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
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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.

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to

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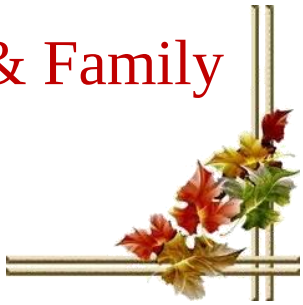

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Date: 23-10-2020

Place: Varanasi ***(Vivek Kumar Pandey)***

Contents

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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
λ_{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 (5μm 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