VITAMIN D LOADED ELECTROSPUN CELLULOSE ACETATE/POLYCAPROLACTONE NANOFIBERS FOR DRUG DELIVERY APPLICATIONS

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DEDICATION

This thesis is dedicated to my father, 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 mother, 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

Vitamin D is a steroid hormone that plays a crucial role in regulating physiological functions in the human body. Its supplements have been used to prevent and treat vitamin D deficiency. Although many approaches are currently available for delivering vitamin D, the low bioavailability and loss of bioactivity of vitamin D remains a challenging task. Therefore, this study aims to introduce a new implantable drug delivery system (IDDS) for delivering vitamin D. An IDDS offers many advantages over other routes of drug administration due to direct delivery into the body. The IDDS was developed from the electrospun cellulose acetate (CA) and polycaprolactone (PCL) nanofibrous membrane, in which the core of the IDDS consisted of vitamin D_3-loaded CA nanofiber (CAVD) and was enclosed in a thin layer of the sintered PCL membrane (CAVD/PCL). The morphological surface and physicochemical properties of the produced electrospun nanofiber of the vitamin D_3-loaded CA and PCL membranes were characterized using a scanning electron microscope (SEM), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). The vitamin D loading efficiency and vitamin D stability were characterized by high-performance liquid chromatography (HPLC) and UV-Visible spectroscopy. Mechanical properties, drug release studies and *in vitro* cytotoxicity studies were also performed in this study. Vitamin D_3 in three different concentrations, including 6, 12, and 20% (w/w) based on the weight of the CA polymer, was efficiently loaded into the CA nanofibrous membrane using electrospinning. The surface morphologies of CA nanofiber and vitamin D_3-loaded CA nanofiber were smooth and bead-free, while their average diameters increased from 324 nm to 428 nm when the weight ratios of vitamin D_3 were increased. The results from HPLC and UV spectra showed that the vitamin D_3 compound in CA nanofiber has a stable structure and did not degrade during electrospinning incorporation. The drug release study and tensile testing showed that the PCL membrane as the outer layer around the core's implants plays a crucial role in the improved mechanical properties and kinetic drug release. The Young modulus and tensile strength of CAVD/PCL were significantly increased as compared to CAVD. The kinetic drug release of CAVD followed the first-order model and converted to the zero-order model in the CAVD/PCL at the first stage of the drug release. The CA and PCL nanofibers are non-cytotoxic based on the results of *in vitro* cytotoxicity studies. In conclusion, based on the outcomes and methods outlined in the present study, the vitamin D-loaded CA nanofibrous membrane and the PCL nanofibrous membrane are suitable for developing an IDDS for delivery of vitamin D.

ABSTRAK

Vitamin D adalah hormon steroid yang memainkan peranan penting dalam mengatur fungsi fisiologi dalam tubuh manusia. Pengambilan vitamin D sebagai sumber makanan tambahan boleh mencegah kekurangan vitamin D. Walaupun banyak pendekatan yang ada setakat ini untuk menyampaikan vitamin D, bioketersediaan yang rendah dan kehilangan bioaktiviti vitamin D tetap menjadi isu utama. Oleh itu, kajian ini bertujuan untuk memperkenalkan sistem penyampaian ubat implan yang baharu (IDDS) untuk penyampaian vitamin D. Kaedah baru (IDDS) mempunyai banyak kelebihan berbanding kaedah penyampaian ubatan yang lain kerana penyampaiannya adalah secara langsung ke dalam badan. IDDS dihasilkan daripada membran serat nano elektrospun selulosa asetat (CA) dan polikaprolakton (PCL), di mana teras IDDS terdiri daripada serat nano CA (CAVD) yang dimuatkan vitamin D_3 dan dilapisi lapisan nipis membran PCL yang disinter (CAVD/PCL). Membran serat nano PCL dijadikan kapsul pada teras melalui proses sintering termal. Pencirian morfologi permukaan dan sifat fisikokimia membran CA dan PCL yang dimuatkan vitamin D_3 telah di analisa menggunakan mikroskop elektron imbasan (SEM), spektroskopi inframerah Fourier transformasi (ATR-FTIR), difraksi sinar-X (XRD) dan kalorimetri pengimbasan pembezaan (DSC). Tahap keefisienan dan kestabilan pemuatan ubat telah dianalisis menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC) dan spektroskopi UV. Analisis mekanikal, kajian pelepasan ubat, dan kajian kesitotoksikan *in vitro* juga dilakukan. Vitamin D_3 dalam tiga kepekatan yang berbeza merangkumi 6, 12, dan 20% (b/b) berdasarkan berat polimer CA, telah dimasukkan ke dalam membran nanofiber CA melalui proses elektrospinning. Morfologi permukaan dari pempentukan CA dan serat nano CA yang dimuatkan dengan Vitamin D_3 adalah halus dan bebas manik, sementara purata diameter nanofiber meningkat dari 324 nm kepada 428 nm apabila nisbah berat vitamin D_3 meningkat. Keputusan spektrum HPLC dan UV menunjukkan sebatian vitamin D_3 dalam CA mempunyai struktur yang stabil dan tidak berkurangan semasa proses penggabungan. Kajian pelepasan ubat dan kajian tegangan menunjukkan bahawa membran PCL sebagai lapisan luar implan teras memainkan peranan penting dalam meningkatkan sifat mekanikal dan pelepasan kinetik. Modulus Young dan kekuatan tegangan CAVD/PCL didapati meningkat dengan ketara berbanding dengan CAVD. Pelepasan kinetik CAVD mengikuti model susunan pertama dan ditukarkan ke model susunan sifar bagi CAVD / PCL pada peringkat pertama pelepasan ubat. Serat nano CA dan PCL bersifat tidak toksik berdasarkan hasil kajian kesitotoksikan secara *in vitro*. Kesimpulannya, berdasarkan hasil dan kaedah yang digariskan dalam kajian ini, membran serat nano CA yang dimuatkan dengan vitamin D dan membran serat nano PCL sesuai untuk menghasilkan IDDS untuk penyampaian vitamin D.

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LIST OF ABBREVIATIONS

AC	-	Acetone
CA	-	Cellulose Acetate
CANF	-	Cellulose acetate nanofiber
CAVD	-	Cellulose acetate loaded with vitamin D
CAVD/PCL	-	Cellulose acetate loaded with vitamin D/ Polycaprolactone
DDS	-	Drug delivery system
DCM	-	Dichloromethane
DMAc	-	N,N-dimethylacetamide
DMEM		Dulbecco's Modified Eagle Medium
DMF	-	N,N-dimethylformamide
EtOH	-	Ethanol
FDA	-	Food and Drug Administration
FESEM	-	Field emission scanning electron microsco
HDF	-	Human dermal fibroblast
M.W	-	Molecular weight
MTS	-	(3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay
NF	-	Nanofiber
PBS	-	Phosphate buffered saline
PCL	-	Polycaprolactone
SEM	-	Scanning electron microscope
Vit.D_3	-	Vitamin D_3
VD	-	Vitamin D_3
DS	-	Degree of substitution
^{13}C NMR	-	Carbon-13 (C13) nuclear magnetic resonance
XRD	-	X-Ray Diffractometer
FT-IR	-	Fourier Transform Infrared Spectroscopy
UV	-	Ultraviolet

LIST OF SYMBOLS

°C	-	Degree Celsius
2Θ	-	Bragg angle
%	-	Percentage
T_v	-	Temperature of water evaporation
Tg	-	Glass transition temperature
Td	-	Thermal decomposition
Tm	-	Melting point
Et	-	Young's modulus
σm	-	Tensile strength
εb	-	Elongation at break
ŋ	-	Viscosity
ΔH_m	-	Melting enthalpy
ΔH_v	-	Enthalpy of water vaporization
λ	-	Wave length
nm	-	Nanometre
X_m	-	Degree of crystallinity
kV	-	Kilovolt
h	-	Hour
cP	-	Centipoise
µS	-	Micro Siemens
MPa	-	Mega Pascal
Sec	-	Second (s)
g	-	Gram
mg	-	Milligram
mL	-	Millilitre
L	-	Litter
µm	-	Micrometre
S^{-1}	-	1/second
cm	-	Centimetre (s)
d	-	Diameter

Min	-	Minute (s)
t	-	Time
v/v	-	Volume per volume
w/v	-	Weight per volume
w/w	-	Weight per weight
wt %	-	Weight percentage
log	-	logarithm
J/g	-	Joule per gram

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Drug delivery systems (DDSs) are defined as formulations or devices that have been used to introduce pharmaceutical agents into the human or animal body to achieve a desirable therapeutic effect (Jain, 2020). Moreover, drug delivery is a drug carrier for effective therapeutic delivery of drugs into the body via various administration routes, such as the implantable drug delivery system (IDDS), oral drug delivery, injectable drug delivery, pulmonary drug delivery, and nasal drug delivery (Kohrs et al., 2019). IDDS offer many advantages over other routes of drug delivery. Most significantly, it reduces the dosage of medication needed for treatment, and enhances the medication's bioavailability due to the drug being directly delivered to the site of action, avoiding first-pass metabolism (Stewart et al., 2020). It can also improve the efficacy and safety of an administered drug by controlling the release rate, protecting the drug level, and reaching the target site selectively while avoiding non-target cells (Pons-Faudoa et al., 2019).

A relatively new system known as the nano-based delivery system (NDDS) uses nanoscale biomaterials to deliver therapeutic agents to specific sites in a controlled manner (Patra et al., 2018). Nanofibers are nanoscale biomaterials that are generally electrospun from a variety of polymers via one of the most flexible techniques known as electrospinning (Shahriar et al., 2019). Electrospinning has become an exciting approach to developing NDDSs due to its increased surface-to-mass ratio, ease of operation, and cost-effectiveness (Iacob et al., 2020; Torres-Martínez et al., 2018). Also, drug-loaded electrospun-polymer nanofibers could overcome several key challenges, such as low solubility of drugs, loading efficiency, and irregular drug-release behavior (Wsoo et al., 2020).

Vitamin D_3 (cholecalciferol) is a secosteroid hormone that is crucial in regulating physiological functions in the human body (Grant, 2020). It plays a meaningful role in reducing the risk of Coronavirus disease 2019 (COVID-19) and other acute respiratory tract infections (Ali, 2020; Ilie et al., 2020). Vitamin D supplements are widely used as an exogenous candidate for treatment or prevention of vitamin D deficiency via either oral administration or injectable drug delivery (Glowka et al., 2019). Vitamin D deficiency is a worldwide health problem in all age groups, especially in those living in countries with inadequate exposure to sunlight (Palacios & Gonzalez, 2014). Despite the fact that many approaches are currently available for delivering vitamin D, the low bioavailability and loss of bioactivity of vitamin D due to taken via oral administration remains a challenging task. Little is known about using electrospun-polymer nanofibers for the treatment of vitamin D deficiency. Therefore, this study used electrospun-polymer nanofibers in formulating IDDS or long-term delivery of vitamin D_3 for the treatment of vitamin D deficiency.

Cellulose acetate (CA) and polycaprolactone (PCL) were used as carrier polymers to produce electrospun-polymer nanofibers. In the current study, CA was chosen owing to its high solubility in organic solvents, renewability, affordability, and ease of mass production (Mehrabi et al., 2017). It is also biocompatible, semi-biodegradable, non-irritating, and non-toxic (Dos Santos et al., 2021; Wsoo et al., 2020). Meanwhile, PCL is more applicable for long-term IDDS due to its slow biodegradation, and the US Food and Drug Administration has approved PCL's biocompatibility (Rezk et al., 2019). PCL could also be fabricated into nanofibers using electrospinning (Sattary et al., 2019), and modified to become a capsule by thermally sintering to improve its long-term tensile properties. This attribute is particularly advantageous for controlling drug releases due to the reduced electrospun pore size and porosity (Chaparro et al., 2019; Nelson et al., 2014). In this study, CA nanofiber loaded with vitamin D_3 was used to develop the core of a rod-shaped IDDS, while PCL polymer was used to produce a rate-limiting membrane around the core. Up to date, no studies have been conducted to investigate the possibility of electrospun polymer nanofiber to produce an IDDS. Furthermore, the use of IDDS for delivery of vitamin D via implanted in subcutaneous tissue is expected to overcome low bioavailability and bioactivity of vitamin D due to vitamin D directly delivered into the site of action.

1.2 Problem Statement

Vitamin D supplements are widely used as an exogenous candidate for treatment or prevention of vitamin D deficiency. However, the low bioavailability and loss of bioactivity of vitamin D remains a challenging task (Glowka et al., 2019). Drug bioavailability is the fraction of active drugs that reach the site of action. The absolute availability of a drug may be determined by comparing the respective areas under the plasma concentration curves after oral and intravenous administration (Bhosle et al., 2017).

Today, vitamin D is mostly taken via either oral administration (McCullough et al., 2019) or subcutaneous injection (Gupta et al., 2017). The oral route of vitamin D administration is problematic for some reasons. First, vitamin D reduces some bioactivity when it reaches the stomach because it is sensitive to gastric acid (Hasanvand et al., 2018). Second, it also has low intestinal drug absorption because vitamin D is a hydrophobic molecule with limited solubility in the aqueous environment (Glowka et al., 2019). The third one related to the oral administration of vitamin D is first-pass metabolism, which is the phenomenon that occurs when a medication is taken orally, reaches the liver, and undergoes substantial biotransformation, resulting in a significant reduction in bioavailability, and it is linked to low vitamin D bioavailability in the body (Maurya & Aggarwal, 2017). As a result, low bioavailability and loss of bioactivity of vitamin D are critical challenges for oral administration (Glowka et al., 2019).

Subcutaneous injections (SC) may avoid some of the problems related to oral administration, such as first-pass metabolism, gastric acid degradation, and low GIT absorption. This route, however, is not without its drawbacks. The problem with SC revolves around the drug's dosage and frequency of administration. Vitamin D toxicity would become a major concern if it is administered in high doses at a low frequency, whereas needle phobia and a reduction in the patient's compliance would be the main limitations if it is administered in low doses but at a high frequency. It has been reported that levels of vitamin D_3 fluctuated in serum after a high single dose injection (Einarsdóttir et al., 2010). Another danger of high-dose vitamin D injections is

intoxication, which occurs when levels of vitamin D_3 plasma exceed 100 ng/ml, or 250 nmol/L (Jones, 2008).

Therefore, this study aims to improve the bioavailability and bioactivity of vitamin D in the body by introducing a new IDDS. IDDS is an alternative system that can achieve a therapeutic effect with lower concentrations of drugs. As a result, it minimizes side-effects whilst increasing patient compliance (Stewart et al., 2018). On the other hand, IDDS can overcome the issues associated with oral and SC delivery by site-specific implantation. The sites of specific implantation can bypass first-pass metabolism; stomach degradation; or poor solubility and distribution phase of oral administration, resulting in higher drug concentrations in targeted areas (Pons-Faudoa et al., 2019).

Drug-loaded electrospun-polymer nanofibers could overcome several key challenges as well, such as low solubility of drugs, loading efficiency, and irregular drug-release behaviour (Wsoo et al., 2020). Based on the aforementioned information, a rod-shaped IDDS is an ideal idea to deliver vitamin D via subcutaneous tissue. It was produced from CA nanofiber loaded with vitamin D_3 and an electrospun-PCL-nanofibrous membrane. In which, the core implants consist of vitamin D-loaded CA nanofibrous membrane, while PCL polymer was used to produce a rate-limiting membrane around the core. In addition, the IDDS was formulated as rod-shaped because implantable biomaterials with rounded edges are easier to implant and more biocompatible than those with sharp edges when they react with host cells at the implant site. According to Ratner (2015), when the sharp edges of an implant come into contact with tissue, undesirable reactions may occur. A mechanical mismatch between a hard biomaterial and soft tissue may damage or irritate the soft tissue (Ratner, 2015).

Regulating long-term drug release, long-term biodegradation and biocompatibility, and suitable tensile properties of IDDS are all important aspects to consider when designing an IDDS. In this respect, both CA (Wsoo et al., 2020) and PCL (Chaparro et al., 2019) would be suitable biopolymers for producing electrospun

nanofiber. However, before moving forward to mass production, the following questions will first need to be answered.

1. What are suitable solvent systems and process parameters to produce electrospun-CA and electrospun-PCL nanofibers?
2. Is electrospinning a good technique for loading vitamin D into electrospun-polymer nanofibers?
3. To what extent can the release of vitamin D_3 from the IDDS be regulated?
4. Do CA and PCL biopolymers have good biocompatibility to formulate an IDDS?

1.3 Research Objectives

This study aimed to develop a rod-shaped IDDS from the electrospun-CA-and electrospun-PCL-nanofibrous membrane, and the core of the IDDS would consist of CA nanofiber loaded with vitamin D (CAVD) and enclosed in a thin layer of the PCL membrane (CAVD/PCL). Therefore, this study aimed to achieve the following objectives:

1. To optimize the solvent and process parameters for the fabrication process of electrospun-CA and electrospun-PCL nanofibers using electrospinning method.
2. To characterize morphological, structural, stability, and physicochemical properties of electrospun-CA nanofiber, vitamin-loaded CA nanofiber, and electrospun-PCL nanofibers.
3. To study the influence of the outer layer PCL on drug release kinetics.
4. To study *in-vitro* cell cytotoxicity and cell viability of electrospun polymer nanofibers against human dermal fibroblast cell lines.

1.4 Scope of Research

The scope of the first objective was to optimize electrospinning conditions by studying the effects of solvent systems and process parameters on the production of smooth and beadless CA-and PCL-nanofibers for effectively loading vitamin D into the electrospun-CA-nanofiber. The optimization included using mixing two solvents as binary solvent systems, such as acetone/dimethylacetamide (DMAc) and dichloromethane (DCM)/dimethylformamide (DMF) at different ratios, as well as a ternary solvent system consisting of acetone, ethanol, and DMAc. Likewise, the optimization of process parameters included the applied voltage, flow rate, and working distance. The morphological surface of the produced CA-and PCL-nanofibrous membrane was characterized by Scanning Electron Microscopy (SEM).

The second objective was defined with the following scope: loading vitamin D_3 into the CA nanofiber. Then, Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), X-ray diffraction (XRD), and Field Emission Scanning Electron Microscopy (FESEM) were used to characterize the produced vitamin D-loaded CA nanofibrous membrane and PCL nanofibrous membrane. Formulation of two samples of rod-shaped IDDS such as CAVD and CAVD/PCL from vitamin D-loaded CA nanofiber membrane and sintered PCL membrane.

The third scope was conducted to investigate the effect of the outer layer sintered PCL membrane on the *in-vitro* kinetic drug release from CAVD/PCL compared to CAVD. The Franz diffusion cell was used for drug release studies.

The final scope was conducted to investigate electrospun-CA and electrospun-PCL nanofiber biological properties using an *in-vitro* cytotoxicity and cell viability assay against HDF cells using the MTS assay and Trypan Blue Exclusion Test.

1.5 Significance of the Study

In this study, the IDDS was designed as a rod-shaped entity for subcutaneous administration by using an applicator implant device that had already been used for Nexplanon implantation (Palomba et al., 2012; Pons-Faudoa et al., 2019). Subcutaneous tissue is an ideal location for implanting vitamin D-depot devices due to the high-fat content that promotes the release and storage of drugs in the body's adipose (fat) tissue for a longer time, and the vitamin would slowly spread through the body by the systemic circulation.

The subcutaneous tissue has already been determined as a lower reactive site for insertion of foreign materials and the probability of localizing inflammation (Kumar & Pillai, 2018). This system offers many advantages over other routes of drug delivery. In particular, it would efficiently carry vitamin D at lower doses while potentially reducing side effects and increasing the patient's compliance. Direct site-localized drug delivery would bypass gastrointestinal tract (GIT) absorption barriers, first-pass metabolism, and prevent gastrointestinal degradation, thereby improving the bioavailability of vitamin D. The level of vitamin D could be regulated in the therapeutic window by managing the drug release and sustained release, resulting in a reduced dosage therapeutic effect and avoiding a high concentration of drugs from being exposed to the systemic environment. It is expected the IDDS will treat vitamin D deficiency more effectively. It might also be used to carry other hydrophobic pharmaceutical substances in the future.

1.6 Thesis Outline

This thesis consists of 7 chapters. Chapter 1 outlines the research context, problem statement, objectives, scope, and significance of the study. Chapter 2 reviews the available literature on the drug delivery system, electrospinning technique and its process parameters, vitamin D, cellulose acetate, and PCL that are relevant to this study. The experimental procedure and characterization techniques are discussed in Chapter 3. Chapter 4-6 results and discusses the data generated from various tests.

Chapter 4 reports on the effect of process parameters and solvent systems on the production of CA nanofiber and PCL nanofiber membranes and their morphological surfaces. Chapter 5 reports on loading vitamin D into the CA fibrous membrane and characterization of vitamin D-loaded CA nanofibrous membrane and PCL fibrous membrane using various techniques. Chapter 6 studies *in-vitro* biocompatibility, *in-vitro* drug release, tensile properties, *in-vitro* swelling and degradation of the CAVD and CAVD/PCL. The final chapter concludes the study while providing suggestions for future work.

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LIST OF PUBLICATIONS

1. Wsoo, M. A., Shahir, S., Bohari, S. P. M., Nayan, N. H. M., & Abd Razak, S. I. (2020). A review on the properties of electrospun cellulose acetate and its application in drug delivery systems: A new perspective. *Carbohydrate Research*, 491, 107978. (Q2, IF: 1.87).

2. Wsoo, M. A., Abd Razak, S. I., Bohari, S. P. M., Shahir, S., Salihu, R., Kadir, M. R. A., & Nayan, N. H. M. (2021). Vitamin D_3-loaded electrospun cellulose acetate/polycaprolactone nanofibers: Characterization, *in-vitro* drug release and cytotoxicity studies. *International Journal of Biological Macromolecules*, 81, 82-98 (Q1, IF: 6.95).

3. Wsoo, M. A., Abd Razak, S. I., Shahir, S., Ahmed Abdullah Al-Moalemi, H., Rafiq Abdul Kadir, M., & Hasraf Mat Nayan, N. (2021). Development of prolonged drug delivery system using electrospun cellulose acetate/polycaprolactone nanofibers: Future subcutaneous implantation. *Polymers for Advanced Technologies*, 32(9), 3664-3678. (Q2, IF 3.66).