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Nanomedicine in bone healing: A review of innovative silver nanoparticle therapies and theranostic advances

Paula V. Messina*

Department of Chemistry, Southern Chemistry Institute–National Scientific and Technical Research Council (INQUISUR-CONICET), National University of The South, Bahía Blanca, Buenos Aires, Argentina

Abstract

Nanomedicine, at the convergence of nanotechnology and medicine, holds transformative potential for bone healing through advanced diagnostic and therapeutic strategies. Among various nanomaterials, silver nanoparticles (AgNPs) have gained attention due to their broad-spectrum antimicrobial, anti-inflammatory, and anticancer properties. This review examines the principles of nanomedicine and theranostics, AgNP synthesis methods and physicochemical characteristics, and their application in bone regeneration for infection control, scaffold design, osteoinduction, and angiogenesis. The integration of AgNPs into theranostic platforms for simultaneous therapy and imaging is critically evaluated, highlighting modalities such as micro-computed tomography, magnetic resonance imaging, and photoacoustic imaging. The preclinical and early clinical evidence is analyzed, addressing critical limitations including toxicity, biocompatibility, pharmacokinetics, manufacturing reproducibility, and regulatory hurdles. This review outlines future trends, such as green synthesis, stimuli-responsive systems, and personalized scaffolds. A comprehensive understanding of these multidimensional facets is crucial for advancing AgNP-based nanomedicine from the bench to the bedside in bone healing applications.

Keywords: Bone defects; Tissue imaging; Personalized medicine; Antimicrobial scaffolds; Photothermal therapy; Bone regeneration

***Corresponding author:**
Paula V. Messina
(pmessina@uns.edu.ar)

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1. Introduction

Bone is a highly specialized and dynamic connective tissue that plays essential structural, metabolic, and protective roles in the human body. Under normal physiological conditions, it possesses a remarkable capacity for self-repair and remodeling through coordinated actions of osteoblasts, osteoclasts, and osteocytes.[1,2] However, this intrinsic regenerative ability is often insufficient when confronting large-scale bone defects.[3] Such critical-size defects may arise from a variety of clinical scenarios, including high-energy trauma, chronic infections such as osteomyelitis, surgical resection of bone tumors, or developmental anomalies. In these cases, the defect exceeds the regenerative threshold of the body, necessitating medical intervention to restore bone integrity and function. Conventional strategies for bone defect repair primarily rely on bone grafting

techniques, which include autografts (the patient's own bone), allografts (donor bone from another human), and synthetic grafts composed of bioceramics or polymers. Each of these approaches, while valuable, is accompanied by significant drawbacks.[4] Autografts remain the gold standard due to their osteoconductive, osteoinductive, and osteogenic properties, but they are limited by donor site morbidity, pain, and volume constraints.[5] Allografts and xenografts introduce risks of immunogenic rejection and disease transmission, and often lack sufficient biological activity to fully support regeneration. Synthetic materials, though customizable and scalable, frequently suffer from poor integration, limited vascularization, and susceptibility to infection, especially in compromised patients.[6]

In response to these limitations, nanomedicine has emerged as a promising interdisciplinary field at the intersection of nanotechnology, biology, and medicine. It enables the engineering of nanoscale materials and systems designed to interact precisely with cellular and molecular processes involved in bone regeneration.[7] Nanoscale platforms offer unique advantages, including increased surface area for protein adsorption and cell attachment, enhanced control over drug and ion release, and the capacity to integrate therapeutic and diagnostic functions into a single device. One of the most innovative directions in this field is the development of theranostic systems, nanoscale constructs that simultaneously deliver therapeutic agents and provide diagnostic feedback.[8,9] In the context of bone repair, theranostic nanomaterials enable real-time tracking of healing progression, early detection of infection or inflammation, and site-specific delivery of drugs, growth factors, or antimicrobial agents. These capabilities align well with the goals of personalized medicine, which seeks to tailor treatments to individual patients based on dynamic physiological cues.[10-12]

Within the array of nanomaterials under investigation, silver nanoparticles (AgNPs) have garnered significant attention due to their multifaceted therapeutic properties.[13] They are well known for broad-spectrum antimicrobial activity, including efficacy against multidrug-resistant bacteria, a feature particularly valuable in orthopedic settings where infection poses a major threat to implant success. Beyond their antimicrobial role, AgNPs have been shown to modulate local immune responses, reducing excessive inflammation while supporting tissue healing. Emerging evidence also points to their potential in inhibiting tumor cell proliferation, making them attractive candidates for post-oncologic bone reconstruction. Importantly, AgNPs can be readily incorporated into various delivery platforms, including porous scaffolds, hydrogels, bone cements, and injectable nanocomposites, allowing flexible application across diverse clinical scenarios.[14-17] Together, these features position AgNP-based nanomedicine as a frontier technology for the regeneration of complex bone defects, addressing limitations of current therapies while opening new avenues for integrated, responsive, and effective clinical solutions.[15,18] Accordingly, this review addresses the following aspects:

(i) The core principles of nanomedicine and theranostic technology
(ii) AgNP synthesis, surface engineering, and mechanism of action
(iii) Application of AgNPs in bone repair for antimicrobial defense, osteoinduction, angiogenesis, and bone imaging
(iv) Integration into multifunctional platforms merging therapy and diagnosis
(v) Preclinical and early clinical insights
(vi) Key challenges in safety, production, and regulatory compliance
(vii) Future directions emphasizing customized, responsive nanoplatforms for bone regeneration.

By synthesizing existing knowledge and pinpointing gaps, this review aims to chart a roadmap for translating AgNP-based theranostic systems into practical, clinical bone-healing solutions. It synthesizes current evidence, considers often-overlooked practical factors, and incorporates perspectives from nanomedicine, theranostics, and bone-repair engineering. It also highlights important knowledge gaps that need to be addressed to transform AgNP-based theranostic systems into effective clinical solutions for bone healing.

2. Fundamentals of nanomedicine

2.1. Definition and historical evolution

Nanomedicine is defined as the medical application of nanotechnology, specifically, the use of materials and systems at the nanoscale, typically within the range of 1–100 nm, to diagnose, monitor, treat, and prevent disease.[19,20] This emerging field represents a paradigm shift in the way biomedical challenges are addressed, offering unprecedented precision, control, and multifunctionality at the molecular and cellular levels. The nanoscale dimension enables materials to interact with biological structures, such as proteins, DNA, and cell membranes, in unique ways that are not possible with conventional macroscale or microscale tools.[19,21,22] These interactions enable novel approaches for targeted drug delivery, non-invasive imaging, real-time diagnostics, regenerative therapies, and combined therapeutic and diagnostic (theranostic) applications. The origins of nanomedicine can be traced back several decades to early research on drug delivery systems, particularly liposomal formulations. In the 1990s, major clinical milestones

were achieved with the approval of Doxil®, the first Food and Drug Administration-approved polyethylene glycol (PEG)-coated liposomal formulation of doxorubicin, used for treating Kaposi's sarcoma and various solid tumors.[23] Doxil demonstrated how nanoparticle encapsulation could improve pharmacokinetics, reduce toxicity, and enhance drug accumulation at tumor sites through the enhanced permeability and retention effect. Another landmark was the development of Abraxane®, an albumin-bound nanoparticle form of paclitaxel, which enabled solvent-free formulation and improved bioavailability of a poorly soluble chemotherapeutic agent.[24] These early successes marked the beginning of clinical nanomedicine and underscored the potential of nanoscale materials to overcome longstanding limitations in drug solubility, biodistribution, and systemic toxicity.

Over time, the scope of nanomedicine has expanded far beyond liposomes to encompass a wide variety of engineered nanostructures, including polymeric nanoparticles, dendrimers, solid lipid nanoparticles, metallic and metal oxide nanomaterials, quantum dots, and nanogels.[25,26] More recent developments have incorporated biologically inspired and biomimetic nanodevices, such as cell membrane-coated nanoparticles that evade immune detection and DNA origami structures capable of logic-gated drug release.[27,28] The evolution of nanomedicine has also paralleled advances in nanofabrication techniques, surface functionalization strategies, and high-resolution imaging technologies.[17,21,29-32] Today, nanomedicine represents a cornerstone of precision medicine. It plays a pivotal role in oncology, cardiovascular therapy, neurology, infectious diseases, and regenerative medicine. As regulatory frameworks evolve and more nanoparticle-based formulations enter clinical trials or receive market approval, nanomedicine continues to transform both diagnostic and therapeutic paradigms, opening pathways toward safer, more effective, and patient-specific healthcare solutions.[33]

2.2. Classification of nanoparticles

Nanoparticles exhibit a broad spectrum of physical, chemical, and biological properties, making them highly versatile tools in nanomedicine.[34] However, not all nanoparticles behave alike. Their composition, morphology, and surface chemistry significantly influence their interaction with biological systems. Differences in material type—whether metallic, polymeric, or biomimetic—translate into varied capabilities in drug loading, release kinetics, cellular uptake, immune response modulation, and biodistribution. Therefore, understanding the distinct characteristics of each nanoparticle class is crucial for designing effective therapeutic and diagnostic systems tailored to specific clinical applications. These distinctions are particularly important in the context of bone regeneration and disorders affecting mineralized tissues, where a delicate balance between antimicrobial protection, inflammation control, osteogenesis, and angiogenesis must be achieved. The local microenvironment in bone injuries or pathologies such as osteomyelitis, osteosarcoma, or osteoporotic fractures presents unique challenges. Here, nanoparticle-based systems can be engineered to meet specific regenerative and therapeutic needs, for example, by delivering antibiotics directly to infected bone tissue, releasing osteoinductive factors to promote bone formation, or enabling localized chemotherapeutic activity in bone malignancies. Within this context, nanoparticles can be broadly classified into several categories.[34-36]

2.2.1. Inorganic nanoparticles

This group includes metal-based nanoparticles,[37,38] such as silver, gold, and copper, as well as metal oxide nanoparticles like zinc oxide and titanium dioxide,[39] and silica-based[40] systems. In the context of bone healing, AgNPs are widely used in bone scaffolds and coatings for orthopedic implants due to their potent antibacterial properties, which help prevent or treat bone infections, such as osteomyelitis.[41,42] Zinc oxide nanoparticles have been shown to stimulate osteoblast proliferation and mineralization,[43] making them valuable in bone tissue engineering. Similarly, gold nanoparticles have been explored for their anti-inflammatory and pro-osteogenic effects,[44] and their use in photothermal therapy for osteosarcoma[45] is under active investigation. Ceramic[46] and hydroxyapatite[47-51] nanoparticles play a pivotal role in bone therapies due to their excellent biocompatibility, bioactivity, and structural similarity to natural bone mineral. These nanoparticles support osteoconduction and promote bone regeneration by serving as scaffolds that enhance cell adhesion, proliferation, and differentiation. Hydroxyapatite, in particular, is widely used to coat implants or deliver therapeutic agents, improving integration with surrounding bone tissue and accelerating healing in cases of fractures, defects, or orthopedic surgeries. Their tunable surface properties also enable functionalization with drugs or growth factors, allowing for targeted and sustained therapeutic effects.[47-51] Recent advances in inorganic nanomaterials for bone cements include the addition of ceramic and carbon-based materials. These additives have shown significant improvements in mechanical performance and functionality. Ceramic components, such as zirconia, hydroxyapatite, and bioactive glass, can enhance compressive strength, radiopacity, and interfacial bonding. They also support bone growth responses. Carbon-based additives, such as carbon nanotubes,

graphene, and carbon fibers, significantly strengthen the cement matrix by increasing toughness, crack resistance, and load distribution. The series "Effect of Various Admixtures on Selected Mechanical Properties of Medium Viscosity Bone Cements: Parts 1–3" provides solid evidence on how these ceramic and carbon materials affect key properties.[52-54] These properties include bending strength, impact resistance, elastic modulus, and fatigue behavior. This research highlights how these additives can improve cement formulations beyond traditional fillers. These findings enhance our understanding of how inorganic nanostructures can be utilized to develop next-generation bone cements with improved mechanical reliability and multifunctional properties.

2.2.2. Polymeric and liposomal nanoparticles

These biodegradable systems (e.g., based on poly[lactic-co-glycolic acid] [PLGA], PEG, or chitosan) are widely used to deliver osteoinductive agents such as bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), or antiresorptive drugs like bisphosphonates.[55-57] For example, PLGA nanoparticles loaded with BMP-2 have been used to enhance bone formation in critical-size defects,[58] while liposomal formulations have been designed to encapsulate antibiotics or anti-inflammatory drugs, allowing for targeted and sustained release in infected or inflamed bone tissue.[59,60]

2.2.3. Biomimetic nanoparticles

Biomimetic nanoparticles include lipoprotein-based carriers,[61] exosome-like vesicles,[62] or nanoparticles cloaked with bone cell-derived membranes.[63] Their surface composition mimics native biological structures, enhancing compatibility and enabling targeted delivery to bone tissue. For instance, exosome-mimetic nanovesicles derived from mesenchymal stem cells (MSCs) have shown promise in promoting osteogenic differentiation and angiogenesis,[64,65] key processes in bone regeneration. In addition, bone-targeting peptides can be conjugated to nanoparticle surfaces to enhance accumulation at sites of demineralization or fracture.[66,67]

2.2.4. Hybrid composite nanoparticles

These systems combine the mechanical or imaging advantages of inorganic cores with the biocompatibility and versatility of polymeric shells. A common example is hydroxyapatite-coated polymeric nanoparticles, which offer both osteoconductivity and drug delivery capacity.[49,68,69] Hybrid systems have also been developed to co-deliver antibiotics and growth factors,[70] targeting infected bone defects with simultaneous regeneration and antimicrobial functions. Critical physicochemical features such as size, shape, surface charge, and surface functionalization govern the *in vivo* behavior of these nanoparticles. For bone-related applications, nanoparticles in the 50–200 nm range are often preferred, as they strike a balance between efficient cellular uptake and systemic circulation time. Surface modifications, such as conjugation with alendronate[71] or other bone-targeting ligands,[49,72,73] enhance selective accumulation in calcified tissues, while neutral or slightly negative charges are typically favored to minimize rapid clearance and immune activation.[74]

Optimizing these parameters is essential for enhancing the therapeutic efficacy and safety of nanoparticle-based strategies in bone repair and calcified tissue disorders. These systems represent a critical innovation in regenerative and precision medicine, offering multifunctional platforms that can diagnose, monitor, and treat complex bone pathologies with high specificity and reduced systemic side effects.

2.3. Diagnostic and therapeutic roles

One of the most transformative advantages of nanomedicine lies in its capacity to integrate diagnostic and therapeutic functions within a single platform.[11,20,30,50,59,61,62,73] Nanoparticles can be engineered not only to deliver therapeutic agents precisely where they are needed but also to enhance diagnostic imaging, monitor disease progression, and provide real-time feedback on treatment efficacy. This convergence of functionalities is central to the concept of theranostics, which seeks to bridge the gap between therapy and diagnosis, particularly valuable in complex, dynamic conditions such as bone infections, tumors, or regenerative therapies. Targeted drug delivery is a cornerstone of nanoparticle-based therapy. Nanoparticles can exploit the enhanced permeability and retention effect in inflamed or tumorous tissues, allowing passive accumulation at pathological sites.[75] Moreover, active targeting strategies, involving the functionalization of nanoparticle surfaces with ligands (e.g., peptides, antibodies, aptamers) that bind specifically to cellular markers (e.g., osteoblasts, tumor cells, or pathogens), further refine tissue selectivity.[71,73] In bone regeneration, this approach has been used to direct osteoinductive molecules such as BMP-2[76,77] or anti-resorptive drugs like alendronate[71] to sites of injury or disease, minimizing systemic side effects. In parallel, nanoparticles significantly enhance diagnostic imaging. Iron oxide nanoparticles, for example, serve as contrast agents in magnetic resonance imaging (MRI),[78] enabling sensitive detection of inflammatory or neoplastic bone lesions. Gold nano shells and nanorods offer strong absorption in the near-infrared range, making them suitable for photoacoustic and computed tomography (CT) imaging, as well as photothermal therapy.[79] Quantum dots, with their bright

and stable fluorescence, are used for high-resolution tracking of cellular processes and scaffold integration in bone tissue engineering studies.[80]

Nanoparticles also support physical therapy modalities. Magnetic nanoparticles can be used to generate localized hyperthermia under alternating magnetic fields, effectively damaging tumor cells in bone malignancies.[81,82] Similarly, gold nanoparticles can induce photothermal ablation when irradiated with near-infrared light, while photodynamic therapy is facilitated by nanoparticles that generate reactive oxygen species (ROS) on light activation, offering localized tumor control with minimal invasiveness.[83-85] Importantly, several nanoparticles exert intrinsic antimicrobial and anti-inflammatory effects. AgNPs and zinc oxide nanoparticles are particularly noteworthy in the context of bone healing, where bacterial infection (e.g., osteomyelitis) can severely compromise regeneration.[13-16,41-43] These nanoparticles can eliminate multidrug-resistant pathogens, modulate the immune response, and support osteogenic activity, making them especially suitable for incorporation into bone scaffolds or injectable hydrogels. Taken together, these multifaceted capabilities are converging into theranostic systems—multifunctional platforms capable of simultaneous drug delivery, imaging enhancement, and real-time monitoring of therapeutic outcomes.[50] In bone-related applications, such systems are being developed to monitor infection status, assess bone regeneration progress through imaging, and adapt drug release profiles in response to local stimuli (e.g., pH, enzymatic activity, or temperature changes). The integration of diagnostic and therapeutic roles in nanoparticle systems represents a paradigm shift in how bone diseases and injuries are managed, moving from static, one-size-fits-all approaches to adaptive, personalized interventions.

3. Theranostics in bone healing

3.1. Concept and rationale

Theranostics refers to the integration of therapeutic and diagnostic functionalities within a single nanoplatform, offering a highly synergistic approach for bone healing applications.[86] In the context of orthopedic regeneration, this dual functionality is particularly advantageous for real-time, *in situ* tracking of healing processes, detection of complications such as infection or delayed union, and localized therapeutic delivery. The rationale behind this approach lies in its ability to provide clinicians with continuous feedback on treatment efficacy, allowing for dynamic adjustment of therapies. By combining diagnostic imaging with therapeutic modalities, theranostic systems enhance precision medicine and reduce systemic side effects. Key advantages of theranostics in bone healing include:

(i) Longitudinal monitoring of scaffold integration and bone regeneration over time, enabling clinicians to assess healing progression or failure without repeated invasive procedures
(ii) Early detection of complications, such as bacterial infection, inflammation, or non-union, through responsive imaging signals
(iii) Localized and controlled drug delivery, which ensures the therapeutic agents act precisely where needed, while imaging signals verify the success of the delivery in real time
(iv) Simultaneous therapeutic feedback allows the clinician to monitor both anatomical and functional outcomes during treatment.

3.2. Imaging modalities

Several imaging techniques have been integrated into theranostic platforms for bone healing, each offering specific advantages based on resolution, depth penetration, and biocompatibility.[87,88]

Micro-CT offers high-resolution, three-dimensional visualization of bone architecture, mineral density, and scaffold integration. Its performance is significantly enhanced by the use of metallic nanoparticles, such as silver or gold, which act as radiopaque contrast agents. These nanoparticles enhance imaging clarity, enabling a detailed assessment of mineralized tissue regeneration.[89,90]

Magnetic resonance imaging provides excellent soft-tissue contrast and is especially useful for assessing inflammatory responses and soft-tissue integration around bone scaffolds.[78] Superparamagnetic nanoparticles, including those doped with gadolinium or iron oxide, are commonly used. AgNPs co-functionalized with these magnetic elements have demonstrated the ability to support both MRI and CT imaging, enabling multimodal diagnostic capabilities.[91,92]

In addition, photoacoustic imaging utilizes the plasmonic resonance of metallic nanostructures such as AgNPs or gold–silver alloy nanoparticles, combining optical and ultrasonic modalities for high-contrast, deep-tissue imaging.[93,94] It offers excellent resolution at significant depths, which is valuable for non-invasive monitoring of scaffold behavior and infection sites.[95,96]

Although less commonly used in bone tissue applications, fluorescence and near-infrared II imaging with rare-earth-doped nanoparticles have shown potential for visualizing inflammatory responses within the bone marrow.[97] This modality allows for sub-millimeter resolution without exposing the tissue to ionizing radiation, making it a promising tool for long-term studies.[17,32,98]

3.3. Dual-function nanosystems

Advanced theranostic systems often rely on AgNPs due to their intrinsic antimicrobial properties and imaging compatibility.[99,100] These platforms can be further functionalized with polymers, peptides, or other biocompatible coatings to enhance targeting and responsiveness to environmental stimuli such as pH shifts or ROS, which are common in infected or inflamed bone tissues.[101,102] A notable example includes silver–strontium mesoporous bioactive glass nanoparticles,[103] which serve multiple functions: they enhance CT imaging due to their high atomic number, release therapeutic silver ions to combat infection, and promote osteogenic differentiation, thereby supporting bone regeneration. Moreover, multifunctional scaffolds that combine antimicrobial and imaging capabilities have been validated in preclinical models.[104] In small animal studies, such scaffolds have demonstrated not only effective bone tissue formation but also visible suppression of infection through real-time imaging feedback.[105-107] These integrated systems exemplify the potential of theranostic platforms to revolutionize bone healing by bridging therapeutic intervention with continuous diagnostic monitoring.

4. Synthesis, properties, and mechanisms of AgNPs

4.1. Synthesis methods

The synthesis of AgNPs can be achieved through various chemical, physical, and biological approaches, each offering unique advantages and limitations regarding particle size, shape, purity, and environmental impact.[108]

4.1.1. Chemical methods

Chemical methods are the most commonly used strategy for AgNP synthesis. They typically involve the reduction of silver ions (Ag^+), often derived from silver nitrate, using chemical reducing agents such as sodium borohydride or trisodium citrate.[109] These methods enable the formation of well-dispersed colloidal nanoparticles with controlled sizes ranging from approximately 5 to 100 nm. While highly reproducible and tunable, a key drawback is the potential presence of toxic chemical residues from the reducing or stabilizing agents, which can limit biomedical applications unless extensive purification is carried out.

4.1.2. Physical methods

Physical methods include top-down techniques such as laser ablation,[110] evaporation–condensation, and ball milling. For instance, laser ablation, for instance, involves irradiating a silver target submerged in a liquid medium, producing nanoparticles with minimal chemical contamination.[110] These methods often yield AgNPs with high purity and good crystallinity, although they frequently exhibit a broad size distribution (polydispersity) and are limited in terms of scalability and cost-efficiency.[111]

4.1.3. Green synthesis

In response to concerns regarding toxicity and environmental sustainability, green synthesis approaches have gained prominence. These leverage natural reducing and capping agents such as plant extracts (rich in polyphenols, terpenoids, or alkaloids), microorganisms, or biomolecules like proteins.[112,113] Recent advances include the use of extracellular matrix proteins, such as fibronectin[114] or silk fibroin,[115] which can simultaneously reduce silver ions and stabilize the resulting nanoparticles. These biomimetic approaches yield biocompatible AgNPs with applications in tissue engineering and regenerative medicine, particularly as osteoinductive surface coatings that support cell adhesion and proliferation.[116]

4.2. Physicochemical properties

The functional performance of AgNPs in biomedical or environmental applications is closely linked to their physicochemical characteristics, which can be tailored through synthesis conditions and post-synthesis modifications.[17,101,102,108]

4.2.1. Size and shape

The size of AgNPs plays a critical role in determining their biological activity.[22,117] Smaller nanoparticles, particularly those under 10 nm, exhibit an enhanced ability to penetrate bacterial biofilms and cellular membranes due to their high surface area-to-volume ratio.[118] However, these same properties also increase their reactivity and potential cytotoxicity toward mammalian cells. Conversely, AgNPs in the range of 20–50 nm strike a balance between antimicrobial efficacy and reduced cytotoxicity, making them more favorable for clinical use.[119] In addition to size, shape (spherical, rod-like, triangular) influences their surface plasmon resonance and interaction with biological interfaces.[22,120]

4.2.2. Surface charge

The zeta potential of AgNPs governs their colloidal stability and interaction with cell membranes.[121,122] Positively charged nanoparticles exhibit stronger electrostatic interactions with negatively charged bacterial membranes, enhancing antimicrobial action.[123] However, they also have a higher propensity for internalization by eukaryotic cells, which can lead to unintended cytotoxic effects. To mitigate these issues, surface modification with neutral or slightly negative coatings such as PEG can be employed to modulate cellular uptake and prolong systemic circulation.[124]

4.2.3. Surface coatings and functionalization

Stabilizing ligands or coatings are crucial for maintaining nanoparticle dispersion and conferring specific biological functions.[108,125] Common capping agents include citrate, PEG, polyvinylpyrrolidone, and dextran, each influencing solubility, immune recognition, and biodistribution.[124] Furthermore, bioactive molecules such as BMP-2,[76] fibronectin,[114] or peptides[61,102] can be conjugated to AgNP surfaces to enhance cellular targeting, osteoinduction, and integration with host tissues, particularly in the context of implantable scaffolds or drug delivery systems.

4.3. Biological mechanisms of action

AgNPs exhibit multifaceted biological activities, making them attractive for a wide range of biomedical applications, including antimicrobial therapies, tissue engineering, and oncology.[126-128]

4.3.1. Antimicrobial activity

AgNPs are known for their potent, broad-spectrum antimicrobial properties.[129] They act through several mechanisms: disruption of bacterial cell wall and membrane integrity, induction of oxidative stress through ROS generation, and sustained release of Ag^+, which interferes with microbial DNA replication and protein synthesis. Minimum inhibitory concentrations for AgNPs are typically below 4 mg/L, and silver-impregnated materials, such as wound dressings or bone grafts, have demonstrated high efficacy against pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.

4.3.2. Anti-biofilm effects

One of the most challenging aspects of bacterial infections is the formation of biofilms, which provides pathogens with resistance to antibiotics and host immune responses.[129] Immobilized AgNPs, particularly when integrated into scaffolds or coatings, can inhibit early-stage microbial adhesion and reduce surface colonization. These effects prevent the maturation of biofilms, making AgNP-based materials especially useful in medical device coatings and surgical implants.

4.3.3. Osteogenic and angiogenic stimulation

Beyond antimicrobial action, AgNPs also influence cellular pathways related to bone regeneration and vascularization.[15] They can modulate MSC behavior by activating the hypoxia-inducible factor 1-alpha pathway through ROS-mediated signaling, leading to increased expression of VEGF and osteogenic markers such as runt-related transcription factor 2 (Runx2) and osteocalcin (OCN). *In vitro* studies have shown enhanced alkaline phosphatase (ALP) activity and mineral deposition in cultures treated with AgNPs, supporting their potential in bone tissue engineering.

4.3.4. Anti-inflammatory and anticancer potential

At controlled doses (typically below 100 μg/mL), AgNPs have been shown to reduce the expression of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6, which may aid in controlling chronic inflammation associated with implant rejection or autoimmune conditions.[130,131] In addition, preliminary *in vitro* studies indicate that AgNPs can inhibit the proliferation of certain bone cancer cells, such as osteosarcoma, by inducing apoptosis and oxidative damage. However, further research is necessary to fully elucidate the therapeutic window and long-term safety of AgNPs in oncological applications.

5. Bone healing and the role of AgNPs

5.1. Bone healing: Inflammatory, reparative, and remodeling phases

Bone healing is a tightly regulated, multi-phase biological process that involves the coordination of cellular and molecular events aimed at restoring skeletal integrity and function after injury. This process unfolds in three overlapping phases: Inflammation, repair, and remodeling.[18,132]

Immediately following a bone injury, a local inflammatory response is initiated. Neutrophils are the first responders, clearing microbial contaminants and necrotic tissue, followed by the recruitment of monocytes that differentiate into macrophages. These immune cells release a cascade of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which serve to recruit and activate MSCs to the site of the defect.[133]

Recruited MSCs undergo osteogenic differentiation under the influence of local growth factors (e.g., BMP-2, transforming growth factor beta). These osteoprogenitor cells deposit a collagen-rich extracellular matrix, which subsequently mineralizes, forming immature woven bone. Concurrently, angiogenesis is triggered by hypoxia-induced expression of VEGF, ensuring an adequate supply of oxygen, nutrients, and osteogenic cells to support tissue regeneration.

Over time, immature bone is remodeled into mature lamellar bone through the coordinated activity of osteoblasts and osteoclasts. This phase restores the structural and mechanical properties of the original bone tissue.

However, this intricate process can be severely compromised in the presence of infection, extensive tissue

damage, or chronic inflammation—conditions frequently encountered in critical-sized bone defects or trauma-related injuries. These clinical challenges highlight the limitations of traditional bone grafts, which often lack the capacity to simultaneously support bone regeneration, promote vascular ingrowth, and prevent or control infection. AgNPs, due to their multifunctional biological activities, are being explored as a novel solution to address these limitations.[18]

5.2. AgNP-based systems in bone therapy

Among the diverse nanomaterials employed in theranostic strategies, AgNPs occupy a prominent position due to their potent antimicrobial properties, biocompatibility at controlled concentrations, and emerging roles in immunomodulation and osteogenesis (Figure 1).[14-17,21,58,67,102] These characteristics make them particularly attractive for applications in bone healing and repair, where the need to simultaneously control infection, reduce inflammation, and support tissue regeneration presents a significant clinical challenge. AgNPs demonstrate broad-spectrum antimicrobial efficacy, including against multidrug-resistant bacteria such as *S. aureus*, *E. coli*, and *P. aeruginosa*, which are often implicated in osteomyelitis, peri-implantitis, and post-operative infections. Their bactericidal activity, which involves membrane disruption, the generation of ROS, and interference with DNA replication, does not rely on classical antibiotic pathways, making them especially valuable in an era of growing antimicrobial resistance.

When incorporated into bone scaffolds, implant coatings, or injectable hydrogels, AgNPs can create an antimicrobial microenvironment at the injury site, preventing bacterial colonization and biofilm formation. Beyond infection control, AgNPs have shown potential in modulating inflammatory responses, which are critical to both the initiation and resolution phases of bone healing. Studies have demonstrated their ability to downregulate proinflammatory cytokines, such as TNF-α and IL-6, while also modulating macrophage polarization toward the M2 phenotype, which is associated with tissue repair.[134-136] This dual role—antibacterial and anti-inflammatory—makes AgNPs uniquely suited for multifunctional regenerative platforms. In addition, emerging evidence suggests that AgNPs can directly influence the behavior of bone cells. At optimized concentrations, AgNPs have been

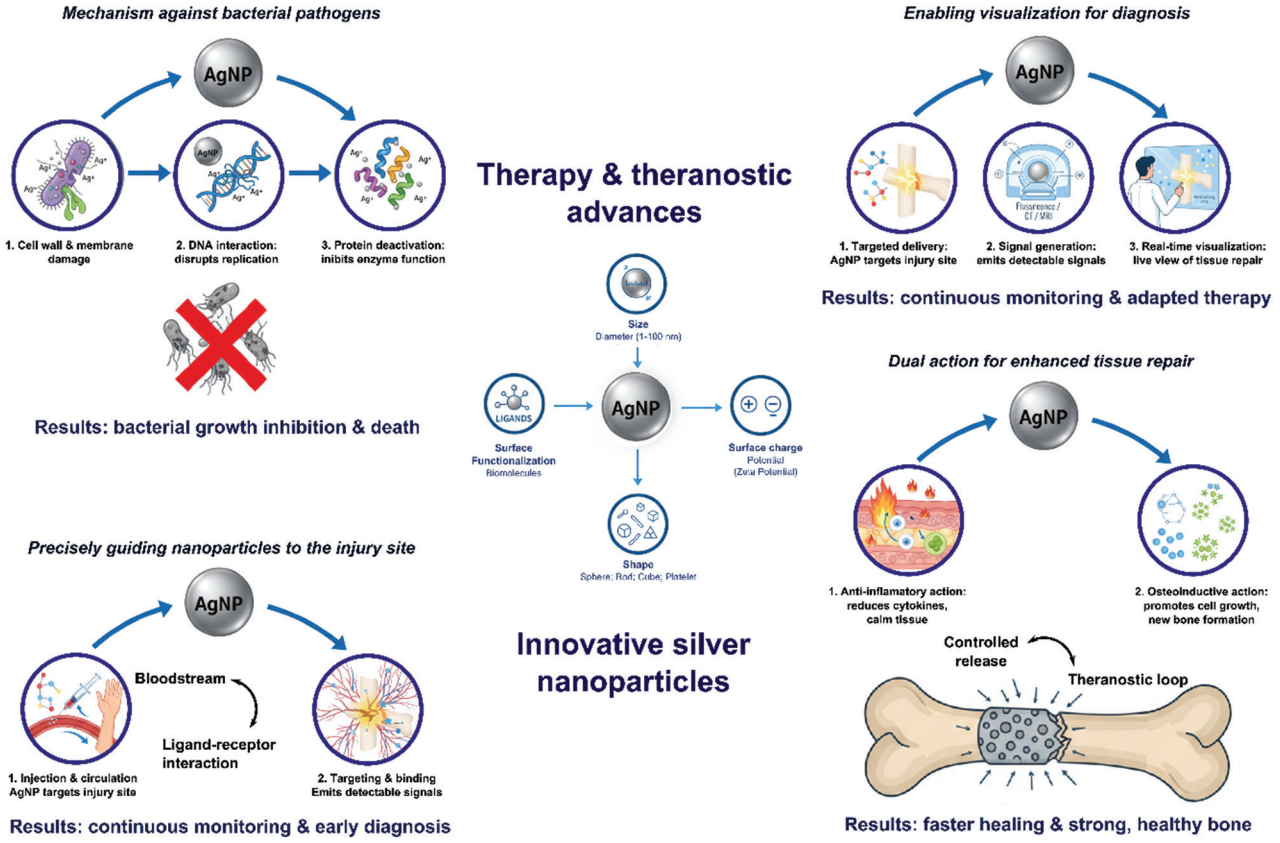


Figure 1. Silver nanoparticle-driven theranostic approaches in bone tissue regeneration

reported to promote osteoblast adhesion, proliferation, and differentiation, while avoiding cytotoxic effects. This is particularly promising when AgNPs are used in synergy with osteoconductive or osteoinductive materials such as hydroxyapatite, collagen, or bioactive glass. Hybrid scaffolds incorporating AgNPs not only offer mechanical support and structural mimicry of bone extracellular matrix but also enhance local tissue integration while deterring infection. Theranostically, AgNPs are increasingly being integrated into stimuli-responsive systems, where their release or activity is triggered by environmental changes such as pH shifts, enzymatic activity, or oxidative stress—conditions commonly present in inflamed or infected bone tissue. Moreover, the plasmonic and conductive properties of AgNPs have potential applications in biosensing and photo-responsive therapies, such as photothermal ablation of osteosarcoma or real-time monitoring of infection resolution. In summary, AgNP-based systems represent a versatile and powerful class of theranostic agents for bone therapy. Their ability to simultaneously disinfect, modulate inflammation, and support bone regeneration, while also being adaptable for diagnostic integration, aligns closely with the goals of precision and regenerative medicine. Continued advances in nanoparticle engineering, surface functionalization, and dose optimization will be essential to fully unlock their clinical potential in complex orthopedic and maxillofacial scenarios.

5.3. Antimicrobial scaffolds and grafts

Theranostic platforms combining therapeutic and diagnostic capabilities are an emerging frontier in bone tissue engineering.[11] As noted earlier, AgNPs, with their potent antimicrobial properties and compatibility with various imaging agents, are central to several innovative dual-function systems. These platforms are designed to enhance bone regeneration, prevent infection, and enable real-time monitoring of treatment progress. A growing body of preclinical evidence demonstrates that scaffolds and bone graft substitutes functionalized with AgNPs possess a dual therapeutic function: providing antimicrobial protection while also supporting osteogenesis.

5.3.1. Rabbit cranial 6-mm defect model

In a controlled study, AgNP-loaded scaffolds composed of either bovine bone xenografts or gelatin methacryloyl (GelMA) hydrogel matrices were implanted in rabbit cranial defects. Approximately 100 μg of AgNPs per scaffold was sufficient to inhibit bacterial proliferation without eliciting any signs of local tissue necrosis or inflammation. Histological analysis revealed enhanced osteoblast proliferation and mineral deposition, confirming both the safety and efficacy of this approach for bone regeneration applications.[137]

5.3.2. Bovine cancellous bone granules functionalized with AgNPs (~50 nm)

These porous bone substitutes exhibited minimum inhibitory concentrations ranging from 0.25 to 4 mg/L against a spectrum of clinically relevant pathogens, including *S. aureus*, *P. aeruginosa*, *E. coli*, *Enterococcus faecalis*, and *C. albicans*. In a murine calvarial defect model, the AgNP-functionalized granules maintained excellent radiolucency, biocompatibility, and structural integrity with reduced resorption rates.[138]

5.3.3. Strontium–silver-coated titanium implants

These multi-metallic implants demonstrated synergistic effects in both *in vitro* and *in vivo* systems. In rat femoral defect models, the implants significantly enhanced MSC osteogenic differentiation markers, including ALP and Runx2. Simultaneously, antibacterial efficacy was demonstrated against *S. aureus* and *E. coli*, indicating their suitability for use in infected or high-risk surgical sites.[139]

5.3.4. Silver–strontium mesoporous bioactive glass nanoparticles

These nanoparticles combine silver and strontium within a mesoporous silica matrix, allowing for the sustained release of therapeutic ions. Silver offers antimicrobial effects, while strontium supports osteoblast activity and bone matrix mineralization. Furthermore, the presence of both metals enhances X-ray attenuation, providing improved contrast in CT imaging. This integration supports non-invasive monitoring of graft incorporation and infection control.[103]

Together, these studies suggest that AgNP-loaded bone constructs can effectively integrate antimicrobial protection with osteoconductive and osteoinductive properties, making them promising candidates for managing contaminated or compromised bone defects.

5.4. AgNP–polymer composites and hydrogels

The incorporation of AgNPs into biodegradable and biocompatible polymer matrices has emerged as a powerful strategy to engineer multifunctional bone scaffolds.

Commonly used materials include PLGA,[58,68] silk fibroin,[101,102,115] chitosan,[100] and GelMA.[137,140] These polymers provide a supportive framework with tunable mechanical properties, controlled porosity, and inherent bioactivity, which is conducive to bone tissue engineering.

AgNP–polymer composites are designed to release silver ions within a therapeutic window (typically 5–30 μg/mL), sufficient to exert antimicrobial effects without inducing

cytotoxicity in host cells. Sustained release profiles have been achieved through nanoparticle encapsulation or surface functionalization strategies.[141,142]

These composites facilitate the adhesion, spreading, proliferation, and differentiation of MSCs. Injectable formulations, particularly GelMA-based hydrogels loaded with AgNPs, have demonstrated effectiveness in addressing irregular or critical-sized defects, such as rodent femoral fractures.[137,140,143] Preclinical outcomes show accelerated bone regeneration, improved vascularization, and reduced infection rates compared to control scaffolds. This approach offers a versatile platform for delivering AgNPs in a spatially and temporally controlled manner, maximizing therapeutic benefit while minimizing systemic exposure.

5.5. Nanoparticle–cell interactions in bone tissue engineering

Nanoparticles engage dynamically with bone-related cells, including osteoblasts, osteoclasts, and MSCs, influencing proliferation, differentiation, and remodeling pathways.[144,145] Osteoblast-like lineages (e.g., MC3T3-E1, Saos-2) internalize nanoparticles via endocytosis; their fate depends on the size, charge, and surface chemistry of the nanoparticles. The review by Zhang *et al.*[145] highlighted that compositional nuances (e.g., metal oxide vs. polymeric) and surface modifications modulate bone cell behavior through signaling cascades such as ERK/MAPK and Wnt/β-catenin.[145] Notably, AgNPs at low concentrations (~5–30 μg/mL) have induced osteogenic gene expression (*RUNX2*, *ALP*, *OCN*) and stimulated angiogenic factors like VEGF in MSC cultures, though higher doses may impair proliferation.[146-148] Concerning therapeutic intent, nanoparticle scaffolds must strike a balance between antimicrobial efficacy and osteogenesis. Researchers have fine-tuned the kinetics of ion release, for example, by doping bone grafts with silver and strontium, to promote osteoblast differentiation while reducing bacterial colonization.[149] Multifunctional composites such as silk fibroin–silver–nanohydroxyapatite achieved excellent osteoblast adhesion while resisting *S. aureus* biofilm formation. Nonetheless, precise dosing and kinetic profiles are crucial; overdose risks ROS overproduction and apoptosis.[150]

5.6. Osteoinduction and angiogenesis

One of the most promising aspects of AgNPs' use in bone regeneration is their ability to simultaneously promote osteogenesis and angiogenesis, provided that dosing is carefully optimized.[15]

At sub-toxic concentrations, AgNPs induce a moderate level of intracellular ROS, which in turn activates the hypoxia-inducible factor 1-alpha pathway. This leads to the upregulation of VEGF and other pro-angiogenic and osteogenic signals. Enhanced ALP activity, collagen Type I synthesis, and extracellular matrix mineralization have all been observed in MSC cultures treated with AgNPs.[151]

The biological activity of AgNPs is highly dose-dependent. Concentrations between 5 and 30 μg/mL are generally considered optimal for stimulating osteogenic differentiation without compromising cell viability. However, at higher concentrations (>50 μg/mL), excessive ROS production may lead to oxidative stress, apoptosis, or necrosis in both progenitor and mature cells, emphasizing the importance of precise dose control in scaffold design.[15,152]

The integration of AgNPs into bone tissue engineering platforms thus provides a dual-function system—combining antimicrobial activity with pro-regenerative signaling. This multifunctionality is particularly advantageous in treating infected bone defects, where traditional grafts may fail due to the hostile inflammatory environment.[153]

5.7. Combined drug delivery and targeting

In addition to their antimicrobial and imaging functions, AgNP-based systems are increasingly designed for combinatorial therapies. These include the localized, controlled delivery of secondary therapeutic agents and active targeting to bone-specific sites.

AgNPs–BMP-2–PLGA composites[154] integrate BMP-2, a potent osteoinductive agent, within a PLGA matrix containing AgNPs. The composite ensures the sustained release of BMP-2, stimulating osteogenesis, while the AgNPs provide continuous antimicrobial protection—a dual action particularly beneficial for infected bone defects or post-surgical implant sites.

Furthermore, AgNPs can be embedded in pH-sensitive or ROS-responsive matrices that trigger the release of drugs under pathological conditions such as infection or inflammation.[155] These "smart" systems offer site-specific and condition-triggered release of antibiotics or anti-inflammatory agents, thereby minimizing systemic toxicity and maximizing local therapeutic effects.

Surface modification of AgNPs with bone-seeking peptides, such as AspSerSer, enhances their affinity for hydroxyapatite-rich regions.[156] This targeting strategy improves the retention of therapeutic agents in the defect zone, thereby optimizing efficacy and minimizing off-target effects.

By integrating antimicrobial, imaging, and therapeutic functionalities within a single platform, these advanced

systems represent a major step toward personalized, image-guided regenerative therapies. Such platforms are particularly promising for treating complex conditions such as osteomyelitis, peri-implant infections, and bone tumors, where localized and multifunctional approaches are essential.

5.8. Preclinical and clinical advances in AgNP-based bone therapies

The growing interest in AgNP-based systems for bone regeneration and infection control has translated into a rapidly expanding body of preclinical research and a smaller but promising number of early clinical studies.[157,158] These investigations aim to validate the therapeutic efficacy, biosafety, and multifunctionality of AgNP-containing platforms in models of bone injury, osteomyelitis, and implant-associated complications. In preclinical settings, AgNPs have been integrated into a wide range of biomaterials designed to mimic the structure and function of native bone.[159] For example, hydroxyapatite–AgNP composites have shown superior antibacterial properties and enhanced osteointegration in rabbit tibial defect models, compared to hydroxyapatite alone.[157,160] Similarly, AgNP-loaded collagen and chitosan scaffolds have demonstrated accelerated bone healing and reduced inflammation in rat calvarial and femoral defect models, confirming the dual osteoconductive and antimicrobial properties of AgNPs.[161-163] The inclusion of growth factors (e.g., BMP-2) or MSCs into AgNP-containing matrices further amplifies regenerative outcomes, supporting a synergistic approach that addresses both biological and infectious barriers to bone repair.[164] Studies have also examined dose-dependent effects and nanoparticle characteristics, such as size, shape, and surface coating, on both efficacy and cytotoxicity. Smaller AgNPs (<20 nm) typically exhibit stronger antimicrobial activity but may pose a greater risk of oxidative stress or mitochondrial dysfunction at high concentrations.[49,70,71,86,137,165] Thus, surface modification strategies (e.g., PEGylation, silica coating, or peptide conjugation) are employed to improve biocompatibility, prolong circulation, and enable targeted delivery *in vivo*.[73,124] In implant-associated models, AgNP coatings on titanium and stainless-steel surfaces have significantly reduced bacterial adhesion and biofilm formation, while maintaining compatibility with osteoblasts.[17,32,102,139,162] These findings are particularly relevant to orthopedic and dental implants, where infection and aseptic loosening remain major causes of failure. While clinical translation is still in early stages, several products incorporating AgNPs for wound healing and infection prevention are already in clinical use (e.g., silver dressings, bone cement additives).[166,167] A few pilot clinical studies have reported the use of AgNP-modified bone grafts or implants in maxillofacial and orthopedic surgeries, showing favorable outcomes in terms of infection control, graft stability, and early tissue integration.[168] However, large-scale, controlled clinical trials are still lacking, and regulatory concerns (particularly related to nanoparticle toxicity, long-term accumulation, and manufacturing reproducibility) remain key hurdles for broader clinical acceptance.

Nonetheless, the consistent preclinical evidence and growing clinical interest suggest that AgNP-based nanotherapeutics are approaching clinical readiness, especially when incorporated into multifunctional, scaffold-integrated platforms designed for complex bone pathologies, as shown in Table 1. Future work will need to focus on standardizing formulations, optimizing dose–response profiles, and designing theranostic protocols that integrate real-time imaging and adaptive drug release for personalized regenerative therapies.

6. Toxicity, biocompatibility, and pharmacokinetics

The clinical translation of AgNP-based platforms in bone regeneration critically depends on their safety profile. While AgNPs are effective antimicrobials, their biological interactions at the cellular and systemic levels must be carefully balanced to avoid adverse effects. Understanding their cytotoxicity thresholds, biodistribution patterns, and immunological interactions is essential for designing safe and effective theranostic systems; results are discussed below and summarized in Table 2.

6.1. *In vitro* cytotoxicity

Cell-based assays consistently demonstrate a dose-dependent cytotoxic response to AgNPs, which varies according to particle size, coating, concentration, and exposure duration. MSCs and pre-osteoblast MC3T3-E1 cells tolerate AgNP concentrations up to

Table 1. Preclinical evidence of silver nanoparticle-based strategies in bone repair and implant integration

Study type	Model/system	Main outcomes
Preclinical (Rabbit) [160,169]	Tibial defect/Hydroxyapatite–AgNPs scaffold	Enhanced osteointegration, infection prevention
Preclinical (Rat) [170]	Calvarial defect/Collagen–AgNPs composite	Accelerated bone healing, reduced inflammation
Preclinical (Mouse) [171]	Femoral fracture/AgNPs	Improved callus formation, synergistic osteoinduction
Implant model (Rat) [172,173]	Titanium implant coated with AgNPs	Reduced bacterial adhesion, biocompatible interface

Abbreviation: AgNP: Silver nanoparticle.

Table 2. Comparative summary of toxicity, biocompatibility, and pharmacokinetics of silver nanoparticle-based systems

Characteristic	Key findings	Determinants/variables	Bone-regenerative applications
In vitro cytotoxicity[15,108,174-178]	• MSCs and MC3T3-E1 cells remain viable up to~30 μg/mL • Toxicity increases sharply above 50–100 μg/mL due to ROS accumulation and mitochondrial dysfunction	• Particle size and coating (polyethylene glycol, polyvinylpyrrolidone, chitosan, silk fibroin) • Concentration and exposure duration • Release kinetics of Ag^+ions (e.g., poly[lactic-co-glycolic acid])/AgNP composites)	• Surface engineering reduces non-specific protein adsorption and ROS • Controlled release supports MSC proliferation, osteogenic differentiation, and matrix mineralization • Highlights the need for precise dose control to balance antimicrobial effects with cytocompatibility
In vivo biodistribution and accumulation[148,179-182]	• Implanted AgNP-loaded scaffolds retain most nanoparticles locally • Small amounts of Ag^+may reach the liver, spleen, and lymph nodes—mainly early after implantation. • No organ damage reported at therapeutic doses in rabbit defect models • Long-term (>8 weeks) biodistribution is still poorly understood	• Administration route (localized scaffold vs. systemic) • Particle dissolution rate and ion release • Implant location and vascularization	• Localized delivery minimizes systemic exposure and toxicity • Need for long-term studies on clearance, metabolism, and excretion • Essential for evaluating repeated or high-dose applications
Dose-dependency and safety thresholds[14,59,101,102,126,129,130,150,153,155,161]	• Strong antibacterial activity at 0.25–4 mg/L (below cytotoxic range) • Cytotoxicity generally occurs above 50–100 μg/mL • HepG2 IC_{50}≈250 μg/mL • Localized slow-release systems have wide safety margins; systemic and topical routes have narrower ones	• Delivery route (local vs. systemic) • Carrier systems (scaffolds, gels, injections) • Exposure frequency and cumulative dose	• Clear therapeutic window for localized delivery • Necessity for pharmacokinetic optimization in systemic applications • Supports development of slow-release bone scaffolds for safer dosing
Immunogenicity and inflammatory responses[186-189]	• Low–moderate doses trigger mild oxidative stress that may support angiogenesis and early repair • High concentrations (>100 μg/mL) induce proinflammatory cytokine (tumor necrosis factor alpha, interleukin 6) • *In vivo* grafts with 100 μg AgNPs show minimal leukocyte infiltration and normal healing	• Concentration and exposure time • Local microenvironment (infection, immune status) • Particle chemistry and surface modifications	• Mild inflammation may enhance regenerative cascades • High-dose inflammatory risk underscores tight dose control • Need for further study in immune-compromised or infection-prone models

Abbreviations: Ag^+: Silver ions; AgNP: Silver nanoparticle; MSC: Mesenchymal stem cell; ROS: Reactive oxygen species.

approximately 30 μg/mL without significant loss of viability.[15,174] At levels above 50–100 μg/mL, elevated intracellular levels of ROS and mitochondrial dysfunction lead to apoptosis or necrosis.[175,176]

Poly(lactic-co-glycolic acid)/AgNP composites, commonly used in scaffold fabrication, are engineered to release Ag^+ ions gradually over time. This controlled release strategy helps maintain silver concentrations below toxic thresholds, supporting sustained MSC proliferation, osteogenic differentiation, and matrix mineralization. Surface modifications play a crucial role in mitigating the toxicity of AgNPs. Coatings such as PEG, polyvinylpyrrolidone, chitosan, and natural biopolymers like silk fibroin help reduce non-specific protein adsorption, improve colloidal stability, and reduce ROS-related cellular stress.[108,177] Moreover, these coatings can facilitate targeted delivery to osteoprogenitor or immune-modulating cells, enhancing therapeutic specificity. These *in vitro* findings underline the importance of dose control and surface engineering in ensuring biocompatibility while preserving antimicrobial functionality.[178]

6.2. *In vivo* biodistribution and accumulation

Animal models have provided insight into the spatial and temporal distribution of AgNPs following localized or systemic administration. When AgNPs are incorporated into bone scaffolds and implanted at defect sites, they tend to remain primarily localized within the implant region, exerting localized antimicrobial and osteoregenerative effects.[179,180] This spatial confinement minimizes systemic exposure. However, trace amounts of silver, mainly in ionic form (Ag^+), may enter circulation and accumulate transiently

in the liver, spleen, and lymphatic tissues, especially during the early post-implantation period.[148] At the same time, therapeutic doses used in rabbit cranial defect models have not been associated with histologically observable organ damage or signs of systemic toxicity. Despite promising short-term findings, comprehensive studies on long-term biodistribution, metabolism, and excretion of AgNPs remain scarce, particularly beyond 6–8 weeks post-implantation. Understanding whether AgNPs are fully cleared or persist in organs is essential for risk assessment, especially for repeated or high-dose applications.[181,182]

6.3. Dose-dependency and safety thresholds

Preclinical evaluations have helped delineate a therapeutic window where AgNPs are both effective and safe. Antibacterial activity is generally observed at concentrations as low as 0.25 mg/L, with biofilm disruption occurring in the 1–4 mg/L range against pathogens such as *S. aureus*, *E. coli*, and *P. aeruginosa*. These concentrations are typically lower than those required to induce cytotoxic effects in mammalian cells.[14,59,101,102,126,129,130,150,153,155,161]

Cytotoxic thresholds for mammalian cells, including osteoblasts, fibroblasts, and hepatocytes, are generally higher, typically exceeding approximately 50–100 μg/mL, indicating a feasible therapeutic window when AgNPs are delivered locally and in a controlled manner (e.g., embedded in slow-release scaffolds). In contrast, topical or systemic delivery systems (e.g., AgNP gels, injections) pose a narrower safety margin. For example, in HepG2 liver cells, the half-maximal inhibitory concentration of AgNPs is approximately 250 μg/mL. Therefore, systemic delivery must be approached with caution, emphasizing the need for pharmacokinetic optimization and refinement of the delivery route.[43,73,122,129,138,151-153,165,166,173,175,181,183-185]

6.4. Immunogenicity and inflammatory responses

AgNPs not only interact with bone and stem cells but also influence various components of the immune system. Their immunomodulatory properties can be beneficial or detrimental depending on the dose and exposure context. At low to moderate concentrations, AgNPs induce mild oxidative stress, which can activate signaling pathways that promote angiogenesis, immune cell recruitment, and early-stage tissue repair. This mild proinflammatory response may be advantageous in bone healing. At high concentrations (>100 μg/mL), AgNPs trigger the release of proinflammatory cytokines, including TNF-α and IL-6, which can lead to excessive inflammation and tissue damage if not properly controlled. In *in vivo* rabbit studies, grafts containing 100 μg of AgNPs did not elicit significant leukocyte infiltration, necrosis, or chronic inflammatory reactions. Histological analyses typically reveal normal healing patterns with minimal immune cell activation, suggesting a favorable immunological profile under therapeutic dosing conditions.[186-189] Future research should aim to further characterize the dose-dependent immunological effects of AgNPs, particularly in immune-compromised or infection-prone environments, to ensure safe clinical translation.

In summary, despite their well-documented antimicrobial and regenerative potential, AgNPs have several important limitations and adverse effects that must be critically discussed when considering clinical translation. At the cellular level, AgNP toxicity is primarily mediated by oxidative stress, mitochondrial dysfunction, and DNA damage, resulting in dose- and size-dependent cytotoxicity and genotoxic risk in various cell types.[190] Neurotoxic outcomes have been reported in multiple models and are associated with oxidative inflammation and disrupted autophagy pathways, raising specific concerns for systemic exposure or accumulation in neural tissues.[191] *In vivo* biodistribution studies show that although locally implanted AgNPs often remain concentrated at the defect site, Ag^+ and small particulates can translocate to the liver, spleen, kidneys, and brain, with clearance kinetics that are incompletely characterized beyond early post-implantation windows; this persistence poses long-term safety uncertainties, including organ accumulation and functional impairment.[192] Mechanistically, many of these adverse effects stem from ROS overproduction and mitochondrial damage (reported across cell and animal studies), which can cause inflammation, cell death, and impaired tissue function if exposure is excessive or prolonged.[193] Finally, although "green" biosynthesis of AgNPs may reduce reagent costs and environmental impact, such methods often yield variable particle size, surface chemistry, and batch reproducibility that complicate Good Manufacturing Practice (GMP)-compliant scale-up and quality control, factors that directly affect both safety and efficacy.[194] Taken together, these limitations suggest a conservative, evidence-based approach to clinical use. Rigorous dose-finding studies, long-term biodistribution and clearance experiments, standardized particle characterization, and the employment of mitigation strategies (e.g., robust surface coatings, controlled-release matrices, targeted delivery) should be required to minimize systemic exposure while preserving antimicrobial efficacy.

7. Manufacturing, scaling, and regulatory considerations

7.1. Reproducibility in synthesis

Reliable and scalable synthesis of AgNPs is fundamental to clinical translation.[195] Reproducibility in terms of

particle size, shape, surface charge, crystallinity, and functionalization is crucial, as these factors significantly influence both biological activity and safety. Chemical reduction methods, such as those using sodium citrate or sodium borohydride, provide fine control over nanoparticle size and monodispersity. However, residual reducing agents or capping chemicals can pose biocompatibility issues, necessitating the use of extensive purification protocols. Physical techniques, such as laser ablation or evaporation–condensation, produce high-purity AgNPs with minimal contamination. However, they often result in broader size distributions and lower yields, which can complicate downstream applications. Green synthesis, leveraging biological reducing and capping agents (e.g., plant polyphenols, microbial enzymes, silk fibroin, or fibronectin), generates biocompatible AgNPs with inherent functional coatings. These methods also reduce environmental impact and reagent toxicity. However, batch-to-batch variability due to natural source heterogeneity and seasonal or geographic differences in raw materials remains a significant limitation for industrial scalability. Future strategies to improve synthesis reproducibility include automated microfluidic reactors,[17,22,113] real-time monitoring with ultraviolet–visible spectroscopy and dynamic light scattering, and artificial intelligence-based process control systems that optimize reaction parameters dynamically.

7.2. Quality control and standardization

Quality control is crucial for ensuring the consistency, safety, and regulatory compliance of AgNP-containing biomaterials.[196] Standard quality control parameters should include:

(i) Particle characterization:[167] Dynamic light scattering for hydrodynamic size, zeta potential for surface charge, transmission electron microscopy for morphology, X-ray diffraction for crystallinity, and surface composition analysis via Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy
(ii) Ion release profiling: Quantification of Ag^+ ion release over time in physiological conditions using inductively coupled plasma mass spectrometry or atomic absorption spectroscopy, with emphasis on achieving therapeutic yet non-cytotoxic levels
(iii) Scaffold evaluation: In composite systems (e.g., AgNP/PLGA foams or hydrogels), mechanical integrity (e.g., compressive strength ≥18 MPa), porosity (>70%), biodegradation rate (tailored to defect healing timeline), and sustained antimicrobial function must be validated
(iv) Sterility and endotoxin testing, per International Organization for Standardization and United States Pharmacopeia standards, is also required for clinical applications.[197]

Establishing inter-laboratory standard operating procedures and adopting international nanomaterial reference standards would significantly advance reproducibility and global standardization.

7.3. Regulatory pathways

AgNP-based bone repair platforms are generally classified as medical devices or combination products, depending on their primary mode of action. Regulatory approval typically requires:[19,166,198] (i) Preclinical studies adhering to ISO 10993 standards to evaluate cytotoxicity,[199] sensitization, irritation, systemic toxicity, genotoxicity, and implantation responses;[200,201] (ii) *In vivo* validation using standard animal models of bone defects, such as critical-size rabbit cranial or rat femoral defects, to demonstrate both biocompatibility and efficacy in promoting bone regeneration and infection control; (iii) Chemistry, manufacturing, and controls documentation that details nanoparticle synthesis, purification, batch reproducibility, and stability; and (iv) Clinical trials designed to confirm scaffold integration, functional bone recovery, absence of systemic silver accumulation, and lack of adverse effects such as chronic inflammation or allergic reactions.

Given the dual-function nature of theranostic AgNP platforms, regulators may impose additional requirements to address long-term biocompatibility, nano-bio interactions, and potential environmental toxicity or nanoparticle leaching. Interdisciplinary collaboration and early engagement with regulatory agencies (e.g., pre-investigational new drug application or pre-submission meetings) are crucial for successful translation.

8. Challenges and clinical translation

8.1. Resistance development

While AgNPs possess a broad-spectrum, multimodal mechanism of antimicrobial action, including membrane disruption, ROS generation, and DNA interference, concerns over microbial adaptation are rising. Emerging reports suggest that bacteria chronically exposed to sub-lethal AgNP doses can develop adaptive mechanisms, including the upregulation of efflux pumps, altered membrane permeability, and enhanced biofilm formation.[202] Mitigation strategies include alternating nanoparticle coatings, co-delivery of silver with other antimicrobials or enzymes (e.g., lysozyme), and responsive release systems that minimize prolonged sub-therapeutic exposure. Monitoring microbial resistance patterns during preclinical and clinical use will be critical for ensuring sustained efficacy and informing future design.[203-205]

A critical comparison with current clinical standards is important to understand the potential of AgNP-based systems in bone repair. Although antibiotic-loaded bone cements remain the standard choice for treating infected or high-risk defects, their effectiveness faces challenges.[206] These challenges include an increase in antimicrobial resistance, limited penetration into biofilms, and the gradual loss of mechanical strength that comes with antibiotic release. Recent studies by Karpiński *et al*.,[52-54] particularly their work on how different mixtures affect the mechanical properties of medium-viscosity bone cements, demonstrate that even well-optimized traditional cements are susceptible to mixing inconsistencies, porosity, and decreased long-term stability. These issues hinder their overall clinical performance. In contrast, AgNPs offer broad-spectrum antimicrobial activity, prolonged effectiveness against biofilm-forming pathogens, and the potential for integrating therapeutic and diagnostic functions into multifunctional scaffolds.[108] However, their production costs are higher due to nanoparticle synthesis, surface treatment, and sterilization requirements.[207] Overall, current evidence suggests that AgNP-based platforms could be most beneficial in situations with a high risk of infection or when conventional antibiotic-enhanced cements offer limited effectiveness. Still, further cost–benefit analyses and direct comparative studies are necessary before these can be widely used in clinical practice.

8.2. Long-term effects

Although short-term *in vivo* studies (up to 12 weeks) generally demonstrate favorable outcomes, long-term exposure scenarios are still poorly characterized. Ag^+ and particles may accumulate in bone, liver, or lymphatic tissues, and over time, may be converted into less soluble compounds, such as silver sulfide, which could disrupt the balance between osteoblasts and osteoclasts or trigger chronic inflammation. Potential downstream effects include altered bone remodeling, delayed healing, or immunogenic reactions. These risks underscore the importance of extended-duration studies (>6–12 months) in large animal models, including toxicokinetics and organ histopathology. Understanding the clearance kinetics, degradation pathways, and long-term retention of AgNPs will be essential for establishing safe chronic-use protocols.[208,209]

8.3. Cost-effectiveness

Cost remains one of the major barriers to the widespread clinical adoption of AgNP-based platforms for bone repair. Although green synthesis routes offer a more sustainable alternative and can reduce dependence on expensive chemical reagents, they often suffer from batch-to-batch inconsistency, variability in particle size and surface chemistry, and difficulties in reproducing results at an industrial scale. These limitations significantly restrict their commercial feasibility, especially in medical applications where reproducibility and regulatory compliance are essential. Moreover, the incorporation of high-value biomolecules, such as BMP-2, VEGF, antimicrobial peptides, or cell-targeting ligands, substantially increases production costs. These biologically active components require rigorously controlled, sterile, and GMP-compliant synthesis environments, which add layers of cost and regulatory oversight to an already complex manufacturing pipeline.

The urgency of cost control is underscored by foundational work in traditional bone cement systems. Karpiński *et al*.[52-54] have demonstrated that even well-established polymethylmethacrylate (PMMA) cements are subject to significant manufacturing challenges. For example, contamination by physiological fluids, such as blood or saline, significantly degrades mechanical performance.[210] Their studies[210,211] demonstrate how porosity, reduced strength, and instability arise when cement is processed under non-ideal conditions. Moreover, their more recent research on the incorporation of glassy carbon in PMMA cements[212] reveals that adding functional fillers is not a trivial solution. Particle size, concentration, and distribution have profound impacts on the final material's performance, which in turn demands more stringent quality control and potentially higher manufacturing costs.

Emerging technologies offer promising avenues to offset these economic challenges. Three-dimensional printing and modular scaffold fabrication enable precise control over architecture and material composition, reducing material waste and enabling the production of patient-specific constructs without the need for large inventories.[213] Likewise, point-of-care biomanufacturing units, designed to operate directly in hospital settings, could decentralize scaffold production and significantly reduce transportation, storage, and sterilization costs.[214,215] These advances support a "just-in-time" fabrication model that aligns well with personalized medicine and may help close the cost gap between nanotechnology-enhanced scaffolds and conventional alternatives.[216]

To justify clinical integration, robust cost–benefit analyses are needed that compare AgNP-based systems with current treatment standards such as antibiotic-laden PMMA cements, autografts, or allografts. While traditional therapies are generally less expensive and benefit from extensive clinical experience, they suffer from limitations,

including antibiotic resistance, donor site morbidity, incomplete regeneration, and inadequate biofilm control, which AgNP-based systems may help overcome. A systematic comparison of long-term outcomes, infection recurrence rates, implant longevity, and overall economic impact will be essential to demonstrate whether the added technological complexity of AgNP-based constructs translates into measurable clinical and economic advantages.

8.4. Clinical protocol integration

For successful clinical implementation, AgNP scaffolds must integrate seamlessly into existing surgical workflows. Materials should exhibit high moldability, sterilization compatibility (e.g., resistance to gamma irradiation or autoclaving), and mechanical robustness during implantation. Compatibility with orthopedic hardware, such as plates or screws, and co-administration with systemic antibiotics or anti-inflammatories should be evaluated to avoid cross-reactivity or interference. Tools for patient monitoring, such as non-invasive Ag^+ detection in urine or serum, could allow real-time tracking of systemic exposure. The development of pre-packaged, off-the-shelf scaffold kits tailored to specific bone defects (e.g., cranial, mandibular, or long-bone injuries) would simplify clinical adoption and improve procedural consistency.[105,143,158,195]

9. Emerging trends and future directions

9.1. Green synthesis and advanced surface engineering

Green synthesis strategies utilizing natural reducers (e.g., microbial metabolites, seaweed extracts, biopolymers) are gaining traction for producing AgNPs with minimal environmental impact and bioactive coatings.[43,61,112,126,130,184,195] Surface engineering advances allow for the functionalization of AgNPs with bone-homing peptides (e.g., AspSerSer), tumor-targeting ligands, or immunomodulatory proteins. Coatings incorporating stimuli-responsive polymers (e.g., pH- or ROS-sensitive hydrogels) provide smart targeting, releasing silver only under pathological conditions such as infection or inflammation.

9.2. Stimuli-responsive and smart scaffolds

Next-generation AgNP-based scaffolds incorporate smart release mechanisms: pH-responsive hydrogels,[155] ROS-degradable linkers, and enzyme-sensitive matrices ensure that silver and co-delivered therapeutics are only released when needed. Light-responsive systems, such as those triggered by near-infrared irradiation, enable spatially and temporally controlled Ag^+ release, minimizing systemic toxicity and maximizing local efficacy. These innovations may be particularly useful in oncological or chronically infected bone sites, where precise control of therapeutic release is critical.

9.3. Multifunctional nanoparticles

Multicomponent AgNP systems are being developed to deliver therapeutic, diagnostic, and regenerative functionalities in a single platform. Silver–gold hybrid nanoparticles enable photoacoustic or CT imaging, while simultaneously providing plasmonic heating and antimicrobial action.[217,218] Silver–iron oxide composites allow for magnetic targeting and MRI visualization.[219] Strontium-incorporated AgNPs enhance osteoinductive activity while preserving antibacterial properties.[103] These multifunctional platforms are ideal candidates for personalized, image-guided bone therapies.

9.4. Personalized bone repair

Three-dimensional printing and computer-aided design technologies enable the fabrication of patient-specific scaffolds tailored to the defect's size, shape, and anatomical constraints. Electrically conductive AgNP–polycaprolactone scaffolds have been demonstrated to enhance osteogenic differentiation through electromechanical stimulation, with potential applications in bioelectronic sensing and stimulation systems.[220,221] Point-of-care additive manufacturing, using portable bioprinters or preloaded scaffold cartridges, may revolutionize emergency bone repair in trauma centers or military field hospitals.[222-225]

10. Conclusion

AgNP-based materials represent a new frontier in regenerative medicine, combining antimicrobial protection, stimulation of bone growth and blood vessel formation, imaging capabilities, and controlled release of therapies in a single multifunctional platform. In various preclinical models, AgNP–enabled constructs have shown strong potential to tackle the many challenges involved in bone repair, especially in cases complicated by infection, reduced blood flow, or chronic inflammation. This review offers an integrated perspective on AgNP-based bone therapies through a theranostic lens. We present, in a unified analysis, how surface engineering methods, interactions between nanomaterials and cells, immune system modulation, imaging compatibility, and smart release mechanisms work together to create next-generation platforms with combined therapeutic effects. In addition, this review connects fundamental concepts in nanomedicine with practical considerations, including manufacturing processes, regulatory pathways, cost assessments, and comparisons with current clinical

standards, such as antibiotic-loaded cements. We also highlight several important gaps in knowledge that need to be filled for successful clinical application, such as (i) The development of consistent, standardized, and GMP-compliant synthesis methods, (ii) Long-term studies on how these materials spread in the body, accumulate, and trigger immune responses, (iii) Optimization of safety profiles based on dosage, (iv) Techniques to reduce antimicrobial resistance through smart, multifunctional designs, and (v) Alignment of regulatory frameworks for complex nanotheranostic systems.

Advancing in these areas requires coordinated efforts among materials scientists, clinicians, nanotechnologists, regulatory bodies, and industry. Only through this collaborative approach can breakthroughs in the lab become reliable technologies in clinical settings. By combining insights into mechanisms, technological developments, translational challenges, and future research priorities, this review offers a roadmap for guiding the next generation of AgNP-based theranostic scaffolds toward practical use in clinical settings. In doing so, it positions AgNP-enabled platforms as potential key elements in the new approach to precision bone regeneration.

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