SYNTHESIS AND CHARACTERIZATION OF HYBRID MULTIWALLED CARBON NANOTUBE-POLYCAPROLACTONE-SELENIUM NANOPARTICLES NANOFIBRES AND ITS ANTIBACTERIAL PROPERTIES

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DEDICATION

This thesis is specifically dedicated to:

My beloved

Dad and Mum
Kamaruzaman Abd Rasid and Nor Arbaayah Sharif

Husband
Hafsham Mohd Ali

Daughters
Nurul Izzul Islam and Nurul Arisha Ayra

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ABSTRACT

Antibacterial materials are particularly important nowadays in many applications such as disinfecting surfaces and maintaining a healthy, clean, and safe environment. This is to prevent any bacterial infection and kill potentially harmful microbes that can cause morbidity and mortality. Hybrid poly-(ε-caprolactone) (PCL) nanofibres are biodegradable antibacterial biomaterials that are essential for preventing and combating dangerous bacterial infections. Hybrid PCL nanofibres were synthesised by incorporating multi-walled carbon nanotubes (MWCNTs) or/and selenium nanoparticles (SeNPs) with PCL nanofibres. Firstly, MWCNTs were purified and functionalised using mild acid, followed by the synthesis of SeNPs by oxidising selenious acid (H_2SeO_3) with ascorbic acid. The carboxyl group was attached to the MWCNTs surface while trigonal SeNPs were successfully synthesised with a purity of 97.15%. The synthesis of PCL nanofibres with nanoparticles at different concentrations by electrospinning was optimised, having concentrations of 0.08 wt.% and 0.6 wt.% of MWCNTs and SeNPs, respectively. FESEM images showed the formation of aligned fibres with a size of less than 530 nm. The FESEM images confirmed that PCL-MWCNTs-SeNPs nanofibres degraded faster followed by PCL-SeNPs, PCL-MWCNTs, and PCL nanofibres. The presence of nanoparticles enhanced the biodegradation process by the agglomeration of nanofibres before holes appeared, degrading the nanofibres. The inhibition zone for PCL-MWCNTs, PCL-SeNPs, and PCL-MWCNTs-SeNPs nanofibres against *Escherichia coli* was around 9–13 mm and 10–16 mm for *Staphylococcus aureus*. The synergistic effects of MWCNTs and SeNPs in PCL-MWCNTs-SeNP nanofibres began degradation in the fourth month and are more effective in inhibiting *E. coli* and *S. aureus*. The characteristics of PCL nanofibres are still maintained and capable of decreasing the hydrophobicity and enhancing the biodegradation rate, as well as antibacterial properties of the hybrid PCL nanofibres. From this study, hybrid PCL nanofibres have the potential to be used in broad and various applications, from daily products to specific applications, especially in healthcare and medical applications.

ABSTRAK

Bahan antibakteria sangat penting pada masa ini dalam banyak aplikasi contohnya membasmi kuman pada permukaan dan mengekalkan persekitaran yang sihat, bersih dan selamat. Ini adalah untuk mengelakkan sebarang jangkitan bakteria dan membunuh mikrob yang berpotensi memudaratkan yang boleh menyebabkan morbiditi dan kematian. Nanogentian poli-(ε-kaprolakton) (PCL) hibrid merupakan bahan bio antibakteria terbiodegradasi yang diperlukan untuk mencegah dan memerangi jangkitan bakteria ini. Nanogentian PCL hibrid disintesis dengan menggabungkan nanotiub karbon dinding berbilang (MWCNTs) atau/dan nanopartikel selenium (SeNPs) dengan nanogentian PCL. Pertama, MWCNTs ditulen dan difungsikan menggunakan asid lemah, diikuti dengan sintesis SeNPs dengan mengoksidakan asid selenus (H_2SeO_3) dengan asid askorbik. Kumpulan karboksil terletak pada permukaan MWCNTs sementara SeNPs trigonal berjaya disintesis dengan ketulenan 97.15%. Sintesis nanogentian PCL dengan nanopartikel dioptimumkan pada kepekatan yang berbeza menggunakan elektroputaran dengan kepekatan masing-masing 0.08 wt.% dan 0.6 wt.% bagi MWCNTs dan SeNPs. Imej FESEM menunjukkan pembentukan gentian sejajar dengan saiz kurang daripada 530 nm. Imej FESEM mengesahkan bahawa nanogentian PCL-MWCNTs-SeNPs mengalami degradasi lebih cepat diikuti oleh nanogentian PCL-SeNPs, PCL-MWCNTs dan PCL. Kehadiran nanopartikel meningkatkan proses biodegradasi dengan aglomerasi nanogentian sebelum lubang muncul, seterusnya mendegradasi nanogentian. Zon perencatan bagi nanogentian PCL-MWCNTs, PCL-SeNPs dan PCL-MWCNTs-SeNPs terhadap bakteria *Escherichia coli* adalah sekitar 9-13 mm dan 10-16 mm bagi *Staphylococcus aureus*. Kesan sinergi MWCNTs dan SeNPs di dalam nanogentian PCL-MWCNTs-SeNP memulakan degradasi pada bulan keempat dan lebih berkesan dalam menghalang *E. coli* dan S. *aureus*. Ciri-ciri nanogentian PCL masih dikekalkan dan berkemampuan untuk mengurangkan kehidrofobikan dan meningkatkan kadar biodegradasi serta sifat antibakteria hibrid nanogentian PCL. Daripada kajian ini, nanogentian PCL hibrid berpotensi untuk digunakan dalam aplikasi yang luas dan pelbagai, dari produk harian ke aplikasi yang khusus, terutamanya dalam aplikasi penjagaan kesihatan dan perubatan.

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LIST OF ABBREVIATIONS

Ag	-	Silver
TiO_2	-	Titanium oxide
ZnO	-	Zinc oxide
Fe_3O_4	-	Iron oxide
CuO	-	Copper oxide
NO	-	Nitric oxide
MWCNTs	-	Multi-walled carbon nanotubes
SeNPs	-	Selenium nanoparticles
PCL	-	Poly-(ε-caprolactone)
PLA	-	Polylactide acid
PGA	-	Polyglycolic acid
PVA	-	Polyvinyl alcohol
PLGA	-	Polylactide-co-glycolide
PU	-	Polyurethane
PLACL	-	Poly(L-lactide acid)-co-poly-(ε-caprolactone)
CNTs	-	Carbon nanotubes
AgNPs	-	Silver nanoparticles
ZnO	-	Zinc oxide
AuNPs	-	Gold nanoparticles
GNPS	-	Graphene nanoplatelets
SBF	-	Simulated Bodies Fluid
DDT	-	Disc Diffusion Test
CO_2	-	Carbon dioxide
H_2O	-	Water
H_2SeO_3	-	Selenious acid
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonucleic acid
FDA	-	Food and Drug Administration
HA	-	Hydroxy apatite

PEG	-	Poly ethylene glycol
PEO	-	Poly ethylene oxide
ISO	-	International Organization for Standardization
POSS	-	Polyhedral oligomeric silsesquioxanes
a-Se	-	Amorphous Se
t-Se	-	Trigonal Se
PMMA	-	Polymethyl methacrylate
PBT	-	Polybutylene terephthalate
FESEM	-	Field Emission Scanning Electron Microscopy
EDX	-	Energy-Dispersive X-ray spectrometry
THF	-	Tetrahydrofuran
DMF	-	Dimethylformamide
CCVD	-	Catalytic Chemical Vapor Deposition
HCl	-	Hydrochloric acid
HNO_3	-	Nitric acid
H_2SO_4	-	Sulphuric acid
H_2SeO_3	-	Selenious acid
$C_6H_8O_6$	-	L(+)-ascorbic acid
$NaHCO_3$	-	Sodium bicarbonate
KCl	-	Potassium chloride
$MgCl_2.6H_2O$	-	Magnesium chloride hexahydrate
$CaCl_2$	-	Calcium chloride
Na_2SO_4	-	Sodium sulphate
$K_2HPO_4.3H_2O$	-	Potassium phosphate dibasic trihydrate
$C_4H_{11}NO_3$	-	Tris(hydroxymethyl) aminomethane
$BaCl_2$	-	Barium chloride
FTIR	-	Fourier Transform Infrared Spectroscopy
TGA	-	Thermogravimetric Analyser
XRD	-	X-ray Diffractometer
GSH-Px	-	Glutathione peroxidase
TrxRs	-	Gthioredoxin reductase
E. coli	-	*Escherichia coli*

P. aeroginosa	-	*Pseudomonas aeroginosa*
S. pneumonia	-	*Staphylococcus pneumonia*
S. aureus	-	*Staphylococcus aureus*
B. subtilis	-	*Bacillus subtilis*
B. cereus	-	*Bacillus cereus*
DSS	-	Dioctyl Sodium Sulfosuccinate
ROS	-	Reactive Oxygen Species

LIST OF SYMBOLS

n	-	reflection order
λ	-	X-ray wavelength
d	-	lattice spacing
θ	-	angle
V	-	volume
S	-	surface area
°	-	degree
°C	-	Degree Celcius
g	-	gram
v	-	volume
V	-	volt
A	-	Ampere
mL	-	millilitre
M	-	molarity
µm	-	Micrometre
h	-	hour
kV	-	kilovolt
rpm	-	revolutions per minutes
cm	-	centimetre
%	-	percentage
w	-	weight
wt.%	-	percentage by weight
mA	-	milliamperes
cm^{-1}	-	reciprocal centimetre
nm	-	nanometre
mm	-	milimetre

CHAPTER 1

INTRODUCTION

1.1 Problem Background

The current healthy lifestyle leads to a billion-dollar in pharmaceutical industry dedicated to antibacterial products in daily life. Health threat of bacterial infections for centuries is a major cause of morbidity and mortality worldwide (Plotkin et al., 2018). It causes serious issues that affects human health and environment via bacterial contamination of air, water, surfaces, medical devices and implants. Thus, investment in antibacterial materials shows huge potential in many application areas especially as protective clothing for medical and chemical workers (Bhattacharjee et al., 2019; Karim et al., 2020; Morris and Murray, 2020), sportswear (Feng, 2021), household appliances (Goodyear et al., 2015), food packaging (Huang et al., 2019) and health related products. Furthermore, the increasing trend of demand for antibacterial agents in medical and industrial materials to prevent bacterial contamination due to the aging population also induce the evolution of antibacterial-resistant bacterial strains.

Over decades, the use of nanoparticles as antibacterial material has shown a growth trend in many applications (Wang, Hu and Shao, 2017; Aggarwal et al., 2019). Metal nanoparticles such as silver (Ag), gold (Au), titanium oxide (TiO_2), zinc oxide (ZnO), iron oxide (Fe_3O_4), copper oxide (CuO) and nitric oxide (NO) are widely used as antibacterial materials due to their unique physical and chemical properties i.e. nano scale size and high surface area (Wang, Hu and Shao, 2017; Jeevanandam et al., 2018). Besides, bacteria such as *Escherichia coli* (*E.coli*) and *staphylococcus aureus* (*S. aureus*) will be killed when the nanoparticles inhibit the bacterial growth by permeating into the bacterial cell membrane and destroying it (Guisbiers et al., 2016; Aggrawal et al., 2019). Furthermore, antibacterial (Wang and Webster, 2012; Srivastava and Mukhopadhyay, 2015) and anticancer (Geoffrion et al., 2020; Luo et al., 2012) properties of selenium nanoparticles (SeNPs) particularly suitable for application in the medicine and it also can act as potent chemopreventive (Menon et al., 2018) and chemotherapeutic (Khurana et al., 2019).

Synthetic polymer such as poly-(ε-caprolactone) (PCL), polylactide acid (PLA), polyglycolic acid (PGA), polylactide-co-glycolide (PLGA), polystyrene, polyurethane (PU), poly(L-lactide acid)-co-poly-(ε-caprolactone) (PLACL) have been successfully studied for biodegradability performance (Manavitehrani et al., 2016; Conte et al., 2018). In addition, an ideal nanofibre not only has antibacterial properties but also required for suitable mechanical properties and its biodegradability can be controlled. PCL nanofibres with different additives such as carbon nanotubes (CNTs), silver nanoparticles (AgNPs), zinc oxide (ZnO), gold nanoparticles (AuNPs), graphene nanoplatelets (GNPs) and other nanoparticles have been reported for improvements of PCL electrospun nanofibre (Chiesa et al., 2020; Prado-Prone et al., 2020).

Although previous study has been done on PCL nanofibre incorporated with various of nanoparticles, it is potentially to incorporate MWCNTs or/and SeNPs with PCL nanofibres for antibacterial materials. Therefore, hybrid PCL nanofibres was fabricated via electrospinning by incorporated MWCNTs or/and SeNPs in the PCL nanofibre. The presence of MWCNTs or/and SeNPs in the PCL nanofibre will enhance the characteristic of PCL nanofibre as well as degradation rate and antibacterial properties (Chung et al., 2016; Geoffrion et al., 2020). Then, the performance hybrid PCL nanofibres were investigated on degradation study in Simulated Bodies Fluid (SBF) environment for 52 weeks. Then, antibacterial test was carried out on *E. coli* and *S. aureus* using Disc Diffusion Test (DDT).

1.2 Problem Statement

Morbidity and mortality are highly recognized as global challenges resulted from bacterial infection either by bacterial adhesion or colonization on the surfaces. Concern about health problems is a worldwide issue where the antibacterial materials have attracted a high degree of interest in all applications. Antibacterial materials shows high demand especially in medical, packaging, filtration and consumer products. Therefore, it is important to prevent infection and contamination by common bacteria such as *E. coli* and *S. aureus* in the products which necessary to maintain and ensure health.

Although the methods such as template method, self-assembly, smelting and phase separation have been successful in the synthesis and fabrication of nanofibres, these methods have constraints in large-scale nanofibre production. Accordingly, the discovery of the electrospinning method in overcoming this problem has attracted the interest of researchers to make extensive studies on the synthesis or fabrication of nanofibres. In addition, this method is also capable of varying the nanofibre diameter from nanometers to micrometers and vice versa, which is seen to give an advantage compared to other methods.

Poly (ε-caprolactone) (PCL) is non-toxic, biodegradable and biocompatible with great potential and giving advantages to be used in the medical and healthcare applications. In addition, PCL nanofibre can be designed based on specific applications as it is flexible and can be adapted to the composition and structure of this polymer. Unfortunately, the poor dispersion of MWCNTs in the solution during the electrospinning process makes PCL to be difficult to synthesize a uniformly aligned fibre. The inability to maintain the continuous electrospinning is due to the absence of functional groups on the MWCNTs surface which will facilitate the MWCNTs to be agglomerated with each other that cause the jets are easy to clog. Therefore, MWCNTs functionalized with carboxyl groups will results a good dispersion in solution to reduce the spraying jet from clog when nanofibres are synthesized. Plus, instability of spraying jets resulting in non-uniform of morphological and size of fibres that can overcome by introducing SeNPs to increase the conductivity of the solution.

Even PCL nanofibre has good mechanical properties, the absence of antibacterial properties will bring disadvantages and limit its use as an antibacterial material. However, the PCL surface can be modified by binding or adhesion with antibacterial agents eventhough it does not have antibacterial properties by itselt alone

(Michael et al., 2018). Thus, MWCNTs and SeNPs can acts as antibacterial agents by penetration into cells and leading to complete destruction of microbial cells.

In addition, PCL is safe to use and poses no danger during degradation in the body which is the most important criterion for the product in healthcare and medical applications (Azimi et al., 2014; Dwivedi et al., 2020). But, the long shelf life of PCL nanofibre caused the time of degradation becomes slowly that almost take two years and above to degrade which suitable for applications that necessary long degradation times. The incorporated of MWCNTs and SeNPs resulting in hydrophobicity degree lower and amorphous region is increased, that causing hydrolysis process of PCL easier to be attacked by water molecules. Thus, the synergistic effect of MWCNT and SeNPs which helps to accelerate the hydrolysis process on PCL nanofibres during degradation. Therefore, antibacterial materials introducing in this study by synthesis PCL nanofibre with MWCNTs and SeNPs can reduce the degradation time by enhanced degradation rate as well as has antibacterial characteristics.

1.3 Research Objectives

The objectives of the research are:

(a) To synthesis, purify and functionalise multi-walled carbon nanotubes (MWCNTs) and selenium nanoparticles (SeNPs) followed by characterizations using various analytical instruments for physico-chemical studies.

(b) To optimise and study the effect of MWCNTs or/and SeNPs in the hybrid PCL nanofibres through surface morphology and physico-chemical properties.

(c) To investigate the biodegradability and anti-bacterial properties of the hybrid PCL nanofibres.

1.4 Scope of the Study

The scope of present study covers the synthesis of MWCNTs by catalytic chemical vapor deposition (CCVD). Then, MWCNTs was purified with hydrochloric acid (HCl) followed by oxidative functionalisation through acid treatment with sulphuric acid (H_2SO_4) and nitric acid (HNO_3). SeNPs was synthesised by the oxidation of selenious acid (H_2SeO_3) with the ascorbic acid ($C_6H_8O_6$). The structural and chemical characteristics of MWCNTs and SeNPs were analysed using various analytical instruments; X-ray diffractometer (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, thermogravimetric analyser (TGA), derivative thermogravimetry (DTG), Field Emission Scanning Electron Microscope (FESEM) and energy dispersive X-Ray (EDX).

The optimization of synthesised of hybrid PCL nanofibres were done using PCL solution at different concentration of nanoparticles (0-0.1 wt.% MWCNTs or/and 0-1.0 wt.% SeNPs) through an electrospinning process in this study. The hybrid PCL nanofibres were synthesised at the optimized concentration of MWCNTs or/and SeNPs while the synthesis of PCL nanofibre without nanoparticles was done as comparison. The characterizations of its morphology, structural and physico-chemical properties of PCL and hybrid PCL nanofibres were performed by XRD, FTIR, FESEM, TGA, Raman Spectroscopy and Optical Contact Angle.

Particular attentions were given on the scope of degradable study of PCL and hybrid PCL nanofibres in the Simulated Body Fluid (SBF) solutions at 37°C for 52 weeks. Effect of nanoparticles in hybrid PCL nanofibres during degradation were investigated through physical and morphology analyses. The efficiency of hybrid PCL nanofibres with antibacterial properties were studied through positive *S. Aureus* (ATCC 6538) and negative *E. coli* (ATCC 11229) gram bacteria using Disc Diffusion Test.

1.5 Significance and Original Contributions of This Study

Antibacterial materials are crucial nowadays for many applications mainly used in protective clothing, sportswear, home appliances and health products. It is significant to maintain healthy health, clean and safe environment by preventing the bacteria infection that can cause morbidity and mortality. Therefore, a new nanofibre product is a solution to overcome this problem by designing and fabricating new biomaterial nanofibre with suitable characteristics such as non-toxic, safe to human and environment, easy to handle, resistance to bacteria and importantly it is a biodegradable material.

This study also deals with the modification of PCL nanofibre with MWCNTs and SeNPs to investigate the fabrication of an antibacterial biomaterial as compared to PCL nanofibre. The disadvantage of using PCL nanofibres is the longer degradation time for almost two years and above which limit its application in certain applications. Therefore, PCL nanofibres with the presence of nanoparticles can be enhanced by shorten the time to degrade less than two years. Besides that, the incorporation of nanoparticles such as MWCNTs and SeNPS in the PCL nanofibres is necessary in order to improve the physical and mechanical properties to be used in many applications. With the incorporation of nanoparticles, hybrid PCL nanofibres can be a suitable material for antibacterial biodegradable material as well as inhibit positive-gram and negative-gram bacteria. Most importantly, the presence of nanoparticles in the single nanofibre of these hybrid PCL nanofibres becomes not toxic and more safety to aquatic life when release into water. Therefore, the hybrid PCL nanofibres was fabricated in this study are necessary and promising to have better results such as degrades faster, enhanced physicochemical as well as mechanical properties of PCL itself also with antibacterial properties. In addition, hybrid PCL nanofibres are required for making it able to withstand shear, prevents fragility of the product, user friendly by easy to handle and safe to human and environment.

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LIST OF PUBLICATIONS AND AWARDS

In this work, several reports have been published and presented in the conferences, journals and technology and innovation expo, as listed below:

Indexed Journal

1. Nurul Asyikin Kamaruzaman, Abdull Rahim Mohd Yusoff, Nik Ahmad Nizam Nik Malek and Marina Talib. Fabrication, Characterization and Degradation of Electrospun Poly(ε-Caprolactone) Infused with Selenium Nanoparticles. *Malaysian Journal of Fundamental and Applied Sciences*, Vol. 17 (2021) 295-305. **(Indexed by SCOPUS)**

Non-Indexed Conference Proceedings

1. Nurul Asyikin Kamaruzaman, Abdull Rahim Mohd Yusoff, Nor Aziah Buang and Nik Ghazali Nik Salleh. Effects on diameter and morphology of polycaprolactone nanofibers infused with various concentrations of selenium nanoparticles. *AIP Conference Proceedings*. Advanced Materials Conference (AMC) 2016, 28-29 N2017.

Technology and Innovation Expo

1. Nurul Asyikin Kamaruzaman, Abdull Rahim Mohd Yusoff, Nik Ahmad Nizam Nik Malek and Marina Talib. *PCL-SeNPs Nanofiber: Innovative Hybrid Polymer Nanofiber*. Malaysia Technology Expo (MTE) 2019. 21-23 February 2019, Putra World Trade Centre, Kuala Lumpur.

Awards

1. Bronze Award in Malaysia Technology Expo (MTE) 2019. 21-23 February 2019, Putra World Trade Centre, Kuala Lumpur for product of *PCL-SeNPs Nanofiber: Innovative Hybrid Polymer Nanofiber.*