

**ELECTROSPUN POLY (ϵ -CAPROLACTONE) NANOFIBER MEMBRANES
BLENDED WITH NATURAL ANTIBACTERIAL AGENTS FOR POTENTIAL
WOUND DRESSING APPLICATIONS**

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Choose an item.

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DEDICATION

This thesis is dedicated to my late father, Alhaji Adamu DanMalam, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my late mother, Malama A'isha Muhammad who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

The present study aims to produce natural drug-loaded poly(ϵ -caprolactone) (PCL) nanofibrous membranes as potential wound dressing material using the electrospinning technique. The production of continuous jets that yield beads-free, smooth, and uniform fibers is an indication of good nanofiber morphology. Poly(ϵ -caprolactone)/eggshell membrane (PCL/ESM), poly(ϵ -caprolactone)/eggshell (PCL/ES), poly(ϵ -caprolactone)/curcumin (PCL/Cur), and poly(ϵ -caprolactone)/ ϵ -poly-L-lysine (PCL/ ϵ -PL) nanofiber membranes of various concentrations have been successfully fabricated. Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), tensile strength test (TT), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and water contact angle measurement (WCA) were used to investigate the physical, chemical, and mechanical properties of each of the nanofiber membranes. Similarly, the membranes were evaluated for their water uptake ability, degradability, water vapour transmission rate, water retention capacity, cell viability, and antibacterial activity. The FESEM results of the membranes revealed bead less and uniform fibers, suggesting good dispersion of the active agents. The various formulations have confirmed molecular interactions via a change in peak intensities, shifting of peak positions, and the appearance of the additional peak as demonstrated by the ATR-FTIR and XRD analyses. The membrane incorporated with 6% ES showed the highest tensile strength of 4.85 ± 0.63 MPa than the other active agents. The WCA of various formulations decreased with the addition of active agents such as PCL/ES 6% nanofiber membrane demonstrated the least WCA of $99.98 \pm 2.70^\circ$. The results of the water uptake ability of the various membranes indicated a decrease according to their active agents and their concentrations with PCL/ ϵ -PL 3.5% demonstrating the highest water uptake of 429% after 72 h due to the presence of hydrophilic functional groups. The degradation process of the membranes occurred mainly on the membrane's surface and the highest weight loss was exhibited after 14 days by the degradation of PCL/ ϵ -PL 3.5%. Overall porosity is $79.79 \pm 2.47\%$, as indicated by PCL/ ϵ -PL 1.5%, while PCL revealed the lowest value, which is $37.85 \pm 7.41\%$ due to the material's strong hydrophobicity. The highest antibacterial activity was shown by the membranes with the highest concentration in the various formulations, in the following order: PCL/ESM 2.5% < PCL/Cur 18% < PCL/ES 2% < PCL/ ϵ -PL 3.5%. The results have shown that PCL/ESM 2.5%, PCL/Cur 18%, PCL/ES 2%, and PCL/ ϵ -PL 3.5% can be used as potential wound dressing materials, with PCL/ ϵ -PL 3.5% nanofiber membranes demonstrating the most favourable performance. This is based on the physicochemical, mechanical, and biochemical properties of the electrospun nanofiber membranes. The L929 fibroblast cells did not exhibit any cytotoxic effects and had the maximum antibacterial activity above 99% against bacterial strains, with cell viability values of $92.42 \pm 1.63\%$ and $81.57 \pm 0.52\%$ after 24 h and 74 h of incubation, respectively. According to the study, the PCL/ESM 2.5%, PCL/Cur 18%, PCL/ES 2%, and PCL/ ϵ -PL 3.5% membranes can be possible candidates for potential wound dressing applications.

ABSTRAK

Kajian ini bertujuan untuk menghasilkan membran nanofiber poli(ϵ -kaprolakton) (PCL) yang dimuatkan dadah semula jadi sebagai bahan pembalut luka yang berpotensi menggunakan teknik pemutaran elektro. Penghasilan jet berterusan yang menghasilkan gentian bebas manik, licin dan seragam adalah petunjuk morfologi gentian nano yang baik. Membran nanofiber poli(ϵ -kaprolakton)/membran kulit telur (PCL/ESM), poli(ϵ -kaprolakton)/kulit telur (PCL/ES), poli(ϵ -kaprolakton)/kunyit (PCL/Cur), dan poli(ϵ -kaprolakton)/ ϵ -poli-L-lisin (PCL/ ϵ -PL) pelbagai kepekatan telah berjaya direka. Spektroskopi inframerah transformasi fourier (FTIR), mikroskopi elektron pengimbasan pelepasan medan (FESEM), ujian kekuatan tegangan (TT), pembelauan sinar-X (XRD), spektroskopi fotoelektron sinar-X (XPS), dan pengukuran sudut sentuhan air (WCA) telah digunakan untuk mengkaji sifat fizikal, kimia dan mekanikal bagi setiap membran nanofiber. Begitu juga, membran dinilai untuk keupayaan pengambilan air, kebolehan terdegradasi, kadar penularan wap air, kapasiti pengekal air, daya maju sel, dan aktiviti antibakteria. Keputusan FESEM membran mendedahkan gentian tidak manik dan seragam, mencadangkan penyebaran agen aktif yang baik. Pelbagai formulasi telah mengesahkan interaksi molekul melalui perubahan dalam intensiti puncak, peralihan kedudukan puncak, dan penampilan puncak tambahan seperti yang ditunjukkan oleh analisis ATR-FTIR dan XRD. Membran yang digabungkan dengan 6% ES menunjukkan kekuatan tegangan tertinggi iaitu 4.85 ± 0.63 MPa berbanding agen aktif yang lain. WCA dari pelbagai formulasi berkurangan dengan penambahan agen aktif sehingga membran nanofiber PCL/ES 6% menunjukkan WCA paling sedikit sebanyak $99.98 \pm 2.70^\circ$. Keputusan keupayaan pengambilan air pelbagai membran menunjukkan penurunan mengikut agen aktif dan kepekatan dengan PCL/ ϵ -PL 3.5% menunjukkan pengambilan air tertinggi sebanyak 429% selepas 72 jam, disebabkan kehadiran kumpulan berfungsi hidrofilik. Proses degradasi membran berlaku terutamanya pada permukaan membran dan penurunan berat tertinggi ditunjukkan selepas 14 hari oleh degradasi PCL/ ϵ -PL 3.5%. Keliangan keseluruhan ialah $79.79 \pm 2.47\%$, seperti yang ditunjukkan oleh PCL/ ϵ -PL 1.5%, manakala PCL mendedahkan nilai terendah iaitu $37.85 \pm 7.41\%$ disebabkan oleh hidrofobisiti bahan yang kuat. Aktiviti antibakteria tertinggi ditunjukkan oleh membran dengan kepekatan tertinggi dalam pelbagai formulasi dalam susunan berikut: PCL/ESM 2.5% < PCL/Cur 18% < PCL/ES 2% < PCL/ ϵ -PL 3.5%. Keputusan telah menunjukkan bahawa PCL/ESM 2.5%, PCL/Cur 18%, PCL/ES 2%, dan PCL/ ϵ -PL 3.5% boleh digunakan sebagai bahan pembalut luka yang berpotensi, dengan membran nanofiber PCL/ ϵ -PL 3.5% menunjukkan prestasi yang paling menggalakkan. Ini berdasarkan sifat fizikokimia, mekanikal dan biokimia membran nanofiber elektroputaran. Sel fibroblast L929 tidak menunjukkan sebarang kesan sitotoksik dan mempunyai aktiviti antibakteria maksimum melebihi 99% terhadap strain bakteria, dengan nilai daya maju sel masing-masing 92.42 ± 1.63 dan $81.57 \pm 0.52\%$ selepas 24 jam dan 74 jam pengeraman. Menurut kajian itu, membran PCL/ESM 2.5%, PCL/Cur 18%, PCL/ES 2%, dan PCL/ ϵ -PL 3.5% boleh menjadi calon untuk aplikasi pembalut luka yang berpotensi.

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LIST OF ABBREVIATIONS

ϵ -PL	-	ϵ -poly-L-lysine
CA	-	cellulose acetate
Cur	-	Curcumin
CS	-	Chitosan
DMAc	-	Dimethylacetamide
DSC	-	Differential scanning calorimetry
ES	-	Eggshell
ESM	-	Eggshell membrane
EMNM	-	eggshell membrane-based nano wrinkled microfibers
FESEM	-	field emission scanning electron microscopy
FTIR-ATR	-	Fourier transforms infrared spectroscopy fitted with attenuated total reflectance
NaCl		Sodium Chloride
PCL		Poly(ϵ -caprolactone)
PVDF		polyvinylidene difluoride
PVC		polyvinyl chloride
PVA		polyvinyl alcohol
PVAc		polyvinyl acetate
PVDF		polyvinylidene difluoride
PAN		polyacrylonitrile
PEO		polyethylene oxide
PLA		poly (lactic acid)
XPS		X-ray photoelectrons spectroscopy
XRD		X-ray diffraction analysis
WCA		water contact angle measurement

LIST OF SYMBOLS

°C	-	Degree Celsius
cm	-	Centimeter
g/mol	-	Gram per mol
kV	-	Kilovolt
K Da	-	Kilo Dalton
mL/h	-	Milliliter per hour
Mw	-	Molecular weight
nm	-	Nanometer

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

The human skin has been one of the body's essential organs, which comprises three primary layers: the stratified epidermis, the dermis, and the hypodermis (Borena et al., 2015). It separates the human body from the environment, provides the body with some basic protections against microbial infections, and performs additional functions, including metabolic, immune, and biochemical. However, when a skin sustained acute or chronic injuries, the human body immediately begins repairing the affected portion of the skin (Akbik et al., 2014) by adopting the following steps: hemostasis, inflammation, proliferation, and remodeling of the tissue (Harper, Young, and McNaught, 2014; Liu et al., 2018). Nonetheless, when wounds receive inadequate treatment, they can be susceptible to high population of bacteria that results in pathogenic infections, thereby making the wounds seems chronic. So, it became imperative to develop ideal wound-dressing materials with potent antibacterial property to protect wounds from bacterial infiltration, prevent pathogenic infection, outstanding biocompatibility and biodegradability, excellent mechanical strength with enhance flexibility, good water retention capacity, and induce cell attachment, migration, and proliferation to enhance the reconstruction of the affected tissue (Dhivya et al., 2015; Perumal et al., 2017).

There is quite a plethora of various wound dressing materials available in markets currently used. These include among others, polymeric films (Tegaderm, Silon-TSR), polymeric forms (Flexzan, Biopatch), polymeric hydrogels (Cultinova gel, Biolex), polymeric alginates (AlgiSite, Kaltostat), and polymeric hydrocoloides (Iodosorb, Debrisan) (Kamoun et al., 2017). All the current wound dressing materials have some inherent limitations associated with wound healing.

Over time, various techniques have been reported as being utilized for producing polymeric nanofiber. These include, among others, solvent casting (Kamel et al., 2018), solvent-casting/particulate-leaching technique combined with thermally induced phase separation (Kucinska-Lipka et al., 2019), electron beam irradiation technique (Jawun et al., 2017), pulsed laser deposition technique (Li et al., 2016), freeze-drying technique (Mehrabani et al., 2018), freeze-thawing method (Khorasani et al., 2018), and electrospinning technique (Kamel et al., 2018). Among these methods, electrospinning technique has recently emerged as the technique widely used in fabricating nanofibers from a variety of materials, including polymers, composite structures, and inorganic materials. This could have been because the technique is used in producing continuous polymer fibers with dimensional control within the nanometer scale, variety of polymers are used in the formation of the electrospun nanofiber membranes, it allows the nanofiber formation in different orientations, as well as, it produces the nanofibers in large quantities (Quirós, Boltes, and Rosal, 2016).

There are currently many studies of electrospun nanofibrous membranes using various carriers, which included natural or synthetic polymers, such as polyvinyl alcohol, poly- ϵ -caprolactone, polyurethane, polylactic acid, poly-ethyl oxide as well as hybrid blends of two polymers. Poly(ϵ -caprolactone) (PCL) has been widely used by researchers due to its excellent biocompatibility, sustained biodegradability, processability with different polymers, and enhanced mechanical properties (Liao et al., 2015). The continued prominence of the PCL due to its potential applications in biomedical and wound dressing applications has been widely investigated (Bartnikowski et al., 2019). Natural antibacterial agents, Eggshell membrane (ESM), Eggshell (ES), Curcumin (Cur), and ϵ -poly-L-lysine (ϵ -PL) electrospun nanofibrous membranes have also been widely used in biomedical and wound dressing applications due to their high antibacterial efficiency (Alibolandi et al., 2017; Li et al., 2016; Ushimaru et al., 2017). Nonetheless, PCL/ESM (0.5%, 1.5%, 2.5%), PCL/ES (2%, 4%, 6%), PCL/Cur (12%, 15%, 18%), and PCL/ ϵ -PL (1.5%, 2.5%, 3.5%) nanofiber membranes were not yet found in literature. Therefore, this study aims to fabricate PCL, PCL/ESM (0.5%, 1.5%, 2.5%), PCL/ES (2%, 4%, 6%), PCL/Cur (12%, 15%, 18%), and PCL/ ϵ -PL (1.5%, 2.5%, 3.5%) nanofiber membranes using the electrospinning technique, characterize, and evaluate their antibacterial efficacy and biocompatibility to determine their potentials for wound dressing applications.

1.2 Problem Statement

Chronic wounds are vulnerable to the risk of infections by a complex population of bacteria, most especially if proper care is not given to the wounds. The longer the wound receives insufficient medical attention, the more likely, it will be colonized by microbial organisms whose sources may be from the surrounding skin, the environment, or endogenous origin (Khalifa, 2016). Hence, tissue inflammation in chronic wounds can further be prolonged due to the immune response and the presence of bacteria, thereby delaying the healing process. Therefore, the pains due to the chronic wound makes the life of the patients unbearable (Simões et al., 2018

Nowadays, there are various wound dressing materials currently available in markets. For instance, the use of polymeric films has been identified as a means of treating wounds in our various hospitals. However, the dressings have some limitations which include adherence to wound bend, and poor exudates absorption. Similarly, polymeric foams are known for their poor stability, semi-permeability to gases, and its adherence to wounds. Polymeric hydrogels are also known for been poor in protecting bacterial infiltration and sometimes poor mechanical stability. Other dressings applicable in the therapeutic approaches are polymeric alginates and polymeric hydrocolloids. Nevertheless, the risks associated with these approaches includes very costly, have unpleasant odor, impermeable to gases and have poor exudates absorption (Kamoun et al., 2017).

To overcome the aforementioned limitations, the use of naturally based antibacterial agents such as ESM, ES, Cur, and ϵ -PL of particular concentrations will not only prevent pathogenic infections but will also safely promotes rapid wound healing mechanisms.

1.3 Research Goal

The research goal was to produce PCL nanofiber membranes blended with natural antibacterial agents (ESM, ES, Cur, and ϵ -PL), for potential wound dressing applications, using the electrospinning technique.

1.3.1 Research Objectives

The objectives of the research are:

- (a) To synthesize natural antibacterial agents loaded PCL nanofiber membranes.
- (b) To investigate the physical, chemical and mechanical properties of the electrospun nanofiber membranes.
- (c) To determine water uptake capacity, degradation ability, water vapour transmission rate, and water retention capacity of the nanofiber membranes.
- (d) To evaluate the cells viability and antibacterial efficiency of the nanofiber membranes using in vitro approach.

1.4 Scope of The Study

To achieve the research objectives, the following scope was considered: The first stage involved the fabrication of the nanofiber membranes. The fabrication of PCL/ESM and the nanofiber blends of other antibacterial agents were prepared using the electrospinning technique. The various concentrations for the polymer and the antibacterial agents were carefully selected. The antibacterial agents used to blend the PCL at different concentrations are ESM, ES, Cur, and ϵ -PL. The various

electrospinning parameters, including the solution, process, and ambient, were adequately controlled to fabricate the nanofiber membranes.

The second stage of the research was the characterization of the different fabricated nanofiber membranes. The nanofibers were characterized by using Field emission scanning electron microscopy (FESEM), Fourier transforms infrared spectroscopy fitted with Attenuated total reflectance (FTIR-ATR), Mechanical test, Differential scanning calorimetry (DSC), X-ray diffraction analysis (XRD), X-ray photoelectrons spectroscopy (XPS), and Water contact angle measurement (WCA). The characterization of the nanofiber membranes seeks to determine the physical, chemical, and mechanical properties of the membranes and their interactions with water.

The third stage involves the in vitro water uptake study, degradability study, water vapour transmission rate study, and porosity measurement. In this case, the water uptake study was performed to determine the water uptake ability of the nanofibrous membranes. At the same time, the degradability analysis was carried out to determine the degree of degradation of the membranes. Similarly, the water vapour transmission rate measures the degree of transmission of water vapour through the membranes, and porosity was also measured to determine the water retention capacity of the as-spun nanofibrous membranes.

The fourth stage involves the cell viability and antibacterial activity analyses. In this case, the in vitro cell viability study was performed on the electrospun nanofiber membranes to determine the cells viability nature of the membranes using L929 mouse fibroblast cells. The percentage cell viability was used to demonstrate the degree of cells viability of the membranes. The antibacterial activity test is the analysis carried out against bacterial strains. The Colony count measurement was used as a test for antibacterial activity. The bacterial strains that were used are the gram-positive ATCC 6538 *Staphylococcus aureus* (*S. aureus*) and the gram-negative ATCC 11229 *Escherichia coli* (*E. coli*). Percent Reduction was used to determine the antibacterial activity for the various membranes. The flow chart of the research is presented in Fig. 1.1.

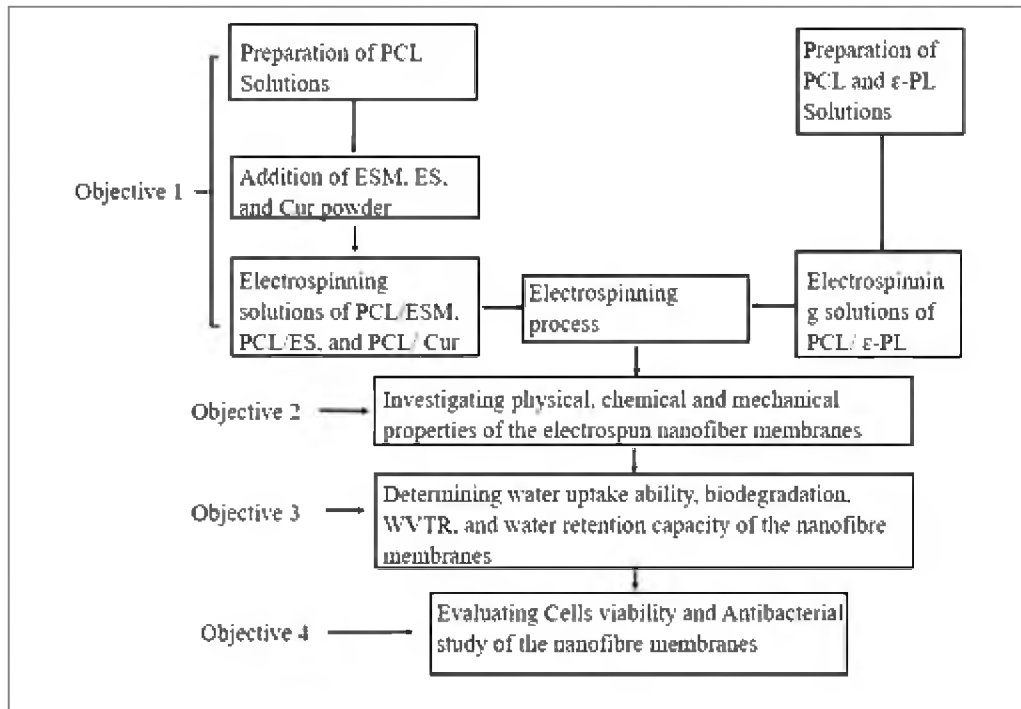


Figure 1.1 Flow chart of the research

1.5 Significance of The Study

The overuse of antibiotics and metallic nanoparticles in the production of nanofibers, which most often leads to the formation of antibiotic-resistant bacteria (Super bacteria) and the risk of contracting pulmonary and heart diseases, will be significantly reduced by the use of natural antibacterial agents for wound dressing application (Grigore et al., 2017).

Furthermore, the community, particularly those with chronic wounds, will benefit from the innovative membranes because of their exceptional capacity to ease their pain by fostering speedy wound healing. If successfully produced, using the electrospinning technique, the blended ESM-loaded PCL and the PCL/ES nanofiber membranes will provide a complete view on the possibility of converting waste derived from eggs into functional biomedical materials to be used for wound dressing

applications. Therefore, our environment will become friendlier by converting large quantity of egg wastes into biomedical materials for wound dressing applications.

1.6 Limitations of The Study

Despite the wide scope of this research, however, the study was bedeviled with some cogent limitations as outlined as follows:

1. The antibacterial property of wound dressing material has been one of the essential requirements for wound healing. However, this research was limited to the use of natural antibacterial agents.
2. In this research, the nanofibrous membranes' fabrication would have employed both the uniaxial and coaxial electrospinning techniques. However, the research was limited to using the uniaxial electrospinning technique due to its simplicity, versatility, and cost-effectiveness.
3. The research was also limited to developing wound dressing devices that can be proved to exhibit cytocompatibility and antibacterial activity via in vitro analysis.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Bello, M.**, Abdullah, F., Yusoff, A. R. M., Mahmood, M. A. W., Yong Chee, T., Malek, N. A. N. N., Jemon, K., & Sathishkumar, P. (2022). Curcumin-loaded electrospun poly (ϵ - caprolactone) nanofibrous membrane: an efficient and biocompatible wound- dressing material. *Material Letters*,315, <http://doi.org/10.1016/j.matlet.2022.131910>. (Q2, IF: 3.423)
2. **Bello, M.**, Abdullah, F., Yusoff, A. R. M., Mahmood, M. A. W., Yong Chee, T., Malek, N. A. N. N., Jemon, K., Siddiquee, S., K., & Sathishkumar, P. (2022). Electrospun poly (ϵ - caprolactone)-eggshell membrane nanofibrous mat as a wound- dressing material. *Biochemical Engineering Journal*, <http://doi.org/10.1016/j.bej.2022.108563>. (Q2, IF: 4.447)

Non-Indexed Conference Proceedings

1. **Bello, M.**, Yusoff, A. R. M., Malek, N. A. N. N., Jemon, K., Abdullah, F., Yong Chee, T., & Pui Pui, W. (2020). Fabrication of Polymeric Nanofibers of Poly(ϵ - caprolactone) blended with Curcumin using Electrospinning Technique. In 2020 IGCESH 8th International Graduate Conference on Engineering, Science and Humanities (pp. 273-276)