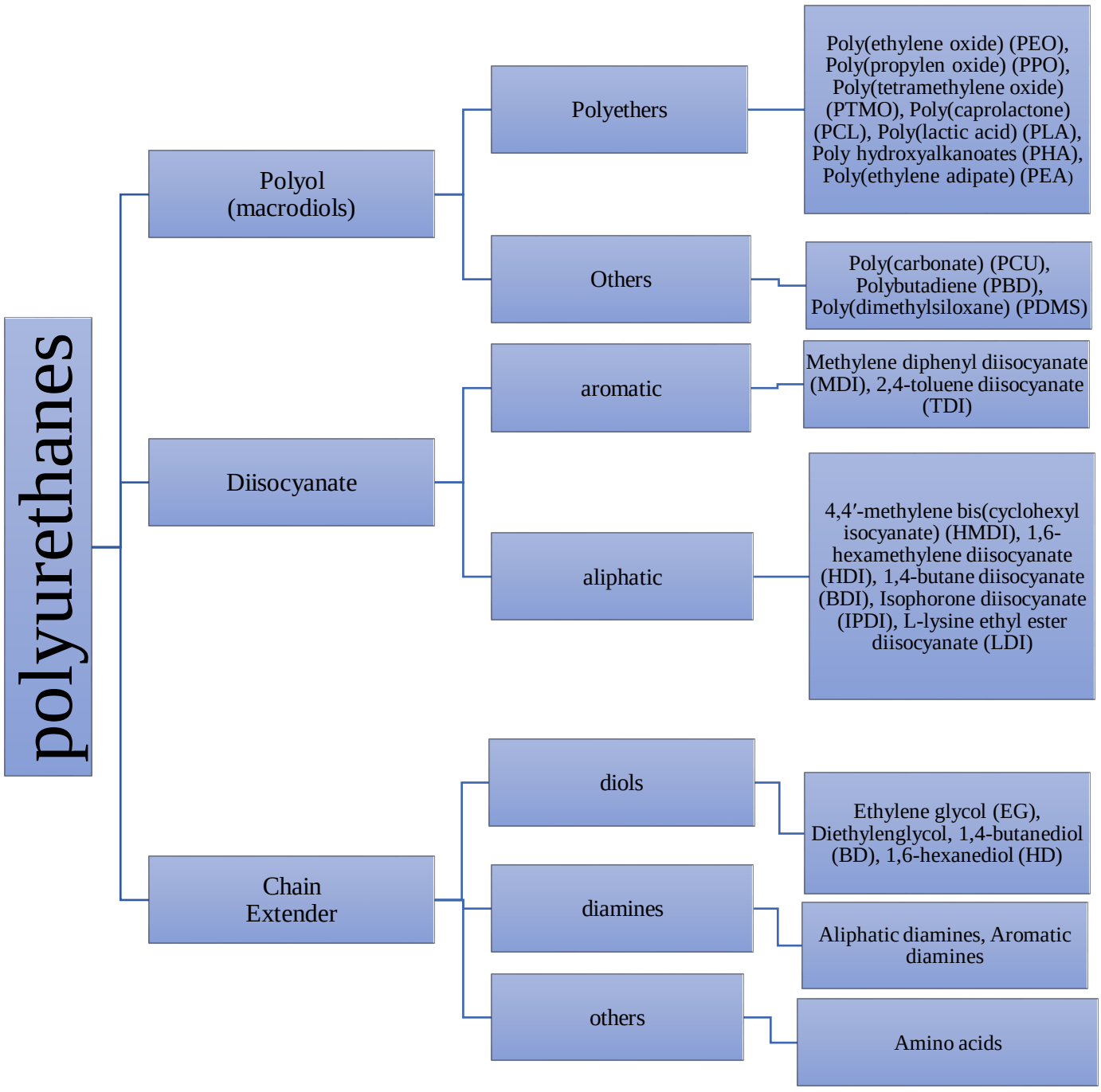


Figure 23:Monomers used in the synthesis of polyurethanes [48].

Vinoy Thomas et al synthesized polyurethane using a cycloaliphatic diisocyanate, 4,4′-methylene bis(cyclohexyl isocyanate) (H_{12}MDI), an aliphatic unsaturated hydrocarbon polyol hydroxyl-terminated polybutadiene (HTPBD), and hexamethylene diamine (HDA). This PU showed polymers' three-dimensional physical crosslinking allows for high flex life and bio durability, in a rat animal model, excellent flexing endurance and long-term in vivo biostability were achieved, less calcification, minimal platelet consumption in whole blood, optimum growth of endothelial cells [51]. That's why this study choosing 4,4′-methylene bis (cyclohexyl isocyanate) (HMDI) based Polyurethanes.

Poly(caprolactone) (PCL) and Poly (lactic acid) (PLA) are coming in biodegradable polyurethane. Poly (tetramethylene oxide) (PTMO) and Poly (ethylene oxide) (PEO) coming in biostable polymer, this shows strong stable properties in biological conditions [48].

Baker et al. co-electrospun PCL (a slow-degrading polyester) and poly(ethylene-oxide) (PEO) (a water-soluble polymer) from two distinct spinnerets to create a dual-polymer composite scaffold. PEO's quick response created space for cell invasion. However, the use of much PEO resulted in the scaffold's structural integrity being compromised. This group used a tri-jet electrospinning technique to create a multicomponent nanofibrous scaffold with an optimum degradation rate to retain scaffold integrity. PEO, PCL, and poly(lactide-co-glycolide) (PLGA) (medium-degrading) were among the fibers tested, each with a variable rate of degradation. Following the rapid dissolution of the PEO fibers, the PCL and PLGA fibers would initially help to maintain scaffold integrity, with the PLGA fibers subsequently degrading to increase pore size and porosity. The inclusion of the three types of fibers in the composite scaffold gave time-dependent characteristics and could also be employed to fine-tune the mechanical properties [49].

Chan-Chan et al, was used Poly(caprolactone) diol was used as a soft segment, 4,4-methylene bis (cyclohexyl isocyanate) (HMDI) as a chain extender, and either butanediol or dithioerythritol as a chain extender in biodegradable segmented polyurethanes. He used to make biodegradable PU used HMDI: BD or DTE: PCL with molar ratio 02:05:1:1. Also concluded that these biodegradable materials showed non-toxicity, supportive to cell adhesion of human body, viability, and spreadable cells. Also commented that this material dominant candidate in cardiovascular application [50].

A. Silvestri et al, in their experiment Polyurethane-based biomaterials for shape-adjustable cardiovascular devices, mentioned as innovative biomaterials for cardiovascular applications, some biostable polyurethane (PUR) formulations, including one using clay as a filler, were developed. Because of their mechanical qualities and excellent hydrolysis resistance, poly (dimethyl siloxane) and poly (tetramethylene oxide) were chosen as macrodiols [67].

3.1.2 Solvents used for PU in electrospinning

Selected solvent has a direct impact on electrospinning solution properties. The morphology of the electrospun nanofiber mat was significantly affected by the form of solvent and its concentration. The polymer solution must be stretched between the two poles of a high voltage supply to form nanofiber by electrospinning; thus, the conductivity of the polymer solution is one of the main factors deciding the spinning current. Highly polar organic solvents such as N, N-dimethylacetamide, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N-Methylpyrrolidone, and tetrahydrofuran are commonly used to dissolve PU[51].

Because of the rapid evaporation of the solvent in the electrospinning process, fiber skin forms easily. Since electrospinning is a quick solvent evaporation process, solvent volatility is equally essential for effective nanofiber electrospinning. If the solvent is not fully removed by the time the fiber jet hits the target plate, fiber fusion and the formation of microfibers can be facilitated. Except in the most extreme situations, the creation of a flat membrane rather than a nanofiber mat occurs [51].

3.1.2.1 DMF

DMF is commonly used as a solvent for PU Generally, DMF is used as a solvent for PU, DMF is shown cytotoxicity. But is volatile which makes it still better to choose as a solvent for electrospinning of PU [52,48].

THF - Mondal et al, used 100% THF as a solvent, THF is a low conductive solvent and resulting Bed- formation on a string and non-uniform diameter throughout the length of nanofiber. It's possible to spin fibers from low conductive solvent, but it will be non-uniform in structural properties. The beaded structure harms nanofiber membrane filtration, reduces the contact area for cells in scaffolds, and other unique properties that are necessary for many other important applications [53].

3.1.2.2 DMAC & DMSO

DMAC has a higher viscosity and boiling temperature than DMF. Viscoelastic forces associated with viscosity will cause thicker fiber formation and fusing of fibers. DMSO solvent evaporates slowly In the short traveling path, As a result of curly, wavy, and smooth nanofibers deposition on the resulting mat, the viscosity and surface tension of the DMSO-containing polyurethane solution induces excessive stretching of the polymer solution between two poles, resulting in the wavy, curly and flat nanofibrous membrane [53].

Looking over the limitation of above all solvent's limitations concerning producibility and toxicity. This thesis suggesting a new solvent that can replace DMF, and solvent names as Cyrene® (di hydro leva glucose none). This solvent is commercially available, non-toxic, made from renewable waste and nonfood cellulosic sources, It is rapidly establishing itself as a viable polar aprotic solvent alternative to DMF [54].
Roxana et al, fabrication of water filtration membrane did by using Cyrene, the membranes created using the bio-based solvent Cyrene® had a higher total porosity, pore size, and thermal stability. This solvent is used for polyester, not for PU. So in future work, it will boost my project idea [54].

3.1.3 Electrospinning of PU

Electrospinning of polyurethane can be carried for different reasons and formulated resultant product shows different results as per the production technique. For most applications diameter of the nanofibers formed by electrospinning is a critical parameter. The structural features of nanofibers, such as pore sizes and unique surface areas, are described by formed fiber diameter. These characteristics influence filter selectivity, permeability, catalytic activity in systems that use nanofibers to immobilize catalysts, and cell proliferation in tissue engineering using nanofiber-based scaffolds [55,56].

3.1.3.1 Needle electrospinning of PU

Cigdem Akduman and Emriye Perrin Akçakoca Kumbasar et al, did electrospinning of polyurethane fiber. According to the study, the experiment used the parameters- Concentration of solution – 8 to 14%. Distance between the liquid surface to collector distance – 10-20cm. Feed rate – 0.3 -1.5 ml/h. Applied voltage - 10-13kV[55].

Zdraveva et al, in electrospinning of polyurethane, used NT-ESS-300, which consists of a high voltage supply, web winding system, electrospinning nozzle, syringe pump, and control unit. DMF and THF are used as solvents with a ratio of 2:3 respectively. The solution is made at room temperature and stirred over a night. Room temperature kept around 20 degrees and relative humidity 40%. They prepared three samples named PU-1, PU-2, and PU-3 with different process parameters [57].

Polymer concentration for all three-material kept on 10 Wt.%. volume flow rate 0.5 cm^3 / h and 1 cm^3 / h. Tip to collector distance 18,15 and 12cm. electrical voltage 13kV,16kV and 19kV respectively PU-1,2,3 [57].

The result for PU-1 is fiber dimeter ranged from 300-500 nm for both flow rates, where PU-2 shows dimeter range from 300-450nm. At last PU-3 diameter ranged for bot flow rates is 350-500nm. Where overall study says that PU-3 shown a minimum diameter of 212mm and a maximum diameter of 732nm with higher structural uniformity and non-bed formation during the process. Used parameters for PU-3 are, applied voltage -19kV, distance -12cm, flow rate 0.5 and 11 cm^3 / h [57].

Zhuo et al, synthesized PU resin for electrospinning by using an average number molecular weight 180000 g/mol. Where resin-based PCL -75% as soft segment content and its produced from bulk polymerization method. Where Methylene diphenyl diisocyanate (MDI) is used as a hard segment and 1,4-butanediol (BDO) as a chain extender. DMF as the solvent used. With different concentrations Zhuo did electrospinning. Where 3,5,7,10 and 12% of concentration used. Applied voltage ranging from 12-25kV. Solution flow rate 0.4 to 0.1 mm/min and spinning distance kept 15cm with ambient temperature condition [58].

Zhuo stated up to 10kV applied voltage PU solution could not spin into nano fibers. On 12kV found uniform nano fiber diameter throughout the length and without bed formation. Also stated that on larger fiber diameter on higher feeding rate and smaller and uniform fiber diameter distribution found on lower feeding rate(0.06 mm/min). on 12 weight % concentration of solution shows high viscosity which results in electro spraying instead of electrospinning of solution. Continuous fibers are cannot be prepared from low viscosity. Clearly stated about lower and upper concentration level is 3 and 12 weight%. Concluded following parameters for best nanofibers are –Solution concentration - 5%, applied voltage-12kV, feeding rate – 0.08 mm/min, tip to collector distance – 15cm and resultant fiber diameter range – 50 to 700nm [58].

Meltem Yanilmaz et al, studied the Effect of Process Variables on Polyurethane Nanofiber Diameter Using a Factorial Design. The effects of process parameters on nanofiber diameters, the voltage applied, and the distance from the tip to the collector was investigated using a factorial experimental design. A 10% concentration solution of polyurethane tetrahydrofuran was used to make nanofibers. The effects of solution factors on nanofiber diameters are well understood. He stated that distance and applied voltage have significant effects on the fiber diameter. he used polymer–thermoplastic polyurethane with 2,70,000 gm/mol. PU:THF -10:10 ratio, Volume flow liquid – 2ml/hr and Voltage – up to 30kV[59].

3.1.3.2 Needleless electrospinning of PU

Yalcinkaya et al, prepared different nanofibrous layers by different polymers, where polyurethane used DMF as solvent at Total concentration of polymer in solution (% wt.) – 13%. Where spinning condition kept as – Applied voltage/distance (kV/cm)- 3.88, Relative

Humidity-Temperature – 26% and 22 degree, Speed of substrate (mm/min) – 13, Results from this experiment is area weight of nanofiber mat – 5.40 g/m^2 [56].

Needleless electrospinning was going carried out by using a 0,2 mm string. Where that string was covered by a polymeric solution using slots in size of 0,5 mm. Formed fibers will move towards the collector, between collector and solution surface mandrel or specified diameter tube/rod is kept, which kept rotating at a constant speed, formed nanofibrous will form fibrous mat around the rod/mandrel/ tube which will result in tubular graft [56].

The temperature was kept at 23°C and relative humidity between 35% to 42%. The used mandrel is 6mm diameter made of stainless steel with 20cm length. The speed of rotation of the mandrel is varied from 250-15000rpm. The reciprocating movement of the spinning electrode kept the motor-driven. The time of electrospinning was adjusted up to the required thickness of the graft [56].

The thickness of produced tubular graft was measured during the fabrication process using a micrometer screw gauge. After electrospinning, the tubular scaffold has to be dried and then removed from the mandrel by manually pushing. It is seen that problem of taking out graft from the mandrel. A suggestion that can overcome the problem is by using a glass rod instead of a steel mandrel or saturated NaCl coating on a steel rod [56].

F. Cengiz et al, studied The Effect of Salt on Polyurethane Nanofiber on Roller Electrospinning This kind of electrospinning coming under needless electrospinning. Where roller has emerged in polymeric solution. On the surface of the roller, Tylor cone formation occurred. Cengiz used polyurethane of 2000g/mol. This PU is aliphatic-based. The solvent was DMF. Solution concentration 15%. Voltage used -81.2 kV with distance between electrode kept at 11cm [60].

Wannes et al produce polyurethane nanofiber-based nonwoven filters by needleless electrospinning. They electro-spined polyurethane nanofiber for filter nanofibrous filter. They used DMF as solvent for PU, PU is based on 4-4 methylene bis(phenyl isocyanate)(MDI), Poly(3-methyl1,5pantanediol)alt(adipic acid)(PAIM) and 1,4-butanediol(BD) with molar ratio 9:1:8 at 90 degree. The concentration of solution kept around 13.5 weight%, viscosity around 1.53Pa and conductivity 146.2 μS/cm. electrospinning machine used rotating electrode with 4 cotton spinning elements. Temperature and humidity are around 22 degrees and 36%. Distance kept 180mm, electrode speed around 7rpm, electric voltage given 75kV [62].

3.1.3.3 Electrospinning of PU for vascular graft

Gostev et al studied Electrospun polyurethane-based vascular grafts: physicochemical properties and function in vivo. Stated the physicochemical properties of vascular grafts (VGs) electrospun from Tecoflex (Tec) with gelatin (GL) and bivalirudin (BV) solutions are investigated. The electrospun VGs of Tec-GL-BV and expanded polytetrafluoroethylene (e-PTFE) implanted in the abdominal aorta of 36 Wistar rats were monitored for up to 24 weeks at varied time intervals[68].

Gostev used - 10% PU Tecoflex-80A (Tec; Lubrizol Advanced Materials, EC), 5% gelatin (GL) solution, and 1.5% bivalirudin (BV) solution and fabricated on the NF-103 electrospinning device, cylindrical steel collectors with diameter 2.7 mm and length, 190 mm, feed rate 1.0 ml/h, voltage 19kV, collecter speed 300rpm, the distance between drum collector and spinneret 19cm. He concluded, produced graft shown high durability, good mechanical properties comparable with a native blood vessel. Under 24-week observation, it showed a high petiancy rate up to 94% with excellent biostability [68].

Jing, X et al, Electrospinning thermoplastic polyurethane/graphene oxide scaffolds for small diameter vascular graft applications. In vascular tissue engineering, the fabrication of narrow diameter vascular grafts is crucial. In his study, electrospinning was used to manufacture thermoplastic polyurethane (TPU)/graphene oxide (GO) scaffolds with various GO contents as possible candidates for small diameter vascular grafts. He mentioned PU has great potential to be used in tissue engineering [69].

Jing used medical-grade thermoplastic PU, DMF graphite, sodium nitrate mainly. He used needle electrospinning with voltage 18kV, working distance 150mm with flow rate 0.5ml/hr. for making a tubular graft he used a grounded aluminum tube with 3.18mm outer dia rotating at 1500rpm[69].

Uttayarat, P et al, produced vascular graft with microfibers and micro-grooved surface. He used PU as a polymer for needle electrospinning. Used PU is polyether PU with a 5% weight ratio in the solution where electrospinning ran at 12kV. Spinning distance kept at 12cm. feed rate was 0.8ml/hr. used needle made up of stainless steel with 18 gauge[70].

To get tubular graft aluminum mandrel used, Mandrell has 4mm inner diameter and 5 cm in length. The morphology of the produced graft was checked on the scanning electron microscope. At the last uttayarat concluded, durable, micropatterned, small-diameter polyurethane grafts capable of encouraging the generation of aligned endothelial cell monolayers can be made using electrospinning and spin casting processes in tandem[70].

3.2 Characterization of needleless electrospun polyurethane

Morphology of vascular graft layer is to assessed by following parameters in this thesis-

- Surface area

- The thickness of the produced electrospun layer
- Average fiber diameter

Surface area – the surface area of the produced nanofibrous layer is going measured by the cutting weighting method. Where samples made of 1×1 cm. Ten samples are sufficient for the correct result. The result of surface area will be in mg/cm^2 .The thickness of sample- The thickness of the nanofibrous layer is measured by a digital micrometer screw gauge. The sample was placed between two jaws of screw gauge to measure the thickness. Where ten samples were tested for thickness. Thickness measured in –μm [63].

Average Fiber diameter – The fiber diameter of the layer is going to decide the important properties of the graft. Where morphology of the fibrous layer is assessed by scanning electron microscope (SEM) and image analysis software is used to characterize average fiber diameter. For SEM analysis, samples are required sputter coating of gold on it and analysis carried further by TESCAN Vega 3SB Easy probe (Czech Republic) or Phenom FEI scanning electron microscope (USA). Appropriate magnification is done to get proper correct values of samples. Image analysis is carried by using software NIS Elements (LIM s.r.o, Czech Republic) or NIH Image J software. Average fiber diameter calculated from 100 results. Measured average fiber diameter in -μm [63].

For surface area, average fiber diameter and thickness mean and the standard deviation is calculated for better result and testing analysis. For surface area, average fiber diameter and thickness mean, and the standard deviation is calculated for better result and testing analysis [63].

3.3 Testing of elasticity and strength

Mechanical testing of tissue-engineered scaffolds is important because it's hypothesized that mechanical properties will mimic the behavior of native blood vessels. To mimic native vessels the produced scaffold should be sufficient strength and elasticity.

To measure the mechanical properties of electrospun graft universal tensile testing machine TIRA Test 2810 going to use. Were during the test stress-strain curve was measured. Planer samples are going to test. Planer samples are in a rectangle shape with a width of 20mm and length of 50mm [63].

To calculate engineer stress (σ), the Thickness of the sample is measured before each mechanical test. To measure thickness micrometer screw gauge is used. active length for the test is 30mm. samples are clamped between two jaws of the tensile tester. Testing was carried out at 100 mm/min of the rate of loading. During the tensile strength test force required to break (F) and elongation at break (Δl) was noted to draw stress Vs strain graph. Calculation of stress is done by using the following formulae

$$\text{Stress } (\sigma) = \frac{force(F)}{cross\ sectional - area}$$

$$(\sigma) = \frac{force}{Thickness \times standard\ width}$$

Strain or relative deformation(ε) calculated by using the following formulae

$$\varepsilon = \frac{extended\ length\ (\Delta l)}{original\ length(l_o)}$$

Engineered tension or stress is measured in MPa and strain is measured in %. The number of samples for this testing is three. From mechanical testing of produced graft, we can compare the mechanical behavior of engineered vascular graft for native blood vessel mechanical behavior [63].

According to ISO 7198:2016(E), pressurized burst strength and longitudinal strength tests are suitable for a tubular vascular prosthesis. Prob burst strength test and circumferential strength test are preferred if the pressurized burst is not available [64].

Pressurized burst strength test be done in two ways as follow –

- Filling a gas or fluid in the prosthesis until the bursting of the sample takes place. During this pressure, change is measured
- Filling an elastic or non-permeable material inside the prosthesis and fill the measured pressure change inside the prosthesis until it breaks [64].

Castillo et al, took an 80 mm long specimen for testing of burst strength. Hear they pressurized prosthesis by controlled pressure unit (1 psi/ s). The radial displacement of a prosthesis is measured by the optical sensor. Pressure at which specimen breaks it was measured while compliance "C" measured by taking universal slope between internal pressure Vs circumferential strain within 0.10% to 0.20% strain range. To get accurate results number of testing is 10 [65].

Uniaxial strength test –

To find compliance and circumferential strength (corresponds to max strength) of a specimen, elastic modulus (E), poisons ratio (ν), and uniaxial tensile strength must be calculated. The testing equipment should be calibrated before the testing. Elastic modulus calculated by slope between stress and strain curve in the small deformation zone of 0 to 10%. Where Poisson's ratio is calculated by determining the linear slope between stress Vs strain curve. Tensile strength was calculated by conducting several loading and unloading of stress on the specimen [65].

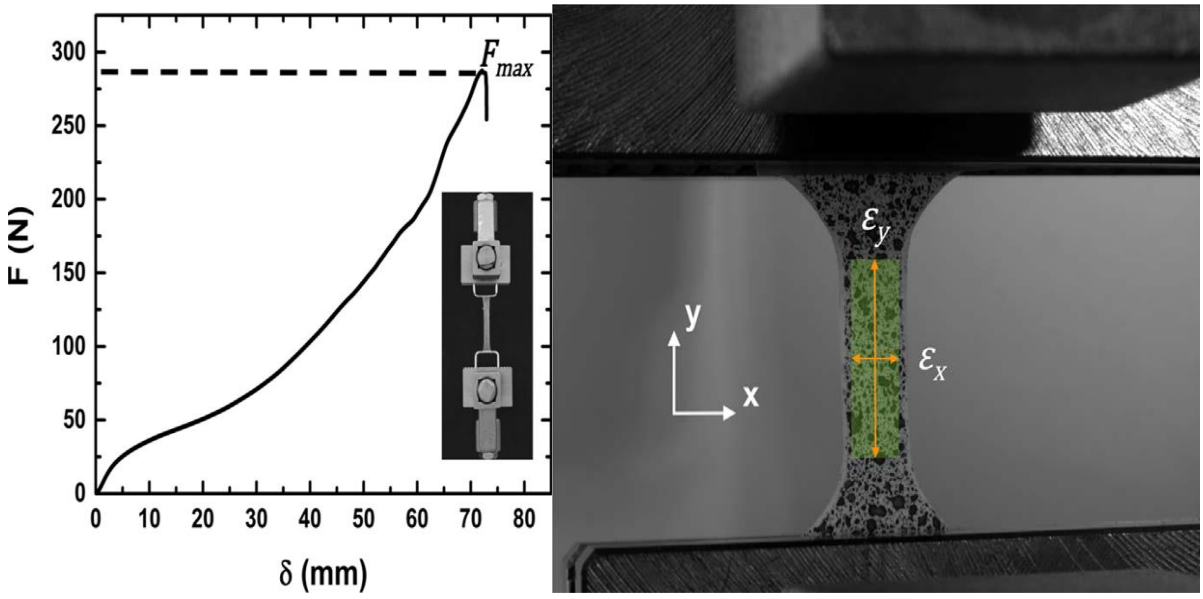


Figure 24:Stress Vs strain curve under uniaxial strength test [65].

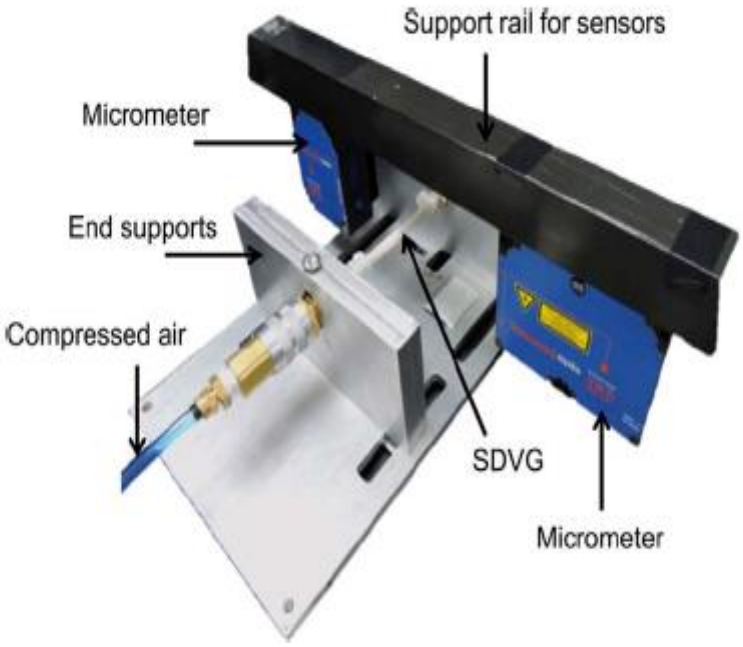


Figure 25:Burst strength set up[65].

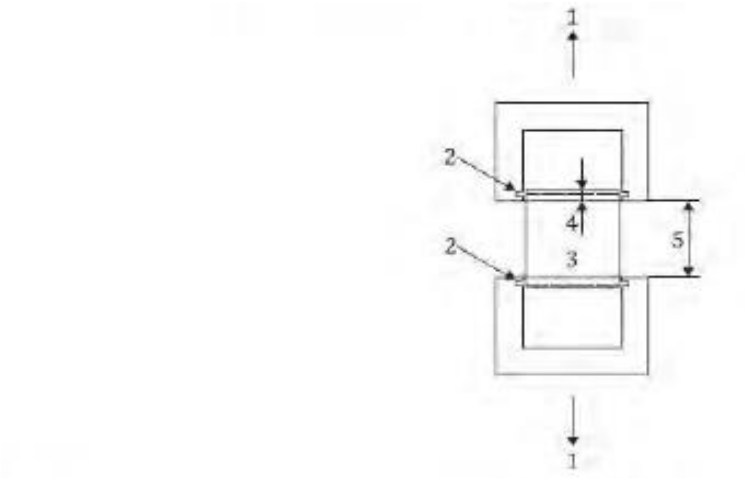
Burst strength test – This test is carried out to determine the pressurized burst strength of graft using a feeling of gas or fluid into graft at a measured rate until the graft tears down or burst completely. Materials used inside the test should provide measuring and recording the pressure with accurate data. A graft can be used for this test with elastic, non-permeable linear inside it or directly can be used. samples are directly attached to the apparatus. Feed gas or fluid should have proceeded with a steady flow rate at 10kPa/s and 70kPa/s. measure the burst pressure take readings and calculate the standard deviation.

The rate of pressure should be in kilopascals per second and bursting pressure should be in kilopascals [64].

Circumferential strength test - this test was carried out to find out the circumferential strength of the tubular graft utilizing clamping the graft between the two rounded pins and stretched out at a uniform rate. Accuracy of apparatus should +-2%, apparatus should be move with the constant traverse. Sample length should be more than the relaxed diameter of the graft. Remove if any crimp is present and then measure the length of the specimen.

Specimen placed between two pins without any twist, slackness, and stretched condition. Stretching of samples done at 50 to 200 mm per min rate. Record the yield of the break.

Calculate the circumferential tensile strength by dividing the maximum load (Tmax) by the original length of the sample. Result strength should be in kilonewtons per millimeter [64].



b) Front view

Key
1 tensile tester
2 split bar
3 sample
4 pin adapter
5 pin separation

Figure A.1 — Split bar tester

Figure 26:Circumferential strength test [64].

Sampling for testing – samples should be representing the whole concerning design attributes and minimum variability. Minimum 3 samples should be taken from every three slots. For every test, this thesis focused to take 5-10 readings of each testing [64].

4 CONCLUSION

Over the years, science growing faster and intelligently. Tissue engineering making a great future for humankind. Tissue engineering creating artificial organs for humankind. Those organs mimic as original organs of the human body.

This thesis is focused on the production of vascular grafts because looking at the current scenario of the world, most human deaths are caused by vessel damages. To mimic the original human graft, textiles give different structures which we can use as artificial grafts. Weaving, knitting, expanded polytetrafluoroethylene These kinds of technics are used to produce grafts previously. This technique has some drawbacks. These drawbacks are overcome by the electrospinning technique. That's why this thesis focused on the electrospinning technique.

Plenty of polymers are used to produce synthetic grafts. in those polymers, polycaprolactone polymer attracts researchers over the years. But PCL has one of the drawbacks about the degradability. To achieve mechanical, biological, and proper degradability polyurethane is used in this thesis. Polyurethanes are very versatile polymers that found application in the biomedical field, es-specially in cardiovascular applications. Despite their good physicochemical and mechanical properties and acceptable biocompatibility, they are prone to degradation under different conditions.

There are different types of electrospinning like multi-jet, melt spinning, centrifugal spinning, needleless spinning. Over this multi, melt and centrifugal spinning are undercome in needle spinning. Needle spinning has a lot of problems during production, those problems as clogging of needle holes regularly which will impact on properties of the end product. Needleless electrospinning is the solution to this problem. Under the thesis study, no one did a study on this technique for the production of synthetic grafts.

Many researchers did a study on this topic by using different materials, production techniques. Looking at those studies this thesis has chosen the given process. This study clearly shows chosen material, production technique has the potential to mimic the graft.

The experimental part shows material selection, electrospinning technique, and testing of the end product. Material selection was done based on biodegradability and biostability. Solvent for PU has to be chosen properly, DMF is the dominant candidate, a recent study shown "cyeren" solvent shown better results than DMF.

Electrospinning parameters have a great impact on the properties of produced nanofibrous graft. For that reason, proper parameters are chosen from the literature.

Testing of the synthetic graft is done mechanically, biologically and morphological structure of the graft. Different kinds of literature are studied well and using that literature, productions of graft and testing of produced graft have to be done.

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