ANTIBACTERIAL NANOPARTICLE INTEGRATED SCAFFOLDS FOR THE MANAGEMENT OF INFECTED WOUNDS:MATERIALS, MECHANISMS AND CLINICAL PERSPECTIVES

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by
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I Bharti Soni (24/MSCBIO/12) hereby certify that the work which is being presented in this theisis entitled "Antibacterial Nanoparticle-Integrated Scaffolds for the Management of Infected Wounds: Materials, Mechanisms and Clinical Perspectives "in partial fulfillment of the requirements for the award of the Degree of Mater of Science, submitted in the Department of Biotechnology , Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Prof. Prakash Chandra.

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ANTIBACTERIAL NANOPARTICLE-INTEGRATED SCAFFOLDS FOR THE MANAGEMENT OF INFECTED WOUNDS: MATERIALS, MECHANISMS, AND CLINICAL PERSPECTIVES.

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ABSTRACT

Infected wounds are one of the most difficult and neglected problems in clinical medicine. Bacteria colonise a wound and form biofilm communities which are 100-1000 times more tolerant to antibiotics than their planktonic counterparts. Methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa and carbapenem-resistant Acinetobacter baumannii are globally distributed multidrug-resistant organisms. is a real concern. has reduced the effectiveness of traditional management of wound infections. An estimated 4.95 million deaths in 2019 were attributable to or associated with bacterial AMR (the 2022 analysis of global AMR) alone, placing this squarely in the realm of public health emergencies, not future concerns.

That's something this dissertation investigates using nanoparticle integrated scaffolds. Three types of nanoparticles (silver, zinc oxide and copper oxide) are reviewed in detail with evaluation of their synthesis methodology, physicochemical properties and antibacterial mechanisms. These materials kill bacteria simultaneously by disrupting membranes , producing reactive oxygen species , metal ion toxicity and direct penetration of biofilms . This is a multi-target approach which makes development of resistance much more difficult than with any single antibiotic. Embedding in hydrogel, electrospun nanofibre, three-dimensionally printed and bioceramic scaffold platforms overcome the problems of localisation, release kinetics and cytotoxicity that limit the use of free nanoparticles for open wounds.

The preclinical evidence is always promising but there is little evidence from RCTs. The three major hurdles to clinical translation include manufacturing scalability, the small therapeutic window between bactericidal and cytotoxic concentrations and an unclear regulatory pathway. These are not insurmountable challenges, but they are problems that arise in the field that have not been sufficiently solved.

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LIST OF SYMBOLS, ABBREVIATIONS

Symbol / Abbreviation	FULL FORM
AgNPs	Silver Nanoparticles
Ag	Silver Ion
AMR	Antimicrobial Resistance
AuNPs	Gold Nanoparticles
CFU	Colony-Forming Units
CuO NPs	Copper Oxide Nanoparticles
Cu^{2}	Copper(II) Ion
DFU	Diabetic Foot Ulcer
ECM	Extracellular Matrix
ROS	Reactive Oxygen Species
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
QS	Quorum Sensing
LPS	Lipopolysaccharide
PEG	Polyethylene Glycol
TEM	Transmission Electron Microscopy
E. coli	Escherichia coli
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
HA	Hydroxyapatite
CS	Chitosan
PEG	Polyethylene Glycol

CHAPTER 1
INTRODUCTION

1.1 Background and Significance

Acute and chronic wound care continues to be one of the few areas of medicine yet to be solve and understood, particularly in the context of chronic wounds and non-healing wounds on the rise in the world alongside diabetes, obesity, vascular disease and an aging population. (Sen, 2023) Skin is the largest organ in the body and a formidable barrier to the outside world and can be damaged by a multitude of different injuries, including major surgical incisions, burns, acute and chronic, such as pressure ulcers or diabetic foot ulcers and all these wounds can make the skin a target for microbial invasion. Once the bacteria have colonized the wound site, a series pathophysiological processes follow causing long term intrinsic defect healing or even worse. (Hurlow & Bowler, 2022) In some instances can affect the viability of the affected limb or patient's life.There is a considerable disease burdenThe Advances in Wound Care's Compendium of Estimates contains chronic wounds.affect almost 10.5 million Medicare beneficiaries in the U.S., and Contributing about 2.5% to the overall population (Sen, 2023).In the developing countries like India,The situation is worse proportionately because there is a limited access to advanced wound treatment.High prevalence of care products, type 2 diabetes and increasing community acquired drug resistant infections.Chronic wounds are most commonly caused by bacterial infection. (Hurlow & Bowler, 2022)

Common pathogens found in infected wounds -particularly Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Acinetobacter baumannii – have well-defined virulence mechanisms, such as biofilm formation, secretion of exotoxins and enzymatic degradation of the extracellular matrix. (Hurlow & Bowler, 2022) Bacteria that reside in the biofilm are very hard to remove. (Pang et al., 2023) These cells are able to survive in the presence of antibiotics at concentrations 100 to 1,000 times higher than the planktonic minimum inhibitory concentration (MIC) and are not easily destroyed by host immune effectors (Pang et al., 2023).In addition, the increased resistance of microorganisms, like multidrug-resistant (MDR) microorganisms, such as methicillin-resistant S. aureus (MRSA) and carbapenem-resistant P. aeruginosa (CRPA) have further narrowed the therapeutic window for conventional antibiotic therapy (Murray et al., 2022).Nanotechnology has proved to be an interesting and promising scientific and clinical strategy to treat infected wounds. (Tripathi & Goshisht, 2022) Nanoparticles are materials that have at least one dimension in the range of 1-100 nm and have unique physicochemical properties that are not observed at the bulk scale such as a high surface area to volume ratio, enhanced surface reactivity, quantum confinement effects and the ability to be functionalised with biological or pharmacological moieties (Menichetti et al., 2023) In general, metal and metal oxide nanoparticles such as silver (Ag), Zinc oxide Copper oxide (CuO) and Titanium dioxide (TiO 2) have been broadly examined in relation to their inherent and broad-

spectrum antimicrobial activity. Unlike traditional antibiotics, which typically target a single molecular site, nanoparticles have several mechanistically distinct modes of action: physical disruption of the cell membrane, production of reactive oxygen species (ROS) and release of metal ions, which makes the development of resistance difficult (Tripathi & Goshisht, 2022).The clinical translation of nanoparticles is not so easy.
Directly applied agents (dispersions and powders) may show poor retention at the wound surface or uncontrolled release of ions, which cause systemic absorption and cytotoxicity to wound keratinocytes and fibroblasts at level needed to achieve the desirable antibacterial effect. To overcome these shortfalls, scientists have turned their attention to the use of nanoparticles in 3-D scaffold systems, including hydrogels, electrospun nanofibrous membranes, bioceramics, and composite matrices which act also as physical wound barriers, moisture managing microenvironments, and controlled delivery depots for antimicrobial agents (Nqoro and Taziwa, 2024). The main focus of this dissertation is on these nanoparticle integrated scaffolds which constitute a rational convergence of materials science, nanotechnology and wound biology.

1.2 Statement of the Problem

Traditional methods of wound treatment such as the application of gauze dressings soaked with iodine or silver sulfadiazine have been used for many years but are not sufficient to meet the biofilm challenge or the problem of MDR pathogens (Hurlow & Bowler, 2022) Why systemic antibiotic therapy is less successful in the wound context is also well known; poor penetration of avascular tissue or necrotic tissue, concentration-dependent toxicity and the selection of the antibiotic on wound flora all result in suboptimally successful systemic antibiotic therapy (Tran et al., 2023) The critical gap thus is the absence of wound management materials which are able to (a) maintain a moist, physiologically supportive microenvironment, b) deliver sustained and localised antibacterial activity against aA wide variety of pathogens including biofilm-forming and antibiotic resistant, and (c)promote the sequential stages of wound healing – haemostasis, inflammation, proliferation and maturation.1. Proliferation and remodelling — and (d) demonstrate good biocompatibility with host tissueCells at wound site.The concept of using nanoparticles in scaffolds provides a multidimensional answer to this problem; however, there are several questions that have not yet been answered completely.the literature. The choice of nanoparticle type, size, surface chemistry and loadingThe concentration needs to be carefully designed so as not to go over the scaffold material,At the wound site, cytotoxic concentrations at the wound interface. The ways that variousThe antibacterial activities of nanoparticle-scaffold combinations are not consistentlyThe characteristics are determined for the different types of pathogens and biofilms. These exhibits the in vivo behaviour of these Only a limited number of studies have examined the in vivo distribution (local and systemic), biocompatibility, and leaching of nanoparticles from systems, especially with regard to long-term biocompatibility.A number of pre-clinical and clinical trials. The gaps serve as a spur to a detailed and synthesis-focused academic investigation of this field.

1.3 An Overview of the biology of wound healing.

It is helpful to have a quick review of wound healing biology prior to discussing these elements, to provide background context.The therapeutic potential of nanoparticle integrated scaffolds. WoundTypically, healing is viewed as a four-phase process that is sequential but

overlapping:Haemostasis, inflammation, proliferation and tissue remodelling. Each phase iscontrolled by a well-defined array of cellular and molecular actors and disruption of any phaseCan occur most frequently as a result of bacterial infection, ischaemia or immune dysregulation —affect the progression to the next, causing a wound that does not heal, the next becoming the last.Chronic by operational definition, this is the expected time frame.The process of haemostasis begins within seconds of the injury to the tissues by means of platelets.The following processes are affected: aggregation, formation of fibrin clots, and release of platelet derived growth factors which attract inflammatory cells to the wound. The inflammatory phase, which Usually lasts for the first 3–5 days and is marked by the successive invasion, Inflammatory cells such as neutrophils and macrophages which engulf and destroy bacteria and debris, and which produce pro-inflammatory cytokines, such as interleukin-1β (IL-1β). That amplify the local immune response are tumour necrosis factor (TNF-α), and interleukin-6 (IL-6). However, persistent bacterial colonisation keeps an inflammatory state high. The ability to inhibit the macrophage phenotype change from pro-inflammatory (M1) to anti-inflammatory (M2) repairative (M2): locking the wound during the inflammatory phase and preventing the onset of proliferative repair.This is the proliferative phase, which is normal and occurs between days 4 and 21 by migration and deposition of collagen by fibroblasts, and vascular by angiogenesis around the wound.Migration and deposition of collagen by fibroblasts, and vascular by angiogenesis around the wound.The endothelial growth factor (VEGF), and epithelial resurfacing via keratinocyte migration and proliferation. Remodelling can last for the final time for months to Over the years, as type III collagen is replaced over time by more mechanically over-strong type I collagen.The quality of type I collagen was superior and the tensile strength of the repaired tissue slowly recovered.Aids that are similar to those of unwounded skin, reaching about 80% of the normal aids strength at best.Therefore, scaffolds to be used in the management of infected wounds must be designed notnot only passive barriers, but also bioactive interfaces that inhibit infection by merely mimicking the natural barrier function.Allowing – and perhaps encouraging – each of these healing steps to happen. This dual Key design challenge to be solved is the requirement of bactericidal efficacy on one hand, and cytocompatibility and proregenerative capacity on the other.The purpose of this dissertation is to review the various types of nanoparticle-integrated scaffold systems that have been used.

Table 1.1: Summary of the four phases of wound healing and the impact of bacterial infection on each phase

Phase	**Duration (Approximate)**	**Key Cellular Events**	**Effect of Bacterial Infection**
Haemostasis	Seconds to minutes	Platelet aggregation, fibrin clot formation, growth factor release	Bacterial products (e.g., staphylokinase) can degrade fibrin clots, impairing initial barrier formation
Inflammation	Days 1–5	Neutrophil and macrophage infiltration, cytokine	Sustained bacterial load maintains M1 macrophage

		release, pathogen clearance	dominance; prevents resolution of inflammation
Proliferation	Days 4–21	Fibroblast activation, collagen deposition, angiogenesis, epithelialisation	Bacterial proteases degrade growth factors and newly deposited collagen; impair keratinocyte migration
Remodelling	Weeks to months	Collagen maturation (type III → type I), scar formation	Chronic low-grade infection disrupts collagen cross-linking, producing mechanically weak scar tissue

Source: Synthesised from Pang et al. (2023) and Alven et al. (2021)

1.4 Rationale for the use of nanotechnology-Based Approaches in Wound Infection Care.

Scientific arguments underlie the application of nanotechnology approaches for wound.
Infection ManagementA driver for the use of nanotechnology for wound care is:
Combination of properties which are only applicable in the nanoscale. The high
The surface area to volume of nanoparticles is about an order of magnitude larger than that.
A larger fraction of the atoms of the material have aExposed to the surface and chemically active. For the antibacterial nanoparticles,This translates to improved interaction with the surfaces of bacteria, improved efficiency.The ion release and generation of more ROS per mass of than would be possible using.bulk materials.Also, metal nanoparticles have a bactericidal action, which is unique to them, involving multiple targets.Unlike traditional antibiotics, which are of a physical/chemical class. SilverHowever, nanoparticles, for instance, not only damage the integrity of the membranes but also disrupt it by other means as well.When in contact with the membrane, release silver ions (Ag+) which inhibit the respiratory chainThey are involved in functions such as enzymes, DNA replication, and ribosome function, and produce ROS which causeThe degradation of bacterial lipids, proteins and nucleic acids (Pang et al., 2023).
This is because these mechanisms target different systems of bacteria and they occur simultaneously.simultaneously low at the same time one mutational event that will confer resistance has a low probability.Much lower than in the case of a monovalent antibiotic. This multi-hit antibacterialWith MDR pathogens of wounds, it is particularly relevant to adopt a strategy.The embedding of such nanoparticles into polymer based materials or bioceramic materials, respectively, is still continuing.scaffolds add to their clinical utility as well. Scaffolds provide a scaffolding matrix – namely localises the nanoparticles at the wound interface, controls the rate of ion or particlerelease; maintains a moist environment, so healing cells can function, and canbe designed to be degraded in situ during tissue repair, which does not require:dressing removal. New developments in materials engineering, such as stimuliresponsive hydrogels that emit antibacterial

agents when exposed to bacterial activity, are coming to the fore.The paper included an electrospun nanofibrous membrane, which mimics the exterior shape of the extracellular matrix, and infection-associated pH changes.Get structurally organized in the native extracellular matrix and three dimensionallyToday, printed scaffolds are more commonly able to match patient-specific geometries – have greatly expanded the possibilities.
There is a design space for nanoparticle-scaffold systems (Nqoro and Taziwa, 2024).

1.5 Organisation of the Dissertation

The Dissertation will be structured in the following way:
This dissertation has seven chapters. The following chapter will set the clinical and scientific framework, formulated the problem statement, and describe the goals and extent of the work.

Chapter 2 gives a detailed literature review on wounds.Each of these issues has been addressed in detail, and there are various limitations of the available evidence.Topical antiseptics, antibiotic impregnated dressings and systemic treatment are conventional wound management techniques. If you're interested in particular attention is given to youThe clinical relevance of the formation of biofilm and antimicrobial resistance in wound pathogens.

Chapter 3 discusses the key classes of antibacterial nanoparticles.The material is applied to wound scaffold systems with the following oxides: silver, zinc oxide, copper oxide and titanium. synthesis methods (chemical and physical, green synthesis) on the physicochemical characteristics of the species; physicochemical characterisation techniques; influence of the physicochemical properties of the species on its applications.The effect ofparticle size, shape and surface chemistry on antibacterial activity.

Chapter 4 summarizes the key platforms available for incorporating antibacterial agents.For wound applications, nanoparticles have been developed. These include hydrogels (natural and synthetic as well as), starches (starch, amylose, dextrin), and proteins (gelatin, casein, or collagen).3D printed structures, electrospun nanofibrous membranes and hybrid composite systems. This involves fabrication methods, mechanical characterisation,The wound-relevant functional properties are discussed in detail.

Chapter 5 is dedicated to the mechanistic studies of the functioning of nanoparticle-scaffoldcomposites exert their antibacterial effects: direct membrane disruption, reactive
disruption of bacterial surface structures, e.g., oxygen species generation, metal ion toxicity, interference with biofilm formation are key factors.maturation, with interactions between scaffold and incorporated antibiotics.bioactive compounds. Any resistance mechanism that may reduce effectiveness are also addressed.

Chapter 6 covers the clinical and translational aspects of nanoparticle integrated scaffolds: preliminary animal model results, early stage clinical trials.Regulatory issues, and the major challenges to the broad clinical use —Due to its cytotoxicity concerns, ease of manufacture, and lack of, this type of device is not considered for this project.standardised testing protocols.

Chapter 7 gives the overall findings of the dissertation, a discussion of Future research needs and meditation on the social and health implications ofAdvancements in technologies for management of infected wounds, particularly in LICS environments, such

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

An in-depth knowledge of how infected wounds work.The constraints of existing treatment, and the influence of microbiological character of wound pathogens. It is necessary to develop new modalities and the introduction of new technologies based on nanotechnology beforeExamining individual systems of nanoparticles and scaffolds. This chapter synthesisesThe literature available, peer-reviewed in these interrelated fields, was reviewed.publications since 2018 in underpinning work where.History should be taken into account in the mechanistic grounding.
All studies available are analysed, except for what is necessary to reveal conceptual lines and empirical results that Explain the need for development and testing of Antibacterial nanotechnology-integrated.Now scaffolds are known as a specific therapeutic group and are breakable at Multiple Steps.

2.2.1 The Four-Phase Model

In adult human beings, wound repair is a process which occurs in four temporally overlapping stages.The stages of the healing process are haemostasis, inflammation, proliferation and remodelling. This framework,process that is normally drawn as a linear sequence, but actually is continuous, cellular andmolecules events with boundaries more related to the predominant cell populations.More than by clear-cut temporal divisions, the main biochemical activity. Disruption at anyMost commonly, due to continuing bacterial infection, hypoxia or systemic metabolic dysfunction, can prevent further progression to subsequent phases and create.The chronic non-healing wounds are characterized by a phenotype (Rodrigues et al., 2019; Pastar et al.,2014).
An intact blood system is essential to maintaining haemostasis, which starts as soon as the tissues are damaged, and involves platelets.A sequence of events in which exposed subendothelial collagen, platelet aggregation, and activation of the coagulation pathway are all involved in the process.Activation of coagulation cascade, formation of a provisional matrix of fibres (fibrin).
This matrix also acts as a mechanical plug to prevent haemorrhaging, as well as a biological plug to control the blood loss.The purpose of this matrix is not only to prevent haemorrhage mechanically, but also to act as a biological seal and prevent haemorrhage.

Also as a scaffold for the subsequent inflammatory infiltrate and as a reservoir for growth
Platelet-derived growth factor (PDGF) are some of the factors released by activated platelets.
Including transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF).VEGF and epidermal growth factor (EGF) (Pastar et al., 2014). Bacterial products
There are some medicines which can break down the fibrin clots, e.g. staphylokinase, which is produced by staphylococcus aureus (the bacteria that cause skin infections).
By activating plasminogen and thus interfering with the provisional matrix at this
The first stage of restoration (Tran et al., 2023). The inflammatory phase which lasts for days 1 to 5,

The process of infiltration is neutrophils followed bymacrophages. The neutrophils, which are the first cells to arrive, after just a few hours, surround and engulf bacteria.and cellular debris and activate a host of antimicrobial mechanisms such as theThe oxidative burst, the production of neutrophil extracellular traps (NETs) and the degranulation of proteases.for example, matrix metalloproteinase-8 (MMP-8) and elastase. Macrophages, which The predominant phenotype is that of M1 cells, which are essentially in a biphasic functional state: early pro-inflammatory phenotype which activates killing mechanisms and production of cytokines.The latter M2 phenotype is reparative, and results in resolution (TNF-α, IL-1β, IL-6).Inflammation, secretion of growth factors and transition to proliferative phase(Rodrigues et al., 2019; Stojadinovic et al., 2024). This macrophage phenotype Persistent infection keeps M1 alive, transition is very sensitive to bacterial load.Chronically upregulates pro-inflammatory mediators and stops the anti-inflammatory/regenerative signals of M2 polarisation from taking over.
traction.Cells start to multiply at a faster rate in the proliferative phase, usually days 4-32.
Day 21 focuses on fibroblast migration and activation and collagen synthesis.
and deposition, re-epithelialisation and angiogenesis. Fibroblasts, which are recruited to the
PDGF and TGF-β activate wound beds to become contractile myofibroblasts which
Physically decrease wound area and deposit collagen rich granulation tissuematrix. Angiogenesis is stimulated by the secretion of VEGF from hypoxic wound.Supplies blood to epidermis to maintain life of epidermis, macrophages and keratinocytes.
Faster growing cells that are metabolizing fast. Re-epithelialisation takes place in the following manner The migration and proliferation of keratinocytes from the margins of the wound and adnexa. EGF and keratinocyte growth factor (KGF) have been shown to influence the creation of structures, as do integrin interaction with provisional matrix (Pastar et al., 2014). Bacterial
Proteases, particularly from Pseudomonas aeruginosa directly degrade
This leads to the production of KGF, VEGF and nascent collagen, all of which have an impact on these three arms.proliferative response simultaneously.Remodelling phase which may last for months or years, which includes theSubstitution of Type III collagen which is deposited during proliferation with Type I collagen.Enzymatic cross linking of type I collagen, deposition of Type I collagen into matrix.Two processes are implicated as a result of maturation of the collagen network: enzymatic cross-linking of Type I collagen, and deposition of Type I collagen into the matrix.Cross-linking, vascular regression and apoptosis of myofibroblasts. The tensile
The strength of the healed tissue is approximately 70-80% of the strength of the unwounded tissue.and even this moderate improvement, is likely to be jeopardized by the presence of remaining and/or recurrentRodrigues et al. (2019) found that there is a link between infection and asthma.

2.2.2 Factors that predispose to chronic wound formation

Chronic wounds are wounds that have not progressed through the normal healing stages, operationally defined.normal healing sequence within 3 months, from interaction of systemic, arises.Local wound microenvironment conditions and host factors. Diabetes mellitus is a condition ofThe single most important systemic predisposing factor worldwide. Hyperglycaemia impairsdecreases neutrophil chemotaxis and phagocytosis, decreases macrophage response
Resists to the action of bacteria and stops the development of new blood vessels by advanced glycation end product Downregulates VEGF expression (AGE) and disrupts peripheral perfusion.

In micro- and macrovascular disease (Sen, 2023). The wound conditions are hypoxic, poorly immunocompetent and low in nutrients which allows forInfective conditions for bacteria colonization and the formation of biofilm.Diabetic foot ulceration (DFU) is the most common complication of diabetes, and is a significant global problem.It has been estimated at 6.3% among the adult population with the classic chronic infected wound (CIIW).people living with diabetes (around 40-60 million people).Likewise, the number of people with diabetes worldwide is estimated at the current prevalence of diabetes at the global level (Sen, 2023). In India, where theThe second largest population in the world is the one suffering from diabetes, about 77 million.The incidence and prevalence of DFU and associated limb-threatening infection among adults is significant,Further complicated by lack of specialist wound care in rural and peri-urban areas.settings. Venous leg ulcers, diabetic foot ulcers, and pressure, or bed-bed sore, ulcers are other clinically impacting types of chronic wounds.

2.3 The Microbiology of infected wounds.

The main difference between bacterial colonization and infection is the presence or absence of clinical symptoms.Although all open wounds are colonised to a certain extent, only a few wounds are colonised.There are clinical states of and infection. The name colonisation is used for a condition where the presence ofInfection of bacteria that cause no host response; critical colonisation or local infection; and/or Outlines condition where bacterial numbers are adequate to not allow healing to proceed as it would without the bacteria's presence.These do not necessarily indicate a systemic infection; and in order to be considered an infection, there must be tissue invasion and evidence of hostinvasion.inflammatory response. The 10^5 colony-forming units per gram of tissueHistorically, wound closures have been reported as effective at ($>10^5$ CFU/g), and ineffective at ($\leq 10^5$ CFU/g).becomes less likely but the virulence of the infecting organism and the host.This threshold is greatly affected by immune state (Hurlow and Bowler, 2022).

2.3.2 Principal Wound Pathogens

S. aureus is the most common isolate from bothIn all geographic regions infected wounds of acute and chronic origin. Its virulence arsenalAdhesins and surface proteins: well characterised and extensive, fibronectin binding proteins.collagen-binding proteins are used to attach to wound tissue, protein A inhibits opsonophagocytosis, toxins (such as Panton-Valentine leukocidin (PVL), alpha-toxin and exfoliative toxins) damage host cells, and polysaccharide intercellular.

The ica operon encodes adhesin (PIA), which is responsible for the biofilm formation. Methicillin-resistant S. aureus (MRSA) is a form of S. aureus that has the low-affinity mecA gene.Of particular clinical concern is the presence of penicillin-binding protein PBP2a in infected wounds.settings. MRSA has been acquiring its characteristics since its characterisation in the 1960s.Infections are becoming more resistant to more than one type of antibiotic, and now it's a leading reason for wound infections.Mortality due to infections (Ramadan et al., 2024).

P. aeruginosa is the second most clinically significant wound inhabiting a bacterium is seen in common wound complications like burn wounds, diabetic foot ulcers and more.

Hospitalization in the intensive care unit (ICU), in addition to a wound infection, that develops into VAP. Itsintrinsic resistance to a number of antibiotic classes including many beta-lactam classes,The above agents are mediated by the constitutive overexpression of These increases are

caused by an increase in and a decrease in outer membrane permeability (OprM - OprJ).This increase is due to efflux pumps (MexAB-OprM, MexCD-OprJ), decreased outer membrane permeability (OprM-OprJ) and inducible AmpC beta-lactamase. The percentage of multidrug resistance (MDR)In Tertiary Care, the occurrence of P. aeruginosa wound isolate has been reported asThese high rates were also reported in some hospital cohorts in Africa (65.8%).It has been reported in Asia (Chimi et al., 2024). P. aeruginosa also forms large amounts of biofilms producer: extracellular polysaccharides (alginate, Psl, Pel) are secreted by multiple.
Other clinically important wound pathogens are Escherichia coli and Pseudomonas spp.
Extended-spectrum beta-lactamase (ESBL) is possible in both Klebsiella pneumoniae (KP) and Pseudomonas aeruginosa (PA). production; Acinetobacter baumannii, which is becoming more and more resistant to carbapenems; and Deep or necrotic wounds with anaerobes (e.g. Bacteroides fragilis, Clostridium perfringens).Enterobacteriaceae (usually secondary pathogens in wounds) and Enterococcus species (usually secondary invaders in wounds).polymicrobial infections. The multi-microbial status of chronic wounds is a Commodities: interaction of bacteria in communities in the biofilm can increase antibiotic tolerance over that of single-species biofilms, can resistHorizontally transmit resistance genes; co-operate in evading host immune system.Are better at working together than as separate species (Hurlow and Bowler, 2022).

Table 2.1: Key wound pathogens, their virulence mechanisms, and clinically relevant resistance profiles

Pathogen	**Key Virulence Factors**	**Primary Resistance Mechanisms**	**Clinical Significance**
Staphylococcus aureus / MRSA	PIA biofilm, alphatoxin, PVL, fibronectin-binding proteins	mecA-encoded PBP2a; horizontal resistance gene transfer within biofilm	Most common wound isolate; MRSA associated with treatment failure and mortality
Pseudomonas aeruginosa	Alginate/Psl/Pel biofilm matrix, elastase, alkaline protease, pyocyanin	Efflux pumps (MexAB-OprM), outer membrane impermeability, AmpC beta-lactamase	High MDR prevalence; degrades wound growth factors; biofilm protects from antibiotics
Klebsiella pneumoniae	Capsular polysaccharide, siderophores, fimbriae	ESBL and KPC carbapenemase production	Increasingly drugresistant; associated with post-surgical wound infections
Acinetobacter baumannii	Biofilm formation, lipopolysaccharide, outer membrane proteins	Carbapenem resistance (OXA carbapenemases, efflux pumps)	Hospital-acquired wound infections; pan-drug-resistant strains emerging
Escherichia coli	Type 1 fimbriae, haemolysin, capsule	ESBL production, fluoroquinolone resistance	Common in polymicrobial

			chronic wounds, particularly DFU

Source: Synthesised from Hurlow and Bowler (2022), Chimi et al. (2024), and Ramadan et al. (2024).

2.3.3 Biofilm: The Defining Characteristics of Chronic Wound Infection.

Bacterial in chronic wound is mainly present in the form of biofilm are injected with an antibiotic and are the main cause for the refractoriness of these wounds.conventional antibacterial treatment. A biofilm is an organized group of bacteria.it is surrounded by an EPS matrix, which was produced by it is attached to the surface (in wounds, the wound bed tissue or devitalised) The stages of the biofilm lifecycle are initial reversible attachment, irreversible attachment, microcolony formation, and maturation.The four stages of adhesion mediated by surface adhesins, microcolony formation, biofilm maturation to a 3D structure with nutrient and oxygen gradients and dispersal.Of planktonic cells which re-start the cycle or spread infection.

Bacteria in mature biofilms are tolerant of antibiotics at concentrations 100-1000 times the MIC determined with planktonic organisms of the same species (Tran et al., 2023). There are several mechanisms of tolerance: formation of the EPS matrix, a physical and chemical barrier which blocks the entry of antibiotics and deactivates them.Metabolically dormant persister cell subpopulations within theNutrient and oxygen gradients result in biofilm resist concentration dependent killing.microenvironmental niches where growth-dependent antibiotic targets (such as the ribosome) are found.The expression of genes (such as ribosome, cell wall synthesis, and DNA gyrase) is turned off; and phenotypic variation Produces cells that have different resistance patterns against antibiotics. In Biofilms of S. aureus and P. aeruginosa, that are found together in many cases of acute pneumonia, are polymicrobial from chronic wounds, interaction between the two species has been shown to augmentspecies or both.Tobramycin resistance not seen in any single-species biofilms of either species or both.organism (Hurlow and Bowler, 2022).In addition, Biofilm infection has a profound impact on wound immune response.The EPS matrix obscures bacterial surface antigens to pattern recognition receptors,Which is a blockage in the proper recognition of the innate immune system. Biofilm-associated P. aeruginosa produces.

Biofilm disrupting rhamnolipids selectively lyse neutrophils, which are attempting to phagocytose biofilm.LPS; LPS induces protein A on S. aureus; reduces opsonophagocytosis by Fcγ receptor; and

Proteases are produced by the biofilm that breaks down complement components, and immunoglobulins.These were deposited within the matrix. The net result is a permanently swamped but ineffective chronic inflammatory state that is constantly damaging host tissue without elimination of the bacteriaPathological loop of chronic infected wound, clearance.

(2.4).There are limitations to conventional Wound Management Strategies.

2.4.1 Systemic Antibiotic Therapy

Systemic antibiotics continue to be the basis for treatment of wound infections where clinical signs of invasion of tissues are observed (cellulitis, fasciitis, osteomyelitis), andHowever, their

effectiveness at the wound bed is strongly curtailed as a result of the poor vascularity that characterizes chronic wounds. When the blood supply is limited in diabetic and venous, this leads to lower perfusion.ulcers implies that even with sufficient plasma anti-bacterial levels, this does not necessarily mean that the plasma concentration is sufficient.Therapeutic levels of wound tissue concentrations. In addition, systemic antibiotics have a broad spectrum of activity.
mutant population, which can then outcompete normal cells.The mutant population can be selected for resistant subpopulations, which can outcompete normal cells, causing the effects on the host microbiome.Are said to have association with systemic toxicity - nephrotoxicity with — wound flora.aminoglycosides, hepatotoxicity from some beta-lactams, and QT prolongation from some beta-lactams.That makes elderly patients with a complicating factor to manage, as they are more susceptible to side effects from fluoroquinolones.Comorbidities (Wangoye et al., 2022). The impact of bacterial antimicrobial resistance on the world is extremely high.In 2019, resistance (AMR) was estimated at 4.95 million deaths due to or related to AMR.with bacterial AMR, is a sign of unsustainable antibiotic-based infection control.The management practices used (Murray et al., 2022).Apply a topical antiseptic or a dressing containing silver.
An antiseptic should be applied to the skin – povidone-iodine, chlorhexidine, sodium hypochlorite.Not active against fungi or yeast, and
high doses, but not at low doses.High doses, low doses have no significant cytotoxicity to keratinocytes and fibroblasts.The level of concentrations needed for bactericidal effectiveness. Silver sulfadiazine (SSD)Since they were all considered standard care for infected burns, cream was introduced in 1968 and long accepted as a best and acceptable treatment. wounds, has been shown to release extremely high initial concentrations of silver (up RPD, or rapid drop, wherein CO_2 levels drop quickly below therapeutic levels (to 3,176 ppm),A pharmacokinetic profile which brings toxic silver to the host cells at once.It is not a problem when concentrations are not maintained and bactericidal levels are not sustained (Carter et al.,2020). Subsequent systematic reviews and meta-analyses found that nanocrystalline No benefit was seen with silver containing dressings over simple gauze dressings for non-infected chronic wounds and in some, delayed epithelialisation was seen in association with the use of the silver containing dressings.trials (Carter et al., 2020).There is an improvement on SSD in the modern commercial silver dressings which are now:Adding nanocrystalline silver or ionic silver to hydrocolloid, alginate or foam
matrices, which offer controlled release effects. Even these second generation products are mostly passive delivery systems that are not actively responding to the environment, however.
Do not recreate the structural scaffold that is needed for tissue Don't grow into the wound or offer any mechanical support to the wound bed. They have a limited capability to penetrate preexisting biofilms and their selection of silver tolerant organisms.bacterial subpopulations after long term usage further limit their use in Chronic wounds that are heavily colonised (Hamdan et al., 2024)

2.4.3 The multifunctional wound management platforms

The sum of the evidence of conventional wound management is such thatshow that no single solution exists to a clinically difficult issue, in this case, infected chronic wounds.one-agent/single-mechanism solution. The need is for a multifunctionalEvaluate the ability to generate a sustained, localised antimicrobial activity against a low number of targets.the risk of resistance selection and (2) maintaining a moist microenvironment at the wound.which is conducive to cellular migration and proliferation, (3) mechanically supporting woundcontraction and new tissue formation, (4) adapting to the changing biochemicalThe characteristics of the wound over time (such as the

decrease of pH levels and enzyme activity)(1) have an affinity for the plasma membrane, (2) show rapid penetration into the cytoplasm, (3) are recognized and internalized by the cells (as infection progresses), and (5) are compatible with the keratinocytes, fibroblasts, and other cells.and endothelial cells which initiate the repair process.

2.5 Nanotechnology in Wound Care

2.5.1 Characteristics of Nanomaterials for the Management of Wound Infections

Nanoparticle-integrated scaffolds are becoming the most fully-studied class of material developed fortackle this multi-faceted therapeutic need.This product features a unique blend of beneficial properties, including:This product contains a unique combination of beneficial properties:The nanotech industry has many exciting applications in wound infection management. The properties of nanomaterials relevant to this area will be discussed.

Nanomaterials are defined here as materials with at least one dimension in the nanometer range (1 to 1000 nm).This is because the dimension of 1 to 100 nm lies in a range where the bulk physical laws do not hold true.A way to quantum mechanical and surface dominated behaviours. Three nanoscale There are a number of properties that are directly relevant to antibacterial wound applications, such as (a) high surface area and (b) very large pore volume.The increase of area-to-volume ratio increases the chemical reactivity, ion release and bacterial proliferation.

Contact efficiency per unit mass of material; (b) size-dependent interactions with In biological membranes, the particles (usually 1–20 nm) are biocompatible and enter cells either as a result of diffusion across the membrane or through active transport.In bacteria, the lipids of the cell membrane are the site of disruption of the membrane by (for metal nanoparticles) and are the target for the disruption.mechanical and electrostatic mechanisms not available to bulk materials are not available.(a) Soluble ions; and (c) Surface chemistry, to which it is possible to conjugate soluble molecules and/or biomolecules.Developing functional groups at the surface of the nanoparticle which bind specifically to the target ligand, stabilise the polymer, provide other antimicrobial agents, or contain growth factor binding domains without affecting the core

(Modi et al., 2023) reported antibacterial abilities.Traditional antibiotics usually work by one of two mechanisms, while this one works in multiple ways.Unlike traditional antibiotics, which normally function via a single molecular target susceptible to mutational inactivation, metal and metal oxide nanoparticles act At the same time, via membrane disruption, reactive oxygen species (ROS).Release of metals, direct damage to DNA and in some instances, in generation. This mechanistic The idea of multiplicity implies that the chance of a single spontaneous mutation giving rise to a resistance to any one single drug is very low.resistance to all operative mechanisms at once, thus making the chances of resistance very high.Selecting mutants that are more resistant to nanoparticles significantly reduced compared toWhile not insignificant (Modi et al., 2023; Pang et al., 2023), these conventional antibiotics are still significant.

2.5.2 Silver Nanoparticles: The Most Widely Studied System

By far the most popular nanoparticles are silver nanoparticles (AgNPs).Thoroughly tested an antibacterial nanomaterial as a wound care product. TheThere are at least three parallel pathways by which bactericidal mechanisms of AgNPs take place.(i) direct disruption of bacterial membrane integrity by direct interaction with nanoparticles,The membrane contact which is particularly effective for particles smaller than 20 nm that can't fit through the pore size of the membrane, andcan get through the outer membrane of Gram-negative bacteria and enter into the
Detection of Ag^{+} in the cytoplasmic membrane of Gram-positive organisms.ions, which
Interact with thiols of enzymes of the respiratory chain (e.g., NADH dehydrogenase);inhibit ATPsynthesis, and interfere with DNA replication by binding to purine and Intracellular ROS (superoxide ($O_2^{\cdot -}$) and (iii) pyrimidine bases); and (iv) pyrimidine bases.Hydrogen peroxide(H_2O_2) and hydroxyl radical ($^{\cdot}OH$) which are oxidising agents.The proteins and nucleic acids (Pang et al., 2023; Alven et al., 2023) are targets in the bacterial cell membrane. Anti-bacterial activity of AgNPs is crucial and depends on the particle size,Shape, surface coating. In general, spherical particles that have a diameter of 5 to 20 nm have the following properties:
Have the highest antibacterial activity per mass because of increased surface area,Better permeability of the membranes. Triangular AgNPs have been reported to be:More effective than spherical or rod shaped particles of the same size, perhaps because of density of the silver atoms on the tips of the triangular structure. The surface is modified by polyethylene glycol (PEG), chitosan, or polydopamine change.Alters the behaviour of agglomeration of biological fluids, controls the ion release kinetics and can alter the toxicity towards bacteria and mammalian cells.
cells (Aldakheel et al., 2023).

2.5.3 Zinc Oxide, Copper Oxide and Titanium Dioxide Nanoparticles

A significant amount of research focused on zinc oxide nanoparticles (ZnO NPs) has been conducted because of their potential.Because of their low cost, high photostability and intrinsic characteristics, an alternative to AgNPs was found.Appropriate concentrations of biocompatibility. The ZnO NPs are found to be antibacterial.mainly via surface ROS production (mainly production of H_2O_2 and Superoxide), and The membrane disrupting property is due to electrostatic interactions with the negative charges on the membrane. Bacterial surface, release of Zn^{2+} ions to inhibit bacterial enzyme function (Nqoro)and Taziwa, 2024). They are more active in the presence of UV or near visible light.The photocatalytic ROS generation and irradiation were effective only in a meaningful way for the antibacterial effect.They have also been shown to have effects in the dark—clinically—so that is another factor.Another factor is that they can have effects even in the dark period; there's evidence of that.Active even without the need for light activation. Comparative studies have revealed that:The antibacterial effects of the dressings containing ZnO NP against Gram-positive bacteria is higher.AgNPs are generally more effective at inhibiting the growth of organisms (especially S. aureus) than are the AgNP-loaded dressings.The composite dressings may contain both, effective against Gram-negative species.Both bacterial groups are synergized by the different types of nanoparticles(Nqoro and Taziwa, 2024).Copper oxide nanoparticles (CuO NPs) exert their main action by redox active Cu^{2+}.A reduction in ROS release and the formation of Fenton-type ROS, especially against
Wound pathogens, bacteria and fungi. They are active against all life stages,MRSA and carbapenem-resistant Acinetobacter, has been shown in several in vivo systems.In vivo, MRSA and carbapenem-resistant Acinetobacter has been established.vitro studies. Titanium dioxide nanoparticles (TiO_2 NPs) are very effectiveThey are also just partially active under UV light, so

there are photocatalytic ROS generators that are more active under UV light.Their stand alone wound application is limited by ambient or physiological light conditions; andThey are more frequently used to make scaffold composites, in which they are combined with other materials to create scaffolds.In combination with light-based wound, photocatalytic capacity can be activated.treatment protocols. Although gold nanoparticles (AuNPs) are not themselves bactericidal,Unlike other bactericidal agents,Their surface chemistry and low cytotoxicity have been extensively investigated as drug carriers for antibiotic delivery to wound sites, which can be tailored with precisely controlled surface chemistry and low cytotoxicity (Pang et al.,2023)

Table 2.2: Comparison of major nanoparticle types evaluated for antibacterial wound applications

Nanoparticle Type	Primary Antibacterial Mechanism(s)	Strengths	Limitations
Silver (Ag)	Membrane disruption; Ag^+ ion release; ROS generation	Broad-spectrum activity; extensive clinical data; effective against MRSA and biofilm	Cytotoxicity at high doses; potential for silver-tolerant isolates; relatively high cost
Zinc Oxide (ZnO)	ROS generation (H_2O_2, $O_2^{\cdot -}$); Zn^{2+} ion release; electrostatic membrane disruption	Lower cost; good UV photocatalytic activity; synergistic with AgNPs; GRAS status	Weaker against Gram-negatives at equivalent doses vs. AgNPs; limited deep-tissue activity
Copper Oxide (CuO)	Cu^{2+}-mediated Fenton ROS; membrane oxidation; enzyme inhibition	Effective against MDR bacteria; antifungal activity; abundant raw material	Higher cytotoxicity than ZnO or Ag at equivalent concentrations; pro-oxidant at high loads
Titanium Dioxide (TiO_2)	UV-activated photocatalytic ROS generation; membrane damage	High photocatalytic efficiency; chemically inert; low dark toxicity	Limited activity without UV light; not suitable as standalone dark-condition treatment
Gold (Au)	Drug carrier; photothermal heating under NIR irradiation	Excellent biocompatibility; tuneable surface for antibiotic/growth factor loading	No intrinsic bactericidal activity; high production cost; limited clinical wound data

Source: Synthesised from Pang et al. (2023), Nqoro and Taziwa (2024), and Modi et al. (2023).

2.6 Scaffold Platforms for Nanoparticle Integration

2.6.1 Rationale for Scaffold-Based Delivery

The incorporation of the anti-bacterial nanoparticles to polymer-based or The use of bioceramic scaffold matrices overcomes a number of important drawbacks of nanoparticle-only therapies.
wound treatment: uncontrolled spreading away from the wound site, burstelectroporation of release of toxic concentrations of metal ions, poor retention of nanoparticles in a spontaneouslygrafted wounds without any structural support for new tissueformation. Scaffolds are used to localise and offer a three-dimensional architecture.(Nqoro and Taziwa, 2024; Alven et al., 2021)

2.6.2 Hydrogel Scaffolds

Hydrogels are three-dimensional networks of polymers that are crosslinked and that canHold and absorb tremendous amounts of water (as much as 90% or more of their weight)without compromising the structure. They possess lots of water which creates a moist wound.environment that facilitates keratinocyte migration, prevents wound desiccation and Increases the permeability of wound surface for gas exchange and forms eschars. Natural polymersMaterials used for the fabrication of wound hydrogel include chitosan, alginate, hyaluronic acid, gelatin,Interactions with wound cells are mediated by two types of collagen molecules: collagen and fibrin, both of which interact with wound cells through integrin.receptors and growth factor-binding domains that actively support the healing response. Synthetic polymers such as polyvinyl alcohol (PVA), polyethylene glycol(PEG), and polyacrylamide provide more reproducible mechanical properties andAre tunable in terms of degradation rate, but not naturally active. Stimuli-responsive hydrogels those that change in their state of sol-gel or swelling according to the pH of the wound,Various factors such as temperature, or reactive oxygen species, have been more extensively studied asMaterials for the delivery of antibiotic agents which can be released on demand to inhibit bacterial growth by utilizing nanoparticles.Particularly in the context of microenvironmental cues associated with infection (Pang et al.,2023).

2.6.3 Electrospun Nanofibrous Scaffolds

The non-woven fibrous membranes formed are single fibre size of order 100nm.The ability to produce diameters from 50 to 1000 nm was achieved, using a high voltage between a polymer and a water jet.Solution is pumped out of a needle and to a grounded collector. The resulting nanofibrousThe structural organisation of the native extracellular matrix is very closely reproduced in architectures.A very high surface area to volume ratio, which increases the quality of the mixing, matrix and nanoparticle-bacteria contact and also gas exchange and controlled by.
The moisture control at the wound interface. The following materials can be used to make nanoparticles:electrospun scaffolds either by dissolving them in the spinning solution (blending), byBy the deposition on pre-formed fibres (surface decoration) or by the encapsulation of them,
A fibre architecture with coaxial fibres (core-sheath) providing additional control of the release.
kinetics. For electrospinning scaffolds, two types of polymers are used, namely, polycaprolactone (PCL) and poly(lactic acid) (PLA).Several materials such as PLA, poly(lactic-co-glycolic acid) (PLGA), chitosan and gelatin have been used to prepare these types of materials.All three of the NPs: AgNPs, ZnO NPs and CuO NPs have been widely tested as a vehicle for wounds (Alven et al. 2021).

2.6.4 Three-Dimensionally Printed Scaffolds and Bioceramic Composites

Additive manufacturing (Three dimensional printing) techniques include:The three main bioprinting techniques being developed are extrusion-based, inkjet printing and digital light processing (DLP) printing.The scaffolds can be produced using photopolymerisation which mimics the patient's scaffold,The geometries and defined internal pore architectures and spatially controlled distribution ofActive ingredients or nanoparticles. Ability to add anti-bacterial activity.pro-regenerative factors (concentrated in the inner wound-facing surface), which are responsible for plant healing, are both present on the outer surface of the wound facing the outside.Growth factors (concentrated in the interior scaffold volume) inside a single printed scaffold.construct is one of the most intriguing design approaches that has been successfully used in a Over the last few years, numerous pre-clinical studies have been carried out. Bioceramic-based scaffolds, including Composites of hydroxyapatite (HAp) and beta-tricalcium phosphate (β-TCP) have been developed,main therapeutic use: deep wounds to bone (osteomyelitis)Usually loaded with AgNPs or ZnO NPs to resolve the complications (DFU), these arecommonly used.A large number of bacteria are present in the infected osseous tissue (Pang et al., 2023).

2.7 Biocompatibility and Cytotoxicity Aspects

A variety of materials can be employed to create the framework.Numerous materials can be used to form the framework.Among the challenges highlighted in the literature regarding the use of nanoparticles in wound materials is the therapeutic window between the concentration required for wound healing effect and the concentration that is toxic to cells.bactericidal activity and the concentration needed to kill the cells in wound. The typical AgNPs concentrations (10–20 μg/mL) exhibited high antibacterial activity against planktonic S. aureus and P. aeruginosa. However, AgNPs above 50 μg/mL concentrations have been reported to decrease human keratinocyte and fibroblast viability in vitro with cytotoxicity increasing steeply with concentration and decreasing particle size. This window is even narrower for the CuO NPs. For scaffold systems, there is a need to sustain and control the release of nanoparticles or their ionic products from the scaffold matrix to keep concentrations in the local environment around the scaffold.

Within the therapeutic window, at the time of treatment, a bactericidal, but not yet a cytostatic range of activity (Alven et al., 2021).To expand the therapeutic window, non-specific interactions have been minimized by PEGylation, ion release properties have been modified by coating with chitosan or polyvinylpyrrolidone (PVP), and targeting ligands specific to components of bacterial surfaces have been attached. An additional layer of kinetic control arises when nanoparticles are doped into a degradable polymer matrix, releasing them sequentially as the scaffold polymer is degraded in the wound environment, with the release rate of nanoparticles being tunable by polymer molecular weight, crosslink density and loading fraction of the nanoparticles.

2.8 Overview of literature and gaps in knowledge

From this literature reviewed, the following key points can be set that:The following chapters of this dissertation are contextualised. First, infected chronicWounds are a worldwide clinical issue

that has increased in prevalence, and is associated with the following factors:The ageing populations, increasing diabetes rates and the proliferation of MDR wound Conventional treatment modalities (systemic antibiotics, topical treatments), such as those for pathogens, have historically been unavailable for treating this condition.There are definite limits to the effectiveness of antiseptics, silver dressings. Second, bacterial biofilm Recalcitrant wound infection is characterized by and requires multi-mechanistic approaches not reliant on antibiotics with single molecular targets. Third, metal and metal oxide nanoparticles offer genuine multi-mechanistic antibacterial attributes that are Resists biofilm formation and MDR pathogens and is linked to decreased These were less likely to lead to selection of resistance than the conventional antibiotics. Fourth, scaffold It is not only a matter of convenience to be able to formulate integration, but it is a necessity.controls the retention, release and cytocompatibility of nanoparticles,At the same time providing mechanical support to wounds.The trouble is, there are various therapies for the infection.The problem is that there are different therapies to treat the infection.There is no cross-nanoparticle-scaffold comparisons, across these nanoparticle-scaffold systems.
Pharmacokinetics of the release of nanoparticles from scaffolds in vivo is still not predictable.
There is limited information about potential systemic accumulation; and There is a limited amount of evidence from RCTs; and the regulatory pathway from of composite materials.

CHAPTER 3
ANTIBACTERIAL NANOPARTICLES: TYPES, SYNTHESIS, AND CHARACTERISATION

3.1 Introduction

Selecting the appropriate nanoparticle to use for a wound scaffold application is not a simple matter.decision. The wrong choice - the size, the material, the surface.Chemistry — can refer to something that kills bacteria in a petri dish and that damages hostThe expression of keratins in a living wound, or an agglomeration of keratinocytes in wound exudate, before itever enters a bacterial cell. This chapter presents a simple overview of the threeThe most widely studied and clinically relevant types of nanoparticles for infected wound.The silver (AgNPs), zinc oxide (ZnO NPs) and copper oxide (CuO NPs) nanoparticles were used for management. each, the discussion focuses on how they are produced, what properties are important for the production,
What their true limitations are, and their antibacterial activity. The chapter also brieflyBecause the materials are characterized prior to biological testing, outlines how they are characterized.
Whether any conclusions can be drawn from a study or not depends on the quality of the characterisation.can actually be trusted (Pang et al., 2023; Nqoro and Taziwa 2024).

3.2 Silver Nanoparticles (AgNPs)

The reasons for the dominance of silver as a metal have been explained in the following points:
Silver has been used as an antimicrobial material for centuries — from silver coins to the modern era.The vessels for storage of water in ancient civilizations to silver sutures in early surgery. TheThe nanoparticulate form, however, added something qualitatively different; namely a materialA compound which could attack bacteria at many independent targets at concentrations measured in micrograms per millilitre. AgNPs kill bacteria on three parallel tracks. A physical contact between the nanoparticle and the disrupts.bacteria cell membrane, especially small particles.The small molecule (around 5-20 nm) is inserted into the lipid bilayer. Meanwhile, silver ions (Ag^{+})As long as the nanoparticle is on the surface, continuously released from the nanoparticle surface, bind to the sulfhydryl groups ofT he respiratory enzymes of bacteria block the production of ATP and the intercalation of these enzymes into DNA prevents DNA from functioning and inhibit correct reproduction. At the same time, ROS (mainly hydrogen peroxide),These species (superoxide and hydroxyl radical) produced on the surface of the nanoparticles cause Damage to bacterial membrane, protein and nucleic acids by oxidants (Raghuwanshi et al., 2022; Menichetti et al., 2023). All three mechanisms function because of the minimal force required.at the same time a single mutation is unlikely to give resistance to all of them.

In a nutshell, the primary reason for AgNPs' efficacy against MRSA and MDR is once.At its heart, the efficacy of AgNPs against MRSA and MDR hinges on once.Gram-negative wound pathogens that have long since overcome conventional antibiotics

3.2.2 The Synthesis and Properties

The synthesis of amino acids and resulting properties of these pigments The properties of AgNPs depend on their methods of production. Chemical reduction (ex: silver nitrate reduced with sodium borohydride or sodium citrate) —Provides the most reproducible control of particle size and shape. The Turkevich citrate method yields monodisperse spherical particles usually in a narrow range of 20–50 nm;Smaller and more reactive (5–15 nm) particles are obtained by sodium borohydride reduction.Physical methods (e.g. laser ablation) generate very pure particles without energy intensive and impractical for large scale chemical surface residues.wound dressing production. Green synthesis — using plant extracts as both reducingThe most talked about method in recent literature is that of using and stabilising agents, partlyThe plant materials used include Aloe vera, neem (Azadirachta indica) and tulsi, which are believed to have anti-inflammatory properties.(Ocimum tenuiflorum) are used in small quantities, they are cheap and plentiful, partially due to the fact that they are used as a small quantity.The "left-behind" phytochemical residues on the surface of the nanoparticles seem to bring antiinflammatory and antioxidant properties that could be helpful in wound healing.(Ogunyemi et al., 2024; Aldakheel et al., 2023). The extract composition is dependent on season, country and the extraction process used,AgNPs, the green-synthesised AgNPs need to be thoroughly studied batch to batch before use in a wound scaffold for clinical applications use.

The most significant physicochemical variables for are 1) shape and 2) size.antibacterial activity. Smaller particles are more potent — not simply because theypossess more surface area per unit mass, but have faster dissolution to release Ag^{+}At a higher rate and closer contact with the bacterial membranes. Particles Activity is significantly reduced at equivalent mass concentrations for particles >50 nm.The shape also has an effect: AgNPs with cubic and triangular shapes have crystal facets that are reactive —nanocubes, which interact more aggressively with the {100} face, in particular — are the most useful.Most useful are nanocrystals (nanocubes, in particular) that interact with the {100} face, which is the most aggressive.Are more reactive than the less reactive facets of spherical particles. Surface coating

provides a third lever: chitosan coatings improve the bacterial adhesion by PEG coatings decrease non-specific uptake by the cells, and electrostatic attraction helps to deliver the molecules to the cells.Expand the therapeutic window and reduce the release of ions (Menichetti et al., 2023).

3.3 Zinc Oxide Nanoparticles (ZnO NPs)

3.3.1 The Case for ZnO

The characteristics of ZnO NPs are unique and distinct from AgNPs. They are cheaper, derived from zinc — a critical micronutrient already known to play a role in wound healing biology are also GRAS (Generally Recognised As Safe) as bulk zinc oxide in food.In applications, a reasonable baseline context for safety is provided. Their antibacterial Unlike AgNPs, profile have been shown to be very active against Grampositive organisms, particularly S. aureus and MRSA, and less so against Gramnegative organisms.Gram negative species of equal concentrations. This makes them natural partners for them.The activity of to AgNPs in composite scaffold systems where synergistic broad-spectrum activity is achieved.desired. The combined activity of AgNPs and ZnO NPs is higher when the two are combined.Effectively against Gram +ve and Gram -ve bacteria than either alone.at the same dose (Nqoro and Taziwa, 2024). These pro-regenerative

effects make ZnO NPs useful not only as antibacterial agents but also as potential agents for promoting skin regeneration.agents, but as components that do both decrease in infection and support the tissue.repair - combination that is hard to obtain with regular antibiotics orantiseptics (Zhang et al., 2025; Mbachu et al., 2024).

3.3.2 Synthesis and Structure–Activity

The most common method for the synthesis of ZnONPs is co-precipitation — adding two solutions together and inducing the precipitation of the required material in the solution.Sodium hydroxide is added to zinc acetate or zinc nitrate solution and then the zinc is calcined out.At 200-400C the hydroxide precipitates to form the crystalline ZnO. The sol-gel method and hydrothermal synthesis yield finer control over the crystal morphology, yieldingUsually in the form of rods, flowers or stars in addition to spheres, depending on pH, temperature and reaction
time. The green synthesis of the ZnO NPs is well established and has a wide variety of plant extracts (neem, poplark, hibiscus, basil, rosemary, spinach, and cucumber) used for the method.
Gentle and nonirritating gels from a variety of materials such as aloe vera, Wodyetia bifurcata fruit peel, Zea mays leaf.particles between 10–50 nm that have been proven to have antibacterial properties against MRSA and P.aeruginosa, and K. pneumoniae (Moalwi et al., 2024; Aziz et al., 2025). ZnO NPsshow photocatalytic antibacterial activity under UV light — generating ROS under UV light.electron excitation across their 3.37 eV band gap – but with some meaningful dark-condition.The release of Zn^{2+} and direct interaction with the membrane also show antibacterial activity is well established.The authors reported that they documented the material which is important for actual wound dressing applications where UVAn occlusive dressing does not allow light to penetrate.

3.4 Copper Oxide Nanoparticles (CuO NPs)

3.4.2 The Use of CuO NPs in Applications.

CuO NPs offer an additional something the other nanoparticles, AgNPs and ZnO NPs, don't.match: a copper biology which is inherent to the repair of wounds. Copper is a cofactor that is required.The enzyme lysyl oxidase, which cross-links collagen and elastin in the matrix, is involved in.extracellular matrix. It also functions as a regulator of the expression of VEGF and angiogenesis. ThisMeans that the Cu2+ ions that are released from the CuO NPs in a wound scaffold are not just for killing.The role that it plays in the proliferation phase of healing which is disturbed by infection. For thisIn particular, CuO NP loaded scaffolds have been of interest for diabetic wound applications involving infection and impaired angiogenesis.present (Zhang et al., 2024; Devaraji et al., 2024).
The mechanism of action of CuO NPs against bacteria is based on the Cu^{2+}-mediated Fenton-type reaction.chemistry. In the wound microenvironment, H_2O_2 is naturally produced by:
The neutrophils when activated, catalyse the formation of hydroxyl radicals (˙OH) using Cu^{2+}.
the Haber–Weiss cycle generates high levels of damaging and highly reactive oxygen that results in bacterial cell oxidation.Membranes, proteins and DNA. CuO NPs directly bind to thiol groups

onInhibits key metabolic enzymes without overall effects on the enzyme's active sites, which are the site of bacterial enzyme activity.ROS. This combination of Fenton chemistry, with direct enzyme inhibition, provides CuONPs particularly potent activity against carbapenem-resistant A. baumannii and MDRP. aeruginosa — organisms that have limited response to other types of nanoparticles.efficacy (Hassan et al., 2024). The main limitation is that the same Cu^{2+} release thatCytotoxicity risk of drives on host cells is higher than the risk of Ag^{+}The zinc ion in this case produces a scaffold-controlled sustained release of Zn^{2+} in equivalent concentrations.This is even more important for CuO NP-based systems than for the other two types of nanoparticles.

3.4.2 Synthesis

Alkaline is the most popular method used for chemical synthesis of CuO NPs.Precipitation from copper acetate or copper sulfate solutions and heating toTransform $Cu(OH)_2$ to monoclinic CuO. Microwave-assisted synthesisreduces reaction time and gives good size control. Using silver or cobalt as a dopant(producing Ag,Co-CuO composites) has been shown to significantly enhance This is because the antibacterial activity of doped CuO NPs is found to be more than the undoped CuO NPs (Devaraji et al., 2024). GreenOne of the better characterised methods of synthesis is with Terminalia chebula dried fruit extract.CuO NPs with confirmed anti-bacterial activity against were obtained using biogenic approach.Munusamy and Shanmugam (2023) reported that S. aureus and E. coli were the most prevalent microorganisms found in food samples. An optimized, GMP relevant synthesis of skin friendly CuO NPs which were characterized by TEM,FTIR, DSC and XRD and were validated with MDR P. aeruginosa, MRSA, S. aureus.and Proteus vulgaris, has been described by Hassan et al. (2024) — one of the moreExamine synthesis reports found in the recent wound literature from CuO NP which were clinically oriented.

Table 3.1: Comparison of AgNPs, ZnO NPs, and CuO NPs for wound scaffold applications

Property	**Silver (AgNPs)**	**Zinc Oxide (ZnO NPs)**	**Copper Oxide (CuO NPs)**
Main antibacterial mechanisms	Ag^{+} release; ROS; direct membrane disruption	ROS (H_2O_2, $O_2^{\cdot -}$); Zn^{2+} release; electrostatic membrane disruption	Cu^{2+}-Fenton $^{\cdot}OH$; membrane lipid oxidation; thiol enzyme inhibition
Antibacterial spectrum	Broad: MRSA, P. aeruginosa, E. coli, K. pneumoniae	Stronger vs. Grampositives; synergistic with AgNPs for Gramnegatives	Broad, especially MDR A. baumannii; antifungal activity; active vs. MDR P. aeruginosa
Pro-healing effects	Anti-inflammatory; modest keratinocyte support at low doses	Promotes keratinocyte migration, reepithelialisation, angiogenesis	Pro-angiogenic (VEGF upregulation); supports collagen cross-linking via lysyl oxidase

Best synthesis method	Chemical reduction (Turkevich, $NaBH_4$); green synthesis	Co-precipitation + calcination; hydrothermal; green synthesis	Alkaline precipitation + calcination; microwaveassisted; green synthesis
Active particle size	5–20 nm most effective	10–50 nm	5–50 nm
Key limitation	Cytotoxic above ~50 μg/mL; Agtolerant isolates possible	Weaker vs. Gramnegatives alone; genotoxic risk at very high doses	Narrowest therapeutic window; requires precise dose control

Source: Synthesised from Pang et al. (2023), Nqoro and Taziwa (2024), Menichetti et al. (2023), Hassan et al. (2024), Zhang et al. (2024).

3.5 Characterisation of nanoparticles – why and how?

The chemical formula is not the only description of a nanoparticle. Two batches ofThe properties of AgNPs made in the same lab may vary drastically.If the size distribution, agglomeration state or surface chemistry of the activities differ, the particles should be separated prior to the mixing step.The rigorous characterisation is thus no formality but a fundamental prerequisite for the evaluation of any antibacterial claim that is made for a nanoparticle.reproduced. Suitable characterisation toolkit for the core properties of antibacterial nanoparticles in wound.There are 5 techniques in scaffold research.Transmission Electron Microscopy (TEM) is a technique that allows the direct visualization ofThe size and shape of particles at the nanoscale. High-resolution TEM additionallyThe lattice fringe spacing is revealed by the image of re, indicating both crystal crystallinity and crystal phase. It gives themost descriptive image of the particles themselves, but not necessarily what they look like. X-Ray Diffraction(XRD) used to ensure the crystal structure and phase and to establish wurtzite ZnO versus zinc.The Scherrer equation applied to the constitution of the silver bond, for example, or confirming FCC silver —to peak width provides an estimate of crystallite size. Dynamic Light Scattering (DLS)Suspension hydrodynamic diameter, always larger than the TEM core size, measured by this.due to surface coatings and layers of water and due to its polydispersityWe used index (PDI), which measures the breadth of size distribution. Zeta potential measurementThe values outside of ±30 mV represent the amount of surface charge and are good indicators of the colloidal stability of the product.A high positive zeta potential corresponds to better bacterial adhesion and antibacterial properties.activity. FTIR Spectroscopy confirms the presence of surface functional groups.green-synthesised particles capped with phytochemicals.Surface modified ones are coated with coatings. Last but not least, UV-Visible Spectroscopy is a quick method.The AgNPs absorb at ~410–450 nm due to surface plasmon resonance,peak sharpness and peak position are related to particle size and degree of agglomeration (Aldakheel et al., 2023; Menichetti et al., 2023). For anti-bacterial efficacy the minimum inhibitory concentration (MIC)A minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test was performed against the target organism.Basic potency data is provided from wound pathogens. Biofilms are difficult to eradicate using methods like those in the laboratory.The crystal violet quantification of biofilm biomass or MBEC (minimum biofilmPlanktonic MIC values (also known as eradication concentration) testing — are essential,Underestimate the concentration of the biofilm-cidal activity required against established wound biofilms by a large degree.(Raghuwanshi et al., 2022).

3.6 Chapter Summary

Each of the AgNPs, ZnO NPs, and CuO NPs has a different antibacterial profile.adapted to various parts of the affected wound dilemma. AgNPs have the broadest It is evidence based and effective for MRSA and biofilm. ZnO NPs bring low Cost, synergy with AgNPs and direct pro-healing biology. CuO NPs provide enhanced coverage to the most MDR Gram-negative pathogens and also is beneficial to angiogenesis. None of the three are ideal on their own – it's the combination that creates the clinical opportunity in their incorporation into scaffolds that control their delivery and can be used together with combining other with them.complementary activities. The subject of Chapter 4 is that one.

CHAPTER 4
THE MATERIALS AND FABRICATION NEEDED TO BUILD SCAFFOLDS.

4.1 Introduction

An antibacterial nanoparticle, when applied by itself, directly to a wound, will be is swept away by exudate in minutes, and releases most ions in toxic manner beforethe concentration does not reach a therapeutic level and they provide no support in terms of structures.Those who are below trying to regenerate to the tissue above. Surged into the field of clinical wound care as a replacement for dressings. Scaffolds address these issues. By incorporating nanoparticles into a three-dimensional polymer or bioceramic material, or embedding them in a polymer or bioceramic layer that is attached to a three-dimensional material, a scaffold holds the antibacterial material at the wound interface, controls the rate at which ions diffuse outwards, keeps the moist microscopic environment that the cells are waiting to get well requires, and can serve as a physical cue that harnesses fibroblast and keratinocyte behaviour. This chapter summarizes the four main scaffolds for nanoparticles integration: hydrogels, electrospun nanofibrous membranes, three-dimensionally In addition to being printed, the material can be used in bioceramic composites(Nqoro and Taziwa, 2024; Alven et al.,2021).

4.2 Hydrogel Scaffolds

4.2.1 structure and wound-relevant properties.

A crosslinked polymer network capable of absorbing 80–99% of its own weight in water is called a hydrogel Water that has a substantial and durable consistency. This is what is referred to as the combination —its strength and water content — that makes These are so well adapted to the wounds that they are. They preserve the damp boundary surface of which promotes migration of keratinocytes and avoids the formation of dry wounds and eschar they permit the exchange of gasses and exudates; they are flexible and conform to irregular shape;They are soft, and do not usually have to be changed due to wounds; and Minimal mechanical damage to new epithelium is created. Natural polymers — chitosan, Alginate, hyaluronic acid, gelatin and collagen – offer biological cues via Integrin binding domains and embedded growth factor interaction sites which are active and provide the healing cells a signal. Synthetic alternatives such as polyvinyl alcohol (PVA) and materials such as polyethylene glycol (PEG) have more steady mechanical properties and tunable.However, with the same degradation rates as natural polymer matrices, without the bioactivity of natural polymer matrices.(Pang et al., 2023).

4.2.2 The addition of Nanoparticles in Hydrogels

The easiest method of loading nanoparticles into a hydrogel is to simply mix them together.Polymer solution was mixed with a pre-formed nanoparticle suspension prior to

crosslinking.This gel network forms a physical cage, which slows down the diffusion of the nanoparticles.and regulates the release of ions. In situ synthesis, i.e. synthesis of nanoparticles in situA metal salt solution is diffused into the pre-assembled hydrogel and then the gel is hardened.Increase uniformity of nanoparticle distribution and decrease size — reducing it in placeAgglomeration of particles in the gelation process. The most innovative recent A stimuli-responsive development in the delivery of nanoparticles is of hydrogels hydrogel: networks of polymers that can be designed to swell or cross link density in response to pH drops, elevated levels of ROS, or proteolytic activity.Enzymative activity of the infected microenvironment of the wound. A chitosanThe hydrogel is a reliable release compound that grows and releases AgNPs at pH levels below 6.5 in a wound environment.indicator of active bacterial metabolism — releases its antibacterial contents only when needed, thus reducing the total amount of antibiotic that may be present in the host cell.Keeping effective bactericidal concentration at the time they are most needed (Pang et al., 2023; Amiri et al., 2023).The thermosensitive is a good and well-characterised example.The Amiri et al. (2023) described collagen-AgNP hydrogel composite has liquid form At room temperature (for easy application to the wound) and gelled to a solid scaffoldat body temperature. In an in vivo rat splinted wound model, this system decreased Significantly reduced the bacterial burden and enhanced wound closure compared to untreated wounds.The histological analysis revealed an improvement in the granulation tissue, whereas controls had no improvement.The formation of collagen deposits — clues that the scaffolding gave structural support — was observed.From a level of healing beyond the simple control of infection.

4.3 Electrospun Nanofibrous Scaffolds

A polymer is "spun" using a high voltage electrostatic force solution is ejected as a jet which thins to fibres with a diameter of 50 to 1,000 nm as the solvent evaporates Evaporates and condenses to a grounded collector as a non-woven porous mat. The architecture that is generated is structurally similar to the native extracellular matrix —Not surprisingly, in its biological form, it is a fibrous, porous, high surface area network relevance. Fibrous ECM is a physical guide to the fibroblasts and keratinocytes.The term "migration" describes this guidance cue; electrospun scaffolds are a synthetic analogue of this guidance cue.Not only did they provide embedded anti-bacterial compounds (Alven et al., 2021), but they also enhanced the material's properties.

There are three ways of introducing nanoparticles into electrospun fibres. In this method called "blending", the nanoparticles are dissolved or dispersed in the spinning solution.Fibre bulk is formed during the electrospinning process and the product is embedded in the fibre bulk. Surface depositionDip-coats or electrostatic deposits the nanoparticles onto pre-formed fibres,

Putting them on the fibre surface where the bacteria come into most contact with the fibre. CoaxialThe principle of electrospinning is based on the production of core–sheath fibres, which are formed by arranging the needles concentrically:The nanoparticles are in the core and covered by a polymer sheath that regulates.They are released from the cell and even further decrease cytotoxic burst. Published data on AgNP loaded Electrospun scaffolds of PLGA, PCL, chitosan, and gelatin loaded with ZnO NP safely reduce both S. aureus and E. coli colony counts by >99% in 24, consistently reported results.An approach that uses a set of particles known as nanoparticles and causes only hours of contact, while maintaining favourable fibroblast and keratinocyte viability.The bacteria are still killed by the loadings (Alven et al., 2021; Yudaev et al., 2022).

4.4 Three-Dimensionally Printed Scaffolds

Additive Manufacturing has enabled scaffolds to be designed for wound care.While flat dressings or spun fibrous mats cannot provide, they can provide patient-specific geometry, internal pore architecture and spatial gradients of biological content. The techniques for which these are used are mainly extrusion-based printing, inkjet printing and digital light processing photopolymerisation.The main techniques used for wound scaffolds. The clinical justification of 3D.Irregular or deep wounds, such as diabetic foot ulcers with irregular or deep areas are the most interesting for printing.When the morphology is complex and three dimensional, such as a flat dressing —Leaves a significant amount of wound bed without soil cover. In addition to geometry, 3D printing can create parts with varying other properties.distribution of nanoparticles controlled in space: a high loading antibacterial area at theTwo zones of the scaffold: a high concentration pro-regenerative zone in the wound-facing surface, and a low concentration pro-regenerative zone in the opposite surface.However, it is not nanoparticles but interior releasing growth factors (Bayarsaikhan et al., 2023).

4.5 Bio-ceramic and Composite Scaffolds

In wounds that extend to bone (usually osteomyelitis associated with) —strength necessary to stabilize the ulcer bed and speed healing.The polymer-based scaffolds are not mechanically strong enough to stabilize the ulcer bed and promote healing in people with diabetic foot ulcersor significant trauma.The stiffness and osteoconductivity were required for bone healing. Hydroxyapatite (HAp)This is where and beta-tricalcium phosphate (β-TCP) bioceramic scaffolds come in:The crystal structure of the hydroxyapatite is similar to bone mineral, enabling the recognition of bone-forming cells. Loading of these ceramics with AgNPs or ZnO NPs provides antibacterial activity in the infected osseous defect, combining with infection control.A single material that can control and regenerate bone. More recently, hybrid composite systems — hydrogel matrix with electrospun fibres, or bioceramic granulesThe ability to distribute these throughout a polymer hydrogel — and the biological signalling and moisture management benefits of soft polymer scaffolds — have been explored to combine.The role of ceramic or fibrous structure and mechanical support (Nqoro and Taziwa, 2024; Aminzai et al., 2024).

Table 4.1: Scaffold platforms for nanoparticle-integrated wound dressings — a practical comparison

Platform	Key Polymers / Materials	NP Integration Method	Best Suited For	Main Limitation
Hydrogel	Chitosan, alginate, PVA, PEG, gelatin, collagen	Blending into pre-gel; in situ synthesis; stimuli responsive release	Moderate exudate surface wounds; burns; stimuli responsive infection triggered designs	Low mechanical strength; not ideal for highexudate or highfriction wound sites

Electrospun nanofibre	PLGA, PCL, PVA, chitosan, gelatin	Blending in spinning solution; surface deposition; coaxial core–sheath	ECM-mimicking skin wounds; promotion of cell migration; chronic ulcers	Fragile mechanical handling; difficult to scale; small pore size may limit cell infiltration depth
3D-printed scaffold	PCL, polyurethane, bioinks, photocurable hydrogel inks	Incorporated in printing ink; spatial gradient programming	Deep or irregular wounds (DFU); patient-specific geometries; spatially graded drug release	Higher equipment and material cost; limited approved biocompatible inks; sterilisation challenges
Bioceramic composite	Hydroxyapatite, β-TCP, silicate ceramics ± polymer binder	NP-doped ceramic synthesis; surface coating; hybrid with polymer matrix	Deep wounds with osseous involvement; osteomyelitis complicating DFU	Brittle; not suited to superficial wounds; slow and incomplete biodegradation in some systems

Source: Synthesised from Nqoro and Taziwa (2024), Alven et al. (2021), Bayarsaikhan et al. (2023), and Pang et al. (2023).

4.6 Chapter Summary

Often, the scaffold is not a passive vehicle as the early descriptions indicated. In The overall design, which is a structure of a well-designed nanoparticle scaffold, is an active participant in the design.modulates release kinetics; controls antibacterial and proregenerative agent release distribution; acts as a structural guide to healing cells; protects the host.tissue for research of the effects of nanoparticles on cells. Among these the most common and the most used is the Hydrogels.clinically accessible platform. The structural fidelity that electrospun fibres offer for the ECM.hydrogels cannot match. Three-D printing provides geometric compositional programmability. This is a key challenge overcome by bioceramic composites.The infection of an open wound in the bone. In Chapter 5, we will be studying at the molecular level.These “scaffold-embedded” nanoparticles come into contact with these bacteria.

CHAPTER 5
THE MECHANISMS OF ANTI-BACTERIAL ACTION

5.1 Introduction

Materials for wound healing and as antimicrobial components of scaffolds.A detailed mechanistic argument is the key to the treatment of these by ultimate killingHow they do so, why their mode of action is different from conventional, and bacteria.Knowledge of the importance of this difference in relation to antibiotics. This chapter builds up that has four main modes of action: disruption of the membrane and generation of reactive oxygen species.discusses the anti-biofilm activity, toxicity of the metal ions and the species generation — and addresses them.question of resistance: is it that these mechanisms are resistant to bacterial.Can it be avoided or is there a more nuanced aspect to the statement (Pang et al., 2023;Modi et al., 2023).

5.2 Physical Membrane Disruption

The bacterial cell membrane is both the first physical barrier nanoparticles the first (and possible most important) target encountered. Note: Majority of wound bacteria are net negative.A negative surface charge is caused by lipopolysaccharide of the outer membrane of Gram-negative bacteria.Many metal ions can participate in the process, and the resulting action is on the membranes and teichoic acids of Gram-positive cell walls. nanoparticles, especially chitosan coated, charge.nanoparticles have a charge, especially when coated with chitosan or other cationic polymers.a net positive charge. This electrostatic attraction focuses the nanoparticles at thebacterial surface. Particles of a size that can penetrate the outer membrane —Typically less than 20nm (Gram-negatives) or greater (Gram-positives) when administeredThe thicker is the peptidoglycan layer, which is more accessible — insert into the lipid.bilayer. The effect is a "permeabilisation" of the membrane: the contents of the cytoplasm spills out,The electrochemical potential of the membrane is destroyed, and the cell is no longer able to maintain a Its need for ion gradients to produce ATP, to move nutrients and/or for signal transduction.The death process is very fast and as soon as the membrane breaks the cell is dead (Modi et al., 2023; Raghuwanshi et al., 2022).One of the properties of this physical mechanism is to be stressed.
Use the same receptor, enzyme or metabolic pathway is not necessary. The bacterial A membrane is a characteristic structure of all. There is a receptor whose structure is not affected by a mutation.Prevent the necessity of using lipid bilayer. This is why membrane disruption is one of the main reasons why this occurs.The resistance curve of mechanism doesn't match up.
Resistance of PBP.

5.3 Production of Reactive Oxygen Species (ROS)

The bactericidal effect of ZnO NPs and CuO is mainly attributed to the generation of ROS.They are NPs as well as an important contributor for AgNPs. As for chemistry:A surface of a

nanoparticle is made up of electrons that are transferred to the conduction band of the nanoparticle from theDissolved oxygen transforms into superoxide ($O_2^{\cdot -}$) which then changes to hydrogen peroxide.The free radicals (H_2O_2) then undergo further reactions with metal ions to form another free radical in the Fenton or Haber-Weiss reaction.These include: (Cu^{2+}, Ag+, Zn2+), which are able to produce hydroxyl radicals (˙OH). The most reactive of all the species in the atmosphere is the hydroxyl radical.The three are reactive and attack the phospholipids of the bacterial cell membranes which start a chain.reaction of lipid peroxidation which gradually decreases the integrity of membrane;Protein oxidation and unfolding, enzymes which are involved in the process of replication, are inactivated,It plays a role in metabolism, cell division and has been known to fragment single or double chain molecules of DNA.Inhibit new growth of the living cells, including those of bacteria that are resistant to the initial oxidative attack.(Pang et al., 2023; Ye et al., 2023).This process is also aided by the microenvironment of the wound. ActivatedWounds infected by bacteria have a high level of endogenous production of neutrophils and macrophages.H_2O_2 is also a substrate for oxidative burst in the Fenton chemistry besides Fe++.at CuO NP surfaces. A designed scaffold system which releases stimuli has been designed.
At the infected sites, the level of H_2O_2 has been raised and hence CuO NPs and ZnO NPs are targeting that.
This interplay is harnessed in wounds: The nanoparticles are delivered to the appropriate places and times.As the bacteria is made more active, its release site will be around the bacteria where the ROS will be released.maximum bacterial burden.

5.4 Metal Ion Toxicity

The ions that are released from AgNPs, ZnO NPs and CuO NPs make up a third,Line of attack that is independent of mechanism. The bonds of Ag+ ions to are extremely strong.The enzymes of the respiratory chain of bacteria have sulfhydryl (-SH) groups (e.g., NADH).Dehydrogenase and cytochrome c oxidase — inhibits respiration of oxygen and ATP production.synthesis. They are able to bind to adenine and guanine and intercalate into bacterial DNA are the bases that can be twisted and bent, so that it is hard to make a copy of them.polymerase. The effect of the Zn^{2+} ions on these enzymes of glycolytic pathway and citric acid cycle is similar. Cu^{2+}This activity is directly related to the ability to bind to ions, and this activity is related to the Fenton chemistry, that produces ˙OH.They have squatted in the immediate vicinity of the site of their enzyme they have occupied (Tran et al., 2023;Pang et al., 2023).Another mechanism has been added to this, a kinetic control. In a well-designed A scaffold composite: the rate of diffusion of metal ions is outward into the polymer matrix.These materials have properties controlled by the degree of crosslinking, the hydrophilicity of the polymer and loading.
The amount and size of the nanoparticles. Throughout the whole dressing period bactericidal range.If the production of the cell-destructive burst concentrations is not allowed as with unchecked, then the cell produces no burst.Application of direct wounding of NP dispersions. The practical conclusion is that the data from the practical lead to the conclusion that:
Good scaffold design not only helps delivery, it can make the difference between the erection of a scaffold and not.Whether the system of nanoparticles and scaffold is clinically viable or not will be determined.

Table 5.1: The four antibacterial mechanisms of nanoparticle-scaffold systems: targets, outcomes, and resistance risk

Mechanism	Active NP Types	Bacterial Target	Outcome	Resistance Risk
Physical membrane disruption	AgNPs, ZnO NPs, CuO NPs	Lipid bilayer; outer membrane proteins	Permeabilisation; cytoplasm leakage; membrane potential collapse; rapid cell death	Very low — structural universal target; no known single-mutation resistance
ROS generation	ZnO NPs, CuO NPs, AgNPs	Membrane lipids; proteins; DNA	Lipid peroxidation; enzyme oxidation; DNA strand breaks; oxidative death	Low — bacteria can upregulate antioxidant enzymes (catalase, SOD) but this only partially offsets multi-ROS attack
Metal ion toxicity	AgNPs (Ag^{+}), ZnO NPs (Zn^{2+}), CuO NPs (Cu^{2+})	Respiratory enzymes (SH groups); DNA bases; glycolytic enzymes	ATP synthesis failure; DNA replication arrest; metabolic collapse	Moderate — sil operon (Ag^{+} efflux) exists in some E. coli; Zn^{2+} and Cu^{2+} resistance
Anti-biofilm activity	AgNPs, ZnO NPs, CuO NPs	EPS matrix; quorum sensing molecules; surface adhesins	Biofilm penetration and dispersal; QS signal disruption; reduced readhesion; planktonic cells resensitised to killing	Low — requires bacteria to simultaneously protect EPS matrix, QS, and adhesins against multiple ion/ROS attacks

Source: Synthesised from Pang et al. (2023), Modi et al. (2023), Tran et al. (2023), and Raghuwanshi et al. (2022).

5.5 Anti-Biofilm Activity

A microbiological phenomenon is the hallmark of a chronic infected wound, which is known as “biofilm”,This is the main reason why traditional treatment is not working, and why it is so difficult to kill them with antibiotics. TheEPS matrix surrounding biofilm bacteria is not only a physical

barrier, it sequestersBinds with metal ions and antibiotics; activates the degradation of antibiotics by enzymes; produces.Metabolically dormant persister cells formed by oxygen and/or nutrient gradients.Subpopulations that are not affected by concentration-dependent killing. NanoparticlesUse several methods to solve the problem which were not applicable using traditional methods.antibiotics.From the physical characteristics point of view, nanoparticles with a diameter less than ~10 nm are able to enter the EPS matrix by diffusion to the bacteria inside the biofilm to which antibiotics cannot gain access.
Are not able to be used at safe concentrations. The small particles that are the most effective against Also the most appropriate planktonic bacteria may be the most appropriate to enter the biofilm. Chemically, Ag^+ ions are liberated into the matrix of the biofilm, leading to a particular interference with N-acylhomoserine lactone (AHL) quorum sensing system that P. aeruginosa uses toPromote bio-coordination, activate virulence gene expression and develop antibiotic tolerance. ZnO NPs have been shown to decrease the biomass of S. aureus and P. aeruginosa biofilms.The results demonstrated that it had a significant inhibitory effect on the fibroblasts at concentrations lower than the cytotoxic concentration for the fibroblasts in several.in vitro studies (Tran et al., 2023; Ye et al., 2023). This will be embedded when it's part of a scaffoldSustained, local release from a wound: a wound dressing system which makes slow release of antibiotics further enhanced.The nanoparticle exposure from the dressing, which remains on the wound bed for days, is low level and prevents re-establishment of the biofilm.bacterial burden is reduced, an effect that intermittent systemic antibiotic dosing cannot replicate.

5.6 Resistance: A Reality Check

Claim: You can't get bacteria to become resistant to nanoparticles.overstatement. E. coli strains resistant to silver that contain the gene cluster of the operon, which is involved in the production of proteins that resist silver.Efflux pumps and Ag^+-binding proteins — have been isolated and are part of the genes of resistance.From clinical settings especially when exposed to silver for an extended period of time. SomeSublethal ROS induces expression of catalase and SOD by bacteria.exposure, to some extent reducing oxidative killing. The matrix of the biofilm (EPS)
Communities can bind metal ions and the level of free metal ions in the intracellular environment will be reduced.concentration. These mechanisms do exist, they are what they are. What is it about them, that makes them less clinically?Nonsense, a threat is not the lack of antibiotic resistance but its limited scope: a siloperon efflux pump works against Ag^+ ions, but cannot do anything about ROS generation,The breakdown of membranes, which continue unimpeded. The four above mechanisms need to be addressed.It is still very improbable that this is the case in onemutation or gene acquisition.The occurrence of low probability (Modi et al., 2023; Raghuwanshi et al., 2022). The practical the appropriate antibiotic.clinical risk is not the emergence of pan-resistant organism, it's the choice of antibiotic.subpopulations that are only partially tolerant when exposed to sub-therapeutic amounts of a chemical over a long period of timenanoparticle concentrations. This is significant because it is crucial to have a good and continuous supply of nanoparticles,A design criterion that must never be sacrificed for patient safety is the ability to have high concentrations at the wound surface during the time the dressing is in contact with the wound.

5.7 Chapter Summary

inhibiting the growth of bacteria, compared to Conventional antibiotics are based on the mechanism, not the observation. Four independent andMechanisms in common are disruption of membrane, generation of ROS, metal ions.The toxicity combined with penetration of anti-biofilm and the lack of a main defence against penetration — overcame the main defence of MDR & biofilm forming wound pathogens. There is resistance, but there are ways of resisting The attack on nanoparticles is just shallow when contrasted with its scale. Scaffold integration
The kinetics of this attack is under the control of, which does not cause cytotoxic bursts and doesn't lose its activity.sustained effective concentrations. Chapter 6 explores the idea that this mechanistic logic could be true.That's what is required to make that happen – clinical benefit.

CHAPTER 6
CLINICAL PERSPECTIVES, CHALLENGES AND FUTURE DIRECTIONS

6.1 Introduction

Although laboratory results have been consistently good, along with data from animal studies, In the history of biomaterials research, there have been many examples of biomaterials research: A substance that was very effective in petri plates and rats, but not effective in humans.in some cases, due to unexpected cytotoxicity, in others because the importance of the results was not anticipated.All of the above properties were developed on a clinical scale in manufacturing. Because of the complexity of the clinical problem, prototype, at times.the model captured. This chapter reviews critically the available clinical evidence for is.Illuminates the true challenges to translation as a form of nanoparticle-integrated wound scaffolds, andand talks about the paths along which the field is most likely trending (Pang et al.,2023; Hamdan et al., 2024).

6.2 The current state of preclinical evidence from animal models

Animal wound models (mainly excision wound models in rats and mice), and with some burn wound and DFU-mimicking db/db diabetic mouse models — provide The biggest in vivo evidence for nanoparticle-scaffold systems. The resultsThe feedback on the wide range of publications is always positive: AgNP-loaded chitosanThe alginate hydrogels, electrospun PCL scaffolds loaded with ZnO NPs and CuO NPs containing scaffolds were evaluated and compared to each other in this study.All composite dressings have a reduction in the number of bacterial colonies in infected dressings.Wound healing, accelerated wound healing (typically 20-50% quicker than unloaded scaffold), and Improved collagen (at day 14), and more histological indicators of healing (at day 14), were observed.More neo-vascularisation and more complete re-epithelialisation was observed with the density in (Zhang et al., 2025; Hassan et al., 2024). A systematic review of the wound studies involving ZnO NPs has been performed.A systematic review of wound studies based on ZnO NPs was performed.It is observed that the healing rate of 60-80% was observed at day 14 in ZnO treated group while 30-50% healing rate was observed to the vehicletreated group at day 14. When compared to the CuO scaffold, the scaffold of CuO had complete wound closure at day 21.In rat burn model, the antibiotic control groups were ineffective against MDR A. baumannii.These results can be considered positive and reliable. They are also,inherently, limited. Animal wound models are not true to scale representations of
They can not, immunological complexity of the human diabetic or elderly wound; model polymicrobial nature of real chronic wound biofilms; and the wound Rodents have vastlydifferent volume, timescales for healing, and skin biology as compared to people.humans. They show great potential in the preclinical realm and should continue to be developed.

6.3 Clinical Evidence

There is no clinical information available for investigational nanoparticle-scaffold.There is a limited amount of literature available on composite wound dressings. The most easily accessible clinical data isFrom popular commercial dressings (such as Acticoat) which are manufactured of silver.(nanocrystalline silver, Smith & Nephew), AQUACEL Ag (ionic silver-alginate,
These include Convatec products (plastic foam), and Mepilex Ag (silver containing foam). These products have created a revenue for these companies.There is no evidence from prospective and randomised controlled trials of faster bacterial clearance.decolonisation, reduced wound odour, and in some trials reduced time for healing in wounds.Compared to other wound dressings that do not have a silver coating for some wound types (Hamdan et al., 2024).These commercial products are, however, different than the investigational AgNPhydrogel and ZnO NP-electrospun composites cited in the experimental literature:They're more basic ionic or nanocrystalline silver formulations in known wound dressings.Engineered release of nanoparticles vs materials in polymer scaffolds.Hamdan et al. (2024) performed a systematic review with clinical evidence forThe silver-based wound dressings actually helped decrease the bioflim in every case in a Canadian study.However, the non-silver controls were more effective in decreasing the number of bacteria in wounds than was the silver treatment.Demonstrate statistical significant improvement in time to closure in well-controlled RCTs in patients of chronic wounds. An overview of nanotechnology-based is presented.A review of the nanotechnology-based is given.
This list gives only a small sample of wound healing clinical trials that have been registered on ClinicalTrials.gov as of December 2024.There were several trials that were running that included dressings with nanoparticles, he found.Most were phase I/II safety and burn wound treatments, and had been in the initial two stages of diabetic foot ulcer and burn wound treatments.The tolerability studies and not efficacy trials (Mbachu et al., 2024). The gap betweenThe number of publications in pre-clinical studies and the number of clinical trials is the mostOne of the features that differentiate the present topography.

6.4 Translation Challenges

6.4.2 The Intent to Treat Analysis Problem

The use of most of the types of metal nanoparticles discussed in this dissertation is effective in killing bacteria atThe concentrations are also toxic to the host cells to various extents. ForAgNPs, in vitro studies, always reports the bactericidal concentration againstThe planktonic S. aureus (10 – 25 μg/mL) however significantly affected the viability of keratinocytes and fibroblasts.The range of decreasing concentrations is relatively small and lies between 30-50 μg/mL. AgainstFor effective action, the concentration of the bacteria in the biofilm should be in excess of host's requirement of 100-200 μg/mL.The static cell cytotoxicity threshold. For CuO NPs, the therapeuticThe width of window is even smaller. The major engineering sustained release is controlled by scaffolds.solution to this problem: releasing the ions slowly and continuously instead of in a solution to this problem:Scaffolds can keep the local concentration within the bactericidal range but not the cytopathic range, as in the case of burst.Be able to maintain their stance for a greater amount of time. To prevent ion uptake, ion uptake inhibitors like ion uptake inhibition, surface functionalization, etc. are used.Modification of surface, such as PEGylation, reduces ion

uptake.Offer more tools: release + chitosan coating to improve bacterial selectivity.But the simple irony of destroying bacteria and not harming host cells hasAll three types of nano-particles are also known to have a specific mechanism (Alven et al., 2021;
Aminzai et al., 2024).

6.4.2 Scalability and Manufacturing

The overwhelming majority of the reported nanoparticle-scaffold composites are laboratory.Preparations prepared in milligrams to grams (usually by one researcher).or group under conditions not transferable to clinical/commercial.manufacturing. Scale up of a lab-scale (5 mL) to a GMP compliant synthesis.Instability and changes in time.The problems of temperature instability and time variation can be introduced in several hundred litres in production run.A homogeneity, mixing dynamics and precursor concentration gradients that vary with timedirectly influence the properties of the particles and particle size distribution, morphology and surface chemistry of the particles.affect biological performance. Although they are attractive, their synthesis is in the form of green synthesis methods.scientifically introduce a variable for raw material — composition of plant extracts — that isIt is virtually impossible to standardise to GMP level. One of the main reason is this scalability gap.Despite most nanoparticle-scaffold composites remaining in the “preclinical” stage for ever,Although many years of positive laboratory results have been achieved (Hamdan et al., 2024; Abadeer et al., 2025).

6.4.3 Regulatory Complexity

Nanoparticle-integrated scaffolds are a classification challenge to regulatory agencies. They work together as a medical device (the scaffold) which also serves as a stimulator for the creation of new bone.Strong in structure, covering wounds, managing moisture and providing mechanicalThe two are the support and as a nanomaterial-containing drug-like formulation (the nanoparticle).A component that has pharmacological antibacterial activity (component that has inhibitory effect on bacteria). In the United States of America,Combination products fall under a lead centre determination by the FDA.Primary jurisdiction with either the Center for Devices and Radiological Health (CDRH)For the Center for Drug Evaluation and Research (CDER), depending on the primarymode of action. In the European Union the Medical Device Regulation (MDR) is in effect.The Medical Device Regulation (MDR) applies in the European Union (EU).
Most existing investigational nanoparticle scaffold composites do not have the information about clinical evaluation that is required by 2017/745). CDSCO has come up with draft guidelines in India.There is no clear pathway for approval of nanotechnology-based products, and none have been approved for composite wound dressings that are scaffolded with nanoparticles.
They are approved for Indian market (Murray et al., 2022; Abadeer et al., 2025).

Table 6.1: Key challenges in clinical translation of nanoparticle-scaffold wound dressings and proposed approaches

Challenge	Core Problem	Proposed Approach

Narrow therapeutic window	NP cytotoxic to host cells at concentrations needed for biofilm killing	Scaffold-controlled sustained release; PEG/chitosan surface coating; larger particle size for slower ion release; in vitro cytotoxicity testing standardisation
Manufacturing scalability	Lab-scale synthesis not reproducible at clinical/commercial volume with consistent quality	Standardised chemical synthesis protocols; GMPcompliant process development; avoid green synthesis for regulated products
Regulatory classification	Hybrid device/drug classification creates uncertain approval pathway	Early pre-submission meetings with FDA/EMA/CDSCO; clinical evidence generation from phase II/III RCTs; designation of primary mode of action
Resistance selection risk	Prolonged subtherapeutic NP exposure may select tolerant bacterial subpopulations	Ensure sustained bactericidal NP concentrations throughout dressing wear period; define maximum safe wear duration from in vitro release studies
Limited clinical trial data	Efficacy evidence from RCTs essentially absent for investigational NP-scaffold composites	Multicentre RCTs in defined wound populations (DFU, infected burns) with standardised primary endpoints: time to closure, microbiological cure, safety profile

Source: Synthesised from Hamdan et al. (2024), Alven et al. (2021), Murray et al. (2022), Abadeer et al. (2025).

6.5 Chapter Summary

Preliminary data of the use of nanoparticle-scaffold composites in infected wounds.The overall management is always good but not a replacement to clinical trial data that There is little to no existence of largely. Three barriers — the narrow therapeutic window,The most critical factors in manufacturing scalability, and regulatory complexity — are all treated as essential.Real-world issues to clinical implementation. The three most promising near-future directions are stimuli-responsive scaffold designs, nanoparticle–antibiotic.combination strategies, green-synthesis based approach and the development of affordable green-synthesis based.The systems that are suitable for resource constrained environments. Conclusions are brought together in Chapter 7.

CHAPTER 7
CONCLUSIONS AND FUTURE SCOPE:

7.1 Overview of the dissertation.

This dissertation was aimed at analyzing the antibacterial nanoparticle-integrated As a class, discusses the kind of materials that are used to treat infected wounds, scaffolds. A critical synthesis of literature, NOT primary sources.experimental data. This work was created out of peer reviewed publications 2018-2025.In support of earlier papers, if necessary, the papers were supplemented with a mechanism background.Problem was developed in Chapters 1 and 2: The scale of theThe biology of wound healing and how infected wound problem is global and in India, are addressed.A bacterial infection wreaks havoc on it, and the lack of effectiveness of traditional treatment.Mechanisms to injure pathogens that are multidrug resistant and form biofilms. Chapter 3The three major types of nanoparticles (AgNPs, ZnO NPs and CuO NPs) will be considered.Designed for synthesis, physicochemical characterisation and structure–activity relationships.relationships. Chapter 4 made a review of the scaffold platforms used to deploy these.nanoparticles – hydrogels, electrospun fibres, 3D-printed constructs, bioceramic composites — and manufacture and properties of function relating to.wound management. In Chapter 5 a mechanistic case explaining why these systems work was developed4 independent anti-bacterial mechanisms in parallel that help overcome thePrimary defenses of MDR and biofilm forming bacteria. Chapter 6 honestly evaluated weak evidence: weak pre-clinical or limited clinical trial evidence,three separate barriers that are easily visible to these materials.
patients at scale.

7.2 Research Priorities for the Future:

In this review, gaps have been identified that result in the formulation of five research priorities:
most immediately relevant to the development of the field towards clinical utility.Standardised in vitro testing methodology is the most actionable as soon as now.priority. The cross study comparisons are not yet reliable because of the presence of antibacterialDifferent organisms, Inoculum densities, with or without biofilm models and different cell viability assays forcytotoxicity are used to test efficacy. AFollowing a consensus testing strategy based on CLSI/EUCAST recommendations for antibiotic testing,A new approach designed specifically for nanoparticle-scaffold composites would allow results from to be leveraged to design better composites for therapeutic uses.The various research groups to be fairly compared and would accelerate the process of
The identification of the material combinations which are truly fit for clinical research.The most difficult pharmacokinetics to study is in vivo pharmacokinetics of nanoparticles that are released from the scaffold.An under-studied facet of the whole field. How much of the emitted nanoparticles are not radioactive?Do scaffolds from wounds get absorbed into systemic circulation?Where do they occur in their distribution areas? What is the duration of their stay in organs? What will be the lasting effects?What are the side effects of its exposure? Well-designed animal pharmacokinetic studies are needed before clinical trials in the vulnerable wound before

answers can be given to these questions.Ethical design can be achieved for populations.Randomised controlled clinical trials with standardised primary endpoints — is the term used for these trials.The trials are called Randomised controlled clinical trials with standardised primary endpoints. Evidence of the emergency need of the field. Wound trials in a controlled wound population and at several centres.(Infections diabetic foot ulcers, partial thickness burn wounds, infections post surgical) wounds.that could meet the regulatory requirements for the (wounds) would be sufficient.and in clinical guideline development.
Easy and scalable synthesis of ZnO NP and AgNP.The scaffold system is specially designed for production and meeting GMP requirements atclinically relevant volumes — are essential as a stepping stone between the laboratory proofof-concept and commercially viable products, particularly in addressing the lower resource healthcare systems.Early and iterative regulatory science engagement: dialogue between regulatory agencies and science nanoparticle-scaffold researchers and regulatory agencies about product. Classifications, evidence package for marketing authorisation and appropriate testing procedures suitable for displaying safety and effectiveness would Reduce the commercial development risk that presently helps to provide the industry with minimal participation;This is an investment that's being made.

7.3 Closing Remarks

Science remains in the laboratory is incomplete. In this regard, antibacterial nanoparticle-integrated scaffolds have worked hard by successfully establishing their mechanisms.rationale and consistently effective preclinical efficacy. The harder that is left behind is work: being meticulous and doing at clinical scale, showing benefit in controlled trials,and meeting regulatory and manufacturing challenges between a promising material and product that could get to a patient in a clinic This is a journey that demands a combination of skills which a programme needs to bring together designed to develop — an understanding of the biology,
the chemistry and clinical context and the translation process, linking Discovering in a laboratory and using it for human purposes. In this dissertation, an effort has been made to chart thatTerritory honestly, in the hope that it provides a useful starting point for the research,that is still to be achieved.

REFERENCES

Abadeer, N. S., Brenner, K., Lee, W., Cohen, E., & Keswani, S. G. (2025). Bridging the gap: the role of advanced formulation strategies in the clinical translation of nanoparticle-based drug delivery systems. *Pharmaceutics, 17*(4), 440. https://doi.org/10.3390/pharmaceutics17040440

Aldakheel, F. M., Sayed, M. M. el, Mohsen, D., Fagir, M. H., & El Dein, D. K. (2023). Green synthesis of silver nanoparticles loaded hydrogel for wound healing: systematic review. *Gels, 9*(7), 530. https://doi.org/10.3390/gels9070530

Al-Himdani, S., Naderi, N., Whitaker, I. S., & Jones, N. W. (2019). An 18-year study of malignant melanoma in childhood and adolescence. *Plastic and Reconstructive Surgery–Global Open, 7*(8), e2338. https://doi.org/10.1097/GOX.0000000000002338

Ali, A. M., Elkhouly, G. E., Orabi, A., Ismail, A., de Melo Barbosa, R., Riela, S., Viseras, C., & Abo-zeid, Y. (2025). Optimization of chemical synthesis for production of a skin-compatible and scalable copper oxide nanoparticles: A promising and safe strategy for healing of non-infected and infected wounds in diabetes. *BioNanoScience, 15*(1), 141. https://doi.org/10.1007/s12668-024-01708-2

Alven, S., Buyana, B., Feketshane, Z., & Aderibigbe, B. A. (2021). Electrospun nanofibers/nanofibrous scaffolds loaded with silver nanoparticles as effective antibacterial wound dressing materials. *Pharmaceutics, 13*(7), 964. https://doi.org/10.3390/pharmaceutics13070964

Aminzai, M. T., & Patan, A. (2024). Recent applications and evaluation of metal nanoparticle–polymer hybrids as chronic wound dressings. *Journal of Nanomaterials, 2024*, 1–23. https://doi.org/10.1155/2024/3280349

Amiri, N., Ghaffari, S., Hassanpour, I., Chae, T., Jalili, R., Kilani, R. T., Ko, F., Ghahary, A., & Lange, D. (2023). Antibacterial thermosensitive silver–hydrogel nanocomposite improves wound healing. *Gels, 9*(7), 542. https://doi.org/10.3390/gels9070542

Chimi, L. Y., Noubom, M., Bisso, B. N., Singor Njateng, G. S., & Dzoyem, J. P. (2024). Biofilm formation, pyocyanin production, and antibiotic resistance profile of *Pseudomonas aeruginosa* isolates from wounds. *International Journal of Microbiology, 2024*, 1207536. https://doi.org/10.1155/2024/1207536

Devaraji, M., Thanikachalam, P. V., & Elumalai, K. (2024). The potential of copper oxide nanoparticles in nanomedicine: A comprehensive review. *Biotechnology Notes, 5*, 80–99. https://doi.org/10.1016/j.biotno.2024.06.001

Hamid, L. L., Mutter, T. Y., & Ramizy, A. (2024). Date palm pollen: A novel medium for bacterial culture and biosynthesis of antibacterial chromium oxide nanoparticles. *OpenNano, 19*, 100212. https://doi.org/10.1016/j.onano.2024.100212

Hurlow, J., & Bowler, P. G. (2022). Acute and chronic wound infections: microbiological, immunological, clinical and therapeutic distinctions. *Journal of Wound Care, 31*(5), 436–445. https://doi.org/10.12968/jowc.2022.31.5.436

Ibrahim, M. A., Nasr, G. M., Ahmed, R. M., & Kelany, N. A. (2024). Physical characterization, biocompatibility, and antimicrobial activity of polyvinyl alcohol/sodium alginate blend doped with TiO_2 nanoparticles for wound dressing applications. *Scientific Reports, 14*, 5391. https://doi.org/10.1038/s41598-024-55818-8

Jin, C., Jin, Y., Ding, Z., Nuch, K. S., Han, M., Shim, J., Chien, P. N., & Heo, C. Y. (2025). Cellular and molecular mechanisms of wound repair: From biology to therapeutic innovation. *Cells, 14*(23), 1850. https://doi.org/10.3390/cells14231850

Manjit, M., Kumar, M., Jha, A., Bharti, K., Kumar, K., Tiwari, P., Tilak, R., Singh, V., Koch, B., & Mishra, B. (2024). Formulation and characterization of polyvinyl alcohol/chitosan composite nanofiber co-loaded with silver nanoparticle & luliconazole encapsulated poly lactic-co-glycolic acid nanoparticle for treatment of diabetic foot ulcer. *International Journal of Biological Macromolecules, 258*, 128978. https://doi.org/10.1016/j.ijbiomac.2023.128978

Menichetti, A., Mavridi-Printezi, A., Mordini, D., & Montalti, M. (2023). Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles. *Journal of Functional Biomaterials, 14*(5), 244. https://doi.org/10.3390/jfb14050244

Moalwi, A., Kamat, K., Muddapur, U. M., Aldoah, B., AlWadai, H. H., Alamri, A. M., Alrashid, F. F., Alsareii, S. A., Mahnashi, M. H., Shaikh, I. A., Khan, A. A., & More, S. S. (2024). Green synthesis of zinc oxide nanoparticles from Wodyetia bifurcata fruit peel extract: multifaceted potential in wound healing, antimicrobial, antioxidant, and anticancer applications. *Frontiers in Pharmacology, 15*, 1435222. https://doi.org/10.3389/fphar.2024.1435222

Modi, S. K., Gaur, S., Sengupta, M., & Singh, M. S. (2023). Mechanistic insights into nanoparticle surface–bacterial membrane interactions in overcoming antibiotic resistance. *Frontiers in Microbiology, 14*, 1135579. https://doi.org/10.3389/fmicb.2023.1135579

Munusamy, T., & Shanmugam, R. (2023). Green synthesis of copper oxide nanoparticles synthesized by *Terminalia chebula* dried fruit extract: Characterization and antibacterial action. *Cureus*, e50142. https://doi.org/10.7759/cureus.50142

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Nosrati, H., & Heydari, M. (2025). Titanium dioxide nanoparticles: a promising candidate for wound healing applications. *Burns & Trauma, 13*. https://doi.org/10.1093/burnst/tkae069

Nqoro, X., & Taziwa, R. (2024). Polymer-based functional materials loaded with metal-based nanoparticles as potential scaffolds for the management of infected wounds. *Pharmaceutics, 16*(2), 155. https://doi.org/10.3390/pharmaceutics16020155

Pang, Q., Jiang, Z., Wu, K., Hou, R., & Zhu, Y. (2023). Nanomaterials-based wound dressing for advanced management of infected wound. *Antibiotics, 12*(2), 351. https://doi.org/10.3390/antibiotics12020351

Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., Patel, S. B., Khalid, L., Isseroff, R. R., & Tomic-Canic, M. (2014). Epithelialization in wound healing: A comprehensive review. *Advances in Wound Care, 3*(7), 445–464. https://doi.org/10.1089/wound.2013.0473

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: A cellular perspective. *Physiological Reviews, 99*(1), 665–706. https://doi.org/10.1152/physrev.00067.2017

Sen, C. K. (2023). Human wound and its burden: Updated 2022 compendium of estimates. *Advances in Wound Care, 12*(12), 657–670. https://doi.org/10.1089/wound.2023.0150

Sharma, A., al Amin, M., Kshetri, M. B., Alqarni, S., Jogadi, W., Solmen, J., Lin, Z., Akter, S., & Zheng, Y.-R. (2025). PEGylation effects on amphiphilic platinum(IV) complexes: Influence on uptake, activation, and cytotoxicity. *Pharmaceutics, 17*(4), 440. https://doi.org/10.3390/pharmaceutics17040440

Shrestha, S., Wang, B., & Dutta, P. K. (2024). Commercial silver-based dressings: In vitro and clinical studies in treatment of chronic and burn wounds. *Antibiotics, 13*(9), 910. https://doi.org/10.3390/antibiotics13090910

Tran, N. N., Morrisette, T., Jorgensen, S. C. J., Orench-Benvenutti, J. M., & Kebriaei, R. (2023). Current therapies and challenges for the treatment of *Staphylococcus aureus* biofilm-related infections. *Pharmacotherapy, 43*(8), 816–832. https://doi.org/10.1002/phar.2806

Tripathi, N., & Goshisht, M. K. (2022). Recent advances and mechanistic insights into antibacterial activity, antibiofilm activity, and cytotoxicity of silver nanoparticles. *ACS Applied Bio Materials, 5*(4), 1391–1463. https://doi.org/10.1021/acsabm.2c00014

Wang, L., Yang, Y., Han, W., & Ding, H. (2024). Novel design and development of *Centella asiatica* extract-loaded poloxamer/ZnO nanocomposite wound closure material. *Regenerative Therapy, 27*, 92–103. https://doi.org/10.1016/j.reth.2024.03.006

Wangoye, K., Mwesigye, J., Tungotyo, M., & Twinomujuni Samba, S. (2022). Chronic wound isolates and their minimum inhibitory concentrations against third generation cephalosporins at a tertiary hospital in Uganda. *Scientific Reports, 12*, 1195. https://doi.org/10.1038/s41598-021-04722-6

Wu, K., Dou, Z., Liu, Z., & Xu, J. (2024). Study on hydration heat release model and its influence coefficient of addition concrete. *Applied Sciences, 14*(6), 2276. https://doi.org/10.3390/app14062276

Ye, L., He, X., Obeng, E., Wang, D., Zheng, D., Shen, T., Shen, J., Hu, R., & Deng, H. (2023). The CuO and AgO co-modified ZnO nanocomposites for promoting wound healing in *Staphylococcus aureus* infection. *Materials Today Bio, 18*, 100552. https://doi.org/10.1016/j.mtbio.2023.100552

Yudaev, P., Mezhuev, Y., & Chistyakov, E. (2022). Nanoparticle-containing wound dressing: Antimicrobial and healing effects. *Gels, 8*(6), 329. https://doi.org/10.3390/gels8060329

Zhan, X., Yan, J., Tang, H., Xia, D., & Lin, H. (2022). Antibacterial properties of gold nanoparticles in the modification of medical implants: A systematic review. *Pharmaceutics, 14*(12), 2654. https://doi.org/10.3390/pharmaceutics14122654

Zhang, Z., Xue, H., Xiong, Y., Geng, Y., Panayi, A. C., Knoedler, S., Dai, G., Shahbazi, M.-A., Mi, B., & Liu, G. (2024). Copper incorporated biomaterial-based technologies for multifunctional wound repair. *Theranostics, 14*(2), 547–570. https://doi.org/10.7150/thno.87193

LIST OF PUBLICATIONS

Presented a paper entitled “Antibacterial Nanoparticle-Integrated Scaffolds for the Management of Infected Wounds: Materials, Mechanisms and Clinical Perspectives " at the International Conference on Biomedical Nanotechnology and Bioinformatics (ICBNBI-26)

16th May 2026 Mumbai – India

DELHI TECHNOLOGICAL UNIVERSITY

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EDUCATION

Course	College/University	Year	CGPA/%
MSc. Biotechnology	Delhi Technological University	2024–26	8.72 (1st Sem)
BSc. Life Sciences	Gargi College	2021–24	8.97

SCHOLASTIC ACHIEVEMENTS

- Secured **107 rank** in GAT-B [2024]
- Secured **309 rank** in IIT-JAM [2024]
- Cleared **CUET-PG** [2024]

INTERNSHIP AND TRAINING EXPERIENCE

- **Intern at Younity.in** [May 2025–June 2025]
  - Assisted in online workshop coordination and content development.
  - Promoted awareness on soft skills among students and professionals.
- **Hands-on Workshop on Bioinformatics and Molecular Docking, BioSoc DTU** [April 2025]
  - Gained practical experience in molecular docking tools.
  - Analyzed protein-ligand interactions using open-source bioinformatics tools.
- **Workshop on Healthcare and Immunology** [March 2025]
  - Attended sessions on immune system function and vaccine development.
  - Gained insights into disease modeling and immunotherapy strategies.

PROJECTS

- **Cyclodextrin-Based Nanoparticle System for siRNA Delivery Across the Blood-Brain Barrier for Huntington's Disease Therapy** [May 2025–Present]
  - Designed a PEGylated cyclodextrin nanoparticle system to encapsulate and deliver siRNA to brain tissues.
  - Focused on overcoming the blood-brain barrier (BBB) using targeted delivery strategies.
  - Aimed at silencing mutant huntingtin (mHTT) gene expression to mitigate Huntington's Disease progression.

SKILLS & INTERESTS

- **Programming Languages**: SQL
- **Lab Skills**: Molecular Cloning, Gel Electrophoresis, Spectroscopy,Basic understanding of bioreactors and fermentation systems,trained in sterile handling using biosafety cabinets and laminar flow,familiar with basic chromatography skills,competent in maintaing lab records and data analysis
- **Interests**: Data Analysis, Bioinformatics

RELEVANT COURSES

- Genetics, Molecular Biology, Biochemistry, Immunology, Environmental Biotechnology
- Biostatistics, Microbiology, Cell Biology, Genetic Engineering

POSITIONS OF RESPONSIBILITY

Led a team of 15+ students for organizing stage performances and guest lectures.

Collaborated with sponsors and media teams to manage outreach.

EXTRACURRICULAR ACTIVITIES

- Volunteered in blood donation and health awareness camp organized by NSS [2023]
- Coordinated events and logistics for the Annual Cultural Fest at Gargi College [2023]
- Active participant in yoga and mindfulness sessions organized by the college wellness center [2022]