Development of Polymeric Nanomedicine for Accelerating Tissue Regeneration and Biological Activity Assessment of Antimicrobial Components

Thesis submitted in partial fulfilment

for the Award of Degree

Doctor of Philosophy

By

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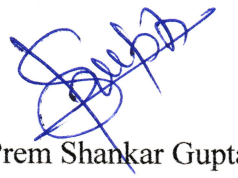

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List of Figures

List of Tables

List of Abbreviations

Ag_2O/Ag NPs or Ag NPs	Silver Nanoparticles
AIBN	A, A′-Azoisobutyronitrile
ANOVA	Analysis Of Variance
AO	Acridine Orange
APTT	Activated Partial Thromboplastin Time
Ar	Argon
ATP	Adenosine Triphosphate
Au NPs	Gold Nanoparticles
BET analysis	Brunauer–Emmett–Teller Analysis
BG	Bioactive Glasses
CAM assay or CEMA	Chicken Embryo Angiogenesis Assay
CDR (%) or %CDR	Cumulative Drug Release
CeO_2 NPs	Cerium Oxide Nanoparticles
CNT	Carbon Nanotube
CO	Carbon Monoxide
CO_2	Carbon Dioxide
CRP	C-Reactive Protein
CuO NPs or Cu NPs	Copper Nanoparticles
DA	Developmental Area
DAMP	Damage-Associated Molecular Patterns
DEPC treated water	Diethyl Pyrocarbonate Treated Water
DFU	Diabetic Foot Ulcers
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium Culture Media
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DVB	Divinylbenzene
ECM	Extracellular Matrix
EDTA	Ethylenediaminetetraacetic Acid
EE%	Entrapment Efficiency
EGF	Epidermal Growth Factor
ELISA	Enzyme-Linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
FBS	Fetal Bovine Serum
Fe NPs	Iron Nanoparticles
FTIR	Fourier-Transform Infrared Spectroscopy
GO	Graphene Oxide
GO NPs	Graphene Oxide Nanoparticles
GTP	Guanosine 5-Triphosphate
H&E	Haematoxylin And Ethyl Eosin
H_2	Molecular Hydrogen
HBOT	Hyperbaric Oxygen Therapy
HD	Hexadecane
HDPs	Host Defence Peptides
He	Helium
HUVEC	Human Umbilical Vein Endothelial Cells

IGF-1	Insulin-Like Growth Factor 1
IL-10	Interleukin 10
IL-1β	Interleukin 1 *Beta*
IL-6	Interleukin 6
IL-8	Interleukin 8
KBr	Potassium Bromide
KOH	Potassium Hydroxide
LC%	Loading Capacity
M1	Pro-Inflammatory Macrophage
M2	Anti-Inflammatory Macrophage
MCP-1	Monocyte Chemoattractant Protein-1
MMPs	Matrix Metalloproteinase
MONPs	Metal oxide nanoparticles
MOs	Metal Oxide/ Metal Oxides
MTT	3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2H-Tetrazolium Bromide
NAG	N-Acryloyl Glycine
NLCs	Nanostructured Lipid Carriers
NMR	Nuclear Magnetic Resonance Spectroscopy
NO	Nitric Oxide
NORM	Nitric Oxide Releasing Molecules
NP/ NPs	Nanoparticle/Nanoparticles
NWCC	National Wound Care Center
O_2	Oxygen
O_3	Ozone
PAMPs	Pathogen-Associated Modifying Proteins
PBS	Phosphate-Buffered Saline
PCL	Polycaprolactone
PDII	Primary Dermal Irritation Index
PEG	Polyethylene Glycol
PEO	Polyethylene Oxide
PI	Propidium Iodide
PLA	Polylactic Acid
PLGA	Poly-Lactic-Co-Glycolic Acid
PNAG	Poly(N-Acryloyl Glycine)
PNAG NPs	Poly(N-Acryloyl Glycine) Nanoparticles
PT	Prothrombin Time
PU	Polyurethane
PVA	Polyvinyl Alcohol
RBCs	Erythrocytes Or Red Blood Cells
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
sGC	Soluble Guanylate Cyclase
Si NPs	Silicon Oxide/ Silica Nanoparticles
SLN	Solid Lipid Nanoparticles

SnO_2 NPs	Tin Nanoparticles
SNP	Sodium Nitroprusside
SO_2	Sulphur Dioxide
SP NPs	Nitric Oxide Releasing Nanoparticles/ Sodium Nitroprusside Loaded PNAG Nps
sqRT-PCR	Semi-Quantitative Reverse Transcription and Polymerase Chain Reaction
TE	Tissue Engineering
TEM or HRTEM	High-Resolution Transmission Electron Microscopy
TGF-β	Transforming Growth Factor-Beta
TiO_2 NPs	Titanium Oxide Nanoparticles
TMB	Tetramethyl Benzidine
TNFα	Tumour Necrosis Factor Alpha
USD	United States Dollars
USP	United States Pharmacopoeia
UV	Ultra Violet Light/ Rays
UV-Vis	Ultraviolet-Visible
VDM	Vascular Density Measurement
VEGF	Vascular Endothelial Growth Factor
VLDM	Vasculature Length Density Measurement
WH	Wound Healing
WHO	World Health Organization
XRD	X-Ray Diffraction Analysis
ZnO NPs	Zinc Oxide Nanoparticles

Preface

The nexus of biopolymers, nanomedicine, and tissue engineering invites us to enter a world where the urgency of healing and the artistic quality of study collide in the always changing field of science. The pages that follow describe a story of creativity, accuracy, and the unwavering research for answers in the fields of wound healing and regenerative nanomedicine as we set out on our intellectual research journey.

Chapter 1: Introduction and background

We begin by delving into the complexities of biopolymers and investigating the exciting field of polymer nanoparticles, nanocapsules, and nanomedicines. Uncovering the many uses of nanoscale particles in tissue engineering, gas transport, nanomedicine, and immunological factors, the canvas widens to show a comprehensive analysis of the body of current work. This chapter lays out the background, highlighting key areas of unmet research need and developing goals to drive our search for solutions.

Objectives of this Dissertation:

Objective I: Synthesis of biopolymeric nanoparticles for enhanced skin regeneration.

Objective II: Wound healing activity potentiation of PNAG NPs for enhanced skin regeneration.

Objective III: Evaluation of biological activity of metal oxide antimicrobial nanoparticles for complex and bacteria infected wounds.

Chapter 2: Materials and Experimental Procedures

This chapter delves into the fundamental components of our study: the substances and procedures that gave rise to the unique biopolymeric nanoparticles. We show off the painstaking workmanship that goes into every step of our research process, from synthesis to characterisation, drug loading to release capabilities, and in vitro to in vivo investigations. Nitric oxide (NO) delivery research is already underway, providing a bright light for improved skin regeneration.

Chapter 3: Results and Discussions

Our laborious experiments are indicated by the complex brushstrokes in Chapter 3. A component of our research goals is revealed in each subchapter, ranging from the creation and testing of PNAG NPs for skin regeneration to the enhancement of wound healing with NO and the thorough biological activity analysis of metal oxides. A colourful display

of scientific investigation is created by the figures and discoveries that dance over these pages.

Chapter 4: The Culmination - Summary, Conclusions, and Future Scope

By the time this dissertation comes to an end, the synthesized PNAG NPs become the heroes in the story of wound healing. Their potential for topical applications in regenerative nanomedicine is indicated by their capacity to promote angiogenesis, decrease inflammation, and accelerate healing rates *in vivo*. The partnership with NO, included in SP NPs, demonstrates synergistic benefits that outperform conventional therapies, opening the door for adaptable formulations that may be used in a variety of wound types.

Our adventure doesn't finish here, though. Metal oxides' biological activities reveal them as possible partners in the battle against microbial contaminated wound and diseases. These nanoparticles hold great potential for medicinal applications, cell compatibility, and antibacterial activities, opening up new research directions.

The Future Awaits

This dissertation serves as both a capstone and an inspiration for future endeavors. Researchers are encouraged to push the frontiers further by means of sophisticated delivery technologies, clinical trials, optimization tactics, and thorough mechanistic investigations. Successful regenerative nanomedicine incorporation into clinical practice requires smart technology integration, teamwork, and public awareness campaigns.

We establish not only a basis in these pages, but also a vision that inspires, presents opportunities, and drives the search for revolutionary breakthroughs in wound care and therapeutic technology. This is a call to action for the scientific community to work together to shape the direction of regenerative nanomedicine, not simply a dissertation. The trip never ends, and there are countless opportunities.

Table of Contents

Abstract

Advanced wound healing research addresses severe clinical issues by developing novel treatments to optimize the process and reduce vulnerability to fatal microbial infections. Effective wound healing requires proficient orchestration between immune and biological systems. This study aimed to formulate poly(N-acryloyl glycine) nanoparticles (PNAG NPs) to enhance skin restoration. PNAG NPs, about c.a. 35 nm in diameter, were synthesized using mini-emulsion radical polymerization and incorporated into an ointment. TNF-α and IL6 levels in the PNAG nanoformulation treated group shifted from 4.10 ± 3.18 to 5.17 ± 3.27 pg mL^{-1} and 11.93 ± 2.34 pg mL^{-1} to 10.33 ± 3.31 pg mL^{-1}, respectively, indicating reduced inflammation while IGF-1 levels increased from 25.41 ± 13.56 ng mL^{-1} to 64.22 ± 16.93 ng mL^{-1}, correlating with enhanced angiogenesis and improved wound healing. PNAG NPs also facilitated coordinated cell proliferation, migration, anti-inflammatory responses, and simultaneous skin tissue regeneration without external synergistic factors. The cytocompatible and hemocompatible PNAG nanoformulation demonstrated 31% higher wound healing efficiency compared to the control group in vivo.

Further, the regenerative potential of PNAG NPs was potentiated by harnessing the regenerative power of Nitric Oxide (NO) by the loading of NO donor [Sodium nitroprusside (SNP): a well-known NO donor]. The SNP loaded PNAG NPs are termed as SP NPs and can release NO in sustained manner for more than 24 hours, crucial for wound healing. SP NPs promoted significant proliferation (~300%) and migration of L929 cells. Haemocompatibility studies in isolated rat blood components confirmed the safety of SP NPs. The SP nanoformulation showed excellent angiogenic activity, non-toxicity, and can closed wounds significantly faster (99.2 ± 1.0%) within 13.23 days than the control group (45.5 ± 3.8%) *in vivo*, demonstrating its clinical potential for tissue regeneration.

Further, the metal oxide (ZnO, ZnO-CuO, ZnO-Ag_2O, ZnO-SnO_2) antimicrobial nanoparticles had been synthesized by keeping in mind that these will be applied in the treatment of complex infected skin wounds. Given the threat of antimicrobial resistance, nanoparticles were utilized for their properties. ZnO and its metal composites, including ZnO-CuO, ZnO-Ag_2O/Ag, and ZnO-SnO_2 NPs, showed excellent antimicrobial properties against various microbes. These nanoparticles have their particle size below

10 nm, demonstrated mesoporous structures and nanocrystallinity, confirming their antibacterial activity in a dose- and time-dependent manner. Hemocompatibility assessments revealed low haemolysis, with ZnO-CuO exhibiting the lowest haemolytic nature. Cytocompatibility studies showed compatibility with L929 cells, particularly for ZnO and ZnO-SnO_2 nanocomposites. Cell migration and proliferation evaluations in scratch wound healing model highlighted the superior performance of ZnO-SnO_2, followed by ZnO, ZnO-Ag_2O, and ZnO-CuO. ZnO-CuO nanocomposite showed the highest angiogenic potential evaluated *in ovo*, suggesting its potential application in tissue regeneration through wound healing. In our work PNAG NPs demonstrated efficacy in coordination of cellular response, anti-inflammatory actions, and angiogenesis which collectively accelerating the skin regeneration. Further, sustained NO release via SP NPs exhibited remarkable proliferation and migration capabilities, proving their clinical potential and metal oxide nanocomposites, especially ZnO-CuO, showed promising antimicrobial, hemocompatible, and cell-compatible properties, making them valuable for therapeutic wound healing applications.