Materials Today Bio 26 (2024) 101016

Contents lists available at ScienceDirect

Materials Today Bio

journal homepage: www.journals.elsevier.com/materials-today-bio

A review on gold nanoparticles as an innovative therapeutic cue in bone tissue engineering: Prospects and future clinical applications

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A B S T R A C T

Bone damage is a complex orthopedic problem primarily caused by trauma, cancer, or bacterial infection of bone tissue. Clinical care management for bone damage remains a significant clinical challenge and there is a growing need for more advanced bone therapy options. Nanotechnology has been widely explored in the field of orthopedic therapy for the treatment of a severe bone disease. Among nanomaterials, gold nanoparticles (GNPs) along with other biomaterials are emerging as a new paradigm for treatment with excellent potential for bone tissue engineering and regenerative medicine applications. In recent years, a great deal of research has focused on demonstrating the potential for GNPs to provide for enhancement of osteogenesis, reduction of osteoclastogenesis/osteomyelitis, and treatment of bone cancer. This review details the latest understandings in regards to GNPs based therapeutic systems, mechanisms, and the applications of GNPs against various bone disorders. The present review aims to summarize i) the mechanisms of GNPs in bone tissue remodeling, ii) preparation methods of GNPs, and iii) functionalization of GNPs and its decoration on biomaterials as a delivery vehicle in a specific bone tissue engineering for future clinical application.

1. Introduction

Bone injury due to trauma, infection, or surgical excision can lead to physical disabilities and other serious problems for public health [1]. Autologous and allogeneic bone graft are the most common treatment methods for restoration and reconstruction of bone [2]. Of these, autologous bone graft is known to be the gold standard clinical method for treating bone deficits [3]. However, treatment strategies that use autologous or allogeneic bone can be limited by the size and shape of the bone deficit, graft rejection, shortage of donors, secondary surgeries and resulting secondary bone loss, immunogenicity, and other factor [4–6]. Over the past several decades, artificial bone scaffolds based on biomaterials and decorated with osteoinductive molecules have been developed as an option for the restoration and reconstruction of damaged bone tissue [7,8]. Biomaterials for bone regeneration based on polymers, ceramics, metal, and composite materials are able to be used

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https://doi.org/10.1016/j.mtbio.2024.101016
Received 2 December 2023; Received in revised form 19 February 2024; Accepted 2 March 2024
Available online 12 March 2024

successfully for bone reconstruction because they possess suitable physicochemical and mechanical properties, they are biocompatible, and they are osteoinductive.

Manufacturing a scaffold with the ideal 3D structure is an essential factor in the reconstruction of damaged bone tissue [9]. In order restore function and anatomical structure and to satisfy aesthetic demands, bioactive scaffolds used in grafts need to utilize all possible benefits [10]. In addition to the needs for mechanical properties, biocompatibility, and precisely controlled biodegradability, the scaffold needs to be able to hold and deliver not only cells, but also numerous biologically active molecules (e.g., cytokines, drugs, antibiotics, growth factors, and other molecules) [11]. Recently, there has been a focus on improving bone regeneration by using 3D scaffolds that contain osteoconductive or osteoinductive agents [12,13]. To develop a system that can efficiently deliver osteogenic agents, it is essential to keep the agents stable at the graft site throughout regeneration of the bone deficit [14]. As such, various types of nano- and microcarrier are being used as biomolecular delivery systems [15,16].

In particular, gold nanoparticles (GNPs) have widely been used in biomolecular delivery systems due to their high biocompatibility, easily adjustable shape and size, and high reproducibility [17–20]. GNPs have also been applied in various biomedical engineering systems including biosensors [21], biomedical imaging applications [22], protein delivery [23], and radiotherapy [24] (Fig. 1a). GNPs have the ability to bind to biomolecules at their surface through gold-thiol bonding [25]. Thiol molecules (R–SH) and gold nanoparticles are adsorbed through weak van der Waals interactions. Upon adsorption, thiol molecules dissociate to form thiolate ions (RS-) and hydrogen ions (H+), which are promoted by the sulfur atom's electron-rich nature and the gold surface's electron-poor nature. Afterwards, the thiolate ion binds strongly by forming a covalent bond between the sulfur atom and the gold atom, known as an Au–S bond, which is very stable [26–28]. Besides being used as a carrier for stable delivery of biologically active molecules, GNPs are an intriguing substance for use in bone tissue engineering given their inherent enhancement of bone regeneration [29]. In fact, GNPs affect bone regeneration by upregulating osteogenic differentiation while inhibiting osteoclast activity [30,31]. In addition, GNPs have been previously investigated for their ability to improve bone regeneration, and they are being actively investigated for the treatment of osteoporosis [32]. Over the past ten years the number of studies using GNPs for tissue engineering and regenerative medicine applications has been increasing which obtained by PubMed (Fig. 1b).

The objective of the present review is to examine the major approaches using biomaterials, including GNPs, which are currently being used strategically in the field of bone tissue engineering (Fig. 2). First, we explain recent reports on the mechanisms by which the cells involved in bone tissue remodeling react with GNPs to affect bone regeneration. Next, we classify GNP-based approaches, from strategies to synthesize GNPs of different shapes and sizes to functional GNP complexes made by surface modifications of GNPs and combinations with organic or inorganic biomaterials, and we discuss the synthesis and structural properties of recently reported GNP-containing biomaterials. We also discuss the recent advanced applications of GNPs that are currently being used in biomedicine. In particular, we review the use of GNPs in bone tissue engineering, including bone regeneration, osteoporosis treatment, and bone cancer treatment. Finally, we conclude the review with a summary of future challenges and outlooks for functional GNPs and GNP-based hybrid materials in the field of biomedicine.

2. Degradation and stability of GNPs for use as a biomedicine

Gold, being a non-toxic and highly biocompatible material, is exceptionally suitable for future clinical applications [33,34]. GNPs are under extensive research in various fields, yet there is limited knowledge regarding their fate within the organism, considering the diversity of application studies (Fig. 3a) [35]. Recently, according to the research

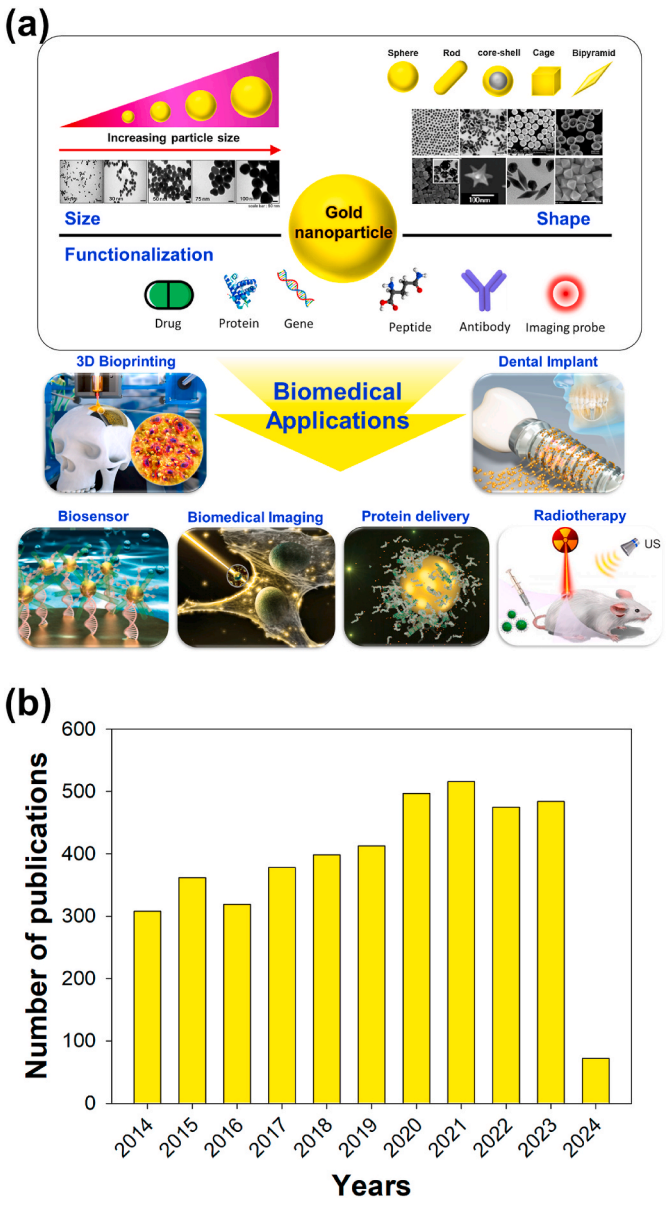

Fig. 1. Schematic representative of functionalized GNPs and their biomedical applications (a), number of published papers in last decade on bone tissue engineering with GNPs (b) (Source: PubMed; https://www.ncbi.nlm.nih.gov, Keywords: gold and bone tissue). Reprinted from Ko et al. [31], Khlebtsov et al. [19], Heo et al. [128], Han et al. [21], Attia et al. [178], John et al. [23], Jiang et al. [179], and Heo et al. [169]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

conducted by Balfourier et al., it was observed that when various-sized GNPs (4, 15, and 22 nm) were administered to primary human fibroblasts, which are the most highly distributed cells within the body, and tracked for six months, smaller-sized particles exhibited relatively quicker degradation [35]. This degradation was found to be induced by reactive oxygen species (ROS) generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), oxidizing GNPs. This challenges the current dogma about the persistence of GNPs in the body, as they are slower to degrade than previously thought. Chen et al. administered GNPs to mice via intraperitoneal injection to achieve efficient absorption, rather than oral intake (Fig. 3b) [36]. The observations indicated a varying location of GNPs within adipose tissue over time, with no evident decrease in the number of GNPs. This suggests a gradual elimination of GNPs from adipose tissue. However, the debate

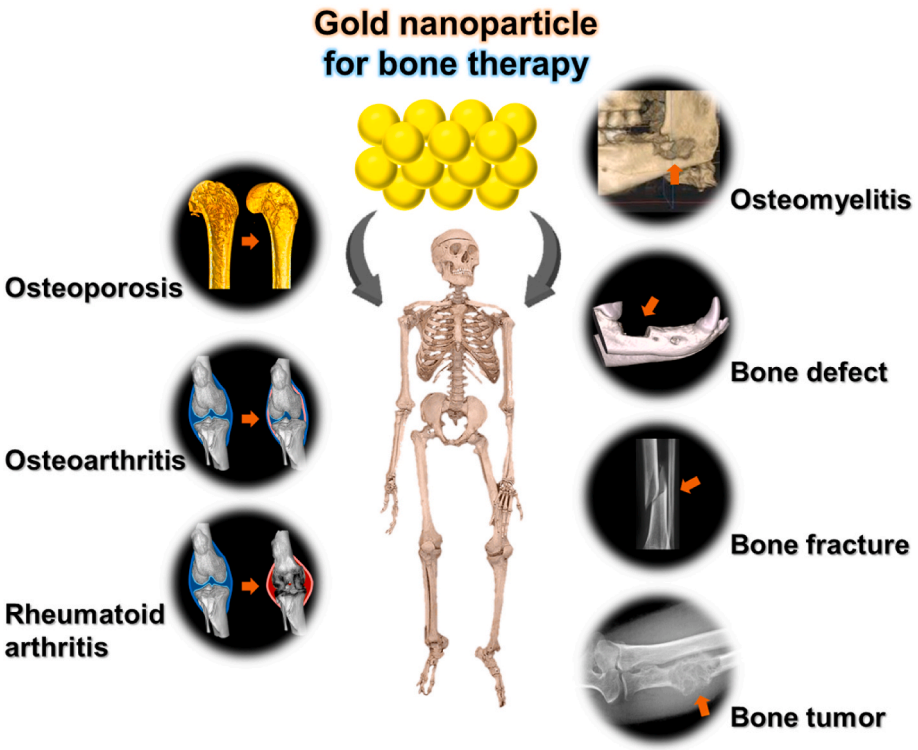

Fig. 2. Illustration depicting the potential application of GNPs in bone therapy. GNPs exhibit biocompatibility, controlled drug release, and imaging capabilities, making them promising candidates for targeted treatment and regeneration of bone diseases such as osteoporosis, osteoarthritis, rheumatoid arthritis, osteomyelitis, bone defect/fracture, and bone tumor.

regarding the degradability of pure spherical GNPs remains unclear, and long-term observations are required to gain a comprehensive perspective.

The storage stability of GNPs is a crucial issue that must be addressed when discussing research or clinical applications. Traditionally, GNPs are known to be stored in dark conditions at temperatures below 4 °C, as described in several studies [37–40]. The GNPs produced through the citrate reduction method can be made in the range of 16–150 nm, depending on the gold-to-citrate concentration ratio [40–42]. 20 nm GNPs produced using the same method exhibit different size stability depending on the storage temperature. When stored at 4 °C, the GNPs remain well-suspended and maintain their size for up to 20 days. However, when compared to storage at room temperature (23 °C), significant aggregation is reported to occur from the 6th day onwards [40]. An evaluation of GNPs stability, monitored over time and at varying temperatures using the Thermal History Indicator (THI), revealed that particle size increases primarily due to storage temperature, while shape alterations are mainly attributed to storage duration [43]. This is manifested by more intense color changes, driven by localized surface plasmon resonance effects, after exposure to higher temperatures [44]. The change in the appearance color of GNPs solution is directly related to the size of the GNPs. This is attributed to the plasmon resonance effect, where exposure to light with oscillating electric fields can be detected by the electrons of the metal nanoparticles like GNPs, leading to observable changes [43–46]. The various effects of GNPs are summarized in Table 1. However, from the several reasons, we are still unable to address the issue of long-term *in vivo* stability of GNPs. To address problems, it is necessary to consider the potential toxicity of accumulated GNPs in the body based on treatment condition. As a result, the issue of *in vivo* safety regarding GNPs is still a topic of discussion, but we can look positively towards the future in this regard. Research on GNP-based formulations that have received clinical trial approval from the FDA is currently ongoing, and it has already been confirmed that several applied particles have been approved [47–49].

3. Preparation and synthesis of GNPs for application

3.1. Traditional synthesis approach of GNPs

In the field of GNP research, there have been numerous studies aiming to produce good quality GNPs with stable and homogeneous size and shape. The citrate reduction method first published by Turkevich in 1951 is a classic example; this method is widely used because it is a simple procedure that can product GNPs of around 20 nm in size [41]. In 1973, Frens presented a method to make GNPs of different sizes by varying the ratio of citrate in Turkevich's method [42]. In the citrate reduction method, citrate acts as a reducer, surfactant, and base. The refined Turkevich-Frens method is used universally because it enables stable synthesis of GNPs with a desired size. However, a purification process is required to obtain homogeneous particles, and this can result in some loss.

Another method is the Brust-Schiffrin method, a two-phase reduction method developed in 1994 that uses alkanethiolate and thiolate ligands to produce highly stable GNPs [50,51]. In this method, a simple process based on rapid reduction of gold ions in water-toluene two-phase system is followed by spontaneous adsorption of alkanethiols. GNPs made in this way are extremely stable and are also soluble in solvents such as toluene, pentane, and chloroform, meaning that they can be sedimented, redissolved, and stored in powder form [52]. In addition, there are methods that use physical stimulation, such as infrared, near-infrared, ultrasound, or microwaves to control the reduction rate or size of GNPs [53–58].

The mechanisms introduced above can be used to manufacture various sizes of GNPs, nanorods, nanoplates, platonic nanoparticles, and branched nanostructures. Recently, there have been studies attempting to adapt these nanomaterials further through surface modifications or conjugation with other materials, with the aim of inducing specific reactions and applying the resulting materials to fields such as drug delivery and regenerative medicine.

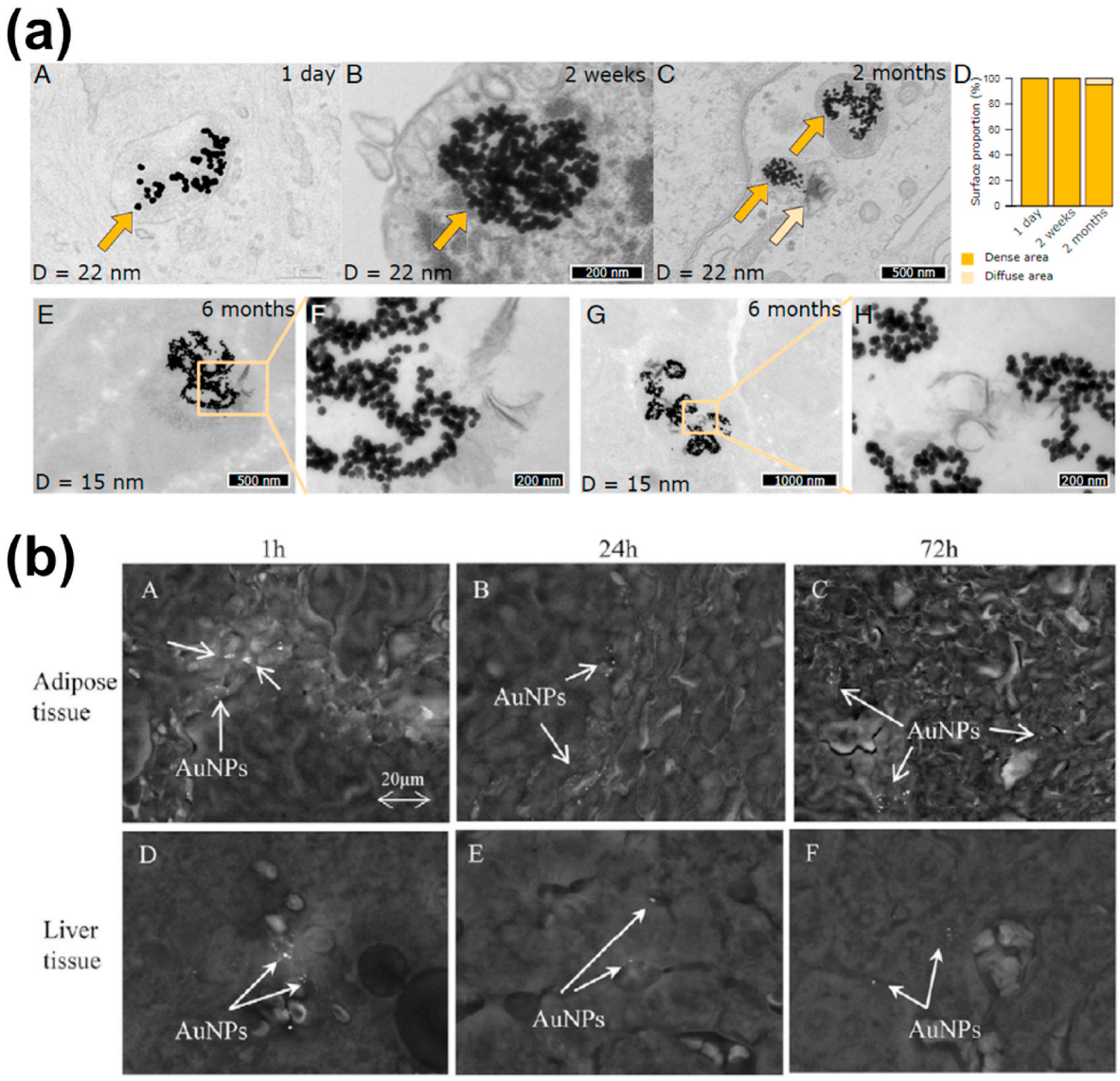

Fig. 3. TEM observations on microtome sections of human fibroblasts exposed to 4-nm GNPs observed 1 day to 6 months after GNPs incubation, evidencing the existence of dense and diffuse electron-dense areas and indicating intracellular crystallization of GNPs (a). GNPs distribution within mouse liver following Intraperitoneal injection. GNPs in the liver connective tissue and a 5 mm capillary within the liver adipose tissue (b). Reprinted from Balfourier et al. [35] and Chen et al. [36].

3.2. Mechanosynthesis of GNPs

There are various methods available to form GNPs using physicochemical processes, including both solid-phase mechanochemical processes as well as top-down and bottom-up approaches [59–62]. Although the manufacturing processes differ, the shape and stability of the produced GNPs are similar. In addition, these methods allow for modifying the particle size directly by controlling the milling time. The mechanosynthesis of GNPs at room temperature was first proposed by the research team led by Geckeler in 2009 [59]. A mixture of 0.11 mM $KAuCl_4$, 0.9 mM polyvinylpyrrolidone, and 0.11 mM $NaBH_4$ was placed in a high-speed vibration mill at room temperature and left to react at 1500 rpm thus producing GNPs. By adjusting the ratio of $KAuCl_4$, reaction time, and molar mass of polyvinylpyrrolidone, GNPs with sizes from 8.8 ± 2.8 to 27.9 ± 6.2 nm can be obtained. This mechanosynthesis method is convenient, can be performed at room temperature, and provides stable GNPs for use over 1 year because there are no solvents required for manufacturing or size control. In 2013, Moores et al. introduced a mechanochemical method to manufacture ultrasmall GNPs (1–4 nm) without solvents [62]. This method does not require any separate reducing agent, and the size of the GNPs can be adjusted by changing the amine ligands or milling time.

3.3. Electrosynthesis of GNPs

GNPs can also be produced by electrolysis. GNPs are produced using an electrochemical reaction called sacrificial anode electrolysis. The electrolyte is prepared by dissolving 0.1 M tetra-alkyl ammonium salt in a solution consisting of a 3:1 mixture of tetrahydrofuran and acetonitrile. Three electrodes are used: 1) a silver/silver nitrate ($Ag/AgNO_3$) reference electrode, 2) a gold anode, and 3) a platinum cathode. Ammonium salts are additionally used to stabilize the particles and replenish the electrolyte. The electrolysis cell is kept under a nitrogen atmosphere, and the electrolysis charge is maintained at 300 °C [63]. Compared with typical GNPs, the GNPs produced by sacrificial anode electrolysis have a lower production cost and can achieve a higher physicochemical stability. Additionally, the particle size can be controlled easily [63–65].

Table 1
Potential applications of gold nanoparticles in biomedicine.

Medical Application	Various effect of GNPs as biomedicine	References
Cancer diagnosis	GNPs enable sensitive cancer diagnosis by selectively binding to cancer cells or biomarkers, leveraging their unique optical properties.	[181,182]
Cell imaging	Leveraging their unique surface plasmon resonance effect, GNPs enable precise tracking and monitoring of cellular processes	[183,184]
Drug delivery	Their tunable properties and surface functionalization enable enhanced cellular uptake and improved therapeutic outcomes, making GNPs promising candidates for advanced drug delivery systems.	[185,186]
Photothermal therapy	When exposed to near-infrared light, GNPs generate heat, leading to localized hyperthermia that selectively destroys cancer cells, providing a highly targeted and efficient therapeutic approach for cancer treatment.	[187,188]
Tissue engineering	GNPs can enhance cell adhesion, proliferation, and differentiation, promoting targeted tissue regeneration	[189,190]
Immunological applications	Through functionalization with antigens and targeting ligands, GNPs enable targeted delivery, enhancing immune responses and facilitating the development of novel immunotherapeutic approaches and vaccines.	[191,192]
Diagnostic device development	their unique optical properties can be leveraged for rapid and accurate detection of various biomarkers, enabling efficient and sensitive diagnostic testing in a wide range of healthcare settings.	[193,194]

3.4. Biological synthesis of GNPs

The biological synthesis of GNPs is being studied as a form of green chemistry by reducing the amounts of harmful substances used or produced during synthesis [66]. As this requires all solvents, reducing agents, and stabilizers to be safe and nontoxic, it is common to use resources such as plant-based compounds and derivatives as well as bacteria, yeasts, or virus [67–70]. The research team led by Shi developed graphene oxide/gold nanorods for applications both in computed tomography and photothermal therapy of cancer. This method uses *Morinda citrifolia* extract with graphene oxide- and gold-seed-mediated methods conducted at room temperature. In 2004, the research team led by Perumal confirmed that this substance has excellent catalytic properties [71].

3.5. Core–shell synthesis of hybrid GNPs

To improve the GNP functionality, GNPs embedded in inorganic materials or polymers in a core–shell structure have been developed. This structure can improve the stability and dispersion of encapsulated particles by reducing the reactivity of the particles while mitigating physical, chemical, and biological effects from the environment. By selecting an appropriate material for the shell, this type of structure can be adapted for specific purposes. Silica, which is relatively easy to control, has been applied to GNPs using various fabrication methods to obtain core–shell particles. By regulating the size and porosity of the nanoparticles, the stability and biocompatibility can be optimized. Core-shell GNPs can be used for targeting, diagnosis, and drug delivery. A pioneering study by Mulvaney in 1996 led to extensive research on core–shell GNPs using silica. Yoon et al. developed a method for synthesizing nanoparticles with high reproducibility while enabling fine adjustment of the thickness of the silica shell [72,73]. Zeng et al. demonstrated the use of a silica shell to reduce the cellular toxicity of GNPs [74]. They made their particle coating by adding tetraethyl orthosilicate and ammonia to an alcohol system using the Stober method to synthesize silica nanospheres in conjunction with a previously prepared GNP solution. The silica/GNP core–shell particles made by this method can be isolated by centrifugation. During toxicity assessments using HepG2 cells, silica-coated GNPs showed a higher cell viability than normal GNPs. The difference was even more pronounced for gold nanorods than for GNPs [74].

GNP core–shell structures using various polymers have also been developed. A polyaniline complex manufactured by Eia et al. and Xu et al. showed biological reactivity due to its unique chemical and electrical properties [75,76]. Poly(N-isopropylacrylamide), which is considered as a smart polymer, has been widely used in tissue engineering due to its ability to induce functional changes according to environmental factors such as temperature, pH, and light. As this biological behavior can be combined with the optical and physicochemical properties of GNPs, core–shell structures can lead to advanced materials that respond to stimulus triggers [77–79]. Choi et al. formed particles with 50 nm diameter and a core–shell structure by treating the GNP surface with polydopamine, which can be easily attached to various surfaces [80]. By fixing fluorescently labeled hairpin DNA that can recognize a specific microRNA sequence to this structure, they developed nanoprobes for long-term tracing for up to 5 days in MSCs and claimed that these nanoprobes have an enormous potential for identification and isolation of specific cell types as well as for high-throughput drug screening. Xing et al. produced a colloidal GNP/collagen nanoconjugate, without a reducing agent or stabilizing agent, by using electrostatic bonds between gold ions and collagen proteins and conducting reduction mediated by hydroxyproline residues [81]. The conjugates manufactured using this biocompatible and solvent-free method promoted effective cell adhesion, growth, and differentiation. These hybrid biomaterials possess advanced features which may be useful for the diagnosis and treatment of diverse health conditions.

3.6. Prepararion of GNPs for nano-sized with various shapes

The nanoscale metal arrangements resulting from the vapor-liquid-solid were first published around 40 years ago, and the development of the seed-mediated growth method has made synthesis considerably simpler [82,83]. Formation of anisotropic particles through seed-mediated growth is the most efficient and best-established protocol for gold nanorod synthesis. Here, the structure of the seed and the silver nitrate are crucial factors affecting the shape and crystalline structure of the gold nanorods [84]. Murphy et al. compared nanorods that had been synthesized using various seeds with different sizes and surface characteristics. The authors found that the aspect ratio of the nanorods increased greatly with decreasing seed size, and that this effect was more pronounced for negatively charged seeds [85]. Based on seed-mediated growth methods and silver nitrate-mediated synthesis methods, it is possible to form various gold platonic solids, including tetrahedra, cuboids, octahedra, and icosahedra [86,87]. In addition, Gold nanorods can also be made using electrochemical methods, photochemical reactions, and seedless one-step methods, etc [88–90].

Dong et al. found that L-amino acids can control the size and shape of gold nanostructures and created nanoplates. In this study, a reaction was induced by mixing for 12 h at 25 °C in an aqueous solution containing 0.1% tetrachloaurate and 0.5 mg/mL amino acid. Because GNPs and gold nanoplates are both formed, when solution is measured using UV/Vis, peaks are observed at 570 nm and 750 nm; analysis using TEM reveals two types of shape and electron diffraction. Since then, several other synthesis methods have been studied to simplify the protocol and obtain homogeneous particles [91,92].

4. Mechanisms of GNPs in bone tissue remodeling: GNPs enhance osteogenic differentiation and inhibit osteoclast differentiation

Bone remodeling is typically a continuous process involving the removal (resorption) of old bone tissue by osteoclasts and the formation of new bone tissue (formation) by osteoblasts [93,94]. Disruption of this balance can lead to bone disorders. For instance, osteoporosis is characterized by decreased bone density and increased fracture risk, often due to increased bone resorption by osteoclasts or decreased bone formation by osteoblasts [95]. This leads to changes in bone microstructure. Osteoarthritis involves cartilage degradation due to inflammation and enzymatic breakdown, as well as subchondral bone sclerosis and osteophyte formation [96,97]. Additionally, Paget's disease of bone, a representative bone disorder, is characterized by excessive activation of osteoclasts leading to increased bone resorption and subsequent overactive bone formation by osteoblasts [98]. This abnormal bone remodeling results in structurally weak bones. These bone disorders often involve defects in collagen synthesis or structure, leading to increased susceptibility to fractures [99–101].

GNPs have several strengths that make them suitable for use in bone regeneration; they show excellent protein adsorption, making them useful protein delivery vehicles via nanobioconjugation, and they can be applied to studies to enhance cellular behavior [102]. Patterns of endocytosis differ depending on the shape and size of the particles, but nanosphere particles with a size of around 50 nm are able to enter cells more efficiently via a receptor-mediated endocytosis pathway compared to smaller particles [103]. In addition, endocytosis-mediated cellular uptake of GNPs decreases when they are in an aggregated form, rather than single particles [104]. Cellular uptake is affected even more strongly by surface ligands than particle size [105]. Endocytosis of GNPs into bone cells is accompanied by various mechanisms. Anatomically, homeostasis of the bone tissue, which supports the structure of the body, is maintained through the bone remodeling cycle, which is a process of absorption and healing (regeneration) of aged and injured bone throughout the lifespan [106]. Through these repeated processes of recovery, bone is able to maintain its normal structure and density. The two key cells contributing to bone metabolism are (1) osteoclasts, which resorb damaged bone tissue, and (2) osteoblasts, which are responsible for neo-osteogenesis. Bone metabolic diseases like osteoporosis develop when the balance between resorption and osteogenesis by these two cell types is disrupted [107]. The reason that GNPs are so attractive for bone tissue engineering applications is because they affect the proliferation and differentiation of the cells that participate directly in the bone remodeling cycle (Fig. 4c) [108,109]. For example, Ko et al. used human adipose-derived mesenchymal stem cells (hADSCs) to study osteogenic

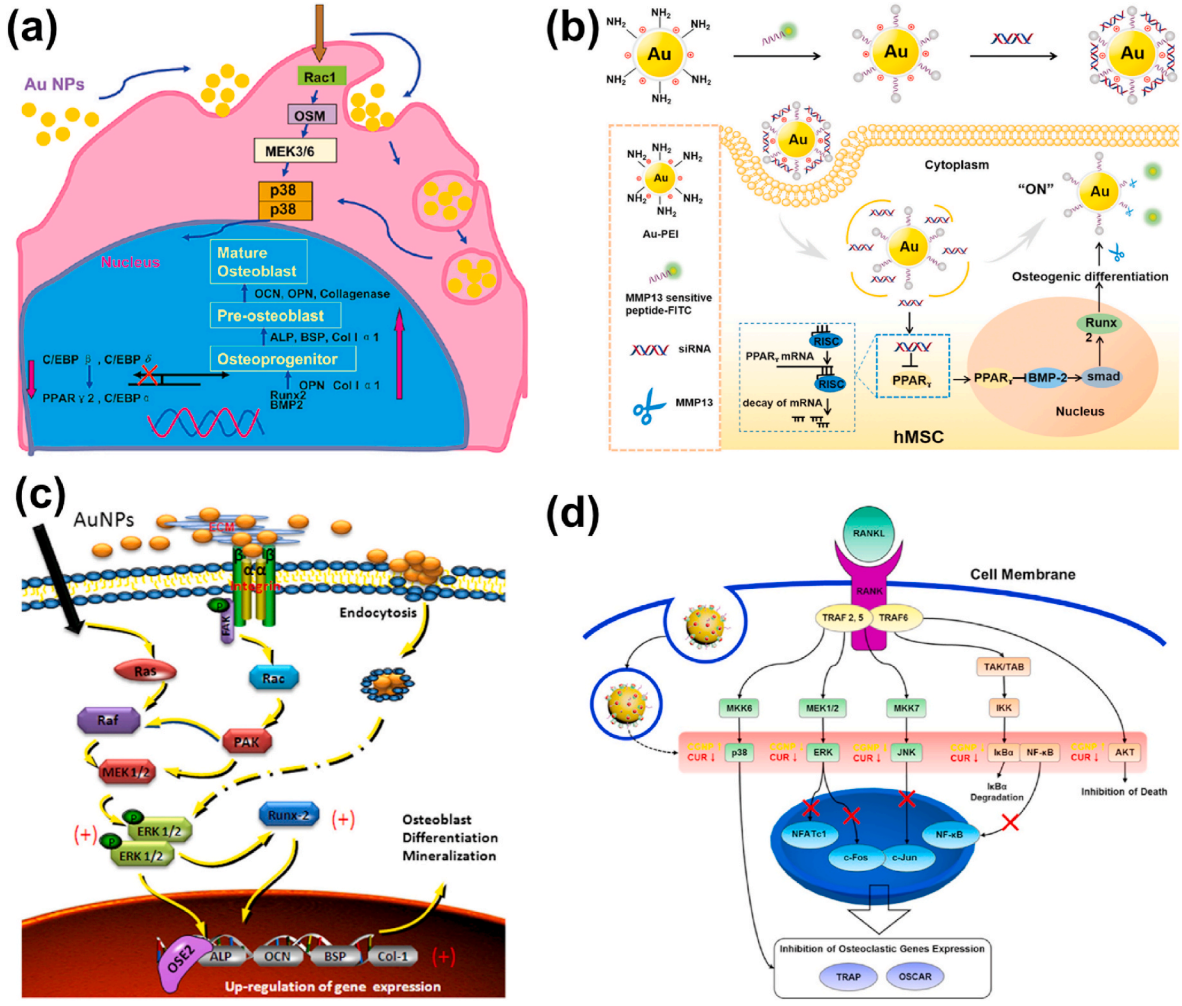

Fig. 4. Various molecular mechanism of the osteogenic and osteoclastogenic function through cellular uptake of GNPs and functionalized GNPs. Osteogenic differentiation of MSCs by GNPs uptake through p38 MAPK signaling pathway (a), enhanced osteogenesis by hydroxyapatite-decorated GNPs by activation of Wnt/β-catenin signaling pathway (b), interactions of GNPs with primary osteoblasts and its gene stimulation for osteogenesis (c), and inhibition of osteoclast differentiation of bone marrow-derived macrophages by curcumin-loaded GNPs through RANKL-induced signaling pathways. Reprinted from Yi et al. [113], Wu et al. [180], Zhang et al. [108], and Heo et al. [32].

differentiation for different sizes of GNPs [31]. When hADSCs were treated with GNPs sized either 15, 30, 50, 75, or 100 nm, the 50 nm GNPs showed the highest rates of cellular uptake, and increased the expression of genes affecting osteogenic differentiation. Zhang et al. described effects of PEGylated GNPs on osteogenic differentiation of various cell types are size-dependent [110]. They defined that 45-nm PEGylated GNPs significantly facilitate osteogenic differentiation of various cell types. On the other hand, 4-nm PEGylated GNPs inhibit osteogenic differentiation of various cell types. GNPs affect osteo-differentiation of mesenchymal stem cells (MSCs) via the mitogen-activated protein kinase (MAPK) signaling pathway. There are three main targets in the MAPK pathway: extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK [111]. Of these, the p38 kinase pathway is known to play a central role in upregulation of osteogenic differentiation in mammalian cells [112]. In a study by Yi et al. published in 2010, 20 nm GNPs were shown to affect osteogenic differentiation of MSCs (Fig. 4a) [113]. According to this study, GNPs interact with the cell membrane and enter the cells by endocytosis, after which they stimulate MSCs by binding with cytoplasmic proteins and causing mechanical stress. This mechanical stress directly upregulates runt-related transcription factor 2 (Runx2), which is believed to be regulated by MAPK cascades, and this is followed by upregulation of osteoblast marker genes. In this process, bone morphogenetic proteins (BMPs) play an essential role affecting cell behavior, and enhance osteoblastic differentiation by upregulating type-1 collagen (Col-1), which is the most abundant protein in organic bone matrix, and osteocalcin (OCN), which accumulates in mineralized bone [114]. Among BMPs, bone morphogenetic protein 2 (BMP-2) is a member of the transforming growth factor-b (TGF-B) super family that plays a key role in bone formation and repair [115]. GNPs have been found to increase expression and activity of BMP-2, and to stimulate expression of alkaline phosphatase (ALP), which plays important roles in bone formation and mineralization. In this way, GNPs were discovered to activate the p38 MAPK pathway and promote osteogenic differentiation.

GNPs have been reported to inhibit adipogenic differentiation while enhancing proliferation of human bone marrow mesenchymal stem cells (hMSCs) and osteogenic differentiation [116]. hMSCs possess multi-differentiation potential, meaning that, under the right culture conditions, they can differentiate into various types of cell that can be induced into different tissues, such as osteoblasts, chondrocytes, adipocytes, keratinocytes, and myocytes; based on this ability, they are being widely used in the field of tissue engineering and regenerative medicine [116–119]. The osteogenic differentiation potential of hMSCs makes them a very important cell source in the bone tissue engineering field, and improving the osteogenic differentiation efficiency of hMSCs is effective for bone tissue regeneration. Kohl et al. reported that, when GNPs were internalized by adipogenic differentiated hMSCs in the process of bone differentiation, they induced lipolysis via damage to the perilipin membrane, where perilipin is a lipid droplet-associated protein [116]. The ability of GNPs to inhibit adipogenic differentiation was also reported in the study by Yi et al., discussed above; GNPs increase the expression of Runx2 while inhibiting peroxisome proliferator-activated receptor gamma (PPAR γ), which destines adipocyte differentiation of MSCs [113,120]. As such, GNPs are very effective in bone tissue engineering using their cellular activity with hMSCs.

GNPs also affect mineralization and cell proliferation of osteoblasts themselves. According to a report by Liu et al., compared to 40 nm GNPs, 20 nm GNPs more effectively enhanced cell proliferation of MC3T3-E1, which is an osteoblast cell line, BMP-2 stimulated Runx2, which is a master regulator in osteogenic differentiation, and OCN expression and mineralization were induced [121]. Zhang et al. reported that the same size of GNP also mediated osteogenic differentiation via upregulation of the ERK/MAPK pathway and Runx2 in primary osteoblasts obtained from NIH mice calvarial (Fig. 4c) [108]. The Wnt/B-catenin signaling pathway is also involved in the stimulation of osteogenic differentiation by GNPs. Wnt is a glycoprotein that binds to receptor complexes, including low-density lipoprotein receptor-related protein (LRP)-5/6 and frizzled proteins [122]. Binding between Wnt ligand and cell-surface receptors in the frizzled family deactivates a protein complex including axin, adenomatous polyposis coli protein (APC), and glycogen synthase kinase 3β (GSK3β) that generally primes phosphorylated B-catenin for ubiquitination and proteasomal degradation [123,124]. As a result, non-phosphorylated B-catenin accumulates in the cytoplasm; this molecule is then translocated into the nuclear zone, where it increases gene expression in association with T-cell factor/-lymphoid enhancer factor (TCF/LEF) transcription factors [124]. As factors like Runx2 are up-regulated, the Wnt/B-catenin pathway affects proliferation of osteoprogenitor cells and osteogenesis. Choi et al. reported that chitosan-conjugated GNPs, fabricated using the chitosan reduction method, cause mechanical stimulation as they are taken up by hADSCs, and this mechanical stimulation leads non-phosphorylated B-catenin to induce osteoblastogenesis of the hADSCs via the Wnt/B-catenin pathway [122]. Studies like those above have demonstrated that GNPs enhance proliferation and mineralization of bone forming cells, thereby triggering osteogenic differentiation. Materials that have been developed based on GNPs to date are being used in the field of bone tissue engineering. The ability to perform surface modification using diverse methods based on surface chemistry has made GNPs viable as nanocarriers for pharmacological agents [125]. Moreover, with the publication of applied research on nanofiber-scaffold [126], hydrogel [127], and 3D-printed scaffold [128,129], using GNPs as an osteogenic agent, studies on the use of GNPs for bone tissue regeneration have continued [130].

GNPs also have direct effects on osteoclasts, which are another key cell involved in the bone remodeling cycle. Osteoclasts are multinucleated cells formed through fusion of mononuclear progenitors in the monocyte/macrophage lineage [131]. Osteoclasts that have undergone differentiation cling tightly to the bone matrix, where they are responsible for bone resorption through the 'sealing zone,' a filamentous actin (F-actin)-rich structure. GNPs inhibit osteoclast differentiation and activation in receptor activator of nuclear factor-kB (RANK) ligand (RANKL)-induced osteoclastogenesis. For instance, Sul et al. reported that 150 nm GNPs inhibit osteoclast differentiation via RANKL/RANK signaling [109]. Binding between RANKL and RANK in osteoclast precursors activates the nuclear factor-kB (NF-kB) pathway, and GNPs suppress osteoclast activation by inhibiting the activation of NF-kB. GNPs also increase production of the antioxidant enzyme glutathione peroxidase 1 (Gpx1), and reduce levels of reactive oxygen species (ROS) produced by RANKL via the RANK/tumor necrosis factor (TNF) receptor-associated factor (TRAF) 6 signal pathway during osteoclast formation from bone marrow macrophages (BMMs) due to RANKL/-RANK binding. Furthermore, numerous studies have shown that GNPs can affect inhibition of osteoclast formation [30,32,132,133].

The ability to inhibit osteoclast activity suggests that GNPs could have clinical applications against bone disease. Multiple myeloma is a type of plasma cell cancer that is often fatal in which myeloma cells form tumors inside bones; this causes osteoclastic bone destruction through interactions with the bone microenvironment, resulting in frequent fractures and reduced bone density [134,135]. Bhattacharya et al. suggested that GNPs could be used as a therapeutic moiety in the treatment of multiple myeloma [136]. Experiments showed that, when GNPs were treated at a concentration of 10 μg/mL or 20 μg/mL in the G1 phase, they induced cell-cycle arrest and inhibited proliferation of the multiple myeloma cells OPM-1, RPMI-8266, and U-266 through up-regulation of the cell-cycle proteins p21 and p27. One mechanism of these effects is that GNPs suppress activity of multiple myeloma cells by selectively deactivating vascular endothelial growth factor 165 (VEGF165), which is a heparin-binding growth factor with a heparin-binding domain [136,137]. In this process, GNPs inhibited proliferation of human umbilical vein endothelial cells (HUVECs) while not inhibit the activity of VEGF121, which does not have a heparin-binding domain [137].

There have also been studies reporting on the clinical applications of GNPs as a drug delivery vehicle, through surface modifications and chemical bindings, with the aim of treating osteoporosis, a major disease of bone metabolism. Fanord et al. found that bisphosphonate-functionalized GNPs were better at inhibiting osteoclast activity than only bisphosphonate, which is the most commonly prescribed drug for osteoporosis treatment [133]. Lee et al. also reported on the ability of GNPs loaded with alendronate, a bisphosphonate class drug, to inhibit osteoclast differentiation for the purpose of treating osteoporosis [30]. Similarly, according to a study by Nah et al., 30 nm GNPs had an inhibitory effect on osteoclast differentiation by themselves, and SH-PEG-vitamin D-conjugated GNPs inhibit expression of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), which is a key transcription factor in osteoclastogenesis [132]. Furthermore, Heo et al. reported that GNPs functionalized with cyclodextrin curcumin complexes (CUR-CGNPs) inhibited osteoclast differentiation as a bone resorption inhibitor to treat osteoporosis [32]. The functionalized GNPs inhibited expression of TRAP and the osteoclast-associated receptor (OSCAR) gene, and also greatly suppressed expression of c-Fos and NFATc1, which are important transcription factors for osteoclastogenesis. In addition, CUR-CGNPs appear to effectively inhibit expression of MAPKs (ERK, JNK, and p38) and I κ Bα proteins, resulting in reduced osteoclast activity in the RANKL-induced signaling pathway. Consistent with these findings, an *in vivo* study using an ovariectomized (OVX) model also showed recovery of bone volume and mineral density in a group that was administered CUR-CGNPs. Bai et al. proposed the clinical applicability of carboxylated GNPs to all to alleviate bone erosion by mitigating the acidic absorption microenvironment formed during bone resorption by osteoclasts [138]. In this way, research on GNPs and osteoclasts is continuing to date.

In the next section, we will discuss in detail how GNPs are synthesized and how their shape and size can be controlled by the choice of synthesis method. As mentioned above, the particle size is extremely important, because it is closely related to the interactions of GNPs at the cell level. GNPs can be fabricated at sizes of several nanometers to 100s of nanometers depending on the method of synthesis. Although differences in endocytosis mechanisms are indiscriminate with respect to particle size, the particle size affects cell cytotoxicity, proliferation, and differentiation. The biological mechanisms of GNPs discussed in this section ultimately indicate the unlimited applicability of GNPs as a bioactive material in the field of bone tissue engineering.

5. Incorporation and functionalization of GNPs for bone tissue regeneration

GNPs are synthesized in various sizes and shapes depending on their intended use. However, GNPs are also imbued with functionality by utilizing their excellent biocompatibility, target efficiency, and surface modifiability to bind GNPs to drugs, macromolecules, and physiologically active substances. These surface-treated GNPs have the advantage that, in addition to the inherent functions of GNPs, they can also exhibit the functions of the substances used for surface modification. The various functionalization methods of GNPs were defined in Table 2. Based on this basic knowledge, in the next section, we describe the functionalization of GNPs and the direct applications of these materials for treatment of bone diseases.

5.1. Biological functionalization of GNPs

5.1.1. Hybrid functionalization of GNPs

As mentioned above, pristine GNPs of a wide range of sizes and shapes can induce osteogenesis of stem cells [31,139]. Based on this, surface-functionalized GNPs have been modified in order to maximize their ability by grafting bioactive molecules and various kinds of ribonucleic acids (RNA). For instance, representative nutritional supplements like vitamin-D promotes osteogenic differentiation. Previously,

Table 2
Functionalization of GNPs for application in biomedicine.

Functionalization Method	Description	References
Surface modification with ligands	GNPs can be functionalized by attaching various ligands, such as thiolated molecules, polymers, or biomolecules, to the gold surface through covalent bonding	[195]
Bioconjugation with antibodies	Antibodies can be conjugated to GNPs to enable targeted binding to specific biomarkers or antigens, allowing for applications in diagnostics, imaging, or targeted drug delivery.	[196]
Polymer encapsulation	GNPs can be encapsulated within a polymer matrix, providing stability, controlled release, and the ability to incorporate additional functionalities through the polymer structure.	[197]
Silica coating	Magnetic nanoparticles can be attached to GNPs, enabling magnetic manipulation, separation, or enhanced imaging capabilities through synergistic effects.	[198]
Peptide-based functionalization	Peptides can be utilized to functionalize GNPs, allowing for specific targeting, cell penetration, or self-assembly properties, thereby expanding their applications in therapeutics or tissue engineering.	[199]
Click chemistry	GNPs can be functionalized using click chemistry, a versatile and highly efficient method for attaching specific functional groups to the nanoparticle surface, enabling a wide range of applications in diagnostics, sensing, and drug delivery.	[200]
Electrostatic adsorption	GNPs can undergo functionalization through electrostatic adsorption, where charged molecules or polymers are adsorbed onto the surface of GNPs via electrostatic interactions, allowing for tailored properties and enhanced stability.	[201]

Nah et al. compared the osteogenic activities of vitamin-D conjugated GNPs and pristine GNPs toward human adipose-derived mesenchymal stem cells (hASCs) [125]. They found that the osteogenic differentiation effects of vitamin-D conjugated GNPs were greater than that of either GNPs or vitamin-D alone, respectively. Additionally, vitamin-D conjugated GNPs showed excellent inhibitory effects on osteoclast differentiation (Fig. 5a) [132]. Peptides may also be attached to GNPs to enhance their properties. For instance, Zhou et al. hypothesized that osteogenic differentiation could be enhanced by coordinating GNPs and Human β-defensin 3 (hBD3) against human periodontal ligament cells in inflammatory microenvironments [140]. The hBD3 possesses both antimicrobial and pro-regeneration properties. They established that GNPs with hBD3 in inflammatory microenvironments upregulated the expression of osteogenic markers via activating the Wnt/β-catenin signaling pathway. Indeed, recent report Yin et al. found that GNPs treatment rescued the osteogenic potential of inflammatory conditioned periodontal ligament stem cells by restoring the inflammation-compromised autophagy–lysosome system. They found GNP's modulation property of inflammation to promote osteogenic differentiation activity [141]. Several researchers have also directly conjugated various ribonucleic acids (RNA) onto GNPs. For example, Abu-Laban et al. developed a variety of miRNA mimics with distinct fluorophores and conjugated these onto GNPs using Diels–Alder chemistry [142]. Their resultant mineralization assays demonstrated that conjugating GNPs with miRNA increased osteogenenesis and promoted gene expressions against hASCs in a dose-dependent manner. They proposed that the potent ability of GNPs to act as a gene delivery system drove osteogenesis. In a similar approach, Xing et al. utilized siRNA-doped GNPs to engineer a hierarchical nanostructured coating on clinically used titanium (Ti) implants for inducing both osteogenesis and angiogenesis (Fig. 5b) [143]. This study focused on the dual function

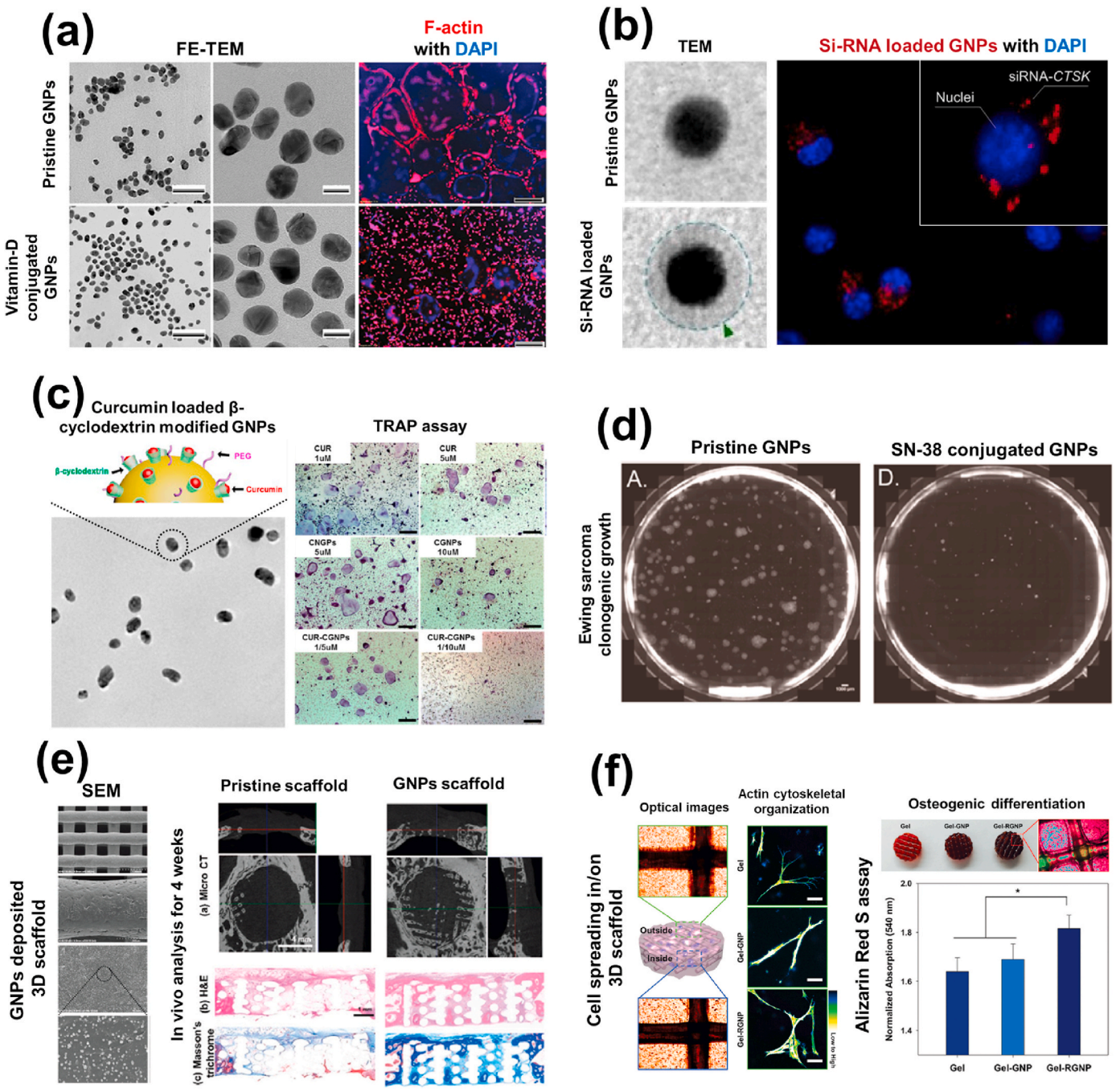

Fig. 5. Surface functionalization and delivery of GNPs to promote osteogenesis and inhibit osteoclastogenesis. Nutritional supplement Vitamin-D conjugated GNPs with its inhibitory effect of osteoclastogenesis (a). The intra-cellular delivery of siRNA-loaded GNPs for synergistic therapeutic effects in vascularization and bone regeneration (b). Inhibition of osteoclast differentiation of bone marrow-derived macrophages by curcumin conjugated GNPs through RANKL-induced signaling pathways (c). Efficient GNPs system for selective SN-38 activation in cancer cells mediated by cancer cell specific mRNA. SN-38 conjugated GNPs significantly inhibited the growth of Ewing sarcoma cells in long-term clonogenic growth assays. (d). GNPs grown on 3D printed polycaprolactone scaffold under mild surface modification and its osteoinductive activity *in vivo* rabbit calvarial defect model (e). A hybrid hydrogel composed of GNPs and human adipose derived stem cells embedded in personalized 3D printed poly-L-lactic acid bone substrate (f). Reprinted from Nah et al. [132], Xing et al. [143], Heo et al. [32], Naumann et al. [165], Lee et al. [172], and Heo et al. [128].

because angiogenesis is crucial to provide for oxygenated blood during bone tissue remodeling [144]. Additionally, they verified the efficacy of the functionalized GNPs in animal models using both rats and beagle dogs. They showed a novel therapeutic approach of GNPs which provides for efficient delivery of siRNA that can accomplish the synergistic goals of revascularization and bone regeneration.

5.1.2. *Medicine/peptide conjugated GNPs*

Bisphosphonates are widely used for the clinical treatment of osteoporosis, such as alendronate, risedronate, and ibandronate for oral administration, or pamidronate or zoledronate for intravenous injection. In the event of chronic medication, these drugs can cause adverse effects such as osteonecrosis of the jaw, atypical femur fracture, atrial fibrillation, or esophageal cancer, but high-dose chronic medication is unavoidable due to their low absorption rate [145,146]. To overcome these

limitations, there have been studies attempting to use GNPs, which have a high cellular uptake rate and inhibit osteoclast activity. Alendronate-conjugated GNPs were synthesized by reacting alendronate solution with GNP solution at room temperature, inducing covalent boding of the amino groups in alendronate to the surface of the GNPs. In cellular and animal models, alendronate-conjugated GNPs showed a superior effect against osteoporosis compared to alendronate only [30,133,147]. Using this type of binding reaction and cellular mechanisms, Nah et al. conjugated vitamin D to GNPs to prevent the toxicity and adverse effects associated with bisphosphonates and confirmed that this method was effective [132].

Even if the drug cannot be bound directly to the GNP surface, cyclodextrin, which forms a pocket with a hydrophobic interior and hydrophilic exterior, can be conjugated to GNPs to enable the loading and release of specific drugs to be controlled while retaining the functionality of the GNPs. Using the mechanism of binding thiol groups to the surface of GNPs, Heo et al. attached fluorescent-labeled cyclodextrin, biotic, and poly(ethylene glycol) (PEG) to the surface of GNPs and loaded paclitaxel into the cyclodextrin to develop a multi-functional nanocarrier for the diagnosis and treatment of cancer cells. Based on these results, another GNP complex was conceived and validated using curcumin to inhibit osteoclast activity and promote osteoporosis treatment (Fig. 4d) [32,148].

Taking advantage of the surface characteristics of GNPs, a research team led by Mirkin used first developed, through a simple synthesis protocol, a polyvalent RNA-GNP complex that could effectively regulate genes. The authors demonstrated that this complex produced a stronger knockdown effect and had higher stability than conventional lipid carriers, showing that GNPs could be utilized as an effective gene regulation material [149]. GNPs have also been used for exocytosis, endocytosis, and nuclear targeting of specific peptides or proteins [150–152].

5.1.3. Natural polymer/GNPs nanocomposites

Nature contains a wide variety of natural macromolecules with different structures and functions, such as gelatin, collagen, fibrin, hyaluronic acid, and chitosan, which can be found in animal, plant, and microbial resources. Natural macromolecules are characterized by their high-stability, low toxicity, non-thrombogenic properties, non-immunogenic properties, water binding capacity, and biodegradability. Depending on the type of molecule, they contain a large number of functional groups (amino, carboxylic, hydroxyl groups) that can bind to different molecules or participate in enzymatic or chemical reactions (hydrolysis, oxidation, reduction, esterification, etherification, and cross-linking reactions). As such, natural macromolecules are being used in the development of diverse substances with customized characteristics in the field of tissue engineering [153–156].

Bhumkar et al. used chitosan to simultaneously act a reducing agent during the synthesis of GNPs and to increase the permeation and uptake of peptide hormone insulin through the mucosa [157]. When the authors compared concentrations of chitosan in the range 0.01–2% in the GNP synthesis process, the GNP/chitosan nanocomposite made with 0.2% chitosan showed the highest electric charge. By loading insulin into the composite and comparing the serum insulin levels between oral administration, nasal administration, and subcutaneous administration, the authors demonstrated that the nanocomposite was a highly efficient drug delivery system.

Natural gum is a high molecular weight, hydrophilic natural polymer typically found inside and outside plant cells; it is composed of monosaccharides linked by glycosidic bonds. Dhar et al. used gellan gum to act as a reducing agent while covering the surface of GNPs [158]. After labeling the biocompatible GNPs that had been reduced by gellan gum, they were demonstrated to have excellent cellular uptake properties in an *in vitro* test using NIH-3T3 and LN-299 cell lines. In a 4-week oral toxicity assessment using rats, these GNPs did not cause any specific hematological, biochemical, or histopathological adverse effects, indicating that they could be used as carriers for the delivery of biologically active substances. Applying a similar chemical mechanism, instead of gellan gum, Pooja et al. made a GNP nanocomposite carrier for anticancer drug delivery using karaya gum, which is used om the food and pharmaceutical industries to increase binding strength and viscosity or to control drug release in oral formulations [159–161]. Karaya gum/GNPs carrier loaded with gemcitabine hydrochloride showed superior anticancer activity, colony formation inhibition, and ROS induction against human lung cancer cells compared to gemcitabine hydrochloride only [159].

It is possible to use more than one material when fabricating GNP nanocomposites. Sobhana et al. synthesized a GNP nanocomposite using gelatin/chitosan solution, and then reacted this, in sequence, with calcium chloride and NaH_2PO_4; compared to the previous protocol, this method could be expected to reduce costs by making hydroxyapatite nanoparticles with a high yield [162].

5.2. Practical application of GNPs in bone diseases

5.2.1. Osteoporosis therapy by functionalized GNPs

Osteoporosis is a degenerative bone disease commonly related to aging. Surface functionalized GNPs has been found to act as promising drug carriers for efficient therapeutic delivery in osteoporosis therapy. Previously, β-cyclodextrin conjugated GNPs were prepared through surface modification and these were found to be capable of transporting curcumin, an osteoporosis drug, via inclusion complex formation (Fig. 5c) [32]. Additionally, drug loaded GNPs were investigated to understand their inhibitory effects on receptor activator of nuclear factor-κb (RANK) ligand-induced osteoclastogenesis in bone marrow-derived macrophages. Functionalized GNPs conjugated with bisphosphonate, such as alendronate (ALD), can be a powerful tool for bone regeneration therapy [30]. This conjugation happens as the citrate of GNPs are displaced by the primary amines of ALD to form partially covalent bonds. The exploratory *in vitro* results demonstrated that the GNPs-ALD system has a synergistic effect leading to higher suppression of osteoclast differentiation than GNPs and ALD alone. In recent study, Xi et al. designed α-Lipoic acid loaded PEGylated hollow GNPs for osteoporosis treatment potential. They successfully synthesized mPEG@HGNPs with a particle size of under 63 nm. It showed not only upregulation of the expression levels of BMP-2, Runx-2 and OCN to regulate osteoblast differentiation, but increased the viability of MC3T3-E1 cells as well as removed reactive oxygen species caused by H_2O_2 injury, which has the prospect of treating osteoporosis [163]. Thus, many drugs and biomolecules can be applied on the GNPs and the use of GNPs can maximize both drug delivery and efficacy for treatment of osteoporosis.

5.2.2. Bone cancer therapy through GNPs

Bone cancer treatment by GNPs can be accomplished by several means including combining the particles with other techniques such as Surface-enhanced Raman spectroscopy (SERS) to analyze their effects. A recent report by Chakraborty et al. demonstrated the size-dependent apoptotic activity of GNPs on osteosarcoma MG63 cells by characterizing these with a SERS signal [164]. They obtained optimal efficacy using a 800 ng/mL concentration of GNPs with a 46 nm particle size. This accelerated the rate of reactive oxygen species (ROS)-induced apoptosis in osteosarcoma MG63 cells by disrupting their mitochondrial membrane potential. Subsequently, the SERS spectra of GNPs-treated cells exhibited molecular signatures related to cellular apoptosis. In another approach, Naumann et al. developed potent topoisomerase I inhibitor such as SN-38 conjugated GNPs (Fig. 5d) [165]. They used SN-38-oligonucleotide, which exhibits significant toxicity against Ewing Sarcoma cells at pico-molar concentrations. Based on this approach, they showed that the functionalized GNPs activated both survivin and EWS-FLI1 mRNA and exhibited toxicity against Ewing sarcoma cells. Sun et al. exploited gold nanorods enclosed inside mesoporous silica nanoparticles, which were then conjugated with zoledronic acid [166].

The conjugated GNPs not only showed anticancer efficiency, but also inhibited the formation of osteoclast-like cells through the promotion of osteogenic differentiation. Furthermore, the combination of conjugated GNPs and photo-thermal therapy triggered by near-infrared irradiation, inhibited tumor growth both *in vitro* and *in vivo* by inducing apoptosis in cancer cells and improving the bone microenvironment. This multi-complex nano-platform presents an exciting strategy for treating breast cancer bone metastasis. Laser-induced photo-thermal therapy is another method for killing cancer tissue by increasing the local temperature of treated cells like osteosarcoma which promotes cellular damage and even necrosis [167]. Tian et al. developed CD271 antibody-functionalized and PEGylated hollow GNPs [168]. After uptake of the functionalized GNPs, the cells were exposed to electromagnetic radiation and cellular morphological changes as well as apoptosis were evaluated to verify the effect of photo-thermal therapy. They found that the surface functionalized GNPs could be targeted by conjugating them with CD271 monoclonal antibody to the osteosarcoma stem cells. The prepared hybrid GNPs achieved excellent cell viability inhibition as compared with a pristine group upon near-infrared (NIR) laser irradiation.

5.2.3. Medical devices decorated with GNPs (scaffolds, hydrogels, titanium implants)

GNPs can play a pivotal role, but how much can be delivered to the defect and/or lesion area is still a challenging research topic. Therefore, many bioengineers have applied the GNPs on or in various natural/synthetic polymer substrates in order to achieve rapid bone tissue reconstruction and tested their systems both *in vitro* and *in vivo*. For instance, immobilizing GNPs onto the surface of Ti through mild surface modifications has been investigated. Previously, Heo et al. prepared a thiol group functionalized Ti surface [169]. Subsequently, a large amount of GNPs was deposited onto the Ti surface directly using simple chemical action. During *in vitro* and *in vivo* evaluations, the Ti–GNP was found to have a significant influence on the osseous interface formation. In a further investigation, Ko et al. developed double layers of GNPs-immobilized (Ti) implants to investigate osseo-integration between bones and Ti implants [170]. The double layered GNPs-Ti showed enhanced osteogenesis as compared to single layered GNPs-Ti for human bone-marrow-derived mesenchymal stem cells (hMSCs). Additionally, GNPs is able to be applied onto electrospun nanofiber. Huang et al. developed citrate-stabilized GNPs encapsulated polyvinylpyrrolidone/ethyl cellulose scaffolds fabricated by coaxial electrospinning technique, which showed great osteogenic bioactivity [171]. Especially, it was observed that GNPs-incorporated scaffolds rapidly accelerated bone regeneration *in vivo*. Furthermore, GNPs also can be applied onto 3D-printed scaffolds. For example, Lee et al. fabricated a 3D polycaprolactone (PCL) scaffold using a rapid prototyping system (Fig. 5e) [172]. Next, polydopamine was coated onto the 3D scaffold in order to grow GNPs on the surface without chemical treatment. The developed 3D scaffold demonstrated potent bone tissue regeneration both *in vitro* and *in vivo*. Similarly, recent report Kim et al. developed a functional bone substitute comprised of a ceramic scaffold maintained by calcium-deficient hydroxyapatite with immobilization of GNPs through 3-aminopropyltriethoxysilane chemistry. It revealed excellent antimicrobial activity that was mediated by the GNPs immobilized on the surface that scavenged microbial ROS. Consequently, the GNPs decorated bone scaffold effectively inhibited the growth of *Micrococcus luteus* (*M. luteus*) to prevent post-surgical infection bacteria [173]. Another approach has been applied to hydrogels thanks to the aqueous delivery effect of GNPs [127]. Lee et al. developed an injectable osteoinductive hydrogel system using enzymatic cross-linkable gelatin and functionalized GNPs [174]. Due to the easy handling of this system, it has the advantage of being able to freely control the amounts of GNPs and cell delivery. In addition, Huang et al. recently described that incorporation of GNPs into the network of bacterial cellulose hydrogel by the in-situ fermentation [175]. The developed hydrogels demonstrated desirable mechanical properties, sustainable release ability of GNPs, favorable biocompatibility and excellent osteogenic activities. However, the hydrogel system has a limitation in regenerating hard tissues like cartilages and bones because the physical properties of the hydrogels are not strong [175–177]. In order to overcome this drawback, Heo el al. Developed a hybrid bio-matrix by combining GNPs encapsulated hydrogel and a 3D-printed biodegradable polylactic acid scaffold (Fig. 5f) [128]. They fused the RGD-bound GNP with cells in an aqueous hydrogel matrix. The 3D hybrid scaffold was found to both retain its shape and promote bone tissue differentiation.

6. The advantages and disadvantages of GNPs with other nanoparticles

In evaluating various nanomaterials for the treatment of bone diseases, it is essential to consider both their advantages and disadvantages. Each nanomaterial possesses unique properties and characteristics that impact their suitability for bone disease regeneration and therapy. When compared to other nanomaterials such as MXene and graphene oxide (GO) for bone disease treatment, gold nanoparticles (GNPs) offer distinct advantages.

(1) Enhanced biocompatibility: GNPs exhibit excellent biocompatibility, making them suitable for biomedical applications. Unlike MXene, which may induce cytotoxicity at higher concentrations, and GO, which has potential toxicity concerns, GNPs have been extensively studied and proven to be biocompatible, ensuring their safe use in bone disease treatments.
(2) Tailorable surface chemistry: GNPs can be easily functionalized with various biomolecules, drugs, or targeting ligands, thanks to their unique surface properties. This flexibility allows for precise control over their bioactivity and interactions with bone cells. In contrast, MXene and GO may require additional modifications or complex functionalization strategies to achieve similar levels of control.
(3) Imaging capabilities: GNPs possess excellent imaging properties, making them valuable tools for bone disease diagnostics. They exhibit strong X-ray attenuation, enabling clear visualization of bone structures in X-ray computed tomography (CT) scans. In contrast, MXene and GO may lack such inherent imaging capabilities, requiring additional contrast agents or modifications for effective imaging.
(4) Photothermal therapy efficacy: GNPs demonstrate outstanding photothermal conversion efficiency, especially in the near-infrared (NIR) region. This property allows for precise localized hyperthermia, ideal for targeted therapies in bone diseases such as bone tumors. MXene and GO, while capable of some photothermal effects, may not exhibit the same level of efficiency or specificity as GNPs in this regard.
(5) Stability and controlled release: GNPs can be easily stabilized and functionalized to provide controlled release of therapeutic agents. This controlled delivery system enables sustained and localized treatment, reducing potential side effects and improving therapeutic efficacy. In comparison, MXene and GO may have challenges in achieving comparable stability and controlled release capabilities.
(6) Established research and applications: GNPs have been extensively studied and have a well-established research base in various biomedical applications, including bone disease treatments. This accumulated knowledge and expertise contribute to a deeper understanding of their properties, interactions, and potential benefits in bone regeneration, diagnostics, and therapy.

Of course, there are certain disadvantages or limitations associated specifically with GNPs when comparing to MXene and GO. Here is a description of the drawbacks of GNPs in comparison to MXene and GO.

(1) Synthesis complexity: While MXene and GO synthesis can be complex, the synthesis of GNPs often requires specialized techniques and equipment as well. The process involves precise control over reaction conditions, reducing the scalability and ease of production compared to MXene and GO.
(2) Higher cost: GNPs can be relatively expensive to produce compared to MXene and GO. The cost of gold and the specialized synthesis methods contribute to the higher production costs, which can limit their widespread application in certain settings or resource-constrained environments.
(3) Limited drug loading capacity: GNPs, compared to MXene and GO, may have a relatively lower drug loading capacity. The size and surface properties of GNPs can restrict the amount of therapeutic agents that can be loaded onto their surface, potentially limiting the efficacy of drug delivery applications.

It is important to note that these limitations are not absolute and ongoing research may address some of these challenges. However, when comparing GNPs to MXene, GO, and other nanomaterials in the context of bone disease treatment, these factors should be considered. Further studies and comparative evaluations are necessary to fully explore the potential of different nanomaterials and determine the optimal options for specific bone disease applications.

7. Outlook

As we described, GNPs possess several advantages and disadvantages when considering their application in bone disease regeneration. The biocompatibility of GNPs ensures their safe use in bone disease treatments, while their tailorable surface chemistry allows for precise control over bioactivity and interactions with bone cells. Additionally, the excellent imaging capabilities of GNPs enable clear visualization of bone structures, and their photothermal therapy efficacy offers targeted treatment options for bone diseases such as tumors. Furthermore, GNPs can be stabilized and functionalized to achieve controlled drug release, enhancing therapeutic efficacy. However, the synthesis complexity and higher cost associated with GNPs may limit their widespread production and utilization. Careful consideration should also be given to potential toxicity concerns, dosage optimization, and the limited drug loading capacity of GNPs compared to other nanomaterials. Surface stability is another aspect that needs to be addressed to maintain the desired properties and bioactivity of GNPs over time. Despite these challenges, further research and development efforts can help optimize the performance of GNPs in bone disease therapy. With ongoing advancements, GNPs hold great promise in revolutionizing bone disease treatment and regeneration. By addressing the limitations and leveraging their unique advantages, GNPs have the potential to significantly contribute to the field of bone disease therapy, improving patient outcomes and quality of life. In summary, while GNPs exhibit both advantages and disadvantages in the context of bone disease regeneration, their versatility, biocompatibility, imaging capabilities, controlled release potential, and photothermal therapy efficacy position them as a promising nanomaterial for the future of bone disease treatments.

CRediT authorship contribution statement

Dae Hyeok Yang: Writing – review & editing. **Haram Nah:** Writing – original draft. **Donghyun Lee:** Writing – original draft. **Sung Jun Min:** Data curation. **Seulki Park:** Data curation. **Sang-Hyun An:** Visualization. **Jianxin Wang:** Validation. **Huining He:** Validation. **Kyu-Sun Choi:** Resources. **Wan-Kyu Ko:** Data curation. **Jae Seo Lee:** Data curation. **Il Keun Kwon:** Software. **Sang Jin Lee:** Writing – original draft. **Dong Nyoung Heo:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2022R1A5A1022977) and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare (HI22C1572, HI22C1988) and the Ministry of Trade, Industry & Energy (MOTIE, Republic of Korea; Grant No. 20026565).

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