

## **CERTIFICATE**

It is certified that the work contained in the thesis titled "*Biodegradable Polymeric Nano-composites for Microbial Growth Control and Wound Healing*" by "*VIVEK KUMAR PANDEY*" has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

**(Prof. Pradeep Kumar Mishra)**

**Supervisor**

Department of Chemical Engineering & Technology

Indian Institute of Technology

(Banaras Hindu University)

Varanasi -221005

## DECLARATION BY THE CANDIDATE

I, "**Vivek Kumar Pandey**", certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of "**Prof. Pradeep Kumar Mishra**" from "**July 2015**" to "**June 2020**", at the "**Department of Chemical Engineering and Technology**", Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, *etc.*, reported in journals, books, magazines, reports dissertations, theses, *etc.*, or available at websites and have not included them in this thesis and have not cited as my own work.

Date: 23-10-2020

Place: Varanasi

(**Vivek Kumar Pandey**)

## CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of my knowledge.

**Prof. Pradeep Kumar Mishra**  
(Supervisor)  
Department of Chemical Engineering &  
Technology IIT (BHU) Varanasi -221005

**Prof. Vijay Laxmi Yadav**  
(Head of Department)  
Department of Chemical Engineering &  
Technology IIT (BHU) Varanasi -221005

## **COPYRIGHT TRANSFER CERTIFICATE**

**Title of the Thesis:** Biodegradable Polymeric Nano-composites for Microbial Growth Control and Wound Healing

**Name of the Student:** Vivek Kumar Pandey

### **Copyright Transfer**

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University), Varanasi, all rights under copyright that may exist in and for the above thesis submitted for the award of the "*Doctor of Philosophy*".

Date: 23-10-2020

Place: Varanasi

**(Vivek Kumar Pandey)**

**Note:** However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the Institute's copyright notice are indicated.



Dedicated

to

My Parents & Family



# Acknowledgements

---

*At the outset, I express my gratitude and indebtedness to “the Almighty”, the “Guru” and the “Parents” for their blessings. This thesis could not have been possibly brought to a successful culmination without the blessings of Lord Shiva and Pandit Madan Mohan Malviya Ji. I am very thankful and proud of the education I received at the Banaras Hindu University.*

*I express my profound obligation and heartfelt gratitude to my supervisor, **Prof. Pradeep Kumar Mishra**, for his continuous support, patience, ideas, valuable suggestions and belief throughout the research. His guidance helped me to focus on research and provided freedom to develop and express my own scientific ideas to raise my individual abilities. He provided me with the opportunity and the resources to be as creative as I like, inspiring me to do my best in progressive way. These things boosted my confidence in my scientific abilities.*

*I also express my heartiest thanks to former heads, Prof. A. S.K. Sinha, Prof. V. L. Yadav (Present Head), Department of Chemical Engineering and Technology, Indian Institute of Technology (BHU) for providing essential research facilities. I am highly thankful to RPEC and all faculty members of Chemical Engineering and Technology Department for their valuable suggestions. I am also grateful to Prof. Sushant Kumar Shrivastava, Head, Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University) for allowing me to use the animal house and departmental facilities. The support staff, Mr. S. K. Verma, Mr. R. C. Singh, Mr. Gopal Ji Srivastava, Mr. Rajiv Nayan Pandey, Mr. Raj Bahadur and all other non-teaching staff of the Department are highly acknowledged for their assistance and co-operation during the Ph.D. tenure. The instrumental facility from CIFIC (IIT-BHU), MCIIE and Department of Chemical Engineering and Technology are gratefully acknowledged. Financial assistance provided by MHRD, New Delhi is also duly acknowledged.*

*I take the opportunity to sincerely acknowledge Department of Biotechnology, India and British Council, United Kingdom for providing Newton-Bhabha funding and the University of Reading, United Kingdom for permitting me as Visiting Research Scholar.*

*It is my deep heartfelt interest to express my regards to my supervisor at University of Reading, United Kingdom, Prof. Keshavan Niranjan, who believed me, and gave the opportunity to carry out the research under his guidance. I am also thankful to Prof. Fred Davis, Department of Chemistry, University of Reading for providing electrospinning facility and Dr. Saeed Mohan for helping during the research.*

*I also owe my sincerest thanks to my seniors Dr. D. B. Pal, Dr. Pardeep Singh, Dr. Neha Srivastava, Dr. Munish Srivastava, Dr. Pratap Srivastava, Dr. Lata Kumari, Dr. Shraddha Awasthi, Dr. Deepika Kushwaha, Dr. Gufran Ajmal for their help, support and encouragement during my research endeavor. I am ardently thankful to my colleagues; Mr. Rohit Srivastava, Ms. Zeenat Arif, Mr. Naresh Kumar, Mr. Mohit Kumar, Mr. Arun Pal, Mr. Anand Gupta, Mr. Madhu Kiran and Mr. Manjit for their valuable help and support in experiment, and presentation in the thesis.*

*I would particularly like to owe my gratitude and special thanks to Prof. Siddh Nath Upadhyay for his encouragement and insightful comments leading me to the completion of the research work. Without his supervision and constant help this dissertation would not have been possible.*

*My deepest gratitude also goes to my family for their unflinching love and support throughout the research. I owe my deepest gratitude specially to my beloved wife "Pragya" and daughter "Mishita" for being a constant source of encouragement and support throughout this journey. Their silent sacrifice, moral support, and encouragement cannot be defined in words. It is their love and trust on my abilities which has made me a person I am today. They backed my decision in all peaks and troughs of my life and made it a smooth journey for me. I could not have come this far without their unconditional love and support.*

*Finally, I thank all those who have helped me directly or indirectly in the successful completion of my thesis. Anyone missed in this acknowledgement are also thanked. I also pray God for the animals which were mortified for the cause of research and advancement of knowledge. I wish for their soul to rest in peace.*

**Date: 23-10-2020**

**Place: Varanasi**

**(Vivek Kumar Pandey)**

# Contents

Description	Page No.
List of Figures	xiv
List of Tables	xvii
List of Abbreviations & Symbols	xviii
Preface	xix
Graphical Abstract	xxiii
<b>Chapter 1: General Introduction</b>	<b>1-13</b>
1.1 Introduction	1
1.2 Problems associated with non-biodegradable food packaging	1
1.3 Bio-based polymers used in food packaging applications	2
1.3.1 Decomposition of bio-based polymers	2
1.4 Nano-composites	4
1.5 Nanofibers by electrospinning	4
1.6 Impact of bio-based biodegradable polymers	5
1.6.1 Food packaging	5
1.6.2 Biomedical and pharmaceutical applications of biodegradable polymers	5
1.6.2.1 Wound dressings	6
1.6.2.2 Nanoparticle-mediated drug delivery system	8
1.7 Research gap	9
1.8 Objectives	9
References	12
<b>Chapter 2: Biodegradable antimicrobial polymeric nanocomposite films for packaging</b>	<b>15-83</b>
2.1 Introduction	15
2.1.1 Bio-plastics	16
2.1.2 Food packaging	17
2.1.3 Foodborne pathogens	17
2.1.4 Antimicrobial packaging	18
2.1.4.1 Chitosan (CH) as antimicrobial packaging	19
2.1.4.2 Silver nanoparticles (AgNPs) as antimicrobial material	20

2.1.5 Electrospinning	22
2.1.6 Packaging of perishable meat products	22
2.2 Literature review	24
2.2.1 Biodegradation of plastic/polymer	25
2.2.2 Antimicrobial packaging	26
2.2.2.1 Polymeric antimicrobial composites of chitosan and their blends	28
2.2.2.2 Utilization of nanomaterials as antimicrobial agents in packaging	30
2.2.2.3 Silver nanoparticles (Ag-NPs)	31
2.2.2.4 Antimicrobial bio-nanocomposite films	34
2.2.3 Nanofibrous packaging	37
2.2.3.1 Electrospinning as a method of choice in packaging	39
2.2.4 Regulation for using nanomaterials in food packaging	43
2.3 Materials and methods	44
2.3.1 Materials	44
2.3.2 Collection & processing for preparation of <i>Ocimum tenuiflorum</i> leaf extract	44
2.3.3 Green Synthesis of silver nanoparticles (AgNPs) via plant extract and Chitosan (CH)	45
2.3.3.1 UV–Visible spectrophotometry	45
2.3.4 Electrospinning solution	46
2.3.4.1 Preparation of CH/PVA/AgNPs solution for electrospinning	46
2.3.4.2 Preparation of fibrous composite nano-layers (FCNLs) through electrospinning	46
2.3.5 Preparation of composite polymeric film	48
2.3.5.1 Diffusion of the active constituents	48
2.3.6 Physico-chemical characteristics	48
2.3.6.1 Electrical conductivity	48
2.3.6.2 Viscosity	48
2.3.6.3 Thickness	49
2.3.6.4 Solubility	49
2.3.6.5 Scanning electron microscopy (SEM)	49
2.3.6.6 Zeta ( $\zeta$ ) potential and particle size distribution	50
2.3.7 Water contact angle (WCA)	50
2.3.8 Fourier transforms infrared (FTIR) spectroscopy	50
2.3.9 X-ray diffraction (XRD)	51
2.3.10 Microbial Analysis	51
2.3.10.1 Culture media and microorganisms	51



2.3.10.2 <i>In vitro</i> antimicrobial activity	52
2.3.11 Antimicrobial application of FCNLs for meat packaging	52
2.3.12 Raw materials	53
2.3.12.1 Chitosan	53
2.3.12.2 Polyvinyl alcohol	54
2.4 Results and discussion	54
2.4.1 Analysis of UV–Visible spectra	54
2.4.2 Solution casting of polymeric film	57
2.4.2.1 The release profile of active constituents	58
2.5 Physico-chemical characteristics of electrospinning solution	59
2.5.1.1 Electrical conductivity	59
2.5.1.2 Viscosity	60
2.5.1.3 Thickness	61
2.5.1.4 Solubility	61
2.5.1.5 The SEM fingerprints	61
2.5.1.6 Zeta ( $\zeta$ ) potential of plant extract-AgNPs	65
2.5.2 Water contact angle (WCA)	66
2.5.3 Fourier Transform Infrared (FTIR) spectroscopy	67
2.5.4 X-ray diffraction (XRD)	68
2.5.5 Microbiological analysis	69
2.5.5.1 <i>In vitro</i> antimicrobial activity of film and FCNLs	69
2.5.6 Antimicrobial application of FCNLs for meat packaging	71
2.6 Conclusion	73
References	75
<b>Chapter 3: Nano-fibrous scaffold with curcumin for anti-scar wound healing</b>	<b>85-147</b>
3.1 Introduction	85
3.2 Literature review	89
3.2.1 Wound care	89
3.2.2 Skin	90
3.2.2.1 Layers of skin	90
3.2.3 Wound	93
3.2.3.1 Types of wounds	93
3.2.3.1.1 Acute wound	93

3.2.3.1.2 Chronic wound	93
3.2.3.2 Types of clinical wound healing	94
3.2.3.2.1 Primary intention wound healing process	94
3.2.3.2.2 Secondary intention wound healing process	95
3.2.3.2.3 Tertiary intention wound healing process	95
3.2.4 Wound healing mechanism	95
3.2.4.1 Hemostasis	97
3.2.4.2 The inflammatory phase	98
3.2.4.3 Proliferative phase	98
3.2.4.4 Maturation or remodeling phase	98
3.2.5 Wound dressings	99
3.2.5.1 Types of wound dressing	100
3.2.6 Components of advanced wound dressings	104
3.2.6.1 Polyvinyl pyrrolidone (PVP)	104
3.2.6.2 Cerium nitrate	104
3.2.6.3 Curcumin as a bioactive agent	105
3.2.6.3.1 Safety	105
3.2.6.3.2 Bioavailability	106
3.2.6.3.3 Application of curcumin	108
3.2.6.3.4 Wound healing action mechanisms of curcumin	108
3.2.6.3.5 Recent studies on curcumin application in wound healing	110
3.3 Materials and methods	111
3.3.1 Materials	111
3.3.2 Preparation of solution for electrospinning of curcumin and cerium loaded nanofibers fabrication	112
3.3.3 Physicochemical characterisation of nanofibers	114
3.3.3.1 Thickness	114
3.3.3.2 Solubility	114
3.3.3.3 Scanning electron microscopy (SEM) of nanofibers	114
3.3.3.4 Fourier Transforms Infrared (FTIR) Spectroscopy	114
3.3.3.5 X-ray diffraction (XRD)	115
3.3.4 Water contact angle (WCA)	115
3.3.5 Nanofiber characteristics	115
3.3.5.1 <i>In vitro</i> free-radical scavenging efficacy of nanofibrous scaffolds	115
3.3.5.2 <i>In vitro</i> antimicrobial activity	116

3.3.6 Evaluation of the biocompatibility of the fibrous scaffolds	117
3.3.6.1 <i>In vitro</i> hemocompatibility assessment	117
3.3.6.2 Cytocompatibility assay (MTT assay)	117
3.3.7 <i>In vivo</i> open-wound healing	119
3.3.8 Histological examination	120
3.3.8.1 Histological examination of granulation tissues	120
3.3.8.2 Anti-oxidant enzyme activity in granulation tissues	120
3.3.8.2.1 Superoxide dismutase (SOD) assay	120
3.3.8.2.2 Catalase assay	121
3.3.8.3 Hydroxyproline content in granulation tissues	122
3.3.8.3.1 Standard curve for hydroxyproline	123
3.3.9 Statistical analysis	124
3.4 Results and discussion	125
3.4.1 Diameter and surface morphology of nanofibers	125
3.4.2 Physicochemical characteristics of nanofibers	127
3.4.3 Water contact angle	129
3.4.4 <i>In vitro</i> free-radical scavenging efficacy of nano-fibrous scaffolds	130
3.4.5 <i>In vitro</i> antimicrobial activity	131
3.4.6 Biocompatibility evaluation of the fibrous scaffolds	131
3.4.6.1 <i>In vitro</i> hemocompatibility assessment	131
3.4.6.2 Cytocompatibility assay (MTT assay)	133
3.4.7 Results of <i>in vivo</i> open-wound healing study	133
3.4.8 Histological examinations of granulation tissues	136
3.4.8.1 Anti-oxidant enzyme activity in granulation tissues	137
3.4.8.2 Hydroxyproline content in granulation tissues	140
3.5 Conclusion	141
References	142
<b>Chapter 4: Nanoparticle-mediated drug delivery for biofilm-associated infections</b>	<b>149-184</b>
4.1 Introduction	149
4.1.1 Biofilm over wounds	149
4.1.2 Biofilm-associated nosocomial infections	150
4.1.3 Strategies for biofilm eradication	150
4.1.4 Externally implanted devices (EIDs)	150

4.1.5 Nanoparticle-mediated drug delivery	152
4.2 Literature review	153
4.2.1 Strategies for biofilm eradication	157
4.2.2 Nanoparticle-based drug delivery systems	160
4.3 Materials and methods	162
4.3.1 Materials and reagents	162
4.3.2 Biofilm formation and quantification	162
4.3.3 Scanning electron microscopy (SEM)	163
4.3.4 Water contact angle (WCA)	163
4.3.5 Preparation of Eudragit-RL100 nanoparticle encapsulated gentamicin sulfate (E-G-S)	164
4.3.5.1 Drug entrapment efficiency	164
4.3.5.2 <i>In vitro</i> gentamicin sulfate release kinetic study	165
4.3.5.3 Standard curve for gentamicin sulfate (G-S)	165
4.3.6 Antimicrobial assessment	166
4.3.7 Determination of minimum inhibitory concentration (MIC) of gentamicin sulfate (G-S)	166
4.3.8 4.3.8 Determination of minimum biofilm inhibitory concentration (MBIC) of G-S and E-G-S	167
4.4 Results and discussion	167
4.4.1 Biofilm formation and quantification	167
4.4.2 Physical characteristic of EIDs by SEM	169
4.4.3 Water contact angle (WCA)	171
4.4.4 Physical characteristic of Eudragit-RL100 nanoparticle encapsulated gentamicin sulfate (E-G-S)	172
4.4.4.1 Drug entrapment efficiency	173
4.4.4.2 <i>In vitro</i> G-S release kinetics	173
4.4.5 Minimum inhibitory concentration (MIC) of G-S	174
4.4.6 Minimum biofilm inhibitory concentration (MBIC) of G-S	175
4.4.7 MBIC of Eudragit RL100 nanoparticle-encapsulated G-S (E-G-S)	176
4.5 Conclusion	178
References	180
<b>Chapter 5: Conclusions and future aspects</b>	<b>185-189</b>
5.1 Conclusion	185
5.2 Future aspects	188
<b>List of Publications</b>	<b>190</b>

---

## *List of Figures*

<b>Fig. No.</b>	<b>Description</b>	<b>Page No.</b>
1.1	Commercial biodegradable thermoplastics	3
2.1	AgNPs utilization in commercial products	21
2.2	The fate of plastics in the aquatic environment	26
2.3	Different approaches for the synthesis of AgNPs	33
2.4	Schematic diagram displaying the active nanocomposite packaging concept	37
2.5	Packaging precursors for antimicrobial packaging in nanofibers	40
2.6	Functional electrospun and food packaging materials	42
2.7	Schematic diagram of electrospinning process and set-up	47
2.8	Absorption spectra of AgNPs under (a) Sunlight; (b) UV-light; (c) Raw materials	55
2.9	Chitosan-mediated green synthesized AgNPs; (a) UV-visible spectra; (b) SEM image of AgNPs; (c) Cumulative size distribution obtained through DLS method	57
2.10	Morphology of antimicrobial active film	58
2.11	Release profile of AgNPs and <i>Ocimum tenuiflorum</i> extract at 427 nm and 335 nm	59
2.12	SEM photo-micrographs of electrospun composite nano-layers; (a) P-100 NL; (b) P70-CH30 NL; (c) P70-CH30-Ag NL; (d) P60-CH40 NL; (e) P50-CH50 NL; (f) CH-100NL	63
2.13	Zeta potential of <i>Ocimum tenuiflorum</i> extract and AgNPs colloidal solution	65
2.14	FTIR spectra of film and composite nano-layers; (a) CH-100 film; (b) P70-CH30 NL; (c) P-100 NL; (d) P70-CH30-Ag NL	67
2.15	XRD pattern of film and composite nano-layers: (a) P70-CH30 NL; (b) P70-CH30-Ag NL; (c) P-100 NL; (d) CH-100 film	69
2.16	Microbial growth pattern in packed meat after 7 days: (a) Normal Plastic; (b) P70-C30-Ag NL; (c) P70-CH30 NL; (d) P-100 NL	72
2.17	Application of PVA/CH/AgNPs composite nano-layer for packaging of fresh meat	72
3.1	Layers of skin	92
3.2	Clinical wound healing (a)Primary intention wound healing process, and (b)Secondary intention wound healing process	94
3.3	Schematic representation of the concept of wound healing process assisted by a dressing material or skin graft	96
3.4	Four phases of acute wound healing	99

<b>Fig. No.</b>	<b>Description</b>	<b>Page No.</b>
3.5	Absorption, metabolism and fate of curcumin after oral administration in rodents and humans	107
3.6	Effects of topical application of curcumin on different stages of wound healing	109
3.7	Schematic diagram of electrospinning set-up for preparing PVP-based curcumin and ceria loaded electrospun nanofiber	113
3.8	Reaction mechanism of hydroxyproline assay	123
3.9	Calibration curve of hydroxyproline	124
3.10	SEM fingerprints of nanofibers; (a) PVP nanofibers, (b) PVP-Ce nanofibers, (c) PVP-Ce-Cur nanofibers	125
3.11	Fiber characteristics: (a) FTIR spectra of PVP, curcumin, PVP-Ce and PVP-Ce-Cur nano-fibers, (b) XRD patterns of PVP-Ce and PVP-Ce-Cur nanofibers	128
3.12	Water contact angle for PVP NF, PVP-Ce-NF and PVP-Ce-Cur NF	130
3.13	Free-radical scavenging efficacy of PVP NF, PVP-Ce NF and PVP-Ce-Cur NF obtained through DPPH assay	131
3.14	<i>In vitro</i> biocompatibility of fibrous scaffolds: (a) hemocompatibility, (b) cytocompatibility of 3 T6-Swiss albino fibroblast cell lines on different scaffolds after 24 h, 48 h and 72 h incubation	133
3.15	Effect of dressing materials on healing of full thickness wound: (a) Images of wound healing on day 8 and 16, (b) percentage of wound healed following treatment with gauze, ciprofloxacin cream, PVP-Ce NF and PVP-Ce-Cur NF on day 4, 8, 16 and 20	135
3.16	Histological images (H&E staining) of granulation tissues with gauze, ciprofloxacin cream, PVP-Ce NF and PVP-Ce-Cur NF treatment on day 8 and 16 at 10X microscopic resolution	137
3.17	<i>In vivo</i> effect of application of different nanofibers on endogenous enzymes viz. (a) SOD, (b) catalase in granulation tissues on day 8 and 16	139
3.18	<i>In vivo</i> effect of different dressings on hydroxyproline content in granulation tissue of Wistar rats on day 8 and 16 of post-wounding	140
4.1	Biofilm-related infections typically found in the human body	155
4.2	Calibration curve of Gentamicin sulfate (G-S) in PBS (pH 7.4)	166
4.3	Growth of <i>E. coli</i> biofilm over different materials	168
4.4	SEM images of catheter inner surfaces before and after <i>E. coli</i> biofilm formation; (a) Rubber catheter; (b) Biofilm over rubber catheter; (c) Foley catheter; (d) Biofilm over Foley catheter; (e) Endotracheal catheter; (f) Biofilm over endotracheal catheter	170
4.5	Contact angle of sessile water drop over different catheter surfaces	171
4.6	Size distribution of Eudragit RL-100 encapsulated gentamicin sulfate (E-G-S) nanoparticles. (a) SEM image; (b) Dynamic light	173

<b>Fig. No.</b>	<b>Description</b>	<b>Page No.</b>
	scattering (DLS) measurement of particle size distribution	
4.7	Drug release profile of E-G-S in PBS	174
4.8	Minimum inhibitory concentration (MIC) of G-S for <i>E. coli</i> by visual detection of colony forming units (CFU)	175
4.9	Biofilm inhibition pattern over different externally implanted medical devices (EIDs) with G-S	176
4.10	Comparison of minimum biofilm inhibitory concentration (MBIC): MBIC50 and MBIC90 for G-S and E-G-S.	177

## *List of Tables*

<b>Table No.</b>	<b>Description</b>	<b>Page No.</b>
2.1	The advantages and disadvantages of bio-based packaging	24
2.2	Natural antimicrobial agents used in food packaging systems	27
2.3	Natural antimicrobial compounds used in the chitosan-based composite for antimicrobial food packaging	29
2.4	Use of bio-nanocomposite films in antimicrobial food packaging applications	35
2.5	Commonly used methods for nanofiber production	38
2.6	Electrospinning parameters (solution, processing, and ambient) and their effect on fiber morphology	41
2.7	Electrospinning parameters used for preparing PVA-based nano-layers	47
2.8	Properties of electrospinning solutions for making fibrous nano-layers	60
2.9	Morphology, thickness, average fiber diameter and water contact angle of fibrous composite nano-layers	64
2.10	Antimicrobial activity of fibrous composite nano-layers and polymeric films against Gram-positive and Gram-negative bacteria	70
3.1	Four phases of healing of a full thickness wound	97
3.2	Function-based classification of available wound dressing products	101
3.3	Main classes of the moisture retentive wound dressings	102
3.4	Action of topical application of curcumin on different stages of wound healing	110
3.5	Parameters used for preparing electrospun PVP-based nanofibers	113
3.6	Morphology, composition, thickness, average fiber diameter and antimicrobial activity of fibrous dressings	126
4.1	Major pathogens involved in biofilm-associated infections	153
4.2	Factors responsible for chronic disease and antibiotic resistance in biofilms	157
4.3	Advantages and disadvantages of conventional therapies against biofilm	159



## *List of Abbreviations & Symbols*

---

%	: Percentage
°	: Degree
µg	: Microgram
C	: Celsius
cm	: Centimeter
h	: Hour
kV	: Kilo volt
mg	: Milligram
min	: Minute
mL	: Milliliter
mm	: Millimeter
s	: Seconds
v/v	: volume/volume
w/v	: weight/volume
w/w	: weight/weight
AgNPs	: Silver nanoparticles
ANOVA	: Analysis of variance
Ce	: Cerium Nitrate Hexahydrate
CH	: Chitosan
Cur	: Curcumin
DLS	: Dynamic light scattering
ECM	: Extracellular matrix
E-G-S	: Eudragit RL100 encapsulated gentamicin sulfate
EPS	: Extracellular polymeric substances
FTIR	: Fourier Transforms Infrared Spectroscopy
FTW	: Full-thickness wounds
G-S	: Gentamicin sulfate
H&E	: Haematoxylin and staining
PVP	: Polyvinyl pyrrolidone
ROS	: Reactive oxygen species
rpm	: Rotation per minutes
SD	: Standard deviation
SEM	: Scanning Electron Microscopy
SOD	: Superoxide dismutase
UV-Vis	: Ultraviolet-Visible
XRD	: X-Ray Diffraction
$\lambda_{\max}$	: Wavelength maxima

## Preface

---

Nanomaterials are already being used in numerous household products as well as in several industrial applications due to their unique properties. The biomedical, pharmaceutical, and food packaging industries worldwide are showing great interest in fabricating and developing nanomaterials based products to address the problems associated with biodegradability, eco-friendly nature and efficacy of various products currently being used.

Excessive use of non-biodegradable plastics as packaging materials has posed serious environmental issues. An alarming condition of the presence of micro-plastics (5 $\mu$ m to 1nm) in the natural rivers and marine ecosystems has been recently reported. These micro-plastics may easily enter the human food chain from marine foods. Bio-based packaging materials derived from renewable resources have several environmental benefits such as biodegradability and nontoxicity. Antimicrobial biodegradable nano-composite packaging films of natural origin for preventing microbial infection and degradation of food may enhance the shelf-life of food products.

Traditional dressings such as bandages, gauzes and cotton wool used offer limited protection against microbial infections, allow moisture evaporation leading to adhesion of the dressing to the wound, causing trauma and scar when removed and resulting in delayed healing. The broad spectrum antibiotics used in dressings to prevent microbial biofilm infection lead to enhanced antibiotic load on patients often resulting in increased bacterial tolerance. Any casual approach towards microbial infection may also result in biofilm-associated iatrogenic infections which contribute significantly to patient's morbidity and healthcare costs. Nearly 80% of human bacterial infections are biofilm-associated. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus*

*epidermidis* and *Staphylococcus aureus* are among the most prominent causative agents. Due to the lack of well-defined treatment protocol for treatment, the biofilm-associated infections are a matter of great concern.

Different wound dressing products are used to protect the wound from environmental threats and microbial penetration whilst simultaneously promoting the process of tissue regeneration. Some inherent problems like higher chances of infection and scar formation have provided stimulus for the development of new advanced multifunctional biocompatible, bio-absorbable nano-composite wound dressings with natural anti-oxidants for anti-scar rapid wound healing. Nanoparticle-mediated targeted drug delivery systems have the potential to provide an effective solution to the control and eradication of biofilm-related infections.

Therefore, in the view of above, the objective of current study was to develop nanomaterial based eco-friendly biodegradable and biocompatible fibrous composite nano-layers loaded with natural antimicrobial and anti-oxidants for providing polymeric nano-composite with antimicrobial property for application as antimicrobial food packaging and bio-absorbable anti-scar wound dressing to achieve accelerated wound healing.

To address these problems and achieve the viable nanomaterial based eco-friendly solutions experiments were planned, executed and tested accordingly *in vitro* and *in vivo*. The relevant subject details and results are presented in this thesis.

**Chapter 1** gives a general background of various areas covered in this work mentioning the need for further work.

**Chapter 2** deals with the development and characterization of AgNPs incorporated electrospun active antimicrobial composite nano-layer for packaging. The developed composite nano-layers inhibit the microbial degradation of packaged food

and extended its shelf-life in an eco-friendly manner. The fibrous nano-layers can release the active constituents and show antimicrobial activity. The nano-layered packed meat displayed extended shelf-life by one week with better organoleptic quality. The biodegradability of composite packaging makes it a suitable replacement for plastic packaging film.

**Chapter 3** focuses on the development of composite biomimetic, bio-absorbable, nanofibrous wound dressing material loaded with curcumin and cerium ion as anti-oxidants to help in anti-scar wound healing by protecting the injured tissues from the reactive oxygen species (ROS) and evaluation of its efficacy for wound healing using animal subjects (Wistar rats). New dressing material has the potential to prevent microbial infiltration, reduce moisture and gaseous exchange rates, and provide high surface area with a microporous skeletal framework for rapid cell proliferation and granulation. It is hemocompatible, devoid of cytotoxicity, and gets bio-absorbed avoiding need for removal and resultant discomfort to the patient.

**Chapter 4** is devoted to the problems associated with biofilm formation such as iatrogenic infections and infection at the open wounded site. The biofilm formation has been shown to depend on the surface characteristics of implanted biomaterials. A novel Eudragit RL100 encapsulated gentamicin sulfate (E-G-S) nanoparticle-mediated drug delivery system has been developed for effective eradication of biofilm-associated infections in an economical manner. The system was found 10-20 times more effective against biofilm-related infections. It has been shown that Eudragit RL100 nanoparticle-mediated drug delivery system provides a promising way to reduce the cost of treatment.

**Chapter-5** presents a summary of the major the results and enlists some suggestions for future work.

Appendix included at the end of the thesis incorporates the publications resulting out of the work presented in this thesis.

# Graphical Abstract

