DEVELOPMENT OF ELECTROSPINNING MACHINE FOR THE PRODUCTION OF HOMOGENEOUS AND FUNCTIONALLY GRADED MULTILAYER POLYMERIC NANOFIBERS

LIM SHING CHEE

A thesis submitted in fulfillment of the requirement for the award of the
Master of Mechanical Engineering

Faculty of Mechanical and Manufacturing Engineering
Universiti Tun Hussein Onn Malaysia

AUGUST 2022

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincerest appreciation to my research supervisor, Dr. Koh Ching Theng, who provided me endless guidance and supports throughout the duration of this project. I am very thankful for all of her valuable comments and guidance during this project. Without her guidance and assistance, this research would not have been possible. Many thanks!

Moreover, I am particularly grateful for the assistance given by Mr. Anuar Ismail and Mr. Tarmizi Nasir from Faculty of Mechanical and Manufacturing Engineering.

And finally, last but not least, my special gratitude also extends to my family members including my wife. Thanks for all the encouragement, guidance and supports given throughout this research.

ABSTRACT

Electrospinning technology has been widely used in producing porous scaffolds consisting of nano- to microfibers. These porous electrospun scaffolds are useful in various applications including medical and filtration applications. The microstructure architecture such as pore size and fiber diameter is able to affect their function and efficiency. In medical applications, the control of pore sizes affects the environment to promote cellular activities. For filtration applications, the pore size can control filtration efficiency. The control of microstructure architecture, however, is a difficult task due to the microstructure of the electrospun being highly sensitive to the electrospinning parameters. One way to manipulate the microstructure architecture is by governing the process parameter and the knowledge in developing electrospinning machines brings the potential to develop novel electrospun scaffolds. This thesis focuses on the design and fabrication of the electrospinning machine. The machine was used to produce gelatin nanofibers with tailored microstructures and functionally graded multilayers. First, an electrospinning machine consists a high voltage supply, a syringe pump and a collector was built to produce homogeneous electrospun scaffolds. Gelatin and Polycaprolactone were spun into porous fibrous networks. The relationship between process parameters and microstructures was studied. These process parameters and microstructure dataset were used to produce the functionally graded multilayer electrospun gelatin scaffolds. A controllable moving stage was developed to precisely control the tip-collector distance and microstructure gradient over scaffold thickness. Microstructure images of functionally graded multilayers electrospun scaffold show the gradual changes of fiber diameters in nano-sized over the scaffold thickness. This study proposes a novel technique for designing the graded electrospun scaffolds which more closely mimic the native tissues.

ABSTRAK

Teknologi *electrospinning* telah digunakan secara meluas dalam menghasilkan perancah yang terdiri daripada gentian yang bersaiz nanometer hingga mikrometer. Perancah yang terhasil ini boleh digunakan dalam pelbagai aplikasi termasuk aplikasi perubatan dan penapisan. Seni bina struktur perancah seperti saiz liang antara gentian dan saiz diameter gentian mampu mempengaruhi fungsi dan kecekapannya bagi satu aplikasi. Dalam aplikasi perubatan, kawalan saiz liang antara gentian boleh mempengaruhi prestasi kegiatan aktiviti selular bagi sel. Untuk aplikasi penapisan, saiz liang antara gentian boleh mempengaruhi prestasi penapisan. Walaubagaimanapun, kawalan struktur untuk menghasilkan perancah adalah satu tugas yang sukar disebabkan struktur perancah amat sensitive dengan *electrospinning* parameter. Antara satu kaedah yang mampu memanipulasikan struktur perancah adalah mengawal proses parameter dan pengalaman untuk membina mesin *electrospinning* membawa potensi untuk menghasilkan perancah yang baru. Objektif utama tesis ini adalah mereka bentuk dan membina satu mesin *electrospinning*. Mesin tersebut digunakan untuk menghasilkan perancah gelatin dan perancah berlapisan yang mempunyai struktur yang berlainan bagi setiap lapisan. Pada permulaannya, mesin *electrospinning* yang terdiri daripada bekalan voltan tinggi, pam picagari dan papan pengumpul gentian disediakan untuk menghasilkan perancah. Gelatin dan *Polycaprolactone* digunakan sebagai bahan untuk menyediakan larutan dan digunakan untuk menghasilkan perancah. Hubungan antara proses parameter dengan struktur dikajikan dan data dicatatkan. Kemudian, data-data yang dicatat digunakan untuk meghasilkan perancah berlapisan. Satu pentas yang bergerak direka dan digunakan untuk mengawal jarak antara papan pengumpul dan hujung picagari dengan jitu. Imej struktur bagi perancah berlapisan menunjukkan perubahan diameter gentian secara beransuran bagi setiap lapisan. Kajian tersebut mencadang satu kaedah baharu untuk menghasilkan perancah berperingkat yang sesuai digunakan untuk menyimulasi tisu asli yang didapati dalam badan manusia.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AC	Alternating Current
BOM	Bill of Materials
d	Tip to Collector Distance
DC	Direct Current
DMF	Dimethylformamide
ECM	Extracellular Matrix
HEPA	High-efficiency Particulate Air
HVAC	Heating, Ventilation, and Air Conditioning
NaCl	Sodium Chloride
PAA	Peracetic Acid
PAN	Polyacrylonitrile
PCL	Polycaprolactone
PEO	Poly (ethylene oxide)
PHBV	Polyhydroxyalkanoate-type Polymer.
PLGA	Poly(lactic-co-glycolic acid)
PVP	Polyvinylpyrrolidone
THF	Tetrahydrofuran
RPM	Rotation per Minutes

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fibers made of polymers have been employed in a variety of sectors, including biomedical field (Yang *et al*., 2018), filtration applications (Sikorska et. al. 2018) and electronic applications (Luzio *et al*., 2014). Polymer fibers are widely employed in medical applications such as drug delivery and tissue engineering. For example, in tissue engineering, polymeric electrospun scaffolds are used to develop three-dimensional functional structures similar to native tissue structures such as the extracellular matrix (ECM) to provide temporary support to cells during native ECM formation (Nemati *et al*., 2019). Filtration applications can be divided into liquid filtration and air filtration. The polymeric nanofibers membranes have been used for the removal of micron-sized particles from the water. Besides, it is also applied as high-efficiency particulate air (HEPA) filters have removal efficiency of 99.97% of particles bigger or equal to 0.3 micrometers (Shabafrooz *et al*., 2014).

There are several techniques to fabricate the polymeric fibers, such as self-assembly, phase separation and electrospinning technique (Nemati *et al*., 2019). Self-assembly used in polymeric materials typically involves the intermolecular association of peptides that immediately assemble into organized and stable structures by electrostatic force. The self-assembly fibers were much thinner compared to scaffold produced by electrospinning technique. Another technique used to produce polymeric fibers scaffold is phase separation. This technique involves three main steps, which are dissolution, gelation, and extracting. First, the polymer is dissolved in a solvent, and then the solution goes through a gelation process. During the gelation process, the polymer fibers formed within the solvent. After that, the solvent is extracted from the gel with water. This technique is simple but involves a long processing time. At the same time, the porosity and the structure of the fibers are difficult to control. The electrospinning technique uses electrostatic force to generate polymeric fibers, and this technique can create continuous polymeric fibers with diameters ranging from micrometers to nanometers. (Wang & Ryan, 2011). Besides this, the diameter of fibers, porosity and structure of the electrospun scaffold that is produced by electrospinning are able to control by varying the electrospinning parameters (Nemati *et al*., 2019). Electrospinning systems can produce various types of polymers, ceramics and composites into microfibers with controlled diameter and surface morphology (Rasel, 2015). Furthermore, through the electrospinning technique and improved collector setups, structures with different compositions, hollow interiors, and functional properties of electrospun fibers have been fabricated.

The electrospinning technique is a simple and versatile method for preparing nanofibers. Electrospinning technology enables nanofibers to be manufactured in small quantities for laboratory research and large-scale production for industrial use. In recent years, electrospinning technology has been widely used in academia and industry due to its unique ability to fabricate fibers from different materials in various assemblies. For example, Elmorco company used the electrospinning technique in air filtration for gas turbine and HVAC industries.

Figure 1.1 shows the growth of published papers and patents containing the electrospinning technology. The result shows the increase in the number of scientists interested in fabricating scaffolds by using electrospinning technology. Figure 1.2 shows the schematic diagram for electrospinning.

Figure 1.1: Number of published papers and patents containing the concept of electrospinning technology between 2010 and June 2021 (Fatirah *et al*., 2021).

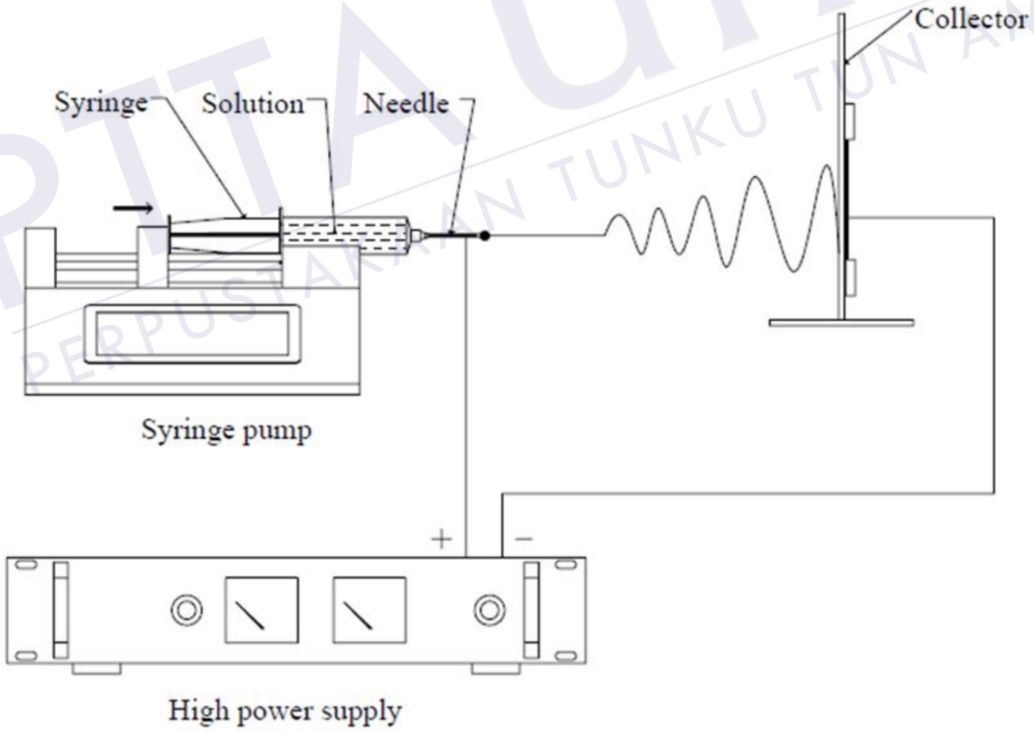

Figure 1.2: The schematic diagram for electrospinning setup.

The electrospinning system is easy to set up and can be modified to meet specific requirements. The typical unit consists of three main components, which are a high power supply, a syringe pump and a metal collector. A syringe filled with polymer solution is mounted on a syringe pump, which pumps the polymer solution at a constant flow rate. A high voltage supply generates an electrostatic force to propel the droplets from the syringe tip, then spinning the droplets into fibers, which are then deposited on a metal collector.

Although the electrospinning system is simple and easy to set up, but this technique is a sensitive process. The electrospinning technique has many operating parameters, including process parameters and solution parameters, that able to affect the structure of electrospun scaffolds. Besides this, it is also sensitive to the environment, such as surrounding temperature and relative humidity (Topuz *et al*., 2021). It is then important to understand the influences of electrospinning parameters in order to fabricate high quality nanofibers. Such understanding of the relative effect of parameters will be useful for the process control during the electrospinning process.

Nowadays, the electrospinning technique is able to produce various types of scaffolds, which are random and aligned electrospun scaffolds. Each type of electrospun scaffolds presented different microstructures and properties and played different roles in tissue engineering. For example, an aligned electrospun scaffold is able to mimic the aligned structure of native extracellular matrix (ECM) tissue such as tendon and cardiac (Jin *et al*., 2018) while a multilayer electrospun scaffolds able to mimic tendon-to-bone insertion area and the structure of articular cartilage (Girão *et al*., 2018) and corneal (Arabpour *et al*., 2019).

1.2 Problem Statement

Nowadays, the electrospinning technique is widely used in various fields. However, many technical issues still exist and a number of fundamental questions need to be resolved. The existence of beads is a problem faced by tissue-engineered scaffolds because the bead affects the mechanical performance of the electrospun scaffold (Huang *et al*., 2004) and hinders cell proliferation (Chen *et al*., 2007). Besides, the structure of electrospun scaffolds is highly sensitive to the electrospinning parameter like process parameters, solution parameters and relative humidity (Khoo and Koh, 2016). Any slight variation of electrospinning parameters such as applied voltage, nozzle-collector distance, solution flow rate, ambient humidity, polymer concentration and solvent volatility not only cause the formation of beads but also influence the structure of electrospun scaffold such as fiber diameter, porosity and pore size of the electrospun membrane. Therefore, producing high quality and beads defects free electrospun scaffolds becomes difficult.

Inhomogeneous structures are presented in native tissues such as articular cartilage. The tissue consists of multiple zones, which are superficial, transitional, deep and calcified zones (Mow *et al*., 1992). These zones have differences in morphology structure (Hwang *et al*., 1992). Therefore, inhomogeneous properties are crucial in tissue-engineered scaffolds to mimic the structures of biological material. Using an inhomogeneous scaffold to mimic the microstructure of native tissue in the human body is a strategy to improve native tissue regeneration. There is a potential in improving cell response when better mimicking the inhomogeneous structure (Di Luca *et al*., 2016). However, it is still a great challenge in the conventional electrospinning techniques to produce electrospun scaffolds with inhomogeneous properties.

1.3 Objectives of Study

The objectives of this study are listed below:

1. To design and develop an electrospinning machine that consisted of a high voltage power supply, a syringe pump, a controllable moving stage and a collector.
2. To fabricate homogenous electrospun gelatin and Polycaprolactone (PCL) scaffolds with different electrospinning parameters and characterize their microstructure morphology.
3. To produce functionally graded multilayer electrospun gelatin scaffold and characterize their microstructure morphology.

1.4 Scope of Study

1. The electrospinning machine consisted of a high power supply, syringe pump, collector and controllable moving stage.
2. There are two types of casing for electrospinning machines which are acrylic and stainless steel casing.
3. A moving stage was included in controlling the tip-collector distance in the electrospinning system.
4. Polycaprolactone (PCL) was used in preparing a homogeneous electrospun scaffold.
5. Fish gelatin was used in preparing homogeneous and multilayer electrospun scaffolds.
6. Electrospinning parameters studied in this work were solution parameters, process parameters and relative humidity.
7. In preparing the homogeneous electrospun PCL scaffolds, solution concentration, process parameters and relative humidity were varied in this work.
8. In preparing the homogeneous and multilayer electrospun gelatin scaffolds, process parameters were varied in this work.
9. The surface morphology of the electrospun scaffold was characterized by using scanning electron microscopy (SEM).
10. ImageJ was used to measure the fiber diameter of electrospun scaffolds.

1.5 Significance of study

An electrospinning machine has been successfully designed and fabricated to produce polymeric nanofiber. The electrospinning machine is able to produce homogeneous and functionally graded multilayer electrospun scaffolds. The electrospun scaffolds can be produced from different polymer solutions at various electrospinning parameters. Visualization under SEM revealed that scaffolds with different microstructure morphologies, i.e., beads and fiber size were obtained.

Through this work, a fundamental understanding of how electrospinning parameters affect the morphology structure of electrospun scaffolds can provide some idea to researchers to produce electrospun scaffolds with desired microstructural morphologies.

In addition, the significance of this study is adopting a new method to produce functionally graded multilayer electrospinning scaffolds to better mimic the inhomogeneous structure of native tissues. A moving stage was designed and fabricated to ensure precise control of the tip-collector distance and duration during the electrospinning process. A patent with the title System and Method for Producing Multilayer and Functional Graded Fibrous Material was file.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Electrospinning

Lord Rayleigh gave the first idea of using electrostatic force to induce droplet formation in 1882 (Rayleigh, 1882). He found that charged droplets are in an unstable equilibrium, forming cones that break apart into smaller droplets when passing through a voltage gradient. With this fact, Rayleigh theorized that the droplet surface tension breakdown was caused by forces created by Coulombic repulsion. After the initial research, several research groups were interested in this technique. They did further research by using aqueous solution, experimented with electrosprays of dilute polymer solution (Dole *et al*., 1968). In 1955, Drozin found droplets that electrospray out resemble a highly dispersed aerosol (Drozin, 1954).

As part of his first patent, Formhals came up with the "Process and Apparatus for Making Artificial Thread" in 1934. (Formhals, 1934). Process and apparatus for making artificial filament utilizing electrical charges are the subject of this invention. A moveable thread collector is used in the spinning process to gather stretched threads. Using acetone or alcohol as the solvent, Formhals is successful in spinning cellulose acetate fibers. In this invention, Formfals has mentioned that this electrospinning method still existed with some shortcomings. Owing to the short distance between the spinneret and collector device, the solvent could not completely evaporate and dry the fibers before the fiber jet was deposited on the collector. This shortage causes the fibers to stick on the collector and causes removal problems due to incomplete solvent evaporation.

When Formfals discovered the short distance between the needle tip and the collector was the main issue with the first invention in 1934, they reworked the second patent in order to overcome the aforementioned shortcoming (Formfals, 1939). The distance between the nozzle and the collector is increased during electrospinning process to allow more time to evaporate the solvent and dry the fibers before depositing them on the collector. Using multiple nozzles and a single polymer solution, the current invention aims to simultaneously spin multiple fibers toward a collector. Subsequently, in 1940, updated method to fabricate the composite fibers webs by using multiple polymer solution direct electrospun onto a moving collector was then patented (Of & Sm, 1940).

Taylor published his work entitled Electrically Driven Jets in 1969 (Taylor, 1969). This published work is related to the shape of the polymer droplets that appear at the needle tip when an electric field is applied. In this study, Taylor found that when electrostatic forces balance the surface tension, the droplet at the tip needle becomes a cone shape, and the fiber jet emerges from the cone's vertices. Other researchers have named this conical shape of the jet as "Taylor cone". A 49.3-degree angle relative to the axis of the cone is required to balance surface tension and electrostatic forces, according to Taylor's research.

In 1971, Baumgarten began to examine the structural qualities of electrospun fibers by altering the process parameters and solution parameters, such as solution concentration, applied voltage, solution flow rate, etc. (Baumgarten, 1971). For the associated work, Baumgarten employed PAN/PDF as the solvent and observed that solution viscosity directly affected the diameter of polymer fibers. With a higher viscosity, the diameter of the fibers will increase. At the same time, he sdemonstrated that the fiber diameter decreased initially with increasing applied electric field until it reached a minimum value. Then the fiber diameter increases as the applied field are increased further. Baumgarten successfully produced electrospun acrylic fibers with diameters between 500 and 1100 nm by varying the solution and processing parameters.

Research into electrospinning of polymer melts began following Baumgarten's initial breakthrough. Electrospinning polyethylene and polypropylene melt fibers by Larrondo and Mandley has proven a success (Larrondo & Manley, 1981a; Larrondo & Manley, 1981b). Fibers electrospun from the melt had bigger diameters than fibers electrospun from the solution, according to the researchers who conducted the study. At the same time, they have demonstrated that the diameter of the fibers decreases with increasing melt temperature. During the same period, several researchers began to investigate the potential applications of electrospun fiber mats, especially in tissue engineering. In 1978, Annis and Bornat published their work examining electrospun polyurethane mats for us as vascular prostheses (Annis and Bornat, 1978). In 1985, Fisher and Annis investigated electrospun arterial prostheses' long-term in vivo performance (Fisher *et al*., 1985). Various applications, such as medication delivery, tissue engineering, filtration, and textiles, have drawn attention to electrospinning technology since the 1980s.

2.2 Electrospinning Setup and Process

A typical electrospinning system consists of three major components, which are a high voltage power supply, a syringe pump with a syringe mounted with a metal needle and a metal collector. All the components were fixed into a casing to set up an electrospinning system. Figure 2.1 shows a schematic diagram of the electrospinning system setup.

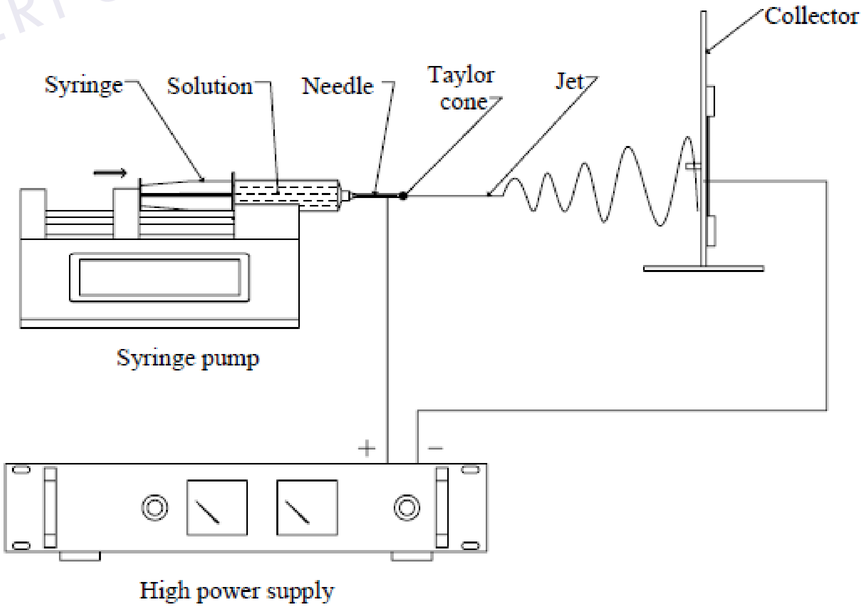

Figure 2.1: Schematic diagram of the basic setup for electrospinning

REFERENCES

Annis, D., Bornat, A., Edwards, R. O., Higham, A., Loveday, B., & Wilson, J. (1978). An elastomeric vascular prosthesis. *ASAIO Journal*, *24(1)*, 209-214.

Arabpour, Z., Baradaran-Rafii, A., Bakhshaiesh, N. L., Ai, J., Ebrahimi-Barough, S., Esmaeili Malekabadi, H., Nazeri, N., Vaez, A., Salehi, M., Sefat, F. & Ostad, S. N. (2019). Design and characterization of biodegradable multi layered electrospun nanofibers for corneal tissue engineering applications. *Journal of Biomedical Materials Research Part A, 107(10)*, 2340-2349.

Barallobre-Barreiro, J., Loeys, B., Mayr, M., Rienks, M., Verstraeten, A., & Kovacic, J. C. (2020). Extracellular matrix in vascular disease, part 2/4: JACC Focus Seminar. *Journal of the American College of Cardiology*, *75(17)*, 2189-2203.

Baumgarten, P. K. (1971). Electrostatic spinning of acrylic microfibers. *Journal of colloid and interface science*, *36(1)*, 71-79.

Beachley, V., & Wen, X. (2009). Effect of electrospinning parameters on the nanofiber diameter and length. *Materials Science and Engineering: C*, *29(3)*, 663-668.

Bosworth, L. A., & Downes, S. (2012). Acetone, a sustainable solvent for electrospinning poly (ε-caprolactone) fibres: effect of varying parameters and solution concentrations on fibre diameter. *Journal of Polymers and the Environment*, *20(3)*, 879-886.

Casper, C. L., Stephens, J. S., Tassi, N. G., Chase, D. B., & Rabolt, J. F. (2004). Controlling surface morphology of electrospun polystyrene fibers: effect of humidity and molecular weight in the electrospinning process. *Macromolecules*, *37(2)*, 573-578.

Chen, F. J., Huang, L. & Lindsay, J. (2006). *Gradient nanofiber materials and methods for making same*. U.S. Patent 2006/0094320 A1.

Chen, J., Li, X., Liu, Q., Wu, Y., Shu, L., He, Z., Ye, C. & Ma, M. (2020). Fabrication of Multilayered Electrospun PLGA/(PVP+ PEO) Scaffolds and Biocompatibility Evaluation. *Journal of Biomedical Materials research. Part A. 109(8)*, 1468-1478.

Chen, M., Patra, P. K., Warner, S. B., & Bhowmick, S. (2007). Role of fiber diameter in adhesion and proliferation of NIH 3T3 fibroblast on electrospun polycaprolactone scaffolds. *Tissue engineering*, *13(3)*, 579-587.

Chu, B., Hsiao, B. S., Fang, D. & Brathwaite, C. (2007). *Biodegradable and/or bioabsorbable fibrous articles and methods for using the articles for medical applications*. U.S. Patent 7172765B2.

Deitzel, J. M., Kleinmeyer, J., Harris, D. E. A., & Tan, N. B. (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, *42(1)*, 261-272.

Demir, M. M., Yilgor, I., Yilgor, E. E. A., & Erman, B. (2002). Electrospinning of polyurethane fibers. *Polymer*, *43(11)*, 3303-3309.

Di Luca, A., Ostrowska, B., Lorenzo-Moldero, I., Lepedda, A., Swieszkowski, W., Van Blitterswijk, C., & Moroni, L. (2016). Gradients in pore size enhance the osteogenic differentiation of human mesenchymal stromal cells in three-dimensional scaffolds. *Scientific reports*, *6(1)*, 1-13.

Dole, M., Mack, L. L., Hines, R. L., Mobley, R. C., Ferguson, L. D., & Alice, M. B. (1968). Molecular beams of macroions. *The Journal of chemical physics*, *49(5)*, 2240-2249.

Doshi, J., & Reneker, D. H. (1995). Electrospinning process and applications of electrospun fibers. *Journal of electrostatics*, *35(2-3)*, 151-160.

Drozin, V. G. (1955). The electrical dispersion of liquids as aerosols. *Journal of colloid science*, *10(2)*, 158-164.

Fadil, F., Affandi, N. D. N., Misnon, M. I., Bonnia, N. N., Harun, A. M., & Alam, M. K. (2021). Review on electrospun nanofiber-applied products. *Polymers*, *13(13)*, 2087.

Fisher, A. C., De Cossart, L., How, T. V., & Annis, D. (1985). Long term in-vivo performance of an electrostatically-spun small bore arterial prosthesis: the contribution of mechanical compliance and anti-platelet therapy. *Life support systems: the journal of the European Society for Artificial Organs*, *3*, 462-465.

Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, *40(16)*, 4585-4592.

Formhals, A. (1934). *Process and apparatus for preparing artificial threads*. U.S. Patent 1,975,594.

Formhals, A. (1939). *Method and apparatus for spinning*. U.S. Patent 2,169,962.

Formhals, A. (1940). *Artificial thread and method of producing same*. U.S. Patent 2,187,306.

Frs, L. R. (1882). On the equilibrium of liquid conducting masses charged with electricity', Lond. *Edinb. Dublin Philos. Mag. J. Sci*, *14*, 87.

Girao, A. F., Semitela, A., Ramalho, G., Completo, A., & Marques, P. A. (2018). Mimicking nature: fabrication of 3D anisotropic electrospun polycaprolactone scaffolds for cartilage tissue engineering applications. *Composites Part B: Engineering*, *154*, 99-107.

Huang, L., Nagapudi, K., P. Apkarian, R., & Chaikof, E. L. (2001). Engineered collagen–PEO nanofibers and fabrics. *Journal of biomaterials science, Polymer edition*, *12(9)*, 979-993.

Huang, Z. M., Zhang, Y. Z., Ramakrishna, S., & Lim, C. T. (2004). Electrospinning and mechanical characterization of gelatin nanofibers. *Polymer*, *45(15)*, 5361-5368.

Hsu, C. M., & Shivkumar, S. (2004). Nano-sized beads and porous fiber constructs of poly (ε-caprolactone) produced by electrospinning. *Journal of Materials Science*, *39(9)*, 3003-3013.

Hwang, W. S., Li, B., Jin, L. H., Ngo, K., Schachar, N. S., & Hughes, G. N. F. (1992). Collagen fibril structure of normal, aging, and osteoarthritic cartilage. *The Journal of pathology*, *167(4)*, 425-433.

Jin, G., He, R., Sha, B., Li, W., Qing, H., Teng, R., & Xu, F. (2018). Electrospun three-dimensional aligned nanofibrous scaffolds for tissue engineering. *Materials Science and Engineering: C*, *92*, 995-1005.

Khoo, W., Chung, S. M., Lim, S. C., Low, C. Y., Shapiro, J. M., & Koh, C. T. (2019). Fracture behavior of multilayer fibrous scaffolds featuring microstructural gradients. *Materials & Design*, 108184.

Khoo, W., & Koh, C. T. (2016). A review of electrospinning process and microstructure morphology control. *ARPN Journal of Engineering and Applied Sciences*, *11(12)*, 7774-7781.

Ki, C. S., Baek, D. H., Gang, K. D., Lee, K. H., Um, I. C., & Park, Y. H. (2005). Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. *Polymer*, *46(14)*, 5094-5102.

Kim, B., Park, H., Lee, S. H., & Sigmund, W. M. (2005). Poly (acrylic acid) nanofibers by electrospinning. *Materials letters*, *59(7)*, 829-832.

Larrondo, L., & St. John Manley, R. (1981). Electrostatic fiber spinning from polymer melts. I. Experimental observations on fiber formation and properties. *Journal of Polymer Science: Polymer Physics Edition*, *19(6)*, 909-920.

Larrondo, L., & St. John Manley, R. (1981). Electrostatic fiber spinning from polymer melts. II. Examination of the flow field in an electrically driven jet. *Journal of Polymer Science: Polymer Physics Edition*, *19(6)*, 921-932.

Leong, K. F., Chua, S. C., Sudarmadji, N., & Yeong, W. Y. (2008). Engineering functionally graded tissue engineering scaffolds. *Journal of the mechanical behavior of biomedical materials*, *1(2)*, 140-152.

Li, H., & Yang, W. (2016). Electrospinning technology in non-woven fabric manufacturing. *Non-woven fabrics*, IntechOpen. 33.

Luzio, A., Canesi, E. V., Bertarelli, C., & Caironi, M. (2014). Electrospun polymer fibers for electronic applications. *Materials*, *7(2)*, 906-947.

Mazoochi, T., Hamadanian, M., Ahmadi, M., & Jabbari, V. (2012). Investigation on the morphological characteristics of nanofiberous membrane as electrospun in the different processing parameters. *International Journal of Industrial Chemistry*, *3(1)*, 1-8.

Megelski, S., Stephens, J. S., Chase, D. B., & Rabolt, J. F. (2002). Micro-and nanostructured surface morphology on electrospun polymer fibers. *Macromolecules*, *35(22)*, 8456-8466.

Mit-uppatham, C., Nithitanakul, M., & Supaphol, P. (2004). Ultrafine electrospun polyamide-6 fibers: effect of solution conditions on morphology and average fiber diameter. *Macromolecular Chemistry and Physics*, *205(17)*, 2327-2338.

Moghe, A. K., Hufenus, R., Hudson, S. M., & Gupta, B. S. (2009). Effect of the addition of a fugitive salt on electrospinnability of poly (ε-caprolactone). *Polymer*, *50(14)*, 3311-3318.
Mow, V. C., Ratcliffe, A., & Poole, A. R. (1992). Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials*, 13(2), 67-97.

Muerza-Cascante, M. L., Shokoohmand, A., Khosrotehrani, K., Haylock, D., Dalton, P. D., Hutmacher, D. W., & Loessner, D. (2017). Endosteal-like extracellular matrix expression on melt electrospun written scaffolds. *Acta biomaterialia*, *52*, 145-158.

Nemati, S., Kim, S. J., Shin, Y. M., & Shin, H. (2019). Current progress in application of polymeric nanofibers to tissue engineering. *Nano convergence*, *6(1)*, 1-16.

O'brien, F. J. (2011). Biomaterials & scaffolds for tissue engineering. *Materials today*, *14(3)*, 88-95.

Patel, S., Kurpinski, K., Wong, Y. C. (2014). *Multilayer fibrous polymer scaffolds, methods of production and methods of use*. U.S. Patent 8,852,621.

Pham, Q. P., Sharma, U., & Mikos, A. G. (2006a). Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue engineering*, *12(5)*, 1197-1211.

Ramakrishnan, R., Ramakrishnan, P., Ranganathan, B., Tan, C., Sridhar, T. M., & Gimbun, J. (2019). Effect of humidity on formation of electrospun polycaprolactone nanofiber embedded with curcumin using needdleless electrospinning. *Materials Today: Proceedings*, *19*, 1241-1246.

Rasel, S. M. (2015). *An advanced electrospinning method of fabricating nanofibrous patterned architectures with controlled deposition and desired alignment*. University of Ontario Institute of Technology (Canada).

Shabafrooz, V., Mozafari, M., Vashaee, D., & Tayebi, L. (2014). Electrospun nanofibers: from filtration membranes to highly specialized tissue engineering scaffolds. *Journal of nanoscience and nanotechnology*, *14(1)*, 522-534.

Sikorska, E., Gac, J. M., & Gradoń, L. (2018). Performance of a depth fibrous filter at particulate loading conditions. Description of temporary and local phenomena with structure development. *Chemical Engineering Research and Design*, *132*, 743-750.

Sill, T. J., & Von Recum, H. A. (2008). Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials*, *29(13)*, 1989-2006.

Son, W. K., Youk, J. H., Lee, T. S., & Park, W. H. (2004). The effects of solution properties and polyelectrolyte on electrospinning of ultrafine poly (ethylene oxide) fibers. *polymer*, *45(9)*, 2959-2966.

Taylor, G. I. (1969). Electrically driven jets. *Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences*, *313(1515)*, 453-475.

Theocharis, A. D., Skandalis, S. S., Gialeli, C., & Karamanos, N. K. (2016). Extracellular matrix structure. *Advanced drug delivery reviews*, *97*, 4-27.

Topuz, F., Abdulhamid, M. A., Holtzl, T., & Szekely, G. (2021). Nanofiber engineering of microporous polyimides through electrospinning: Influence of electrospinning parameters and salt addition. *Materials & Design*, *198*, 109280.

Van der Schueren, L., De Schoenmaker, B., Kalaoglu, Ö. I., & De Clerck, K. (2011). An alternative solvent system for the steady state electrospinning of polycaprolactone. *European Polymer Journal*, *47(6)*, 1256-1263.

Wang, L., & Ryan, A. J. (2011). Introduction to electrospinning. In *Electrospinning for tissue regeneration*. Woodhead Publishing. pp. 33.

Woodfield, T. B., Malda, J., De Wijn, J., Peters, F., Riesle, J., & van Blitterswijk, C. A. (2004). Design of porous scaffolds for cartilage tissue engineering using a three-dimensional fiber-deposition technique. *Biomaterials*, *25(18)*, 4149-4161.

Wu, D., Wang, H., & Cao, J. (2012). *Electrospinning membrane machine in warp and weft directions and application process thereof*. EP2447396A1.
Zhang, X. Y., Fang, G., Xing, L. L., Liu, W., & Zhou, J. (2018). Effect of porosity variation strategy on the performance of functionally graded Ti-6Al-4V scaffolds for bone tissue engineering. *Materials & Design*, *157*, 523-538.

Zeng, J., Haoqing, H., Schaper, A., Wendorff, J. H., & Greiner, A. (2003). Poly-L-lactide nanofibers by electrospinning–Influence of solution viscosity and electrical conductivity on fiber diameter and fiber morphology. *e-Polymers*, *3(1)*.

Zhang, Y., Ouyang, H., Lim, C. T., Ramakrishna, S., & Huang, Z. M. (2005). Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *72(1)*, 156-165.

Zong, X., Kim, K., Fang, D., Ran, S., Hsiao, B. S., & Chu, B. (2002). Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *polymer*, *43(16)*, 4403-4412.

Zou, B., Liu, Y., Luo, X., Chen, F., Guo, X., & Li, X. (2012). Electrospun fibrous scaffolds with continuous gradations in mineral contents and biological cues for manipulating cellular behaviors. *Acta biomaterialia*, *8(4)*, 1576-1585.

Zuo, W., Zhu, M., Yang, W., Yu, H., Chen, Y., & Zhang, Y. (2005). Experimental study on relationship between jet instability and formation of beaded fibers during electrospinning. *Polymer Engineering & Science*, *45(5)*, 704-709.