COTTON CANDY MACHINE: EXPLORE ITS POTENTIAL FOR FABRICATION OF POLYMER MICROFIBER FOR VASCULAR TISSUE ENGINEERING APPLICATION

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by

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DECLARATION

I hereby declare that I conducted, completed the research work and written the dissertation entitled “Cotton Candy Machine: Explore Its Potential For Fabrication of Polymer Microfiber For Vascular Tissue Engineering Application”. I also declare that it has not been previously submitted for the award for any degree or diploma or other similar title of this for any other examining body or University

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LIST OF SYMBOLS

°C	C
d_p	Density of Polymer
d_s	Density of Scaffold
kV	Kilovolt
m_s	Mass of Scaffold
μm	Micrometer
nm	Nanometer
%	Percentage
% T	Transmittance
V_S	Volume of Scaffold
% wt	Weight percent

LIST OF ABBREVIATIONS

CL	ε-capprolactone
CVD	Cardiovascular Disease
DSC	Differential scanning calorimetry
FTIR	Fourier-transform infrared spectroscopy
LA	L-Lactide
PGA	Polyglycolic Acid
PLA	Polylactic acid
PLCL	Poly(L-lactic acid-co-ε-caprolactone)
PLGA	Polylactic-cooglycolic acid
PLLA	Poly-L-lactide
SEM	Scanning Electron Microscope (SEM)
T_{cc}	Crystrallization Temperature
TEVGs	Tissue Engineered Vascular Grafts
T_g	Glass Transition Temperature
T_m	Melting Temperature
VTE	Vascular Tissue Engineering

MESIN GULA-GULA KAPAS: MENEROKA POTENSI UNTUK FABRIKASI POLIMER GENTIAN MIKRO UNTUK APLIKASI KEJURUTERAAN TISU VASKULAR

ABSTRAK

Kajian ini melibatkan fabrikasi perancah dengan menggunakan kaedah penyarian beku dan kaedah mesin gula-gula kapas untuk aplikasi kejuruteraan tisu vaskular. Kepingan perancah tunggal dari mesin gula-gula kapas poli(asid laktik) (PLA) pada perbezaan masa penyepuhlindapan pada 24 jam, 48 jam dan 72 jam pada suhu 80°C untuk menganalisa optimum kepingan perancah tunggal. Kepingan perancah tunggal telah disiasat untuk kepekatan poly(L-laktik asid-co-ε-kaprolakton) (PLCL) dihasilkan dengan menggunakan kaedah penyarian beku. Gabungan kedua-dua kaedah dihasilkan untuk menjadi gandaan dua kepingan dan gandaan berbilang kepingan perancah. Perancah ideal difabrikasi menjadi tisu perancah yang menyerupai tisu asal kardiovaskular. Perubahan morfologi bagi perbezaan kepekatan PLCL dan masa penyepuhlindapan PLA dilihat dengan menggunakan mikroskop imbasan elektron dengan perubahan keliangan dari 21 % hingga 32 % perancah dalam perbezaan penyepuhlindapan PLA. dan 19 % hingga 43 % untuk perbezaan kepekatan PLCL. Penyepuhlindapan PLA pada 72 jam mempunyai diameter yang paling kecil dan saiz keliangan yang paling kecil. Kepekatan PLCL pada 9 wt% adalah kepingan perancah tunggal yang optimum yang difabrikasikan oleh kaedah penyarian beku kerana saiz poros yang besar terbentuk. Dua kepingan perancah menunjukkan ia adalah serasi untuk dijadikan perancah berbanding dengan gandaan berbilang kepingan perancah disebabkan pembentukkan ruang antara kepingan perancah.

COTTON CANDY MACHINE: EXPLORE ITS POTENTIAL FOR FABRICATION OF POLYMER MICROFIBER FOR CARDIOVASCULAR TISSUE ENGINEERING APPLICATION

ABSTRACT

This work presents the fabrication of scaffold by using freeze extraction method and cotton candy machine for application vascular tissue engineering. The single layer scaffold by cotton candy machine was studied on different polylactic acid (PLA) annealing time for 24 hours, 48 hours and 72 hours in 80°C to analyse the optimize single layer scaffold. The single layer scaffold in different concentrations 3 wt%, 6 wt%, and 9 wt% of poly(L-lactic acid-co-ε-caprolactone) (PLCL) produced by using freeze extraction method was investigated. The combination of boths method were produced to form double and multilayer scaffold. The ideal scaffold was fabricated to form tissue scaffold that similar to the native cardiovascular tissue. The morphological changes in different concentrations PLCL and PLA annealing time were observed via scanning electron microscope (SEM) with porosity varied from 21 % to 32 % for PLA scaffold with different annealing time of PLA and 19 % to 43 % for different concentrations of PLCL. The 72 hours of the PLA annealed time were smaller diameter and smaller pore size. The 9 wt% of concentration PLCL was optimum single scaffold by freeze extraction method due to the largest pore size forms compare to others. The double layer scaffold were shows that, it is suitable to form scaffold compared to the multilayer scaffold due to the formation of gap between layer.

CHAPTER 1

INTRODUCTION

1.1 Overview

The news on the organ shortage crisis is known everywhere around the globes. There is a limited supply on organ donor which creates a large gap between supply and demand. Vascular Tissue Engineering (VTE) provides one of the alternative ways to solve the crisis. Cardiovascular disease is among the famous severe type of diseases that cause main reasons of death in the world. Atherosclerosis is the one of severe forms of heart disease that causes tightening of the arteries. Approximately half thousand cases per year performed bypass of surgical replacement of vessel segments or bypass surgery (Jaffery and Grant, 2009). Thus, the artificial tissue, functions and growth potential are new strategy of VTE to provide similar native tissue by using material degradable scaffolds (Naito et al., 2011).

The three dimensional and the porous structures are the physical characteristics of the scaffold that can act as cell growth beds. The increasing of porous structure of the scaffold is the most desired for cell ingrowth and importance for transport the nutrients and gases through the pore. The ideal vascular scaffold should have properties of mechanical strength, blood compatibility, and provide good seam retaining. Three-dimensional scaffolds play an important role because it provides cells with a tissue-specific environment and design for tissue engineering, the vital factors are to create three-dimensional scaffold with degradation rate that suitable with the requirements of new tissue growth, interconnected pores, high porosity, and suitable mechanical stability.

There are numerous fabrication methods for construction of three-dimensional biomimetic scaffolds, such as phase-separation, electrospinning, freeze

drying, and self-assembly. It have been developed for tissue engineering and regenerative medicine. These scaffolds can mimic the design of the native extracellular matrix which provides the initial space for redevelopment of new tissue (Lu et al., 2013). Electrospinning has been widely used to produce scaffold however it has low production rate, limited application, expensive method, the complexity of control parameter (Sarkar et al., 2010) and it is harmful due to a toxic organic solvent that is used during the fabrication process (Khorshidi et al., 2016). Thus the alternative to solve this problem is by introducing a melt-spinning method by cotton candy machine. The fibrous of cotton-like structure that been produced by cotton candy machine can overcome the problem in which it is cost-effective and it is not harmful which is compatible to be use as medical applications (Pangesty and Todo, 2018).

One of the other fabrication techniques is freeze drying. To retain the porous structure of the scaffolds, this technique usually required for solvent removal. The issues encountered in the application of freeze-drying is the existence of surface skin in the preparation of scaffold. The polymer would not be rigid to resist the interfacial tension if the temperature is not controlled low enough that is caused by evaporation of the solvent. Thus, the porous structure collapses and dense skin layers occur in the prepared scaffolds (Loh and Choong, 2013). Thus this problem is investigated by introduced fabrication by using freeze extraction. Frozen polymer solution dip into the non-solvent bath. It will make the process of exchange of solvent and non-solvent that will resulting in same porous structure without the surface skin

Scaffold manufacturing uses several types of materials such as synthetic materials (carbon fiber, teflon and biocative glass) and natural materials (collagen, fibrin and alginate). The advantages of the synthetic materials are help better on control of physical, chemical, mechanical properties, and the degradation rate. The fabrication

methods also can help in the process of synthetic material into scaffolds to form desired anisotropies , porosity, and morphologies, that will improve cell attachment and migration (Jenkins and Little, 2019). The synthetic materials that usually made to form scaffold are polymeric. The linear aliphatic polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), and their co-polymers polylactic-co-glycolic acid (PLGA) are the most popular groups among the polymeric. PLA is favorable on the formation of scaffold due to the characteristic of biodegradable, biocompatible and also an absorbable polyester (Kakinoki and Yamaoka, 2014). PLA also the most attractive material which the implanted in vivo is successful as in human heart-blood vessels (Rasal et al., 2010). Poly(L-lactic acid-co-ε-caprolactone) (PLCL) copolymers is an appealing candidates to offer a range of different degradation behaviors. PLCL are thought to have potential as they can lengthen the degradation period in aqueous environments (Pan et al., 2014).

Three available systems that scaffold categorized were single phase, bi- or multi-layered and gradient structures. It is expected to be a principle for development of scaffold designs to get nearer to an ideal vascular scaffold for clinical practice. Multilayer scaffolds are being broadly researched instead of using single phase materials. This is because it can be designed and made-up to mimic the structure of the native tissue (Qu et al., 2011, Klein et al., 2009, Zhao et al., 2011). The single layer microfibrous scaffold by cotton candy machine and microporous scaffold of freeze extraction studies. The multilayer scaffold by the combination method is fabricated to identify the scaffold properties and the mechanical properties of the multilayer scaffold.

1.2 Problem Statements

Cardiovascular tissue has an important role in circulating blood for the transport of oxygen, carbon dioxide, nutrients, blood cells, and hormones, to maintain the homeostasis of human body. Because it is a vital organ of the human body to sustain life, disease and/or damage of vascular tissue can result in critical health issues. The ideal scaffold biomaterial for a cardiac patch is an elastic material capable of supporting thousands of stretch cycles without constraining heart contractions and relaxation. Therefore, artificial vascular grafts that can substitute for damaged blood vessels have been widely explored to treat these vascular diseases.

Vascular tissue engineering is found as an alternative way to solve the problem (Carrabba and Madeddu, 2018). The solution is by forming a synthetic polymer by using the electrospinning method. However, this process is not productive due to the low production rate and the complexity of the control parameter. Moreover, this process is harmful to the human body because it involves toxic organic solvents during the fabrication (Sarkar et al., 2010, Khorshidi et al., 2016). Therefore, the fabrication scaffold of melt-spinning by using cotton candy machine is investigated in this research. This method is not using any organic solvents thus it is non-toxic to the human body. This report examines the use of a novel technique combining melt-spinning by cotton candy machine with freeze extraction to create a unique multi-layered scaffold with differential morphology and porosity that provides a high level of control to influence cell behavior.

Thus, the purpose of the study is to determine the optimized fabrication parameters of single layer microfiber scaffolds via two different fabrication methods which are by cotton candy machines and freeze extraction methods. This is to ensure the characteristics, properties and mechanical strength scaffold can achieved similar

to the native cardiovascular tissues. There is no research are found on the multilayer scaffold by using both methods. The biomimetic scaffold for vascular tissue would required different pore structure and pore size to meet the mechanical demands. Thus, this research is investigating on the double layer microfiber scaffold's properties using the combined fabrication methods of multilayers microfiber scaffold to achieve suitable properties of scaffold for cardiovascular tissue.

1.3 Objectives of the study

The objectives of this research are:

1. To determine the optimized fabrication parameters of single layer microfiber scaffolds via two different fabrication methods (cotton candy machines and freeze extraction)
2. To investigate the double-layer scaffold's properties using the combined fabrication methods
3. To study the properties of multilayer scaffolds by alternate fabrication methods for each layer.

1.4 Scope of the study

In the first stage, the fabrication of scaffold by using freeze extraction methods was produced. The different concentration PLCL was used in this fabrication method. The fabrication of scaffold by using cotton candy machine was also explored. During the fabrication scaffold of cotton candy machine, different annealing times of PLA was used to produce optimum scaffold.

The second stage of the research involved double-layer scaffold via different fabrication methods in different types of polymer. The first layer consists of the PLCL microporous scaffold and the second layer was the PLA microfibrous scaffold.

Lastly, the fabrication of multilayers scaffold in an alternate fabrication method in four layers with the method of the cotton candy machine and freeze extraction method. The characterization and testing were investigated. The testing used in this thesis are Scanning Electron Microscopy (SEM), tensile test, Differential Scanning Calorimetry (DSC), visual observation, thickness measurement and density and porosity, pore size and fiber diameter test.

1.5 Thesis outline

In Chapter one, a general introduction to the subject is presented, in addition to the problem statement, research objectives, scope of work and outline.

Chapter two covers literature review in support for remainder of the thesis. General understanding of PLA, PLCL, its structure, properties, and the advantages and disadvantages are provided in this chapter. The next part of this chapter is focused on the fabrication scaffold by using cotton candy machine and freeze extraction methods.

Chapter three presents the details of material, the methodology used for preparation of single layer scaffold by cotton candy machine and freeze extraction methods. The fabrication of multilayer scaffold of both methods and the techniques for characterization are also covers in the methodology part in this chapter.

In Chapter four, the experiment results and discussion of both fabrication methods for single and multilayer scaffold was covered. This includes results of the Scanning Electron Microscopy (SEM), tensile test, Differential Scanning Calorimetry (DSC), visual observation, thickness measurement and density and porosity, pore size and fiber diameter test.

Chapter five summarizes this research and also include a few suggestions for future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Cardiovascular Disease (CDV)

Cardiovascular disease (CVD) is the top cause of sickness and mortality, producing massive health and economic burdens in the United States and globally (Huang et al., 2018). In the United States, approximately 84 million American adults have CVD (Go et al., 2014) with heart and cerebrovascular diseases being the first and third leading causes of death, respectively. In 2010, the overall death rate from CVD was 235.5 per 100,000 with one American dying of CVD every 40 seconds (Go et al., 2014) Figure 2.1 shows each year, the number of people on the waiting list continues to be much larger than both the number of donors and transplants, which grow slowly.

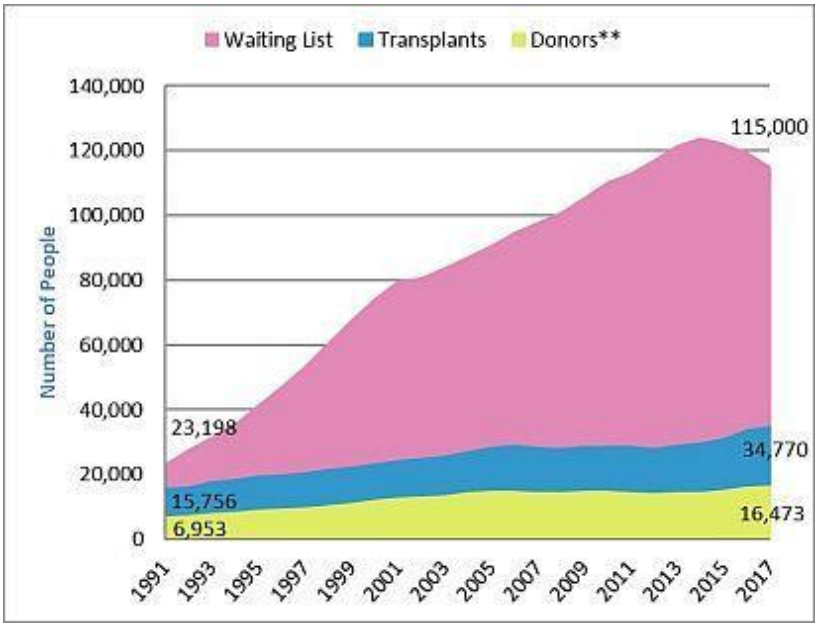

Figure 2.1: The organ donation statistic from the year 1991 to 2017 (Go et al., 2014)

CVD are also economically burdensome. Based on (Go et al., 2014), the direct and indirect costs of CVD were approximately 315.4 billion in 2010. It was an increase of more than 10% compared to 2007 (Roger et al., 2011). Organ shortage is likely to

continue to increase in the near future Allografts have also previously been used, however, they have the added risks of tissue rejection and disease transmission and consequently are no longer used clinically. Artificial vascular grafts, therefore, are seen as the "holy grail" of vascular surgery resulting in an extensive quantity of research into the area.

2.2 Vascular Tissue Engineering (VTE)

Tissue engineering has grown out of our knowledge of tissue formation and regeneration and aims to purposefully induce the growth of new functional tissues, rather than just replace diseased or injured tissues with nonviable implantable spare parts (Chen and Liu, 2016). For this reason, tissue engineering has emerged as a promising approach to treat the loss of function of a tissue or organ without the limitations of current therapies. The most widely used definition of tissue engineering was proposed by Langer and Vacanti in a 1993 issue of Science, as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ." (Langer and Vacanti, 1993). This working definition is still relevant to the application of tissue engineering toward solving cardiovascular diseases.

To address the limitations and complications of current technologies, tissue-engineered vascular grafts (TEVGs) have emerged as an alternative source of vascular conduits. In its most simplified form, vascular tissue engineering is the implantation of a biodegradable scaffold seeded with cells or unseeded within a living host. The biodegradable scaffold initially serves as a site of cell attachment and neotissue formation while acting as a passive conduit for blood flow. Over time, the

biodegradable scaffold loses mechanical strength while the neotissue begins to develop mechanical strength. The culmination is a neovascular conduit composed entirely of autologous tissue with an intima, media, and adventitia in the absence of any scaffold material (Wang et al., 2016).

In tissue engineering strategies, different types of cells have been combined with materials and with bioactive molecules if necessary to again try to recover the injured tissue. The employed materials will support cells, provide them 3D organization, protect them, stimulate and guide its growth, maintain them in the site of interest, etc.; in sum, they will act as an artificial extracellular matrix during the regeneration process. But the use of materials either injectable, or ex vivo conformed (gels –patches- or scaffolds) as shown in Figure 2.2 has an additional and important effect: the implantation of a material in the scarred ventricular wall, increases its thickness and by Laplace's law, this increase leads to a reduction in the wall stress. This side-effect could be by itself very positive, even although regeneration did not arrive to happen, to limit ventricular remodeling and improve the quality of life of cardiac patients (Arnal Pastor, 2013).

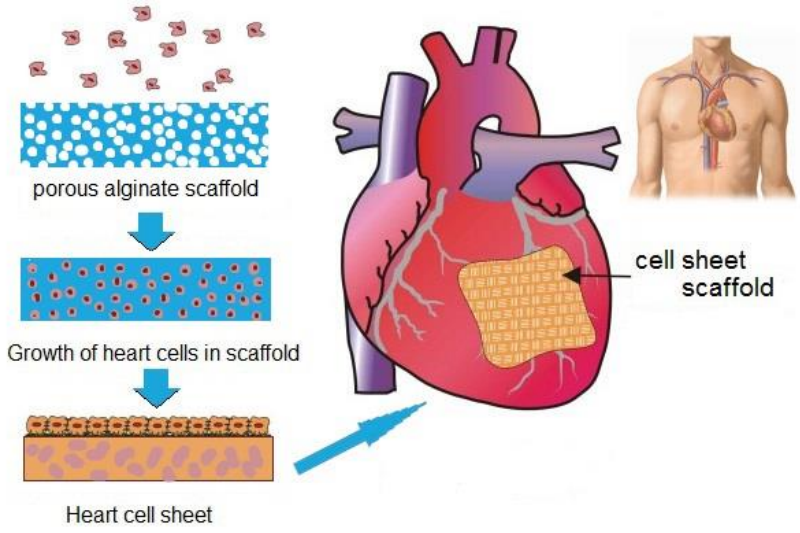

Figure 2.2: Scaffold patch for vascular tissue (Hadasha and Bezuidenhout, 2018)