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Review Article

# Biogenic Metallic Nanoparticles Derived from Agricultural and Biowaste for Advanced Wound Healing Applications: Mechanisms, Therapeutic Potential, Challenges, and Future Perspectives

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ABSTRACT

Chronic and non-healing wounds remain a major global healthcare challenge owing to persistent microbial infections, prolonged inflammation, oxidative stress, impaired angiogenesis, and increasing antimicrobial resistance. Conventional wound management strategies often provide suboptimal outcomes, necessitating the development of multifunctional and sustainable therapeutic approaches. Green-synthesized metallic nanoparticles derived from agricultural and food-processing waste have emerged as promising biomaterials because of their eco-friendly production, cost-effectiveness, and broad spectrum of biological activities. Agricultural residues are rich in bioactive phytochemicals, including polyphenols, flavonoids, proteins, carbohydrates, and organic acids, which act as natural reducing, stabilizing, and capping agents during nanoparticle synthesis. Metallic nanoparticles such as silver, gold, zinc oxide, copper oxide, iron oxide, titanium dioxide, and magnesium oxide exhibit potent antimicrobial, antioxidant, anti-inflammatory, angiogenic, and regenerative properties that collectively promote wound repair. These nanoparticles facilitate healing by disrupting microbial membranes, modulating inflammatory cytokines, regulating oxidative stress, enhancing fibroblast proliferation, stimulating keratinocyte migration, promoting collagen deposition, and accelerating extracellular matrix remodeling and angiogenesis. Despite encouraging preclinical evidence, challenges including variability in biological precursors, reproducibility of green synthesis, long-term biosafety, large-scale manufacturing, and regulatory approval continue to limit clinical translation. This review summarizes recent advances in agricultural waste-mediated green synthesis of metallic nanoparticles, their physicochemical characteristics, mechanisms of wound healing, biomedical applications, safety considerations, and translational challenges, while highlighting their potential to integrate sustainable nanotechnology with circular bioeconomy principles for the development of next-generation wound care systems.



## *Introduction*

Skin is the largest organ of the human body and serves as the primary physical and immunological barrier against environmental insults, microbial invasion, chemical exposure, ultraviolet radiation, and mechanical injury. Beyond its protective role, the skin

contributes to thermoregulation, sensory perception, immune surveillance, maintenance of fluid balance, and metabolic homeostasis. Any disruption of skin integrity compromises these essential physiological functions and initiates a highly coordinated wound healing response involving numerous cell populations, signaling molecules, extracellular matrix (ECM) components, and vascular networks [1–3]. Under normal physiological conditions, wound repair proceeds through a series of overlapping phases—hemostasis, inflammation, proliferation, and remodeling—that collectively restore tissue architecture and function. However, disturbances in any of these stages may delay tissue regeneration, resulting in chronic, non-healing wounds associated with significant morbidity and mortality [1,2].

The global burden of chronic wounds has increased substantially over the past two decades due to population aging, urbanization, sedentary lifestyles, diabetes mellitus, obesity, peripheral vascular disease, pressure injuries, burns, trauma, and immunocompromised conditions [2,4]. It is estimated that millions of individuals worldwide suffer from chronic wounds each year, imposing a considerable socioeconomic burden on healthcare systems through prolonged hospitalization, repeated surgical interventions, and long-term wound management. Patients frequently experience persistent pain, reduced mobility, recurrent infections, diminished quality of life, psychological distress, and increased risk of limb amputation. The rising prevalence of multidrug-resistant (MDR) microorganisms has further complicated wound management by reducing the effectiveness of conventional antimicrobial therapies and increasing treatment costs [4,5].

Successful wound healing depends on precise temporal coordination between inflammatory responses, immune cell recruitment, angiogenesis, extracellular matrix remodeling, and epithelial regeneration. During the inflammatory phase, neutrophils and macrophages eliminate invading pathogens and remove necrotic tissue while releasing cytokines and growth factors that initiate tissue repair [1,6].

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Subsequently, fibroblasts migrate into the wound bed to synthesize collagen and other extracellular matrix proteins, whereas endothelial cells stimulate angiogenesis to restore oxygen and nutrient supply. Keratinocytes proliferate and migrate across the wound surface to re-establish the epidermal barrier. Finally, collagen remodeling and scar maturation improve tissue strength and functionality. Dysregulation of these tightly controlled biological events often results in excessive inflammation, oxidative stress, persistent infection, impaired vascularization, and delayed wound closure [2,3].

Oxidative stress has emerged as one of the principal pathological mechanisms responsible for chronic wound development. Reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are generated physiologically during inflammatory responses and contribute to antimicrobial defense and intracellular signaling [7]. Nevertheless, sustained overproduction of ROS overwhelms endogenous antioxidant defense systems, resulting in lipid peroxidation, protein oxidation, mitochondrial dysfunction, DNA damage, and apoptosis of resident skin cells. Excessive oxidative stress also activates pro-inflammatory signaling pathways, including nuclear factor-kappa B (NF-κB) and the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome, thereby perpetuating chronic inflammation and preventing progression toward the proliferative phase of healing [8,9]. Consequently, therapeutic strategies capable of simultaneously controlling oxidative stress, regulating inflammation, and eliminating microbial pathogens are increasingly regarded as promising approaches for advanced wound management.

Traditional wound care relies on topical antiseptics, systemic antibiotics, surgical debridement, hydrocolloid dressings, alginate dressings, negative-pressure wound therapy, and skin grafting. Although these interventions remain indispensable in clinical practice, they exhibit several limitations. Frequent dressing changes, poor drug penetration into biofilms, development of antimicrobial resistance, inadequate modulation of inflammatory responses, and high treatment costs often compromise healing outcomes, particularly in patients with diabetes or vascular insufficiency [4,10]. These shortcomings have stimulated extensive research into multifunctional biomaterials capable of integrating antimicrobial activity with regenerative properties.

Nanotechnology has emerged as one of the most promising interdisciplinary fields in regenerative medicine by enabling the development of nanoscale

materials possessing unique physicochemical, optical, catalytic, and biological properties. Metallic nanoparticles—including silver (AgNPs), gold (AuNPs), zinc oxide (ZnO NPs), copper oxide (CuO NPs), iron oxide ($Fe_3O_4$ NPs), magnesium oxide (MgO NPs), and titanium dioxide ($TiO_2$ NPs)—have demonstrated broad-spectrum antimicrobial activity, antioxidant capacity, anti-inflammatory effects, angiogenic potential, and the ability to stimulate collagen synthesis and cellular proliferation [11–13]. Their exceptionally high surface-area-to-volume ratio facilitates enhanced interaction with bacterial membranes, cellular receptors, and biomolecules, thereby improving therapeutic efficacy while enabling controlled incorporation into advanced wound dressings such as hydrogels, electrospun nanofibers, polymeric films, sponges, and composite scaffolds.

Despite their considerable biomedical potential, conventional physical and chemical methods employed for nanoparticle synthesis frequently require hazardous reducing agents, elevated temperatures, high energy consumption, toxic organic solvents, and sophisticated instrumentation [14]. Residual chemical contaminants generated during these processes may adversely affect biocompatibility and environmental safety, limiting their suitability for biomedical applications. In response to these concerns, green synthesis has emerged as an environmentally sustainable alternative that utilizes biological resources to produce nanoparticles under mild reaction conditions while minimizing hazardous waste generation.

Agricultural residues and food-processing by-products represent one of the most promising renewable resources for green nanoparticle synthesis. Every year, enormous quantities of fruit peels, vegetable residues, seed husks, cereal bran, sugarcane bagasse, rice husk, tea waste, coffee grounds, coconut shells, banana peels, citrus peels, pomegranate rinds, grape pomace, and numerous other agro-industrial wastes are generated worldwide [15]. Historically regarded as disposal burdens, these materials are now recognized as rich reservoirs of bioactive phytochemicals, including flavonoids, phenolic acids, tannins, alkaloids, terpenoids, proteins, polysaccharides, amino acids, and reducing sugars. These naturally occurring compounds simultaneously function as reducing, stabilizing, and capping agents during nanoparticle synthesis, eliminating the need for toxic chemical reagents while improving nanoparticle stability and biological compatibility [14–16].

The utilization of agricultural biowaste for nanoparticle synthesis aligns closely with the principles of the circular economy, which emphasizes resource recovery, waste minimization, recycling, and sustainable production systems. Rather than treating agricultural residues as environmental liabilities, waste valorization transforms low-value biomass into high-value biomedical products with significant commercial and therapeutic potential. Such strategies not only reduce environmental pollution associated with open burning and landfill disposal but also contribute to sustainable bioeconomy initiatives by creating economically valuable products from renewable resources [15,17]. Integrating green nanotechnology with circular economy principles therefore represents an innovative approach that simultaneously addresses environmental sustainability and global healthcare challenges.

Recent advances in green nanotechnology have demonstrated that biogenic metallic nanoparticles possess multifunctional biological activities extending beyond antimicrobial effects. These nanoparticles have been shown to regulate macrophage polarization, stimulate fibroblast migration, accelerate keratinocyte proliferation, enhance collagen deposition, promote angiogenesis through vascular endothelial growth factor (VEGF) signaling, and modulate inflammatory mediators such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) [12,13,18]. Their incorporation into smart wound dressings enables controlled drug release, moisture retention, oxygen permeability, and sustained antimicrobial protection, thereby creating an optimal microenvironment for tissue regeneration.

Nevertheless, significant challenges remain before these nanomaterials can achieve widespread clinical adoption. Variability in the chemical composition of biological extracts, lack of standardized synthesis protocols, limited reproducibility, insufficient long-term toxicity studies, incomplete pharmacokinetic data, and regulatory uncertainties continue to impede successful clinical translation [13,16]. Addressing these limitations will require multidisciplinary collaboration among materials scientists, microbiologists, clinicians, toxicologists, biomedical engineers, and regulatory agencies to establish standardized manufacturing processes, comprehensive safety assessments, and robust clinical evidence.

The present review aims to provide a comprehensive and critical overview of recent advances in the application of biogenic metallic nanoparticles synthesized from agricultural and biological waste resources for wound healing. Particular emphasis is placed on the biology of wound repair, cellular and molecular mechanisms underlying tissue regeneration, inflammatory and oxidative stress pathways, sustainable waste valorization strategies, green

synthesis methodologies, nanoparticle characterization techniques, antimicrobial and regenerative mechanisms, biomedical applications, biosafety considerations, current challenges, and future perspectives. By integrating developments in regenerative medicine, nanotechnology, and circular bioeconomy, this review highlights the potential of sustainable nanomaterials to transform next-generation wound care and contribute to environmentally responsible biomedical innovation.

## *Skin Structure and Classification of Wounds*

### Skin Structure

The skin is the largest organ of the human body, accounting for approximately 15–16% of total body weight in adults and covering an average surface area of nearly 2 $m^2$. As the primary interface between the body and the external environment, it performs multiple physiological functions, including mechanical protection, immune surveillance, thermoregulation, sensory perception, prevention of excessive water loss, vitamin D synthesis, and maintenance of metabolic homeostasis [19,20]. Owing to its complex structural organization and dynamic cellular composition, the skin possesses remarkable regenerative capacity following injury. However, extensive tissue damage, systemic diseases, aging, or persistent microbial infection may compromise this regenerative potential and lead to delayed wound healing or chronic wound formation.

Anatomically, the skin consists of three interconnected layers: the epidermis, dermis, and hypodermis (subcutaneous tissue). Each layer contains specialized cell populations and extracellular components that contribute collectively to tissue integrity and wound repair.

### Physiological Basis of Wound Formation

A wound is defined as any disruption in the anatomical continuity or physiological function of skin or underlying tissues resulting from physical, chemical, thermal, electrical, radiation-induced, or biological insults. Tissue injury immediately initiates a complex cascade involving hemostasis, inflammation, cellular migration, proliferation, angiogenesis, matrix deposition, and remodeling to restore structural integrity [1].

The severity and duration of tissue injury depend upon multiple factors including:

- Nature and magnitude of trauma
- Degree of microbial contamination
- Tissue oxygenation
- Local blood supply
- Nutritional status
- Age
- Presence of systemic disorders such as diabetes mellitus or peripheral vascular disease
- Immune competence
- Smoking and lifestyle factors

Disruption of these physiological determinants frequently results in impaired wound healing characterized by persistent inflammation, microbial colonization, excessive oxidative stress, extracellular matrix degradation, and defective angiogenesis [2].

## *Classification of Wounds*

Accurate classification of wounds is essential for selecting appropriate therapeutic interventions and predicting clinical outcomes. Wounds may be classified according to duration, mechanism of injury, depth, microbial contamination, or healing pattern.

### Acute Wounds

Acute wounds progress through the normal stages of wound healing within an expected timeframe, generally achieving complete tissue repair within four to six weeks. These wounds are usually caused by surgical incisions, accidental trauma, burns, abrasions, lacerations, or puncture injuries [2].

Acute wounds exhibit:

- Controlled inflammation
- Efficient microbial clearance
- Adequate angiogenesis
- Balanced collagen synthesis
- Minimal scar formation

Under appropriate clinical management, these wounds demonstrate predictable healing with restoration of tissue function.

### Chronic Wounds

Chronic wounds fail to progress through the orderly stages of healing and remain unhealed for more than

four to six weeks. Persistent inflammation repeated microbial infection, excessive protease activity, impaired angiogenesis, oxidative stress, and cellular senescence contribute to delayed healing [3].

Common chronic wounds include:

- Diabetic foot ulcers
- Venous leg ulcers
- Pressure ulcers
- Arterial ulcers
- Non-healing traumatic wounds

Chronic wounds are characterized by elevated concentrations of matrix metalloproteinases (MMPs), pro-inflammatory cytokines, reactive oxygen species, and bacterial biofilms, all of which collectively inhibit fibroblast proliferation, keratinocyte migration, and extracellular matrix deposition [25].

### *Classification According to Etiology*

#### Surgical Wounds

These are intentionally created under sterile conditions during operative procedures and generally heal by primary intention with minimal complications unless secondary infection develops.

#### Traumatic Wounds

Traumatic injuries include abrasions, lacerations, avulsions, puncture wounds, crush injuries, and penetrating trauma. The degree of tissue destruction varies according to the mechanism and energy of injury.

#### Burn Wounds

Burn injuries result from exposure to thermal energy, electricity, chemicals, or radiation and are classified into superficial, partial-thickness, and full-thickness burns depending on tissue involvement. Severe burns are frequently associated with systemic inflammatory responses, fluid imbalance, and increased susceptibility to infection [26].

#### Diabetic Foot Ulcers

Diabetic ulcers develop primarily due to peripheral neuropathy, microvascular dysfunction, impaired immunity, and chronic hyperglycemia. These wounds exhibit prolonged inflammation, defective angiogenesis, reduced collagen synthesis, and increased bacterial colonization, making them among the most difficult wounds to treat [27].

#### Pressure Ulcers

Pressure ulcers arise from prolonged compression of soft tissues over bony prominences, leading to ischemia, hypoxia, and tissue necrosis. They commonly affect immobilized, elderly, or critically ill patients and remain a significant cause of healthcare-associated morbidity worldwide [28].

#### Venous Leg Ulcers

Venous ulcers result from chronic venous insufficiency and sustained venous hypertension. Persistent edema, inflammatory mediator accumulation, and impaired oxygen diffusion contribute to delayed healing and high recurrence rates.

#### Arterial Ulcers

Arterial ulcers occur secondary to peripheral arterial disease and reduced tissue perfusion. Inadequate oxygen delivery, ischemia, and nutrient deprivation severely compromise tissue regeneration and frequently necessitate vascular intervention before wound closure can occur.

### *Classification According to Healing Pattern*

Wounds may also be categorized according to the mechanism by which tissue repair occurs:

**Primary intention** involves direct approximation of wound edges with minimal tissue loss, rapid epithelialization, and limited scar formation.

**Secondary intention** occurs when substantial tissue loss prevents direct closure. Healing depends upon granulation tissue formation, wound contraction, angiogenesis, and epithelial migration, resulting in larger scars and prolonged healing time.

**Tertiary intention (delayed primary closure)** combines features of both mechanisms, where contaminated wounds are initially left open to allow infection control before surgical closure is performed.

#### Clinical Significance for Advanced Nanotherapeutics

The pathophysiological diversity among wound types necessitates therapeutic systems capable of addressing multiple biological targets simultaneously. Chronic wounds, in particular, require interventions that combine antimicrobial activity, immunomodulation, antioxidant effects, angiogenesis promotion, extracellular matrix remodeling, and stimulation of cellular proliferation. Biogenic metallic nanoparticles synthesized from agricultural and biological waste resources have emerged as promising multifunctional nanotherapeutics because they can simultaneously suppress microbial infection, regulate inflammatory signaling, scavenge excessive reactive oxygen species, enhance fibroblast and keratinocyte activity, and promote neovascularization. Consequently, integrating these nanoparticles into advanced wound dressings offers a novel strategy for improving healing outcomes while supporting sustainable biomedical innovation through waste valorization.

## *Phases of Wound Healing*

Wound healing is a highly coordinated and dynamic biological process that restores the structural and functional integrity of damaged tissue following injury. Rather than occurring as isolated events, wound repair progresses through four interconnected and overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Each phase is regulated by precise interactions among platelets, inflammatory cells, fibroblasts, keratinocytes, endothelial cells, extracellular matrix (ECM) components, cytokines, chemokines, and growth factors. Successful healing depends on the timely initiation, progression, and resolution of these phases. Any disruption in this tightly controlled sequence may lead to impaired healing, chronic wound formation, excessive fibrosis, or pathological scarring [1–3].

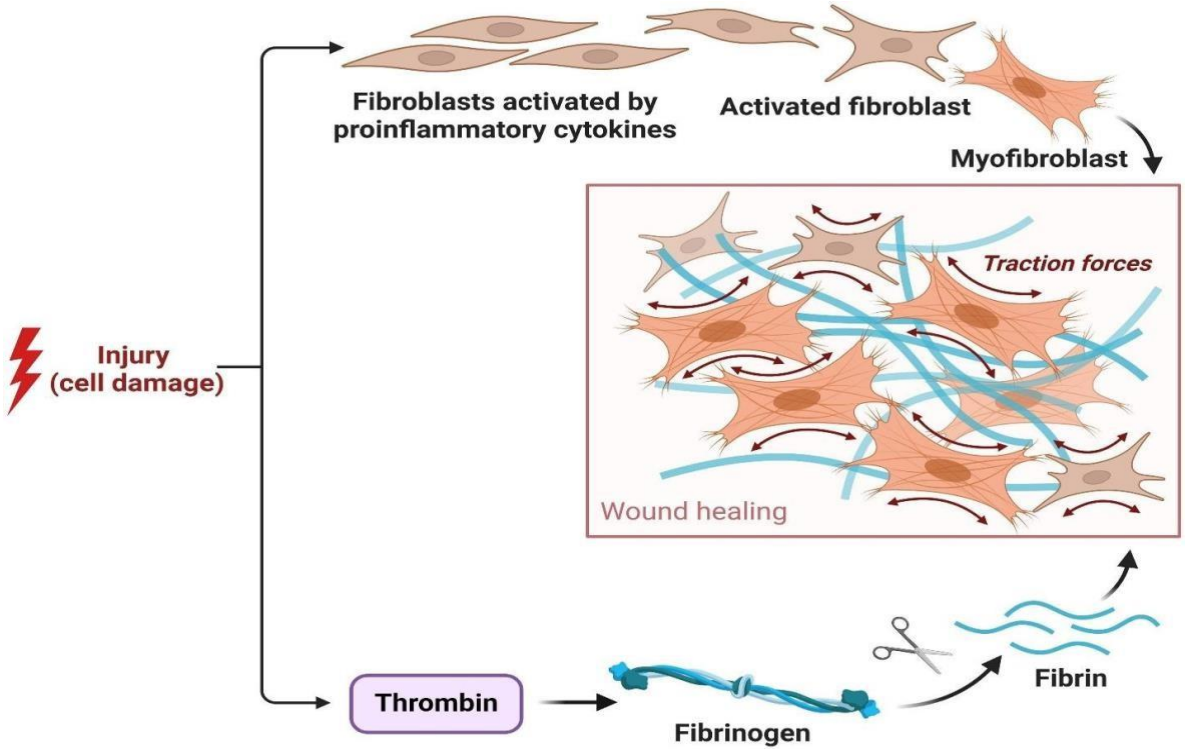


**Figure 1:** Phases of wound healing.

### Hemostasis Phase

The hemostatic phase is initiated immediately after tissue injury and typically lasts from several minutes to a few hours. Its principal objective is to prevent excessive blood loss while establishing a temporary extracellular matrix that supports subsequent inflammatory and reparative events. Vascular injury results in immediate vasoconstriction mediated by endothelin, thromboxane $A_2$, and sympathetic neural reflexes, thereby reducing local blood flow and limiting hemorrhage [29].

Exposure of subendothelial collagen activates circulating platelets, which rapidly adhere to the damaged vascular surface through interactions involving von Willebrand factor and glycoprotein receptors. Activated platelets undergo degranulation and release numerous bioactive mediators, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), serotonin, adenosine diphosphate (ADP), thromboxane $A_2$, and fibrinogen [30]. These mediators initiate coagulation, recruit inflammatory cells, stimulate fibroblast activation, and promote angiogenesis.

Simultaneously, activation of the intrinsic and extrinsic coagulation pathways culminates in thrombin generation and conversion of fibrinogen into insoluble fibrin. The resulting fibrin mesh stabilizes the platelet plug and forms a provisional extracellular matrix that provides structural support for infiltrating neutrophils, macrophages, fibroblasts, and endothelial cells [31]. Beyond its hemostatic function, the fibrin clot serves as a temporary scaffold for cellular migration and reservoir for growth factors essential for subsequent wound repair.

**Inflammatory Phase**

Following successful hemostasis, the inflammatory phase begins within hours after injury and generally persists for 2–5 days under physiological conditions. Controlled inflammation is indispensable for eliminating microorganisms, removing necrotic tissue, and preparing the wound microenvironment for tissue regeneration. However, prolonged or excessive inflammation represents one of the principal causes of chronic non-healing wounds [2,32].

Neutrophils constitute the earliest inflammatory cells to infiltrate the wound, reaching peak concentrations within the first 24–48 hours. Guided by chemotactic mediators such as interleukin-8 (IL-8), complement proteins, and bacterial peptides, neutrophils migrate from the circulation into injured tissue where they phagocytose microorganisms, remove cellular debris, and release antimicrobial peptides, proteases, and reactive oxygen species (ROS) [33]. Although ROS contribute to microbial killing, excessive ROS generation can damage surrounding healthy tissues through lipid peroxidation, protein oxidation, and DNA injury.

As neutrophil numbers decline, circulating monocytes infiltrate the wound and differentiate into macrophages, which become the dominant immune cells during the inflammatory phase. Macrophages perform multiple essential functions, including phagocytosis of apoptotic neutrophils, secretion of pro-inflammatory cytokines, antigen presentation, regulation of extracellular matrix turnover, and orchestration of tissue repair [34]. Initially, classically activated M1 macrophages predominate and produce inflammatory mediators such as tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and nitric oxide, thereby enhancing antimicrobial defense. Subsequently, successful wound healing requires phenotypic transition toward alternatively activated M2 macrophages, which secrete anti-inflammatory cytokines, including IL-10 and TGF-β, stimulate fibroblast proliferation, promote collagen synthesis, and facilitate angiogenesis [35].

Other inflammatory cells, including mast cells, dendritic cells, and lymphocytes, further modulate the inflammatory response through secretion of histamine, cytokines, chemokines, and growth factors. Resolution of inflammation is characterized by clearance of inflammatory cells, restoration of tissue homeostasis, and transition to the proliferative phase. Failure of this transition results in persistent inflammation, continuous protease activity, degradation of extracellular matrix components, and impaired wound healing, which are hallmarks of chronic diabetic ulcers, venous leg ulcers, and pressure injuries [32].

**Proliferative Phase**

The proliferative phase generally extends from the third day after injury until approximately the third week, although its duration varies according to wound size, severity, and patient-specific factors. This stage is characterized by rapid cellular proliferation, extracellular matrix synthesis, angiogenesis, granulation tissue formation, wound contraction, and re-epithelialization [1,36].

Fibroblasts migrate into the wound bed under the influence of PDGF, TGF-β, fibroblast growth factor (FGF), and connective tissue growth factor (CTGF).

These cells synthesize collagen type III, fibronectin, hyaluronic acid, proteoglycans, and other extracellular matrix components that form the structural framework of granulation tissue [37]. As healing progresses, many fibroblasts differentiate into α-smooth muscle actin (α-SMA)-expressing myofibroblasts, which generate contractile forces responsible for wound contraction and reduction of wound dimensions.

Angiogenesis represents another hallmark of the proliferative phase. Tissue hypoxia stimulates expression of hypoxia-inducible factor-1α (HIF-1α), which enhances production of VEGF and other angiogenic mediators. Endothelial cells proliferate, migrate, and organize into newly formed capillary networks that restore oxygen delivery, nutrient transport, and metabolic exchange within regenerating tissue [38]. Adequate angiogenesis is particularly critical for healing diabetic wounds, where impaired neovascularization frequently contributes to delayed tissue repair.

Simultaneously, keratinocytes located at the wound margins proliferate and migrate across the provisional matrix to re-establish epidermal continuity through re-epithelialization. Growth factors such as EGF, keratinocyte growth factor (KGF), and TGF-α regulate keratinocyte proliferation and migration. Successful re-epithelialization restores barrier function and reduces the risk of secondary microbial infection [39].

The coordinated interactions among fibroblasts, endothelial cells, keratinocytes, macrophages, and extracellular matrix components culminate in formation of highly vascularized granulation tissue, which gradually replaces the temporary fibrin scaffold established during hemostasis.

**Remodeling and Maturation Phase**

The remodeling phase represents the final stage of wound healing and may continue for several months or even years following injury. Although the wound appears clinically healed, extensive molecular and structural remodeling continues within the extracellular matrix to improve tissue strength and functionality [1,2].

During this phase, collagen type III deposited during granulation tissue formation is progressively degraded by matrix metalloproteinases (MMPs) and replaced by thicker, mechanically stronger collagen type I fibers. Simultaneously, tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity to maintain extracellular matrix homeostasis [40]. Balanced collagen turnover is essential for preventing excessive fibrosis or chronic tissue degradation.

Myofibroblasts gradually undergo apoptosis after completing wound contraction, resulting in decreased cellularity and vascular density within the repaired tissue. Blood vessels formed during angiogenesis regress through physiological pruning, while collagen fibers become increasingly organized along mechanical stress lines, thereby enhancing tensile strength [37].

Despite extensive remodeling, healed skin rarely regains the biomechanical properties of uninjured tissue. Mature scar tissue typically achieves approximately 70–80% of the tensile strength of normal skin and exhibits reduced elasticity, diminished vascularity, and absence of skin appendages such as hair follicles and sweat glands [2]. Dysregulation of remodeling may lead to pathological outcomes including hypertrophic scars, keloids, or chronic fibrotic disorders.

## *Molecular Coordination of the Wound-Healing Cascade*

The four phases of wound healing are regulated by an intricate network of cytokines, chemokines, growth factors, transcription factors, and intracellular signaling pathways. Key mediators include PDGF, TGF-β, VEGF, FGF, EGF, IL-1β, IL-6, TNF-α, and interferon-γ (IFN-γ), which collectively regulate cellular migration, proliferation, differentiation, angiogenesis, and extracellular matrix remodeling [41]. Emerging evidence also highlights the importance of pathways such as NF-κB, PI3K/Akt, MAPK, Wnt/β-catenin, and the NLRP3 inflammasome in coordinating tissue repair and inflammatory resolution.

Understanding these molecular mechanisms has facilitated the development of advanced therapeutic approaches, including growth factor delivery systems, stem cell-based therapies, gene therapy, biomaterial scaffolds, and nanotechnology-based wound dressings. Among these innovations, biogenic metallic

nanoparticles have attracted particular interest because of their ability to simultaneously modulate inflammation, reduce oxidative stress, inhibit microbial growth, stimulate angiogenesis, and enhance collagen deposition, thereby targeting multiple stages of the wound-healing process.

## *Cellular Components of Wound Healing and Their Therapeutic Modulation by Biogenic Metallic Nanoparticles*

### Cellular Dynamics During Cutaneous Wound Repair

Cutaneous wound healing is orchestrated by a diverse population of resident and infiltrating cells that communicate through cytokines, chemokines, growth factors, extracellular vesicles, and direct cell–cell interactions. Each cell type contributes distinct but interconnected functions that collectively regulate inflammation, tissue regeneration, extracellular matrix remodeling, angiogenesis, and restoration of skin barrier integrity. Disturbance in the function or temporal coordination of these cellular components contributes significantly to chronic wound pathology. Recent advances in nanomedicine indicate that biogenic metallic nanoparticles (BMNPs) can modulate several of these cellular processes, thereby enhancing wound repair while reducing infection and inflammation [42–44].

### Platelets: Initiators of Tissue Repair

Platelets are the first cellular responders following vascular injury and play a pivotal role beyond hemostasis. Upon activation, platelets release numerous growth factors stored within their α-granules, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1). These mediators initiate inflammatory cell recruitment, fibroblast activation, endothelial cell proliferation, and extracellular matrix synthesis.

Platelet-derived chemokines also regulate neutrophil migration and monocyte recruitment, thereby linking coagulation with innate immune responses. In addition, platelets release extracellular vesicles and microparticles containing proteins, lipids, and nucleic acids that influence cellular communication within the wound microenvironment [45].

Emerging studies suggest that biomaterial-based wound dressings incorporating biogenic nanoparticles preserve platelet-derived growth factors while providing antimicrobial protection, thereby supporting early tissue repair without compromising clot stability.

### Neutrophils: First-Line Innate Immune Defenders

Neutrophils represent the earliest inflammatory leukocytes recruited to injured tissue, typically reaching maximal concentrations within the first 24–48 hours after injury. Their primary function is elimination of invading microorganisms through phagocytosis, degranulation, neutrophil extracellular trap (NET) formation, and production of reactive oxygen species (ROS).

Although essential for host defense, prolonged neutrophil persistence contributes to excessive inflammation through continuous secretion of elastase, myeloperoxidase, matrix metalloproteinases (MMP-8 and MMP-9), and pro-inflammatory cytokines. These mediators degrade extracellular matrix components and impair fibroblast function, thereby delaying wound closure [46].

Recent evidence indicates that silver and zinc oxide nanoparticles synthesized using plant-derived phytochemicals can reduce bacterial burden rapidly, thereby shortening the duration of neutrophil activation. Consequently, controlled nanotherapeutic interventions may facilitate earlier resolution of inflammation while minimizing collateral tissue damage.

### Macrophages: Master Regulators of Tissue Regeneration

Among all immune cells participating in wound repair, macrophages exhibit the greatest functional diversity. These cells originate from circulating monocytes and undergo phenotypic polarization according to environmental stimuli.

During the early inflammatory phase, macrophages predominantly exhibit the **M1 phenotype**, characterized by production of TNF-α, IL-1β, IL-6, nitric oxide, and reactive oxygen species. M1 macrophages effectively eliminate pathogens and

remove damaged tissue but may impair healing if their activation persists.

As healing progresses, macrophages undergo polarization toward the M2 phenotype, which secretes anti-inflammatory cytokines such as IL-10 and TGF-β while stimulating fibroblast proliferation, collagen deposition, angiogenesis, and extracellular matrix remodeling [47].

Failure of the M1-to-M2 transition represents one of the defining pathological features of chronic diabetic wounds.

Recent investigations demonstrate that several biogenic metallic nanoparticles—including silver, gold, cerium oxide, and iron oxide nanoparticles synthesized from botanical extracts—promote M2 polarization while suppressing excessive inflammatory cytokine production. This immunomodulatory activity contributes significantly to accelerated wound closure observed in numerous experimental models.

**Fibroblasts and Myofibroblasts**

Fibroblasts constitute the principal mesenchymal cells responsible for synthesis and maintenance of the extracellular matrix. Following injury, fibroblasts migrate into the wound bed in response to PDGF, TGF-β, fibroblast growth factor (FGF), and connective tissue growth factor (CTGF). Once activated, they synthesize collagen, elastin, fibronectin, proteoglycans, and glycosaminoglycans that collectively establish granulation tissue.

Under the influence of TGF-β1 and mechanical tension, fibroblasts differentiate into contractile myofibroblasts expressing α-smooth muscle actin (α-SMA). These specialized cells generate contractile forces that decrease wound size and facilitate tissue approximation [48].

Biogenic metallic nanoparticles have demonstrated the ability to stimulate fibroblast proliferation at therapeutic concentrations while simultaneously reducing oxidative stress-induced apoptosis. Green-synthesized gold nanoparticles, in particular, have been reported to enhance collagen maturation and extracellular matrix organization without inducing excessive fibrosis.

**Keratinocytes and Re-epithelialization**

Keratinocytes comprise approximately 90% of epidermal cells and are indispensable for restoration of the skin barrier. Following injury, keratinocytes at the wound edge undergo phenotypic transformation characterized by reduced intercellular adhesion, increased migratory capacity, and accelerated proliferation.

Migration is regulated by epidermal growth factor (EGF), keratinocyte growth factor (KGF), transforming growth factor-α (TGF-α), and extracellular matrix proteins such as laminin and fibronectin [49].

Chronic inflammation, bacterial toxins, excessive ROS generation, and persistent hyperglycemia inhibit keratinocyte migration, thereby delaying wound closure.

Several studies have demonstrated that nanoparticle-loaded hydrogels enhance keratinocyte migration by maintaining optimal wound moisture, reducing microbial contamination, and modulating inflammatory signaling pathways.

**Endothelial Cells and Angiogenesis**

Formation of new blood vessels represents one of the most critical determinants of successful wound repair. Endothelial cells respond to tissue hypoxia through activation of hypoxia-inducible factor-1α (HIF-1α), which stimulates expression of VEGF and other angiogenic mediators.

During angiogenesis, endothelial cells proliferate, migrate, degrade basement membrane components, and organize into functional capillary networks supplying oxygen and nutrients to regenerating tissue [50].

Insufficient angiogenesis is particularly evident in diabetic ulcers, ischemic wounds, and elderly patients.

Recent investigations indicate that low concentrations of zinc oxide, copper oxide, and iron oxide nanoparticles stimulate endothelial proliferation and neovascularization while maintaining acceptable biocompatibility. Incorporation of these nanoparticles into hydrogel dressings has been associated with improved vascular density and accelerated granulation tissue formation.

**Mast Cells**

Mast cells participate in the earliest inflammatory responses through rapid degranulation and release of histamine, serotonin, proteases, TNF-α, and vascular permeability factors.

These mediators promote vasodilation, leukocyte recruitment, and activation of fibroblasts. Mast cells also regulate angiogenesis by secreting VEGF and fibroblast growth factor.

Although beneficial during early inflammation, excessive mast cell activation may contribute to fibrosis and hypertrophic scar formation. Controlled modulation of mast cell activity therefore represents an emerging therapeutic target in regenerative medicine.

**Mesenchymal Stem Cells (MSCs)**

Mesenchymal stem cells have attracted considerable attention owing to their regenerative, immunomodulatory, and paracrine properties. These multipotent cells can differentiate into fibroblasts, endothelial cells, adipocytes, osteoblasts, and other connective tissue lineages while secreting numerous growth factors involved in tissue repair.

Rather than direct differentiation alone, MSCs primarily facilitate wound healing through secretion of extracellular vesicles, exosomes, VEGF, hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and anti-inflammatory cytokines that stimulate resident cell proliferation and angiogenesis [51].

Recent research indicates that combining MSC therapy with biogenic nanoparticle-based scaffolds may enhance stem cell survival, improve cellular retention within wounds, and prolong regenerative signaling.

**Cellular Crosstalk During Wound Repair**

Efficient wound healing depends not only on the function of individual cell populations but also on continuous communication among immune cells, stromal cells, endothelial cells, and epithelial cells. Cytokines, chemokines, extracellular vesicles, and growth factors coordinate sequential activation and resolution of each healing phase.

Biogenic metallic nanoparticles appear capable of influencing multiple aspects of this cellular network simultaneously by:

- reducing microbial colonization,
- suppressing excessive inflammatory cytokines,
- regulating macrophage polarization,
- enhancing fibroblast proliferation,
- stimulating keratinocyte migration,
- promoting endothelial angiogenesis, and
- protecting cells from oxidative injury.

This multitarget mode of action distinguishes BMNPs from conventional topical antimicrobials and positions them as promising components of next-generation wound dressings.

## *Molecular Signaling Pathways Regulating Wound Healing and Their Modulation by Biogenic Metallic Nanoparticles*

Successful wound healing is governed by an intricate network of intracellular signaling pathways that regulate inflammation, oxidative stress, cell proliferation, migration, angiogenesis, extracellular matrix (ECM) remodeling, and tissue maturation. These signaling cascades ensure that the different phases of wound healing occur in a coordinated manner. Dysregulation of one or more pathways contributes to chronic inflammation, impaired angiogenesis, excessive fibrosis, and delayed tissue regeneration, particularly in diabetic and ischemic wounds. Recent advances in nanomedicine have demonstrated that biogenic metallic nanoparticles (BMNPs) synthesized from agricultural and biological waste can interact with these molecular pathways, thereby promoting a regenerative wound microenvironment.

**Nuclear Factor-kappa B (NF-κB) Signaling Pathway**

The **NF-κB signaling pathway** is one of the most important regulators of inflammation during wound healing. Under physiological conditions, NF-κB remains inactive in the cytoplasm through association with inhibitor proteins known as IκBs. Following tissue injury, inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), bacterial lipopolysaccharides (LPS), and reactive oxygen species activate the IκB kinase (IKK) complex, resulting in degradation of IκB and nuclear translocation of NF-κB.

Activated NF-κB stimulates transcription of numerous pro-inflammatory genes, including TNF-α, IL-6, IL-8, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and several matrix metalloproteinases (MMPs). While transient activation is essential for host defense and microbial clearance, persistent NF-κB activation prolongs inflammation, promotes ECM degradation, and delays wound closure.

Several studies have shown that plant-mediated silver, gold, and zinc oxide nanoparticles can suppress excessive NF-κB activation by reducing intracellular oxidative stress and downregulating inflammatory cytokine production. This controlled immunomodulation facilitates the transition from the inflammatory to the proliferative phase while minimizing collateral tissue damage.

**Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Pathway**

Oxidative stress is a hallmark of chronic wounds, particularly those associated with diabetes mellitus. The **Nrf2 signaling pathway** constitutes the principal endogenous defense mechanism against oxidative injury.

Under basal conditions, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1), which promotes its proteasomal degradation. Increased oxidative stress disrupts this interaction, allowing Nrf2 to translocate into the nucleus where it binds antioxidant response elements (AREs) and induces expression of cytoprotective enzymes, including:

- Heme oxygenase-1 (HO-1)
- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione peroxidase (GPx)
- NAD(P)H quinone oxidoreductase-1 (NQO1)

Activation of Nrf2 reduces oxidative damage, preserves mitochondrial function, limits inflammatory signaling, and promotes survival of fibroblasts and keratinocytes. Biogenic nanoparticles rich in phytochemical surface coatings have demonstrated the capacity to activate Nrf2 signaling while simultaneously scavenging excessive reactive oxygen species, thereby restoring redox homeostasis within chronic wounds.

**PI3K/Akt Signaling Pathway**

The phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) pathway plays a fundamental role in regulating cell survival, proliferation, migration, metabolism, and angiogenesis.

Activation begins when growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), or vascular endothelial growth factor (VEGF) bind their respective receptor tyrosine kinases, stimulating PI3K-mediated generation of phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 subsequently activates Akt, which phosphorylates multiple downstream proteins involved in:

- Cell survival
- Protein synthesis
- Glucose metabolism
- Fibroblast proliferation
- Keratinocyte migration
- Endothelial cell function

Experimental evidence suggests that biogenic gold nanoparticles and zinc oxide nanoparticles enhance PI3K/Akt signaling, resulting in accelerated collagen synthesis, improved epithelial regeneration, and enhanced angiogenesis. Activation of this pathway is particularly beneficial in diabetic wounds, where impaired PI3K/Akt signaling contributes to delayed healing.

**Mitogen-Activated Protein Kinase (MAPK) Pathway**

The MAPK signaling network comprises three major kinase families:

- Extracellular signal-regulated kinase (ERK)
- c-Jun N-terminal kinase (JNK)
- p38 MAPK

These kinases regulate cellular proliferation, migration, apoptosis, differentiation, and inflammatory responses.

ERK signaling primarily promotes fibroblast proliferation and keratinocyte migration, whereas JNK and p38 pathways are activated under cellular stress conditions and regulate inflammatory cytokine production.

Controlled activation of MAPK signaling contributes to tissue regeneration, whereas excessive activation promotes chronic inflammation and apoptosis.

Recent investigations indicate that biologically synthesized metallic nanoparticles may modulate MAPK phosphorylation, thereby enhancing regenerative signaling while limiting excessive inflammatory responses.

**Transforming Growth Factor-β (TGF-β)/Smad Pathway**

Transforming growth factor-β represents one of the most influential cytokines involved in wound repair. Binding of TGF-β to its membrane receptors activates intracellular Smad proteins that regulate transcription of genes associated with extracellular matrix synthesis.

The TGF-β/Smad pathway stimulates:

- Fibroblast proliferation
- Myofibroblast differentiation
- Collagen synthesis
- Fibronectin production
- Wound contraction
- Scar formation

Although essential for tissue repair, excessive TGF-β signaling may contribute to hypertrophic scar formation and fibrosis.

Emerging nanotherapeutic strategies aim to achieve balanced modulation of TGF-β activity, enhancing regenerative repair while preventing pathological fibrosis.

**Wnt/β-Catenin Signaling**

The Wnt/β-catenin pathway regulates epidermal stem cell activation, hair follicle regeneration, fibroblast proliferation, and tissue remodeling.

Activation occurs when Wnt ligands bind Frizzled receptors, preventing degradation of β-catenin. Stabilized β-catenin translocates into the nucleus where it activates transcription of genes controlling cellular proliferation and differentiation.

Appropriate Wnt signaling accelerates re-epithelialization and dermal regeneration. However, sustained activation has been associated with excessive scar formation.

Recent biomaterial studies indicate that nanoparticle-containing scaffolds may indirectly influence Wnt signaling by improving the wound microenvironment and reducing oxidative stress.

**HIF-1α/VEGF Signaling and Angiogenesis**

Adequate vascularization is indispensable for successful wound healing. Tissue injury produces localized hypoxia, leading to stabilization of hypoxia-inducible factor-1α (HIF-1α).

HIF-1α stimulates expression of several angiogenic mediators, including:

- VEGF
- Platelet-derived growth factor (PDGF)
- Angiopoietins
- Stromal-derived factor-1 (SDF-1)

These mediators promote endothelial cell proliferation, migration, and formation of functional capillary networks.

Biogenic iron oxide, zinc oxide, and copper oxide nanoparticles have demonstrated pro-angiogenic activity through enhancement of VEGF expression and endothelial cell function, thereby improving oxygen delivery and granulation tissue formation.

**NLRP3 Inflammasome Signaling**

The NLRP3 inflammasome is an intracellular multiprotein complex that links cellular stress with innate immune activation.

Following activation by microbial products, extracellular ATP, mitochondrial dysfunction, or oxidative stress, NLRP3 recruits apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase-1, resulting in activation of caspase-1.

Activated caspase-1 promotes maturation of:

- Interleukin-1β (IL-1β)
- Interleukin-18 (IL-18)

and induces inflammatory cell death through pyroptosis.

Persistent NLRP3 activation contributes to chronic inflammation, diabetic wound pathology, and delayed tissue regeneration.

Several plant-derived metallic nanoparticles have demonstrated the ability to attenuate NLRP3 activation

by reducing mitochondrial oxidative stress and limiting inflammasome assembly, thereby promoting inflammatory resolution.

### Crosstalk Between Signaling Pathways

Wound healing signaling pathways function as an integrated regulatory network rather than independent cascades. For example:

- ROS activate both NF-κB and NLRP3 signaling.
- Nrf2 suppresses oxidative stress and indirectly inhibits NF-κB activation.
- PI3K/Akt enhances endothelial survival and cooperates with VEGF signaling during angiogenesis.
- TGF-β interacts with MAPK signaling to regulate fibroblast differentiation.
- Wnt signaling influences stem cell activation and tissue remodeling.

Biogenic metallic nanoparticles exhibit a unique advantage because they can simultaneously modulate multiple signaling pathways through antioxidant, anti-inflammatory, antimicrobial, and pro-regenerative mechanisms. This multitarget therapeutic profile distinguishes BMNPs from conventional single-target pharmacological agents and supports their development as multifunctional components of advanced wound dressings.

## Agricultural and Biowaste Valorization for Green Synthesis of Metallic Nanoparticles

### Agricultural Biowaste: An Emerging Resource for Sustainable Nanotechnology

The rapid expansion of agricultural production and food-processing industries has resulted in an unprecedented increase in the generation of agricultural residues and organic biowaste worldwide. According to recent estimates by international organizations, billions of tonnes of agricultural biomass are produced annually, of which a substantial proportion remains underutilized or is disposed of through open-field burning, uncontrolled landfilling, or incineration. These disposal practices contribute significantly to greenhouse gas emissions, environmental pollution, soil degradation, and loss of valuable renewable resources [52,53].

Traditionally, agricultural residues such as fruit peels, vegetable wastes, cereal husks, seed shells, sugarcane bagasse, rice straw, wheat bran, corn cobs, coconut shells, coffee grounds, tea residues, and forestry by-products were regarded primarily as waste materials requiring disposal. However, advances in green chemistry and biorefinery technologies have transformed this perspective by recognizing agricultural waste as a renewable feedstock rich in bioactive phytochemicals capable of generating high-value products, including biofuels, nutraceuticals, pharmaceuticals, biodegradable polymers, and nanomaterials [54].

The concept of **waste valorization** emphasizes converting low-value agricultural residues into economically valuable products while minimizing environmental impact. In the context of nanotechnology, agricultural waste serves not only as a sustainable source of reducing agents but also as a natural stabilizing and capping matrix for the synthesis of metallic nanoparticles. This approach aligns closely with the principles of sustainable development, green chemistry, and the circular bioeconomy by simultaneously reducing waste generation and producing advanced functional nanomaterials for biomedical applications.

### Medicinal Plant Residues

Residues remaining after extraction of medicinal plants often retain considerable quantities of flavonoids, alkaloids, tannins, terpenoids, and polysaccharides.

Reuse of these extraction residues not only reduces industrial waste but also improves the economic sustainability of herbal medicine production.

### Phytochemical Constituents Responsible for Green Nanoparticle Synthesis

The success of biological nanoparticle synthesis depends largely on the diverse phytochemical composition of agricultural biomass. Unlike conventional chemical synthesis, which relies on synthetic reducing agents such as sodium borohydride or hydrazine, green synthesis utilizes naturally occurring biomolecules to mediate reduction of metal ions into stable nanoparticles.

Major phytochemical groups involved include:

**Polyphenols**

Polyphenols are among the most effective reducing agents because their hydroxyl groups readily donate electrons to metallic ions. Simultaneously, oxidized polyphenols adsorb onto nanoparticle surfaces, preventing aggregation and improving colloidal stability.

**Flavonoids**

Flavonoids possess multiple hydroxyl and carbonyl groups capable of chelating metal ions and facilitating controlled reduction reactions. They also contribute antioxidant activity that enhances the biological performance of synthesized nanoparticles.

**Proteins and Amino Acids**

Proteins act both as reducing agents and surface stabilizers. Functional groups including amino, carboxyl, sulfhydryl, and hydroxyl moieties interact strongly with nanoparticle surfaces, forming protective capping layers that improve dispersion and biocompatibility.

**Polysaccharides**

Natural polysaccharides such as cellulose, starch, pectin, chitosan, hemicellulose, and gums provide steric stabilization during nanoparticle formation. Their biodegradable nature makes them particularly attractive for biomedical applications.

**Organic Acids and Reducing Sugars**

Citric acid, malic acid, ascorbic acid, glucose, fructose, and sucrose facilitate electron transfer during metal ion reduction while simultaneously influencing nanoparticle morphology.

**Mechanism of Green Synthesis of Metallic Nanoparticles**

Although the precise mechanism varies according to plant species and metal precursor, green nanoparticle synthesis generally proceeds through four sequential stages.

**Stage I: Activation**

Metal salts such as silver nitrate ($AgNO_3$), chloroauric acid ($HAuCl_4$), zinc acetate, ferric chloride, or copper sulfate dissociate into ionic species within aqueous solution. Phytochemicals extracted from agricultural waste donate electrons that reduce these ions into electrically neutral metal atoms.

**Stage II: Nucleation**

Reduced metal atoms aggregate to form nanoscale nuclei. This nucleation phase determines the initial size distribution and influences subsequent particle morphology.

**Stage III: Growth**

Additional reduced metal atoms deposit onto existing nuclei, resulting in crystal growth. Temperature, pH, precursor concentration, and phytochemical composition strongly influence particle size, crystallinity, and morphology during this stage.

**Stage IV: Stabilization**

Biomolecules adsorb onto nanoparticle surfaces, producing a natural capping layer that prevents excessive aggregation through electrostatic and steric stabilization. Surface-bound phytochemicals also enhance biological compatibility and may contribute additional antioxidant or antimicrobial properties.

**Agricultural Waste Valorization within the Circular Bioeconomy**

The integration of agricultural waste into nanoparticle synthesis exemplifies the transition from a linear economy to a **circular bioeconomy**, in which biological resources remain in productive use for as long as possible. Instead of viewing agricultural residues as waste, circular systems treat them as renewable feedstocks for high-value materials.

Within this framework:

- Agricultural biomass is collected and processed into phytochemical-rich extracts.
- These extracts are used for environmentally benign nanoparticle synthesis.
- The resulting nanoparticles are incorporated into advanced wound dressings, antimicrobial coatings,

drug delivery systems, and tissue engineering scaffolds.

- Residual biomass can subsequently be converted into compost, biochar, or bioenergy, minimizing waste generation and maximizing resource efficiency.

This integrated approach not only reduces environmental pollution but also creates sustainable value chains that benefit agriculture, biotechnology, and healthcare simultaneously.

## *Green Synthesis of Metallic Nanoparticles from Agricultural Biowaste for Wound Healing Applications*

Green synthesis has emerged as a sustainable alternative to conventional physical and chemical methods for the production of metallic nanoparticles. Unlike traditional approaches that often require hazardous reducing agents, high temperatures, and toxic organic solvents, green synthesis utilizes naturally occurring phytochemicals from plant materials, agricultural residues, microorganisms, or other biological resources to reduce metal ions into stable nanoparticles. The process is environmentally benign, energy-efficient, cost-effective, and generally yields nanoparticles with enhanced biocompatibility due to the presence of naturally adsorbed biomolecules on their surfaces [55–57].

Agricultural and food-processing wastes—including fruit peels, vegetable residues, cereal husks, seed coats, sugarcane bagasse, tea waste, coffee grounds, and coconut shells—are particularly attractive as reducing and stabilizing agents because they are abundant, inexpensive, and rich in polyphenols, flavonoids, proteins, carbohydrates, and organic acids. These biomolecules not only facilitate nanoparticle synthesis but also improve colloidal stability and may impart additional antioxidant or antimicrobial properties that are advantageous for wound healing [58].

Among the various nanomaterials investigated, silver (Ag), gold (Au), zinc oxide (ZnO), copper oxide (CuO), iron oxide ($Fe_3O_4$), titanium dioxide ($TiO_2$), and magnesium oxide (MgO) nanoparticles have demonstrated significant therapeutic potential owing to their antimicrobial, antioxidant, anti-inflammatory, and regenerative properties.

### Silver Nanoparticles (AgNPs)

### Green Synthesis

Silver nanoparticles are the most extensively studied metallic nanomaterials for wound management because of their broad-spectrum antimicrobial activity and favorable biological properties. Green synthesis is commonly performed by mixing an aqueous extract of agricultural waste with an aqueous solution of silver nitrate ($AgNO_3$). Phytochemicals present in the extract donate electrons to $Ag^+$ ions, reducing them to elemental silver ($Ag^0$), which subsequently undergoes nucleation and growth to form nanoparticles [59].

Agricultural residues successfully employed for AgNP synthesis include:

- Banana peel
- Orange peel
- Lemon peel
- Pomegranate rind
- Mango peel
- Grape pomace
- Onion peel
- Tea waste
- Rice husk
- Sugarcane bagasse

Reaction conditions—including pH, temperature, extract concentration, silver precursor concentration, and reaction time—significantly influence particle size, morphology, and colloidal stability. In general, alkaline pH and moderate temperatures favor the formation of smaller, more uniformly distributed nanoparticles.

### Physicochemical Characteristics

Green-synthesized AgNPs typically exhibit spherical or quasi-spherical morphology with particle sizes ranging from approximately 10 to 80 nm, although triangular, hexagonal, and rod-shaped particles have also been reported depending on synthesis conditions. Their large surface-area-to-volume ratio enables extensive interaction with microbial cells and host tissues.

Surface-bound phytochemicals act as natural capping agents, improving nanoparticle stability while reducing

the cytotoxicity often associated with chemically synthesized silver nanoparticles.

**Antimicrobial Mechanisms**

Silver nanoparticles exert antimicrobial activity through multiple complementary mechanisms, reducing the likelihood of resistance development.

Key mechanisms include:

- Attachment to bacterial cell membranes, increasing membrane permeability.
- Disruption of membrane integrity and leakage of intracellular components.
- Release of $Ag^{+}$ ions that interact with thiol-containing proteins and enzymes.
- Generation of reactive oxygen species (ROS), leading to oxidative damage.
- Binding to bacterial DNA and inhibition of replication.
- Interference with protein synthesis and cellular respiration.
- Inhibition of quorum sensing and biofilm formation.

Because these mechanisms target several essential cellular processes simultaneously, AgNPs exhibit potent activity against Gram-positive bacteria, Gram-negative bacteria, fungi, and many multidrug-resistant pathogens frequently associated with chronic wounds.

**Role in Wound Healing**

Beyond antimicrobial activity, AgNPs contribute directly to tissue regeneration by modulating inflammation and cellular responses.

Experimental studies have demonstrated that appropriately formulated AgNPs can:

- Reduce excessive inflammatory cytokine production.
- Promote macrophage transition toward the reparative M2 phenotype.
- Enhance fibroblast proliferation.
- Stimulate keratinocyte migration.
- Accelerate collagen deposition.
- Support angiogenesis.
- Shorten wound-closure time in experimental models.

These multifunctional properties have led to the incorporation of AgNPs into hydrogel dressings, electrospun nanofibers, alginate dressings, chitosan films, collagen scaffolds, and polyurethane foams.

Despite their considerable therapeutic potential, excessive silver concentrations may induce oxidative stress, mitochondrial dysfunction, and cytotoxicity in mammalian cells. Long-term accumulation of silver within tissues and the environment also remains an area of ongoing investigation. Consequently, optimization of dosage, particle size, and release kinetics is essential to maximize therapeutic efficacy while minimizing adverse effects.

**Gold Nanoparticles (AuNPs)**

**Green Synthesis**

Gold nanoparticles are synthesized using aqueous solutions of chloroauric acid ($HAuCl_4$) as the metal precursor. Agricultural waste extracts rich in flavonoids, phenolic acids, tannins, and reducing sugars reduce $Au^{3+}$ ions to elemental gold ($Au^{0}$), followed by controlled nucleation and nanoparticle growth.

Common agricultural sources include citrus peels, grape pomace, tea residues, coffee waste, and pomegranate rind. The mild reaction conditions employed in green synthesis preserve the biological activity of surface-bound phytochemicals, enhancing nanoparticle stability and compatibility with living tissues.

**Biological Properties**

Unlike silver nanoparticles, AuNPs exhibit relatively low intrinsic antimicrobial activity but possess excellent biocompatibility and anti-inflammatory characteristics. Their unique optical and surface properties facilitate functionalization with peptides, proteins, growth factors, nucleic acids, and therapeutic drugs.

**Mechanisms Promoting Wound Repair**

Gold nanoparticles influence several biological processes relevant to tissue regeneration:

- Suppression of excessive inflammatory signaling.
- Reduction of intracellular oxidative stress.
- Promotion of fibroblast proliferation.
- Enhancement of keratinocyte migration.
- Stimulation of collagen synthesis.

- Facilitation of angiogenesis through VEGF-related pathways.

Furthermore, AuNPs serve as highly efficient carriers for bioactive molecules, enabling localized and sustained delivery of therapeutic agents within wound dressings.

**Biomedical Applications**

Gold nanoparticles have been incorporated into:

- Injectable hydrogels
- Electrospun nanofibrous scaffolds
- Collagen matrices
- Chitosan-based dressings
- Composite polymer films

These multifunctional biomaterials provide antimicrobial support when combined with additional agents while simultaneously promoting tissue regeneration and reducing inflammation.

**Table 1: Comparison of Silver and Gold Nanoparticles**

| Characteristic | Silver Nanoparticles | Gold Nanoparticles |
|---|---|---|
| Primary function | Potent antimicrobial | Tissue regeneration and drug delivery |
| Antibacterial activity | Very high | Moderate |
| Anti-inflammatory activity | High | High |
| Antioxidant potential | Moderate–High | High |
| Biocompatibility | Good (dose dependent) | Excellent |
| Drug functionalization | Moderate | Excellent |
| Typical wound application | Infection control | Regenerative scaffolds and targeted delivery |

**Zinc Oxide Nanoparticles (ZnO NPs): Green Synthesis, Properties, and Applications in Wound Healing**

Zinc is an essential trace element involved in numerous physiological processes, including cell proliferation, protein synthesis, antioxidant defense, immune regulation, and tissue regeneration. Consequently, zinc oxide nanoparticles (ZnO NPs) have attracted considerable interest as multifunctional nanomaterials for wound management. In addition to their intrinsic antimicrobial activity, ZnO NPs promote fibroblast proliferation, keratinocyte migration, angiogenesis, collagen deposition, and modulation of inflammatory responses. Compared with many other metallic nanoparticles, ZnO NPs exhibit favorable biocompatibility, relatively low manufacturing costs, and excellent chemical stability, making them attractive candidates for incorporation into advanced wound dressings and tissue-engineered scaffolds [60–62].

**Green Synthesis of ZnO Nanoparticles**

Green synthesis of ZnO NPs typically employs zinc salts such as zinc acetate, zinc nitrate, or zinc chloride as metal precursors. Extracts obtained from agricultural and food-processing wastes act as both reducing and stabilizing agents. Phytochemicals—including flavonoids, phenolic acids, tannins, proteins, amino acids, polysaccharides, and organic acids—facilitate hydrolysis of zinc ions, nucleation of zinc hydroxide intermediates, and subsequent formation of crystalline ZnO nanoparticles after controlled heating or calcination.

Agricultural residues frequently used for ZnO nanoparticle synthesis include:

- Citrus fruit peels (orange, lemon, lime)
- Banana peels
- Mango peels

- Pomegranate rinds
- Tea waste
- Coffee residues
- Rice husk
- Sugarcane bagasse
- Corn husk
- Coconut shell extracts
- Onion peel
- Potato peel

The characteristics of the resulting nanoparticles depend on several reaction parameters, including pH, precursor concentration, extract composition, reaction temperature, calcination temperature, and reaction time. Optimizing these variables enables production of ZnO nanoparticles with controlled size, morphology, crystallinity, and surface chemistry.

**Physicochemical Characteristics**

Green-synthesized ZnO nanoparticles generally range from **20–100 nm** in diameter, although smaller particles have also been reported depending on synthesis conditions. They exhibit diverse morphologies, including:

- Spherical
- Hexagonal
- Rod-shaped
- Flower-like
- Needle-like
- Plate-like structures

Their high surface-area-to-volume ratio enhances interactions with bacterial cells and host tissues. Furthermore, phytochemical capping layers derived from plant extracts improve colloidal stability, reduce particle aggregation, and may enhance biological compatibility.

Typical characterization techniques include:

- UV–Visible spectroscopy
- Fourier-transform infrared spectroscopy (FTIR)
- X-ray diffraction (XRD)
- Dynamic light scattering (DLS)
- Zeta potential analysis
- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)
- Energy-dispersive X-ray spectroscopy (EDX)

These analytical methods confirm nanoparticle formation, crystal structure, elemental composition, surface functional groups, morphology, particle size distribution, and colloidal stability.

**Antimicrobial Mechanisms**

ZnO nanoparticles exhibit broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and certain fungal pathogens commonly isolated from infected wounds, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*.

Their antimicrobial effects arise through multiple complementary mechanisms:

1. **Generation of Reactive Oxygen Species (ROS):** ZnO NPs generate superoxide radicals, hydroxyl radicals, and hydrogen peroxide, causing oxidative damage to microbial membranes, proteins, and nucleic acids.
2. **Release of $Zn^{2+}$ Ions:** Dissolution of ZnO releases zinc ions that interfere with microbial enzyme systems, membrane integrity, and intracellular metabolism.
3. **Direct Membrane Interaction:** Electrostatic attraction between ZnO nanoparticles and negatively charged bacterial membranes leads to membrane disruption and leakage of cytoplasmic contents.
4. **Biofilm Inhibition:** ZnO NPs inhibit bacterial adhesion and disrupt biofilm formation, which is particularly important in chronic wounds where biofilms contribute to antibiotic resistance and delayed healing.

The presence of multiple antimicrobial mechanisms reduces the probability of microorganisms developing resistance compared with conventional antibiotics.

**Biological Role in Wound Healing**

Beyond antimicrobial activity, ZnO nanoparticles actively participate in tissue regeneration by influencing several cellular processes.

**Fibroblast Proliferation**

Appropriate concentrations of ZnO nanoparticles stimulate fibroblast proliferation and collagen synthesis, facilitating extracellular matrix formation and granulation tissue development.

**Keratinocyte Migration**

ZnO nanoparticles accelerate re-epithelialization by promoting keratinocyte proliferation and migration across the wound surface, restoring epidermal barrier function.

**Angiogenesis**

Zinc plays an important role in endothelial cell proliferation and expression of angiogenic mediators such as vascular endothelial growth factor (VEGF). ZnO nanoparticles have been reported to enhance neovascularization within regenerating tissue.

**Immunomodulation**

ZnO nanoparticles reduce excessive inflammatory cytokine production while supporting macrophage polarization toward the reparative M2 phenotype. This contributes to timely resolution of inflammation and progression to the proliferative phase of healing.

**Antioxidant Regulation**

Although ZnO nanoparticles can generate ROS within microbial cells, they also activate endogenous antioxidant defense mechanisms in mammalian tissues, including superoxide dismutase and metallothioneins, thereby helping maintain redox balance during tissue repair.

**Incorporation into Advanced Wound Dressings**

ZnO nanoparticles have been successfully integrated into a wide range of biomaterials designed for wound management, including:

- Chitosan hydrogels
- Alginate dressings
- Collagen sponges
- Polyvinyl alcohol (PVA) films
- Electrospun nanofibers
- Polyurethane foams
- Gelatin scaffolds
- Cellulose-based membranes

These nanocomposite dressings provide:

- Sustained antimicrobial activity
- Moist wound environment
- Improved oxygen permeability
- Enhanced mechanical strength
- Controlled release of zinc ions
- Accelerated tissue regeneration

Several experimental studies have demonstrated significantly faster wound closure rates in ZnO NP-containing dressings compared with conventional wound care materials.

**Table 2: Advantages and Limitations of Green-Synthesized ZnO Nanoparticles**

| Advantages | Limitations |
|---|---|
| Broad-spectrum antimicrobial activity | Potential dose-dependent cytotoxicity |
| Promotes fibroblast proliferation | Need for standardized synthesis methods |
| Enhances keratinocyte migration | Variability in phytochemical composition of plant extracts |
| Supports angiogenesis | Limited long-term clinical evidence |
| Excellent chemical stability | Scale-up challenges |
| Low-cost production | Regulatory uncertainties |
| Good compatibility with biomaterial dressings | Need for comprehensive toxicological evaluation |

**Copper Oxide Nanoparticles (CuO NPs): Green Synthesis and Therapeutic Potential in Wound Healing**

Copper is an essential trace element that plays a fundamental role in numerous biological processes, including cellular respiration, connective tissue formation, angiogenesis, antioxidant defense, and immune regulation. Copper-dependent enzymes such as lysyl oxidase, cytochrome c oxidase, and superoxide dismutase are indispensable for collagen cross-linking, mitochondrial energy production, and protection against oxidative stress. Owing to these physiological functions, copper oxide nanoparticles (CuO NPs) have emerged as promising multifunctional nanomaterials for wound-healing applications. In addition to their broad-spectrum antimicrobial activity, CuO NPs promote neovascularization, stimulate extracellular matrix remodeling, and enhance tissue regeneration, making them attractive candidates for incorporation into advanced wound dressings.

**Green Synthesis of Copper Oxide Nanoparticles**

Green synthesis of CuO NPs generally employs copper salts such as copper sulfate, copper acetate, or copper chloride as metal precursors. Extracts prepared from agricultural residues or food-processing wastes provide naturally occurring phytochemicals—including polyphenols, flavonoids, tannins, proteins, reducing sugars, and organic acids—that facilitate reduction of copper ions and stabilize the resulting nanoparticles.

Agricultural resources reported for CuO nanoparticle synthesis include:

- Banana peel
- Citrus fruit peels
- Pomegranate rind
- Tea waste
- Coffee grounds
- Rice husk
- Sugarcane bagasse
- Coconut shell
- Onion peel
- Aloe processing residues

Reaction parameters such as precursor concentration, pH, temperature, extraction solvent, and reaction time significantly influence nanoparticle size, crystallinity, morphology, and biological activity. Mild alkaline conditions generally favor formation of smaller and more uniform nanoparticles.

**Physicochemical Properties**

Green-synthesized CuO nanoparticles commonly exhibit particle sizes between approximately 10 and 80 nm and may display spherical, rod-shaped, spindle-like, or flower-like morphologies depending on synthesis conditions. Surface-bound phytochemicals serve as natural capping agents that enhance colloidal stability and improve compatibility with biological systems.

Comprehensive characterization of CuO nanoparticles typically involves:

- UV–Visible spectroscopy
- Fourier-transform infrared spectroscopy (FTIR)
- X-ray diffraction (XRD)
- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)
- Dynamic light scattering (DLS)
- Zeta potential analysis
- Energy-dispersive X-ray spectroscopy (EDX)

These techniques confirm nanoparticle formation, elemental composition, crystal phase, morphology, particle size distribution, and surface chemistry.

**Antimicrobial Activity**

Copper oxide nanoparticles exhibit potent antimicrobial activity against numerous wound-associated pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Their antimicrobial efficacy arises from several complementary mechanisms:

- Generation of reactive oxygen species (ROS) that damage microbial lipids, proteins, and DNA.
- Release of $Cu^{2+}$ ions, which interfere with enzyme function and membrane integrity.
- Disruption of bacterial cell walls and increased membrane permeability.
- Inhibition of bacterial respiration and energy metabolism.
- Suppression of biofilm formation and microbial adhesion.

Because CuO NPs act through multiple pathways simultaneously, they may reduce the likelihood of

microbial resistance compared with conventional antibiotics.

### Role in Wound Healing

Copper contributes directly to tissue repair through several biological mechanisms:

### Promotion of Angiogenesis

Copper ions stimulate expression of angiogenic mediators such as vascular endothelial growth factor (VEGF) and support endothelial cell proliferation, facilitating formation of new blood vessels within regenerating tissue.

### Collagen Maturation

Copper serves as a cofactor for lysyl oxidase, the enzyme responsible for collagen and elastin cross-linking. This process improves tensile strength and structural stability of newly formed extracellular matrix.

### Modulation of Inflammation

Appropriately formulated CuO nanoparticles can attenuate excessive inflammatory responses by reducing production of pro-inflammatory cytokines while supporting progression toward the proliferative phase of healing.

### Fibroblast and Keratinocyte Activity

Experimental studies have demonstrated that controlled concentrations of CuO NPs promote fibroblast proliferation, extracellular matrix synthesis, and keratinocyte migration, thereby accelerating granulation tissue formation and re-epithelialization.

### Biomedical Applications

Green-synthesized CuO nanoparticles have been incorporated into diverse wound-care biomaterials, including:

- Hydrogel dressings
- Chitosan-based films
- Electrospun nanofibers
- Collagen scaffolds
- Alginate composites
- Polymeric membranes

These nanocomposite systems combine sustained antimicrobial protection with structural support for tissue regeneration and controlled release of therapeutic copper ions.

## Iron Oxide Nanoparticles ($Fe_3O_4$ NPs): Green Synthesis, Biological Properties, and Emerging Applications in Wound Healing

Iron oxide nanoparticles (IONPs), particularly magnetite ($Fe_3O_4$) nanoparticles, have attracted considerable attention in regenerative medicine due to their unique magnetic behavior, excellent biocompatibility, and multifunctional therapeutic properties. Unlike nanoparticles designed solely for antimicrobial activity, $Fe_3O_4$ nanoparticles offer additional advantages such as magnetic responsiveness, imaging capability, controlled drug delivery, and stimulation of tissue regeneration. These characteristics make them promising candidates for next-generation wound dressings capable of integrating diagnosis, targeted therapy, and tissue repair within a single platform [62-63].

Iron is an indispensable micronutrient involved in oxygen transport, cellular respiration, DNA synthesis, and numerous enzymatic reactions. During wound healing, adequate iron availability supports cell proliferation and energy metabolism, whereas iron deficiency may impair collagen synthesis and tissue regeneration. However, excessive free iron can catalyze oxidative damage through Fenton chemistry. Therefore, nanoscale iron oxide formulations are being investigated as controlled delivery systems that maximize therapeutic benefits while minimizing toxicity.

### Green Synthesis of Iron Oxide Nanoparticles

Green synthesis of $Fe_3O_4$ nanoparticles generally involves reduction and controlled precipitation of ferric ($Fe^{3+}$) and ferrous ($Fe^{2+}$) salts using plant-derived biomolecules obtained from agricultural residues or food-processing by-products. Unlike conventional co-precipitation methods that often require synthetic stabilizers, phytochemicals naturally present in plant extracts simultaneously reduce, cap, and stabilize the nanoparticles.

Agricultural wastes explored for $Fe_3O_4$ nanoparticle synthesis include:

- Orange peel
- Banana peel
- Pomegranate rind
- Tea residues
- Coffee waste
- Rice husk
- Sugarcane bagasse
- Coconut shell
- Corn husk
- Grape pomace

Polyphenols, flavonoids, tannins, polysaccharides, proteins, and reducing sugars present in these materials interact with iron ions, regulating nucleation and crystal growth while improving colloidal stability. Parameters such as extract concentration, pH, reaction temperature, iron salt ratio, and stirring conditions significantly influence nanoparticle characteristics.

**Physicochemical Characteristics**

Green-synthesized $Fe_3O_4$ nanoparticles generally possess diameters ranging from 10 to 50 nm, although larger particles may be produced depending on reaction conditions. They frequently exhibit spherical or quasi-spherical morphology and display superparamagnetic behavior at nanoscale dimensions, enabling magnetic guidance without residual magnetization after removal of an external magnetic field.

Typical characterization methods include:

- UV–Visible spectroscopy
- Fourier-transform infrared spectroscopy (FTIR)
- X-ray diffraction (XRD)
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)
- Dynamic light scattering (DLS)
- Vibrating sample magnetometry (VSM)
- Energy-dispersive X-ray spectroscopy (EDX)

Surface phytochemicals remaining after green synthesis improve aqueous dispersibility, reduce aggregation, and enhance interaction with biological tissues.

**Biological Mechanisms Relevant to Wound Healing**

**Antimicrobial Activity**

Although $Fe_3O_4$ nanoparticles generally exhibit lower intrinsic antimicrobial activity than silver or copper nanoparticles, they inhibit microbial growth through:

- Production of reactive oxygen species
- Membrane disruption
- Interference with bacterial metabolism
- Enhanced delivery of co-loaded antimicrobial agents

When combined with antibiotics or antimicrobial peptides, $Fe_3O_4$ nanoparticles may enhance bacterial susceptibility and improve penetration into microbial biofilms.

**Modulation of Oxidative Stress**

Controlled oxidative signaling is essential for normal wound repair. Iron oxide nanoparticles have been reported to influence intracellular redox homeostasis by regulating reactive oxygen species generation. Appropriate nanoparticle concentrations can activate endogenous antioxidant defense mechanisms while avoiding excessive oxidative injury.

Surface phytochemicals acquired during green synthesis may further contribute antioxidant activity, reducing oxidative stress within chronic wounds.

**Promotion of Angiogenesis**

Restoration of blood supply is essential for successful tissue regeneration. Several experimental studies suggest that $Fe_3O_4$ nanoparticles stimulate endothelial cell proliferation and enhance expression of angiogenic mediators, including vascular endothelial growth factor (VEGF). Improved neovascularization increases oxygen delivery, nutrient transport, and removal of metabolic waste from healing tissue.

**Fibroblast Activation and Extracellular Matrix Formation**

Fibroblasts cultured in the presence of appropriately formulated $Fe_3O_4$ nanoparticles demonstrate enhanced proliferation, migration, and collagen production. Improved extracellular matrix deposition contributes to granulation tissue formation and accelerated wound contraction.

**Immunomodulatory Effects**

Macrophages play central roles in coordinating inflammation and tissue repair. Emerging evidence indicates that iron oxide nanoparticles may influence macrophage polarization, promoting resolution of inflammation and facilitating progression toward the proliferative phase of healing. However, these effects depend strongly on nanoparticle size, surface chemistry, and concentration.

**Magnetic Nanotechnology in Wound Care**

One of the most distinctive advantages of $Fe_3O_4$ nanoparticles is their responsiveness to external magnetic fields. This property enables several innovative therapeutic strategies:

- Magnetically guided delivery of nanoparticles to wound sites
- Localized release of antimicrobial agents
- Controlled retention of therapeutic nanoparticles within wound dressings
- Magnetic stimulation of cellular proliferation
- Real-time imaging and monitoring using magnetic resonance imaging (MRI)

Such multifunctional systems may improve treatment precision while reducing systemic exposure to therapeutic agents.

**Incorporation into Advanced Biomaterials**

Iron oxide nanoparticles have been incorporated into numerous wound-healing materials, including:

- Chitosan hydrogels
- Gelatin scaffolds
- Electrospun nanofibers
- Collagen matrices
- Polyvinyl alcohol (PVA) composites
- Alginate dressings
- Cellulose-based membranes

These nanocomposite biomaterials provide:

- Improved mechanical strength
- Controlled drug release
- Enhanced cellular adhesion
- Sustained antimicrobial activity
- Magnetic responsiveness
- Increased structural stability

Several experimental models have demonstrated accelerated wound closure, improved collagen organization, and enhanced vascularization using $Fe_3O_4$ nanoparticle-containing dressings.

## *Clinical Translation, Regulatory Challenges, and Future Commercialization of Green-Synthesized Metallic Nanoparticles for Wound Healing*

The growing body of preclinical evidence supporting the therapeutic potential of green-synthesized metallic nanoparticles (GMNPs) has stimulated considerable interest in their clinical translation for wound management. Numerous in vitro and animal studies have demonstrated that nanoparticles synthesized using agricultural waste possess antimicrobial, antioxidant, anti-inflammatory, angiogenic, and tissue-regenerative properties that collectively accelerate wound repair. Despite these promising findings, only a limited number of nanoparticle-based wound care products have reached clinical practice. The discrepancy between laboratory success and clinical implementation reflects the complex scientific, manufacturing, regulatory, and economic challenges associated with translating nanotechnology into approved medical products.

Successful clinical translation requires not only demonstration of biological efficacy but also reproducible manufacturing, comprehensive safety evaluation, quality assurance, regulatory compliance, cost-effectiveness, and acceptance by healthcare professionals. Green synthesis introduces additional variables related to biological raw materials, including seasonal variation in phytochemical composition, source-to-source variability, and challenges in process standardization. Addressing these issues is essential to realize the full therapeutic potential of agricultural waste-derived metallic nanoparticles in modern wound care.

## *Current Status of Nanoparticle-Based Wound Dressings*

Nanotechnology has already influenced the wound-care market through several commercially available products incorporating metallic nanoparticles, particularly silver. Silver-containing dressings are widely used for the management of infected burns, diabetic foot ulcers, pressure ulcers, venous leg ulcers,

and traumatic wounds because of their broad-spectrum antimicrobial activity and ability to reduce wound bioburden.

In contrast, green-synthesized nanoparticles remain largely at the preclinical stage. Most published studies have evaluated their efficacy in cell culture models or experimental animal wounds. Only a small number of formulations have progressed to early-phase clinical evaluation, highlighting the need for robust translational research.

The next generation of wound dressings is expected to incorporate multifunctional nanoparticles capable of combining antimicrobial protection with controlled drug delivery, antioxidant activity, angiogenic stimulation, and real-time monitoring of wound status.

**Manufacturing Challenges**

**Standardization of Green Synthesis**

One of the principal obstacles to commercialization is the lack of standardized synthesis protocols. Agricultural residues differ markedly in their phytochemical composition depending on:

- plant species,
- cultivar,
- geographical origin,
- climatic conditions,
- harvesting season,
- maturity,
- storage conditions, and
- extraction method.

These factors influence nanoparticle nucleation, growth, morphology, surface chemistry, and biological activity, making reproducibility difficult. Development of standardized extraction procedures and validated manufacturing protocols is therefore essential.

**Batch-to-Batch Reproducibility**

Pharmaceutical manufacturing requires consistent product quality across production batches. Variability in particle size, shape, crystallinity, surface charge, and phytochemical capping can significantly alter therapeutic performance and safety.

Critical quality attributes (CQAs) that require strict control include:

- particle size distribution,
- zeta potential,
- crystallinity,
- morphology,
- metal purity,
- endotoxin levels,
- microbial contamination,
- release profile, and
- long-term stability.

Implementation of Quality by Design (QbD) principles and Process Analytical Technology (PAT) can help ensure manufacturing consistency.

**Scale-Up Production**

Most green synthesis methods have been optimized only at laboratory scale. Industrial production introduces challenges including:

- reactor design,
- efficient biomass extraction,
- solvent recovery,
- purification,
- drying,
- storage,
- quality control, and
- waste management.

Continuous-flow synthesis, automated bioreactors, and artificial intelligence-assisted process optimization may facilitate large-scale production while maintaining nanoparticle quality.

**Safety and Toxicological Evaluation**

Comprehensive toxicological assessment represents one of the most important requirements before clinical application.

**Cytotoxicity**

Nanoparticles interact directly with mammalian cells, and excessive concentrations may induce:

- oxidative stress,
- mitochondrial dysfunction,
- DNA damage,
- membrane disruption,
- apoptosis,
- inflammatory responses, and
- impaired cellular proliferation.

Safety evaluations should therefore include keratinocytes, fibroblasts, endothelial cells, macrophages, and stem cells relevant to wound repair.

**Biodistribution and Systemic Exposure**

Although wound dressings are intended for topical application, nanoparticles may penetrate damaged skin and enter systemic circulation. Important considerations include:

- tissue distribution,
- organ accumulation,
- biodegradation,
- metabolism,
- renal clearance,
- hepatic elimination, and
- long-term persistence.

These parameters should be investigated using appropriate animal models before human use.

**Immunological Safety**

Nanoparticles may activate innate or adaptive immune responses depending on their physicochemical properties.

Potential concerns include:

- hypersensitivity reactions,
- complement activation,
- cytokine release,
- chronic inflammation,
- immune suppression, and
- delayed wound healing.

Green synthesis may improve immunocompatibility through naturally derived phytochemical coatings; however, rigorous immunotoxicity testing remains essential.

**Environmental Safety**

Large-scale production and disposal of nanoparticle-containing dressings raise environmental concerns.

Potential issues include:

- release into wastewater,
- accumulation in soil,
- toxicity to aquatic organisms,
- persistence in ecosystems, and
- development of antimicrobial resistance.

Life-cycle assessment and environmentally responsible disposal strategies should accompany commercialization efforts.

**Regulatory Considerations**

Regulatory approval of nanoparticle-based wound dressings requires compliance with national and international standards governing pharmaceuticals, medical devices, or combination products.

Manufacturers must demonstrate:

- product quality,
- manufacturing consistency,
- sterility,
- biocompatibility,
- toxicological safety,
- clinical efficacy,
- shelf-life stability, and
- post-marketing surveillance.

Because green-synthesized nanoparticles incorporate biologically derived materials, regulators may also require detailed characterization of botanical sources, extraction procedures, impurity profiles, and batch consistency.

International harmonization of regulatory guidelines would facilitate commercialization and reduce barriers to global market entry.

## *Conclusion*

The increasing prevalence of chronic and hard-to-heal wounds presents a significant clinical and socioeconomic challenge, necessitating the development of innovative therapeutic strategies that overcome the limitations of conventional wound-care approaches. Green-synthesized metallic nanoparticles have emerged as a promising class of multifunctional nanomaterials capable of addressing multiple pathological processes involved in impaired wound healing, including microbial infection, excessive inflammation, oxidative stress, poor angiogenesis, and defective extracellular matrix remodeling. By integrating antimicrobial, antioxidant, anti-inflammatory, pro-angiogenic, and regenerative activities within a single nanoscale platform, these materials offer considerable advantages over conventional topical therapies.

Agricultural and food-processing wastes provide abundant, renewable, and inexpensive sources of phytochemicals that can act as reducing, stabilizing, and capping agents during nanoparticle synthesis. This sustainable approach aligns with the principles of green chemistry and the circular bioeconomy by transforming low-value biomass into high-value biomedical materials while reducing reliance on hazardous chemicals and minimizing environmental impact. In addition to improving the ecological sustainability of nanoparticle production, phytochemical-functionalized nanoparticles often exhibit enhanced biocompatibility and may provide synergistic biological activities that further promote tissue regeneration.

Among the various metallic nanoparticles investigated, silver nanoparticles remain the most extensively studied owing to their potent broad-spectrum antimicrobial activity and effectiveness against multidrug-resistant wound pathogens. Gold nanoparticles exhibit exceptional biocompatibility and serve as versatile platforms for targeted drug delivery and modulation of inflammatory responses. Zinc oxide and copper oxide nanoparticles contribute both antimicrobial activity and stimulation of angiogenesis, fibroblast proliferation, and collagen synthesis, whereas iron oxide nanoparticles introduce unique opportunities for magnetic targeting, controlled drug delivery, and theranostic applications. Collectively, these nanomaterials demonstrate complementary biological properties that can be exploited individually or in combination to develop multifunctional wound dressings capable of addressing the complex pathophysiology of chronic wounds.

Despite encouraging laboratory and preclinical findings, translation of green-synthesized metallic nanoparticles into routine clinical practice remains limited. Significant challenges include variability in agricultural feedstocks, lack of standardized synthesis protocols, batch-to-batch reproducibility, large-scale manufacturing, long-term toxicological evaluation, and regulatory approval. Addressing these issues will require multidisciplinary collaboration among materials scientists, chemists, microbiologists, biomedical engineers, clinicians, pharmacologists, toxicologists, and regulatory agencies. Establishment of internationally accepted quality-control standards and Good Manufacturing Practice (GMP)-compliant production methods will be essential for ensuring consistent product quality and patient safety.

Future research should focus on developing multifunctional smart wound dressings that integrate green-synthesized nanoparticles with advanced biomaterials, growth factors, antimicrobial peptides, stem-cell-derived extracellular vesicles, biosensors, and stimuli-responsive drug delivery systems. Emerging technologies such as artificial intelligence-assisted formulation optimization, three-dimensional bioprinting, wearable wound-monitoring devices, and personalized medicine are expected to further enhance the therapeutic potential of nanoparticle-based wound care. In parallel, well-designed multicenter clinical trials and comprehensive health-economic evaluations are required to establish long-term efficacy, safety, and cost-effectiveness in diverse patient populations.

Overall, green synthesis of metallic nanoparticles from agricultural biowaste represents a convergence of nanotechnology, regenerative medicine, and sustainable resource utilization. Continued advances in this interdisciplinary field have the potential not only to improve clinical outcomes for patients with acute and chronic wounds but also to promote environmentally responsible manufacturing practices and support the transition toward a circular bioeconomy. With sustained research, technological innovation, and appropriate regulatory frameworks, agricultural waste-derived metallic nanoparticles are poised to become important components of next-generation wound-care technologies.

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## Conflict of Interest

The authors declare no conflict of interest.

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## Ethical Approvals

This study does not involve experiments on animals or human subjects.

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