FABRICATION OF 3D BIODEGRADABLE PBAT/PLA BLENDS ELECTROSPUN NANOFIBER FOR CARDIAC PATCH APPLICATION

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by

SYAMIL AKHYAR BIN AZMAN

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

cm	Centimetre
T_{cc}	Cold crystallization temperature
C_e	Critical minimum concentration
°C	Degree Celsius
°C/min	Degree Celsius per minute
X_c	Degree of crystallinity
$\triangle H_c$	Enthalpy of cold crystallization
$\triangle H_m$	Enthalpy of melting
G	Gauge
T_g	Glass transition temperature
g	Gram
g/cm^3	Gram per centimetre cubic
g/mol	Gram per mol
kV	Kilo Volt
T_{max}	Maximum degradation temperature
MPa	Megapascal
T_m	Melting point
µL	Microliter
mg	Milligram
mL	Millilitre
ml/H	Millilitre per hour
mm	Millimetre
mm/min	Millimetre per minute
nm	Nanometre

N	Newton
n	Number
cm-1	Per centimetre
T_{1st}	Starting degradation temperature
$\triangle H_m°$	Theoritical Enthalpy melting of 100% crystalline
I_{total}	Volume total
wt	Weight
w/v	Weight per volume

LIST OF ABBREVIATIONS

3D	Three dimensional
ASTM	American Society for Testing and Materials
Ave	Average
BASF	Baden Aniline and Soda Factory
CHD	Coronary heart disease
CHF	Congestive heart failure
CM	Cardiomyocytes
CTE	Cardiac Tissue Engineering
DCM	Dichloromethane
DMF	n-n,dimethylformamide
DMT	Dimethyl terephthalate
EC	Endothelial cell
ECM	Extracellular Matrix
FTIR	Fourier Transform Infrared
HA	Hydroxyapatite
IR	Infrared
LDPE	Low density polyethylene
LV	Left Ventricle
Max	Maximum
MI	Myocardial infarction
Min	Minimum
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene Succinate

PCL	Polycaprolactone
PGA	Polyglycolic acid
PGS	Polyglycerol sebacate
PLA	Polylactic acid
PLGA	Polylactic-co-glycolic acid
PPKBSM	Pusat Pengajian Kejuruteraan Bahan dan Sumber Mineral
PPy	Polypyrrole
RPM	Rotation per minute
SEM	Scanning Electron Microscopy
St dev	Standard deviation
TA	Thermal analysis
TPE	Thermoplastic elastomer
WHO	World Health Organization

PENGHASILAN 3D TERBIODEGRADASI CAMPURAN PBAT/PLA ELEKTROSPUN NANOFIBER UNTUK APLIKASI TAMPALAN JANTUNG

ABSTRAK

Kedua-dua polibutilin adipat tereftalat (PBAT) dan asid polilaktik (PLA) biasanya diadun dengan polimer sintetik terbiodegradasi lain, iaitu polikaprolakton (PCL), asid poliglikolik (PGA), poliglisrol sebacate (PGS) dan beberapa polimer biodegradasi semulajadi lain seperti gelatin, chitosan dan collagen. Walau bagaimanapun, terdapat beberapa kajian dalam literatur mengenai campuran binari PBAT dan PLA bagi kejuruteraan tisu jantung. Oleh itu, dalam penyelidikan ini, campuran antara polimer ini dianalisis untuk menyiasat pengaruh campuran PBAT dan PLA asli serta nisbahnya pada ciri fizikal, mekanikal, dan terma gentian elektrospun. Didapati bahawa campuran dengan kepekatan 12.5wt % adalah parameter pintalan elektro yang optimum untuk penghasilan campuran PBAT/PLA. PBAT/PLA dengan nisbah 60/40 menghasilkan peningkatan yang paling baik bagi ciri-ciri yang disiasat walaupun keputusan yang diperolehi adalah agak rendah daripada PBAT dan PLA tulen. Analisis FTIR menggambarkan bahawa spektrum PBAT/PLA agak serupa dengan spektra PBAT IR tulen. Apabila bahagian komponen PLA yang lebih tinggi ditambah kepada pencampuran; Analisis SEM menunjukkan morfologi gentian yang lancar dan sejajar, pengukuran sudut sentuhan air menggambarkan pengurangan hidrofobisiti, sifat mekanikal (ketegasan dan keliatan) dan kestabilan haba campuran PBAT/PLA telah dipertingkatkan.

FABRICATION OF 3D BIODEGRADABLE PBAT/PLA BLENDS ELECTROSPUN NANOFIBER FOR CARDIAC PATCH APPLICATION

ABSTRACT

Usually both polybutylene adipate terephthalate (PBAT) and polylactic acid (PLA) have been compounded with other biodegradable synthetic polymers, namely polycaprolactone (PCL), polyglycolic acid (PGA), polyglycerol sebacate (PGS) and several other natural biodegradable polymers such as gelatin, chitosan and collagen. However, there are few studies in the literature on PBAT and PLA binary blends for cardiac tissue engineering. Thus, in this work, blends between these polymers were analyzed to investigate the influence of the pure PBAT and pure PLA blend and its ratio on electrospun fibre scaffolds' physical, mechanical, and thermal characteristics. It was found that 12.5wt % solution concentration is the optimum electrospinning parameter for the fabrication of PBAT/PLA blend. PBAT/PLA blend with ratio of 60/40 produced the most feasible enhancement in properties even though the obtained results were comparatively low than pure PBAT and pure PLA. Fourier Transform Infrared (FTIR) analysis depicts that PBAT/PLA spectrum was quite similar to pure PBAT IR spectra. When a higher portion of PLA was added to the blending; Scanning Electron Microscopy (SEM) analysis indicates smooth and aligned fibre morphology, water contact angle measurement illustrates a reduction in the hydrophobicity, mechanical properties (rigidity and toughness) and thermal stability of PBAT/PLA blend was enhanced.

CHAPTER 1

INTRODUCTION

1.1 Background Study

According to WHO (2011), Cardiovascular disorders have been one of the leading causes of mortality over the previous two decades, and they continue to be a severe health concern in the twenty-first century, even in wealthy countries. Cardiac failure occurs as a result of a cardiac arrest in the left ventricle, where cardiac muscle cells have limited ability to regenerate injured tissue (Zhao et al, 2015). Despite being the most suitable therapy option, heart transplantation has not yet become a common treatment option due to a scarcity of donors and an unfavourable immunologic response.

Due to these limitations, cellular treatment and tissue engineering techniques have received a lot of interest. (Zhao et al, 2015 and Kai et al, 2011). According to (Leor et al, 2006 and Kai et al, 2011) one of the most difficult aspects of engineering cardiac tissue is creating a porous nanofibrous scaffold that can match the physical and chemical characteristics of the extracellular matrix (ECM) of the target tissue and hence facilitate cellular adhesion, proliferation, and nutrition supply. ECM is a complex network made up of a variety of multidomain macromolecules that are arranged in cell or tissue in specific ways. The ECM's components connect together to produce a structurally stable composite, which helps tissues' mechanical characteristics (Lu et al, 2019).

Mayoral et al., (2022) stated that various kinds of natural and synthetic polymers are the most prevalent materials used in the production of cardiac patches. Collagen, chitosan, alginate, gelatin, fibrin, and hyaluronic acid are natural polymers used for cardiac tissue engineering. In addition, synthetic polymers such as poly (glycolic acid)

(PGA), polycaprolactone (PCL), poly (lactic acid) (PLA), poly D, L-lactic-co-glycolic acid (PLGA), and an appropriate mix of synthetic, natural, and metallic materials may be excellent for the creation of cardiac patches. These materials are utilised to build a three-dimensional cardiac patch that resembles the ECM and has a comparable configuration to cells as they depart the original organ.

Electrospinning, solvent casting, extrusion, and phase separation methods are some of the standard processes that are employed in the manufacturing of cardiac patches. Electrospinning is the only one of these techniques that can be efficiently scaled up for industrial use. According to Persano et al., (2013), this is shown by the market expansion of electrospinning equipment during the last several years. Kitsara et al., (2017) reported that electrospun nanofiber matrices look a lot like the natural ECM, with continuous fibres ranging from nano to micro scale, a high surface-to-volume ratio, high porosity, and a variable pore-size distribution. Also, efforts have been undertaken to alter nanofiber surfaces with a number of bioactive chemicals to provide cells the essential chemical signals and a microenvironment that is more similar to that found in the body. Furthermore, to fulfil the needs of a specific application, electrospinning settings may be changed to alter the physical and chemical characteristics of nanofiber matrices.

Once the idea of using an electrospun nanofiber scaffold has been accepted, it's important to figure out a few key details. These are the material's composition, its surface, its mechanical properties, its biocompatibility, the rate at which it breaks down, and the conditions for cell cultivation. According to He et. al (2017), Synthetic or natural polymers, or a combination of both, can be used to make electrospun fibres. However, the polymers employed to make the fibres must be biocompatible in order

to be used in biological applications. It reduces the risk of infections and other negative effects caused by the material's incompatibility with the body.

Recent research has focused on biodegradable polyesters such as poly(butylene succinate) (PBS), poly(lactic acid) (PLA), and polybutylene adipate terephthalate (PBAT). The primary disadvantages of bio-based and biodegradable polymers compared to typical polymers include the need for highly regulated processing conditions, greater temperature sensitivity and poor mechanical characteristics. As a result, numerous research efforts are centered on developing biodegradable polymer blend compositions with optimized thermal, rheological, and mechanical qualities.

PBAT is an aromatic-aliphatic co-polyester made from oil-based resources that is biodegradable in the presence of natural microorganisms (Tan et. Al, 2008). It also has good toughness and is mostly used for film extrusion. Polymer blends including brittle polymers, such as PLA, can benefit from PBAT. According Gerard et al, (2016) the main application of PBAT is blown film and Shankar et al, (2018) also reported that another common application of PBAT represented by extrusion coatings. Electrospun PBAT/PLA nanofibers have been not extensively investigated as tissue scaffolds or carriers for drug and gene delivery and their application in engineering cardiac tissue is rarely reported. Hence, this study aims to explore the potential candidates for development of 3D electrospun nanofiber scaffold of PBAT/PLA that can perform and function as cardiac patch which offers advance properties in application of wound dressing on the cardiac tissue engineering.

1.2 Problem Statement

Biodegradable polymers are not currently widely used due to some limitations such as mechanical and physical properties also their thermal stability. Researchers

have been trying to address these issues by utilizing blending techniques to obtain biodegradable blends with tailored properties. As two typical thermoplastic biodegradable polyesters, the blend of PBAT and PLA is of great interest due to their unique properties, which can extend their applications in diversified areas, especially in cardiac tissue engineering applications. Hence, the present work focused more on the fabrication of a high-performance PBAT/PLA blend by using the electrospinning method.

One of the primary concerns of this research is the role that nanofibers' mechanical integrity which plays in their potential applications across a wide variety of promising fields (Boppa, 2009). The mechanical and morphological properties of electrospun nanofiber mats are dependent on the solution and process parameters used to produce them. According to Aussawasathien et al, (2006) these parameters include the gap distance between the capillary tip and the collector, the applied voltage, and the hydrostatic pressure in the solution container. In order to create ideal nanofiber mats, it is necessary to exert careful control over these variables. Abdelwahab et al, (2015) stated that there are several previous reports on blending commercially available PBAT with PLA to solve the problem of the hard and brittle nature of PLA. Hence, in this work, the effect of blends between these two polymers were analyzed to investigate how different solution concentration can affect the overall properties of fabricated fiber scaffold.

It is well known that blending of polymers is the suitable way to obtain material with required properties and performances. Flexibility is one of many properties that need in the fabrication of biodegradable fiber scaffold to be acceptable for cardiac tissue application. Therefore, in this work, blends of PBAT/PLA were studied to observe how PBAT role as one of the best potential candidates to achieve

the high elongation and flexibility to reduces the brittleness and stiffness of PLA hence improves it elongation at break.

1.3 Research Objectives

The objectives for this research are to study and fabricate a biodegradable PBAT/PLA electrospun fiber scaffold for cardiac tissue engineering application. The specific research objectives are as follows:

i. To investigate the effect of electrospinning processing parameters (solution concentration and blend ratio) on the mechanical, thermal, and physical characteristics of PBAT/PLA electrospun fibre scaffolds.
ii. To study the effect of PBAT/PLA ratio on the physical, mechanical, and thermal characteristics of electrospun fibre scaffolds.

1.4 Thesis Outline

There are five key chapters in the thesis. The five chapters are made up of:

Chapter 1: Presented the background information for the study, including the problem statement and research goals.

Chapter 2: A literature review on the previous research was presented, which include a brief introduction to tissue engineering and, more particularly, cardiac tissue engineering, as well as the roles and critical requirements for developing the polymeric-based scaffold. An explanation of the type of fabrication technique that was used to construct the nanofiber scaffold, with a focus on how it can be applied to the treatment of heart tissue.

Chapter 3: The methodology of the study, which includes a comprehensive explanation of the materials and chemicals used, as well as the fabrication of electrospun nanofibers of pure PBAT, PLA, and PBAT/PLA blends, and the

characterization of these nanofibers, which includes chemical characteristic analysis, surface morphology studies, water contact angle analysis, tensile testing, and analysis of its thermal properties.

Chapter 4: Results and discussion obtained from the characterization of electrospun nanofibers, Fourier Transform Infrared (FTIR) analysis of PBAT/PLA blend with pure PBAT and PLA, morphology studies through Scanning Electron Microscopy (SEM), water contact angle analysis and comparison of physical, mechanical and thermal properties between pure materials and blend samples.

Chapter 5: A conclusion on the entire piece of study will be presented, along with a few recommendations for potential enhancements to be made in subsequent work.

CHAPTER 2

LITERATURE REVIEW

2.1 Tissue engineering

Tissue engineering is an area in the field of biomedical engineering that combines biology and engineering to develop tissues or biological products outside the body or to utilize acquired knowledge to effectively control the recovery of tissues inside the body (McClelland et al, 2005). Mashayekhan et al, (2013) reported that since decades, researchers are working in the field of tissue engineering have been attempting to replace damaged tissues or organs in the body with functional created counterparts. Tissue engineering has developed in a new and interesting path when the emergence of stem cell research. Stem cells is known to be able to self-renew and adhere to certain cell lineages in response to the appropriate stimuli, which indicates that they have a huge regeneration potential that will almost probably lead to the capability of tailored tissue.

Generally, from the framework of biology and pathology point of view, the majority of diseases occurred due to the cell behaving in an abnormal manner (Kim, Rutka and Chan, 2010). Tissue and organ complexity and multicellular structure, differentiation of stem cells into various tissues or organs remains a fundamental limiting factor in tissue engineering today (Mashayekhan et al, 2013 and Kim et al, 2010).

2.2 Heart muscle structure and diseases

Approximately 2–3 billion cardiomyocytes (CMs) make up the human myocardium (75% by volume or 30% by number); striated cardiomyocytes are only found in the heart and may be distinguished from skeletal and smooth muscle cells. In

addition to cardiomyocytes, the heart tissue also contains endothelial cells (ECs) and fibroblasts, with the former accounting for around sixty percent of the EC. CMs are controlled by the autonomic nervous system, which is involuntary, as opposed to the somatic nervous system, which is voluntary. Skeletal muscle cells are controlled by the somatic nerve system. Excitatory impulses may be generated by CMs on their own, allowing them to function as a biological pacemaker. Figure 2.1 shows schematics of cardiac muscle structure consist of several components.

Myocardial infarction (MI) and congenital heart failure are two serious heart conditions that might be helped by the availability of cardiac patches for transplantation. MI is the coronary heart disease (CHD) symptom that is seen most often (MI). MI is often brought on by blockages in the primary coronary arteries. These blockages bring about a dramatic reduction in the amount of blood that flows to the working muscle of the heart (primarily the left ventricle (LV)), which in turn causes ischemia and necrosis. Ischemia causes the death of all cell types present in the tissue, including neurons, fibroblasts, and vascular cells, despite the fact that CMs are the most vulnerable cell group (Jessup and Brozena, 2003; Whelan et al., 2007; McMurray, 2010). A decrease in the ability of the heart to contract is brought on by the sudden destruction of a significant portion of the myocardium. In order to compensate for this loss, a number of structural changes take place, one of which is an increase in the LV volume. This results in additional stress being placed on the ventricular wall. The development of noncontractile scar tissue, a weakening of the damaged wall, and a dilatation of the ventricular chamber are the three main factors that contribute to the progression of congestive heart failure (CHF) over time (Jessup and Brozena, 2003; Whelan et al., 2007; McMurray, 2010).

The development of a cardiac patch is very necessary in order to successfully treat heart defects, which are the leading cause of mortality in young children (Martin et al., 2008). Approximately one percent of all newborns are found to have structural myocardial anomalies, which carries a risk of sudden cardiac death that is anywhere from 25 to 100 times higher than that of young patients in the general population (Silka et al., 1998). Major heart conditions are usually connected to other types of birth abnormalities, whether those abnormalities involve the heart or not.

Both of these conditions might benefit from cardiac tissue engineering (CTE), which can be used to create and transplant a contractile cardiac patch that improves heart function.

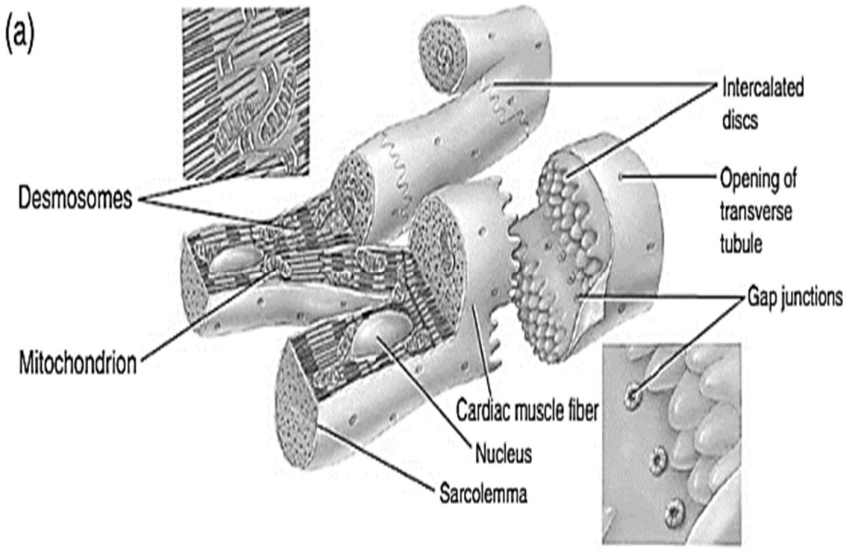

Figure 2.1: Schematics of cardiac muscle structure (Sapir, Polyak and Cohen, 2013)

2.3 Cardiac Tissue Engineering (CTE)

The goal of cardiac tissue engineering, also known as CTE, is to develop contractile heart muscle tissues that are capable of replacing sections of the heart that are either missing (as a result of congenital heart defects) or dysfunctional (as a result

of myocardial infarction), and hence resulting in cardiac repair. The *in vitro* creation of a cardiac patch by seeding cardiomyocytes (CMs) as well as extravascular cells in a biodegradable scaffold is currently one of the most important CTE techniques. This is followed by the application of inductive biological, physical, mechanical, and electrical stimuli to enhance tissue maturation and function.

To perform cardiac repair, a fully-developed cardiac muscle patch must be transplanted. This is necessary both to address congenital heart defects and to replace a large non-contractile scar that develops after a MI. The patch has to be substantial, and it needs to have the same functional and anatomical characteristics as the original heart muscle. After being implanted in the heart, the cardiac patch has to generate a systolic force, which enable it to withstand a diastolic load while maintaining an adequate degree of consistency, and be able to establish an electrical and functional syncytium with the host myocardium.

According to Ruvinov et al. (2008), during the course of the last decade or so, three basic approaches for cardiac patch reconstruction have been established. Each of these methodologies depends on embedding cardiac cells in scaffolds in order to function (hydrogels, polymer layers, and pre-formed macro-porous scaffolds). Cell constructs are developed in a moderate perfusion environment for the purpose of optimizing the supply of dissolved oxygen and nutrients to the growing cells. Concurrently, inductive biological and physical signals are applied in order to produce cardiac tissue that is capable of fully performing its functions. But since cells are the "real tissue engineers," the primary goal of this method is to create the best inductive environment possible that is able to stimulate the growth and maturation phases of the engineered tissue. This can be accomplished by ensuring that the tissue is exposed to a

variety of growth factors. Figure 2.2 shows a schematic illustration of preparation of a decellularized matrix-based patch and its effects on infarcted myocardium.

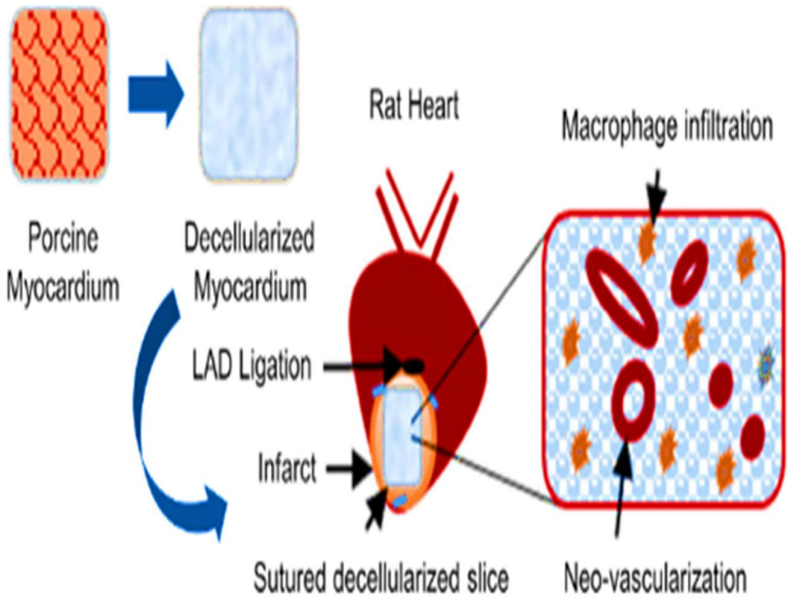

Figure 2.2: Schematic illustration of preparation of a decellularized matrix-based patch and its effects on infarcted myocardium (Chang et al., 2021)

2.4 Polymer-based scaffolds

2.4.1 Role of polymeric scaffold

Scaffolds made of polymers are helpful in the field of tissue engineering because they encourage cell adhesion and proliferation in addition to the emergence of various tissue in three dimensions (3D). It is common knowledge that the pore size and surface area of a scaffold are both significant features. For cell seeding, migration, growth, mass transfer, and tissue regeneration, porous structures provide essential obstacles. Pore geometry, pore wall morphology, and the interrelationship between pores are all important factors (Ma, 2004; Lien et al., 2009).

It is possible for scaffolds to have a solid or porous composition. The process of tissue engineering employs the usage of porous materials as a model for the creation of cells. A scaffold that has a high porosity makes it possible for cells to penetrate and gives room for the multiplication of cells as well as the creation of extracellular matrix (ECM). In addition to this, they transport nutrients and regulating components necessary for tissue repair, medications necessary for certain types of healing, and other biomolecular signals. When it comes to the creation of synthetic scaffolds, consideration has always been given to stability, toxicity, and degradation. Latest developments in fabrication methods have contributed to the development of technology that enables the construction of fully customizable scaffolding that can be tailored to match the needs of a given application. It is now possible to include factors such as regulated drug release, the presence of proteins that induce cell adhesion and regeneration, and multiple degradation mechanisms that have an effect on the pace of disintegration and the strength of the material (Burdick & Mauck, 2011).

A component of the scaffold is known as an open structure, and it has numerous holes that link to one another. This feature enables cells to proliferate inside the scaffold while preserving its mechanical stability. If, for instance, the scaffold is bioresorbable, it will disintegrate over time as new tissue develops and takes over the structure; if it is not bioresorbable, it will remain in place for the purposes of providing support and strength. It is essential to match the pace of degradation to the rate at which tissues regenerate in order to preserve the mechanical stability of the scaffold and to guarantee that the sections of the disintegrated scaffold components are nontoxic and simple to metabolise. The scaffold must possess the appropriate surface chemistry and mechanical strength in order for it to be used under certain applications. For instance, orthopaedic uses of scaffolds require that the scaffolds have a high capacity for load

bearing. In contrast, the scaffolds that are used for skin grafts need to have elastic qualities and be able to be created out of collagen fibres so that they can promote tissue adherence and proliferation. (George et al., 2020)

2.4.2 Key Requirements for designing scaffolds

2.4.2(a) Mechanical properties

More often than not, the mechanical properties of a scaffold are determined depending on the needs of its use. These criteria have an effect on the structural characteristics of the scaffold, such as the pore geometry, size, and shape. (Chen, 2019) This is often owing to the fact that any biopolymer scaffold should provide mechanical characteristics that are ideal for the implantation location, in addition to having the needed level of strength for any surgical procedure that may be required for implantation. It is essential that the created scaffold should be easily manipulable. For example, the strength requirements for scaffolds used in the treatment of soft tissue are lower than those for orthopedic applications. As a direct result of this, the scaffold has to have a level of mechanical strength that is on par with that of the tissue. This strength will continually decrease over time for biodegradable scaffolds; however, since the weakened scaffold material is filled by newly produced tissue, the combined strength of the newly formed tissue and the decaying scaffold should be similar.

2.4.2(b) Biocompatibility

Rejection of a scaffold or implant may occur as a consequence of the immune system's response to the introduction of foreign substances into the body, which is an essential component of the body's defense mechanism. Therefore, any special bioengineered structures should induce a negligent immune response. This will ensure that the inflammation produced by the bioengineered structures does not hinder the

healing process or create any harm *in vivo*. (O'Brien et al., 2008). In addition to that, the structure has to include biomimetic binding sites that the cells can adhere to in order for them to be able to both multiply and differentiate. Aliphatic polymers are the kind of biomaterials that have been chosen for this application. The scaffold must be free of any cytotoxic components that might lead to the death of cells via necrosis or apoptosis. In most cases, the chemicals that were employed to treat the scaffold were the source of such components. These chemicals included organic solvent residues left over after polymer synthesis, initiators that were utilized during polymerization, and macromolecules derived from scaffold material (Mondschein et al., 2017).

2.4.2(c) Biodegradability

Permanent and biodegradable scaffolds are the two types of scaffolds that may be distinguished by their level of degradation resilience. The permanent scaffold must be resistant to deterioration and must have properties that are comparable to those of the soft tissue it is replacing. After it has served its purpose, a biodegradable scaffold is intended to decompose on its own, hence making way for the growth of new cell development over the course of time. Therefore, it is a crucial quality for biodegradable side products to be able to depart the body without the need for a trace of any toxicity that would cause the patient to have issues in the foreseeable future. The degree of crystallinity of a polymer, as well as its glass transition temperature, the existence of hydrolytically unstable bonds, and its molecular weight are all chemical factors that play a significant role in determining how rapidly a polymer can biodegrade (Ye et al., 1997).

2.4.2(d) Manufacturing technology

Due to the great variety of biomaterials, each of which has its own unique features, the technique of biomaterial fabrication in the field of tissue engineering is selected based on the required characteristics of the biomaterials for a certain tissue engineering application. Traditional, electrospinning, and 3D printing are the three different kinds of 3D manufacturing procedures. The processes of gas foaming, melt moulding, porogen leaching, phase separation, and freeze-drying are examples of some of the more classic methods. Electrospinning results in the production of very tiny fibres on the nanoscale scale that may be incorporated into the structure. Printing in three dimensions (3D printing) and decellularization represent the most recent developments in fabrication processes and are thus the latest production technologies. The deposition of biomaterials is done in a layer-by-layer fashion using this approach. In the fabrication process, some of the fast prototyping or 3D printing processes that are employed include those that are based on extrusion, inkjet, or laser assistance (Chen, 2019).

2.5 Electrospinning

2.5.1 Definition of Electrospinning

Williams et al., (2018) stated that electrospinning, which is developed from "electrostatic spinning," is a process of drawing charged threads from polymer solutions or polymer melts up to fibre, with fibre sizes ranging from hundreds to thousands of nanometers and has been first studied by Rayleigh in 1897. To generate the electric field necessary for effective electrospinning, a high voltage is delivered to the spinneret tip while the collector is grounded. A syringe pump is used to continuously deliver a viscoelastic solution, referred to the spinning dope, through the spinneret and into the previously described electric field. When the spinning dope encounters an electric field,

electrical charges accumulate on the fluid's surface until the critical voltage is attained known as Taylor cone which develops at the spinneret's tip, and a charged solution jet flows from the Taylor cone's tip to the grounded collector. Figure 2.3 shows a digital photograph of the Taylor cone and ejected polymer jet observed in a typical electrospinning experiment.

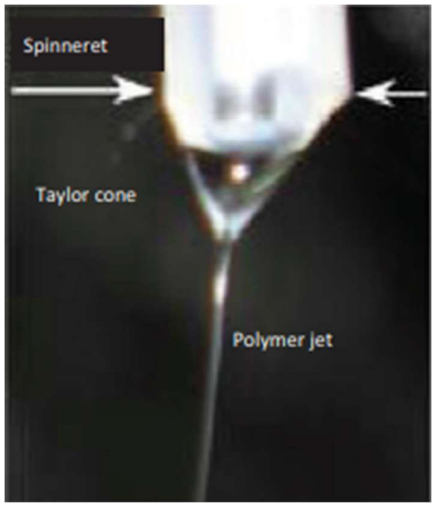

Figure 2.3: A digital photograph of the Taylor cone and ejected polymer jet observed in a typical electrospinning experiment. (Yu et al., 2012)

Nevertheless, the electrospinning process includes a number of different factors, each of which has the potential to influence the production of nanofibers. Polymer concentration, solvent ratio, flow rate of electrospinning solution pumped, needle or nozzle size, electrospinning voltage or electric field between needle and collector, spinning distance or distance between needle tip and collector, drum collector revolutions per minute (RPM), and electrospinning deposition time are the most critical, according to Haider et al. (2018). Figure 2.4 shows a schematic illustration of the basic setup of the electrospinning apparatus.

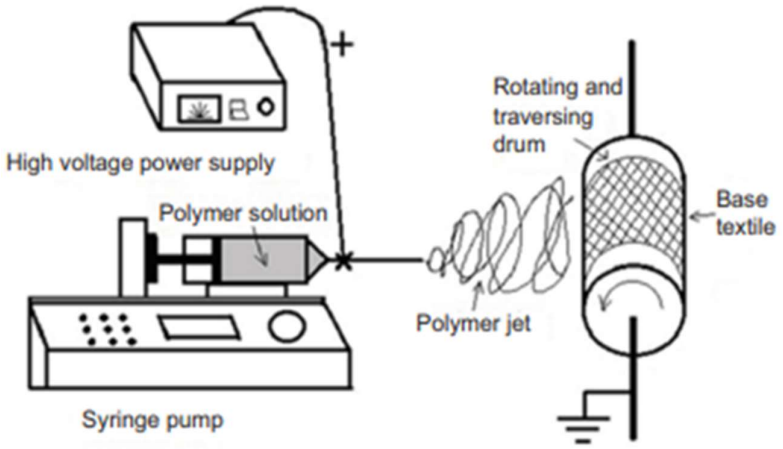

Figure 2.4: Schematic illustration of the basic setup of the electrospinning apparatus. (Lin et al., 2014)

2.5.2 Configuration of electrospun nanofibers

2.5.2(a) Randomly-oriented nanofibers

Nanofibers that are deposited on the surface of a conductive plate or a rotating conductive drum generate a randomly-oriented mess because of the bending instability of the highly charged jet. The deposition produces a nonwoven mat that is formed of nanofibers that are aligned in a random pattern and has a high porosity of around 50 to more than 90 percent. As a consequence of this, the ratio of their surface area to their volume is one of the highest documented values for any cohesive porous material (Burger et al., 2006). On the other hand, the pores in these materials are very fine, ranging in size from a few tenths of a nanometer to several micrometers (Mushi et al. 2016 and Wang et al. 2011)

In general, as porosity rises, so does pore diameter. In the process of wound healing, these structures are often exploited as hydrogels to cover the top of wounds while still enabling air to circulate through them. In addition, research conducted by Han et al. (2019) found that since these fibre membranes are able to absorb excess

exudate and provide a stable habitat that encourages epithelial regeneration, they have the potential to be modified to accelerate the cosmetic wound healing process.

2.5.2(b) Align nanofibers

By substituting the typical electrospun collector with custom collectors, speed-rotating collectors, or aligned pins, continuous, linearly aligned nanofibers may be produced. Some tissues, such as cartilage (Ren et al., 2019), tendons (Brennan et al., 2018 and Deepthi et al., 2016), ligaments (Brennan et al., 2018), blood vessels (Yi et al., 2019, nerve conduits (Zhu et al., 2020 and Zou et al., 2016), are composed of arranged fibres and directed cells.

Random-oriented nanofibers alone are not strong enough to stimulate these structures and unable to control the direction of cell growth and development. So that, nanofibers with align structures can aid tissue healing by regulating the activity of cell development.

Rushmer et al., (1953) stated that the contraction of cardiac tissue is generated by the nonlinear, anisotropic alignment of the ECM. Several ways have been developed to imitate the anisotropy of the myocardial by generating scaffolding materials with aligned nano or microstructures, which are required for duplicating the heart's anisotropic impulse propagation during electrical stimulation. (Bursac et al., 2007) and (Filgueira et al., 2019). Kharaziha et al., (2013) created an aligned, fibrous material utilizing poly(glycerol sebacate) (PGS) and gelatin, allowing for tailoring of a construct's mechanical characteristics to match those of the myocardial, leading in a 30% increase in CM beating rate.

2.5.3 Key Requirements for Electrospinning

2.5.3(a) Solution Parameters

1) Concentration: It is necessary to have a solid understanding of the fact that the concentration of the polymer solution has a significant impact on the diameter as well as the structure of the fibre that are generated by electrospinning. In order for this process to be successful, it is required to attain a critical minimum concentration, often referred to as C_e. Only then will enough molecularly chain entanglement be able to overcome surface tension and prevent the electrospinning jet from breaking apart. Because of an increase in electrospun fibre diameter caused by a greater polymer concentration, the formation of beadles, uniform nanofibers are possible when the concentration is over C_e. This will result in a reduction in the number of bead-on-string defects. The value of C_e is related to the length of the molecular chain, the molecular structure, and the solvent or solvents that were employed to dissolve the polymer.

2) Solution viscosity: Both the molecular weight of the polymer and the solvent's characteristics were profoundly affected by the solution's viscosity. Therefore, it may be altered by changing the solvent or polymer content in the solution. As the viscosity of the polymer solution rises, it will be considerably more difficult to elongate and confine the jet of polymer solution, which will result in thicker fibres. If the polymer solution has a viscosity that is too high, it has the potential to harden on the spinneret, which will cause it to get blocked at the very end of the spinneret. If the viscosity is very low, then electrospinning will occur and the products will comprise particles. An intermediate viscosity will lead to beaded fibres.

3) Surface tension: Together, the surface tension of the polymer solution and its viscosity combine to resist the pulling force that is caused by the electrical current. In order to continue with the electrospinning process, the electrostatic repulsion that exists between the charges in the polymer solution must be strong enough to overcome the surface tension.

4) Solvent choice: The solvent and solvent combination selected has a significant influence on several characteristics such as the solution's viscosity, surface tension, electrical conductivity, dielectric constant, and volatility. According to (Luo et al., 2010) it is essential to choose a solvent that has suitable electrical conductivity, surface tension, and viscosity. Having this combination of properties is vital. In addition to this, the solvent should be able to dissolve the polymer as well as any other functional components that are of significance at concentration levels that are adequate and manageable.

5) Volatility: There are two sensitive parameters that have an effect on the pace at which the solvent evaporates, and those are the fibre diameter and its morphology. The use of a volatile solvent allows the fibre to dry out completely before it reaches the grounded collector, which helps to avoid faults such as merged wet fibres from occurring. Nevertheless, the volatile nature of the solvent has an effect on the processes of phase separation and, as a result, the morphology of electrospun fibres. (Williams et al., 2018)

2.5.3(b) Processing Parameters

1) **Voltage:** Fridrikh et al., (2003) studied that in the process of electrospinning, the magnitude of the voltage that is applied is mostly determined by the properties of the solution, such as its surface tension and viscosity. It is believed that this processing parameter decreases the flight time between the spinneret and the collector. Additionally, the degree of whipping instability and the elongation of the fibre jet can be correlated to the applied voltage. This means that an increase in voltage that exceeds the limit for jet initiation will result in an increase in the amount of both of these factors.

2) **Flowrate:** This electrospinning processing parameter may also be described in other common words such as feeding rate or infusion rate, which refer to the amount of polymer solution fed into the spinneret to feed the spinning cone jet. The magnitude of applied voltage supply, the size, and the diameter of the spinneret orifice all have a significant impact on the flow rate factor (Deitzel et al., 2001). As a consequence of this, a polymer substance has an ideal flow rate range at any certain voltage, and this range shifts depending on the inner diameter of the needle.

3) **Spinneret-to-collector distance:** During the electrospinning process, it stands to reason that a stronger electric field strength will be achieved by using a shorter distance between the spinneret and the collector. The distance between the spinneret and the collector in traditional electrospinning setups is typically between 10 and 20 centimeters. This spacing range is considered to be the most optimal. (Kidoaki et al., 2005) It is critical to understand that

utilizing a shorter collecting distance reduces flight time and, as a result, might result in an incomplete rate of solvent evaporation, which will subsequently alter the surface morphology of the generated fibres.

4) **Spinneret:** The majority of spinnerets on the market nowadays are composed of metal. These needles generally have rather small inner orifice diameters since the production of thin fibers requires this. However, this can also be a reason why it is more likely to become blocked by the solidification of the polymer solution at the tip of the spinneret. Although having a narrow needle orifice can be advantageous in terms of reducing the fiber diameter and assisting in the production of smooth fibers that do not contain any beaded fibres, but is also more prone to becoming blocked (Katti et al., 2004).

2.5.3(c) Environmental Parameters

At the location where the electrospinning process takes place, it is important to take into consideration environmental factors like temperature and humidity. Any and all variances and changes brought on by these environmental conditions may have an effect on the rate of solvent evaporation as well as the stability of the cone jet, all of which might potentially lead to variation in the fiber samples that are collected. Electrospinning should be performed in an area that is completely covered and enclosed in order to ensure the reproducibility of sample production.

2.6 PBAT-based electrospun fibers

2.6.1 Polybutylene Adipate Terephthalate (PBAT)

PBAT is completely biodegradable, which is an exceptional feature for synthetic polymers and to add its outstanding characteristics, it is more flexible than most biodegradable polyesters, such as PLA and PBS, with a Young's modulus of 20–35 MPa, tensile strength of 32–36 MPa, and elongation at break (near to 700%). (Bordes et al., 2009 and Nagarajan et al., 2013).

PBAT has both outstanding biodegradability and great mechanical properties due to the aliphatic unit in the molecular chain. Compared to most biodegradable polyesters such as PLA and PBS, the mechanical properties of PBAT show more flexible, and are similar to those of low-density PE (LDPE) (Bordes et al., 2009 and Nagarajan et al., 2013). These mechanical properties make PBAT a very promising biodegradable material for a wide range of potential applications. In addition, PBAT and its composites are currently used in different areas, such as biomedical (Wei et al., 2016; Wang et al., 2015 and Neto et al., 2015), food packaging (Moustafa et al., 2017 and Livi et al., 2017), environmental industrial applications (Fukushima et al., 2012).

2.6.2 PBAT-based blend

Due to greater production costs and weaker mechanical characteristics when compared to other polymers in the same horizon as PBAT, the properties of pure PBAT are alone not sufficient enough to cope with the consumer adoption. As a result, the growth pattern of PBAT market will be limited and reserved until its production costs reduced or its current properties are enhanced. For example, the combination of low-cost components (such as starch) and reinforcing elements (such as PLA) is an efficient technique to lower the final price while retaining the composites' biodegradability.

PBAT has recently been mentioned in various research as a possible therapeutic use. Several cytotoxicity and cell culture experiments revealed that PBAT is non-toxic and biocompatible with a wide range of cell types. (Wang et al., 2012; Fukushima et al., 2013 and Correlo et al., 2010).

Several research have been conducted on the creation of various forms of PBAT-containing materials, such as extrusion-based scaffolds and fibrous mats. Castro et al., (2016), attempted to create an electrically conductive nanofiber scaffold for bone tissue engineering applications, electrospun PBAT matrices were created in the presence of a conducting polymer, polypyrrole (PPy). It was found that MG-63 cells adhered to and proliferated on PBAT scaffolds alone or in combination with PPy, and the cells on both scaffolds generated significant amounts of alkaline phosphate, which suggesting osteoblastic differentiation.

Moreover, by using electrospinning and spin coating technologies, Neto et al., (2015) created a PBAT and hydroxyapatite (HA) nanoparticles-based biocomposite. In vitro and in vivo data demonstrated that these composite scaffolds facilitated attachment, proliferation, and osteogenic differentiation of human adipose stem cells, as well as a modest inflammatory response.

According to Jian, Xiangbin and Xiangbo (2020), throughout a decade, plethora series of PBAT-based composites have been developed into commercial products. PBAT-based products have been certified as biodegradable according to international standards. These materials may be processed directly on conventional plastic equipment, making them an excellent choice for creating similar application products to conventional plastics. As a result of their high quality, consistent performance, and low cost, PBAT-based products are extensively utilized in various of applications such as packaging, mulch film, and cutlery. Thus, in this study, an attempt to enhanced the