International Journal of Nanomedicine

Dovepress
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REVIEW

Emerging Role of Enzyme-Immobilized Nanocarriers in Osteoporosis: Advances and Challenges

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Abstract: Osteoporosis (OP) is a common bone disease that involves low bone mass and high risk of fracture mainly in older men and women and perimenopausal years. Although conventional therapies provide good therapeutic effects, they have numerous limitations, including poorly targeted and systemic administration and severe side effects. Recent developments in nanotechnology enabled design of enzyme-immobilized nanocarriers as experimental platforms to enhance the delivery of therapeutic agents to bone tissue. This review pays special attention to the development of these multifunctional systems that can transport anti-osteoporotic agents and carry enzymes to stimulate bone formation. Enzymes like alkaline phosphatase for mineralization, superoxide dismutase for reactive oxygen species reduction, and cathepsin K inhibitors for osteoclast regulation are highlighted to demonstrate rationale behind enzyme immobilization. Enzyme immobilization promotes local bone regeneration by increasing enzyme stability and activity at target site offering more sustained therapeutic effect in OP therapy. Polymeric NP and liposomes like nanocarriers are well explained along with their various mechanisms such as stability, bioavailability controlling and release kinetics. Further, we review the current literature for the recent in vivo and in vitro studies highlighting the potential of these systems in stimulating osteoblast function and suppressing osteoclast-mediated bone resorption. Areas for future research include improving carrier design for increased targetability and exploring the clinical translation of these nanocarrier systems for OP management.

Keywords: osteoporosis, nanotechnology, treatment, nanocarriers, enzyme immobilization

Introduction

Osteoporosis (OP) is characterized by a decrease in bone mineral density (BMD) and an elevation in bone fragility. According to the World Health Organization (WHO), it is one of the most prevalent conditions impacting middle-aged and elderly populations. The global prevalence stands at 18.3%, exhibiting a higher incidence among women at 23.1% compared to 11.7% in men.[1,2] Fractures can happen in severe cases, which can cause a lot of disability, depression, a lower quality of life, and even death.[3] Studies have shown that more than one-third of middle-aged and older women (but only one-fifth of men) around the world suffer from fractures because of OP.[4,5] It is anticipated that by the year 2050, the rate of hip fractures will exceed 21 million cases.[6] The concerning trend of OP gets worse by the aging population, as demonstrated by the rising occurrence of clinical cases among middle-aged men. According to census population projections, as the age of the American population rises, the incidence of OP is anticipated to increase by 32%, reaching 17.2 million cases from 2010 to 2030.[7] Furthermore, OP presents a significant economic challenge on

Received: 31 July 2025
Accepted: 19 November 2025
Published: 3 December 2025

a global scale. The estimated expenditure for the prevention and management of OP within the European Union is €37 billion, a projection anticipated to rise by 25% by the year 2025.

Current treatment procedures frequently prescribe bisphosphonates as the primary intervention for all patients, irrespective of specific risk factors. The "step therapy" method fails to account for the unique requirements of high-risk individuals who could derive greater benefit from osteoanabolic drugs or tailored treatment strategies designed to meet specific BMD objectives. Customized treatment according to fracture risk and BMD levels, is not extensively implemented despite data endorsing its effectiveness.[7] Adherence to OP treatments remains suboptimal, often hindered by side effects, lack of awareness, and patient skepticism. These challenges underscore innovative therapeutic strategies by deeper understanding of OP pathophysiology and bone metabolism.[8,9] Bone remodelling hinges on enzymes such as alkaline phosphatase (ALP), essential for mineralization; cathepsin K (CatK), critical in osteoclast-mediated bone resorption; and superoxide dismutase (SOD), which counters oxidative stress that accelerates bone loss. However, direct systemic delivery of these enzymes often suffers from rapid degradation, off-target distribution, and reduced catalytic efficiency. Recent studies reinforce importance of enzyme immobilization like in 2024, an ultrathin ZIF-8 coating-enforced SOD nanoformulation (utZIF-SOD) have been developed which avoids lysosomal degradation, maintains ~1.5× greater ROS-scavenging activity than native SOD, and doubles bone mineral density in a senile osteoporosis mouse model.[10] Another study demonstrated nanozymes regulating ROS in OP therapeutics, underscoring how mimetic or immobilized antioxidant enzyme activity can modulate oxidative damage in bone tissue.[11] Enzyme immobilization onto nanocarrier systems offers mechanistic advantages including enhanced stability under physiological conditions, preserved/enhanced enzymatic activity, improved localization to bone tissue, reduced systemic side-effects, and ability to respond to pathological microenvironments. Recent investigations into polymeric nanoparticles, liposomes, and functional nanozymes highlights importance of immobilized enzymes in osteoblast differentiation (via ALP upregulation, increased RUNX2 and osteocalcin expression in aged/senescent cells treated with Prussian blue nanozyme-based platforms), suppress osteoclast activity (Cathepsin K inhibition using odanacatib or ectosteric inhibitors T06 which also reduce RANKL levels), and protect bone-forming cells from oxidative stress through SOD or CAT like activities of nanozymes in models of OP.[12–16]

Role of Enzyme Immobilization in Nanocarrier System

The process of enzyme immobilization creates a microenvironment that protects against variations in operating conditions, thereby obviating the necessity for tedious and costly removal and purification processes. Furthermore, increased substrate concentrations or by-products may impede enzyme function; nevertheless, this constraint can be addressed through immobilization, leading to improved enzymatic activity. In addition, it is demonstrated that the method of immobilization can affect the enzymatic activity of enzymes with flexible active sites.[17] Nanocarriers have a higher surface area volume ratio than macroparticles, which means that there is increased mass transfer and better access to substrates. This characteristic is, however, vital since it brings about some preliminary easy interaction between the enzyme and the substrate such that the rate of the reaction improves. Due to the small space offered by nanocarriers delivery, there is easier control of the diffusion of substrates, and therefore provides a better chance for enzymes.[18] Enzyme immobilization on the surface of nanocarriers enables controlled targeted delivery of therapeutic agents to the OP affected bone tissue. This is a focused approach that minimizes systematic side effects which are associated with conventional treatments and ensures the active agents operate within the regions necessary. The application of bone-targeted NP has higher therapeutic efficacies in the treatment of OP by delivering enzymes providing better bone formation or by decreasing bone resorption.[19] For example, ALP immobilized on chitosan nanoparticles significantly enhanced osteoblast differentiation by upregulating mineralization-related genes, indicating higher enzymatic stability and functionality compared to free ALP.[20] Nanocarrier systems improve the partially of enzymes through two ways, whereby they protect the enzymes from degradation and a controlled release of the enzymes. Such a delivery system ensures that the enzyme is delivered in a controlled manner at the desired site thus enhancing its potential for bone formation or inhibition of osteoclast activity. Examples include liposomal formulations of enzymes where the liposome has the ability to entrap the enzyme and deliver in a way that enables the enzymes therapeutic value to be optimized.[21] Because enzymes attached to nanocarriers can be designed to release multiple therapeutic agents at the tumor site, the

effectiveness of the treatment can be enhanced through the synergistic effects of the released drugs. For instance, the use of enzymes that promote osteoblast differentiation together with anti-resorptive factors might lead to a better approach to OP treatment strategy. Such an approach is likely to address several aspects of bone metabolism simultaneously thus improving the outcome for patients.[21]

Scope of Synthetic Nanocarriers in OP Therapy

Polymeric NP such as PLGA NP has been also investigated in OP treatment due to its biodegradable and biocompatible nature and its ability to carry drugs of interests. These NP can be designed in such a way that they facilitate the targeted release of the drugs making these more bioavailable and thus therapeutically more effective. The surface characteristics of PLGA NP can be modified this way in order to target the drug to bone tissue and avoid side effects and improve outcomes in patients with OP.[22] The lipid-based nanocarriers, particularly liposomes and solid lipid NP, hold great promises of drug delivery in OP therapy. They also form conditions that allow to create ideal shell for hydrophobic drugs, as well as increase the stability and solubility of the drug. In addition, these lipid carriers can be conjugated with bone targeting ligands which result in enhanced localization of the formulation at the site of action in bone. This targeted strategy not only increases the extent to which the anti-osteoporotic agents are potent in their therapeutic endeavor's but also reduces the incidents of adverse side effects, which accost typical therapies.[23] The method is particularly effective for inorganic NP like calcium phosphate and titanium nanotubes whose recommended usage involves bone regeneration. Moreover, these NP have great potential for inducing osteogenesis and improving bone mineralization while also providing drug carrier functions to achieve localized drug delivery. These are versatile tools that can form part of OP treatment mechanisms since their porous structures are amendable to being tailored to any healing substances. In addition, they can also interact directly with bone cells so as to enhance the elicitation of biological reactions that are helpful in the formation of new bone.[21] Nanoscale extracellular vesicles known as exosomes are gaining attention as natural nanocarriers for drug delivery in the treatment of OP. These materials have natural compatibility with biological systems and enable communication between cells by transporting bioactive substances directly to the intended cells. Researchers can enhance treatment efficacy and minimize off-target effects by loading exosomes with therapeutic agents designed to promote bone health or inhibit bone resorption, utilizing their natural targeting capabilities.[24]

The aim of this review is to seek assess recent developments in the design and application of enzyme-immobilized synthetic nanocarriers for OPs' treatment, its composition, its mode of action, and therapeutic effectiveness in comparison to conventional treatments. It will compare how these nanocarriers improve selective drug delivery and osteoblast stimulation and reduce osteoclast activity; the existing problems such as biocompatibility and stability that plague them. The review will also discuss areas of future development of carrier design and enhance the specificity of targeting and present a synopsis of preclinical and clinical data of their efficacy. Moreover, it will describe multifunctional enzyme systems in which enzymes could be co-administered with other drugs with an enhanced therapeutic effect and the essential regulatory requirements for clinical applications. To these ends, the review aims specifically to offer a comprehensive understanding of the consequences of enzyme-immobilized synthetic nanocarriers on OP management.

Enzyme Immobilization Techniques

Immobilization of enzymes means locking or keeping the enzyme in a selective area to render the enzymes highly efficient, stable and reusable where the soluble enzymes are. They are sharply localized in space or enclosed within a well-defined region: this facilitates their maintenance or enhancement of their catalytic activities and their reuse and recovery. There are certain enzymes which can be identified as insoluble in their immobilized state. This represents another kind of heterogeneous biocatalysis. During enzyme immobilization procedure, some properties of the enzyme such as catalytic activity, thermal stability and storage stability were altered.[25,26] As indicated, there are several benefits associated with enzyme immobilization but there are also several drawbacks. This is one of the major problem where in the activity of the enzyme is decreased when immobilizing the enzymes onto the surface, more so when the enzymes are bound to enormous macromolecular substrates.[27] There are three separate methods to immobilizing enzymes on a support material: adsorption, cross-linking or covalent bonding, entrapment, and/or cross encapsulation,[28] as shown in Figure 1.

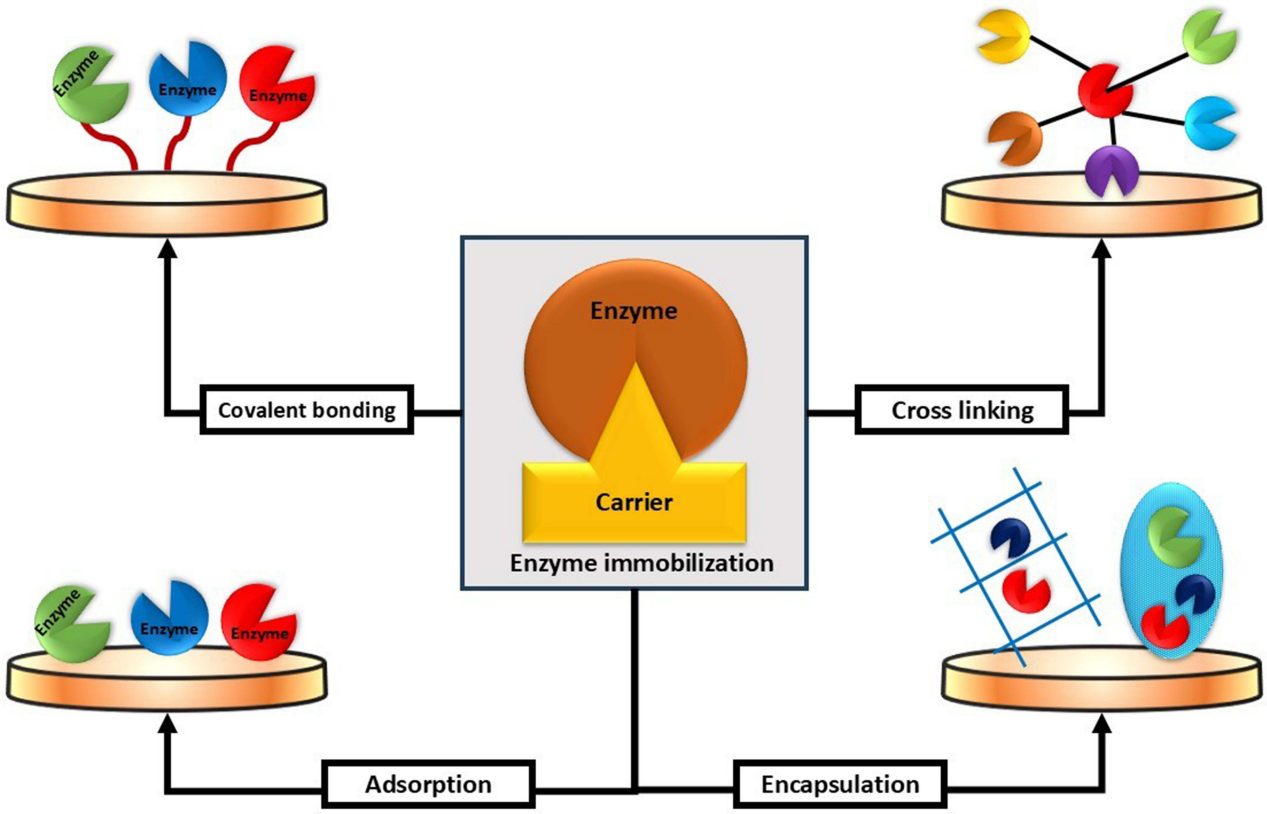

Figure 1 Various enzyme immobilization techniques, that may help enzyme stability and activity, are illustrated. Immobilization methods for Enzyme are covalent bonding, cross linking, adsorption, and encapsulation. Each immobilization technique relies on the carrier material which is a central structure representing the base with an enzyme bound. Enzymes are directly attached to the carrier by covalent interactions in covalent bonding. The invention relates to cross linking, ie, aggregating enzyme molecules together through cross linkers to increase stability and reusability. Enzymes bound reversibly to carriers by adsorption constitute an easy and mild immobilization method. This encapsulation traps enzymes within a network or gel matrix such that release can be controlled but the enzyme activity maintained. Immobilization approaches for these enzymes enable efficient use of enzymes for therapeutic applications, such as osteoporosis treatment.

Adsorption

Adsorption is defined as the ability of an enzyme to become attached on a solid surface support is due to the forces of attraction between the molecules.[29] The nature of the interaction between enzymes and support surfaces is central to the adsorption process. The polarity and charges of the enzyme are critical fundamental characteristics to attain the desired increase in enzyme coverage on the substrate consistently. This gives relatively kind, simple, economical, fast, high output and recyclable value.[30] However, the adsorption method of immobilizing enzymes, there are some disadvantages associated with the process: leakage of the enzymes and relatively lower stability of the enzyme-support interaction as appose to covalent bonding.[31]

Cross Linking

Cross-linking is the chemical process of joining two or more molecules through covalent bonds. Cross-linked enzyme aggregates represent a multifaceted method for enzyme immobilization. The method is often preceded by a surface modification or activation procedure.[32,33] Silanisation, the process of applying organic functional groups to a surface using an organofunctional silane reagent, is a commonly employed method for the preliminary surface modification of inorganic substrates. p-Nitro benzoyl chloride can be utilized to derivatize coating or natural surface amino groups into arylamine or aldehyde groups.[34] The enzyme that has been immobilized through cross-linking demonstrates stability and functionality as a biocatalyst. On the other hand, the drawbacks consist of frequently restricted activity retention, inadequate reusability, diminished mechanical stability, and difficulties in managing the gel-like cross-linked enzymes.[35]

Encapsulation

Enzyme immobilization through entrapment involves the encapsulation of enzymes using either covalent or non-covalent bonds. The matrices effectively minimize conformation change while preserving the properties of the biocatalyst.[36] The enzyme enclosed within a polymer network allows substrates and products to pass through while keeping the enzyme contained.[37] Enzymes remain unattached to the polymeric matrix following entrapment, although their diffusion is limited. An enzyme that is entrapped exhibits greater stability compared to one that is physically adsorbed. Entrapment immobilization is easier to achieve compared to covalent bonding, while still enhancing or maintaining enzyme activity.[38] The physical entrapment of enzymes offers a wide array of applications and is likely to minimize interference with the inherent properties of the natural enzyme.[39] Nonetheless, the strategies for encapsulation need to consider the chemical conditions of the polymerization matrices, the dimensions and volume of the pores, as well as the compatibility of enzymes with these matrices, to guarantee that both the substrate and product can effectively diffuse in and out of the polymer matrices. The retention of catalytic activity is quite common, as numerous enzyme encapsulations tend to show minimal loss in enzyme activity following immobilization.[40] Encapsulated enzymes enhance enzymatic performance through the modification of hydrophobic interactions, the increase of reaction surface area, and the elevation of intermediate concentration. They exhibit greater stability across diverse situations. These applications are prevalent in various fields, including biocatalysis, biosensing, enzyme treatment, biomedicine, and bioremediation.[41]

Importance of Immobilization for Enhanced Stability and Activity

Enzymes that are immobilized show a greater ability to withstand thermal denaturation when compared to those that are free. For example, research indicates that immobilized α-amylase maintains as much as 82% of its activity after extended exposure to high temperatures, whereas free enzymes demonstrate significant activity reduction under comparable circumstances. The use of immobilization techniques, including covalent binding and entrapment, ensures the structural rigidity necessary for preserving enzyme integrity. This holds significant relevance in extreme conditions where unprotected enzymes would generally deteriorate quickly. For instance, particular immobilization techniques have demonstrated the ability to enhance the stability of enzymes such as lipase and α-chymotrypsin when subjected to extreme conditions.[42] Enzymes that are immobilized can maintain considerable activity even after being stored for long durations. Studies show that certain immobilized enzymes retain more than 89% of their original activity even after 35 days when stored at refrigeration temperatures, highlighting their suitability for extended use. When immobilization occurs, it can result in enhanced catalytic rates. For example, enzymes that are fixed on supports show greater maximum reaction rates (Vmax) and enhanced catalytic efficiencies in comparison to their free counterparts. This can be linked to improved substrate accessibility and minimized diffusion constraints.[43] The process of immobilization facilitates improved management of enzyme orientation and positioning, thereby potentially increasing selectivity and specificity in reactions. Methods such as the PRECISE system have shown that managing the orientation of immobilized enzymes can lead to activity enhancements of as much as 73% when compared to those that are immobilized randomly. One of the key benefits of enzyme immobilization is the capacity for reuse across multiple cycles while maintaining a high level of activity. This approach not only cuts expenses but also lessens waste, enhancing the sustainability of processes.[44]

Types of Synthetic Nanocarriers

Some common synthetically engineered nanocarriers have been designed in such a way to improve the drug delivery, stability and effectiveness of the therapeutic compounds in the biomedical field, as illustrated via Figure 2.

Polymeric NP

In recent years, polymeric NPs have gotten a lot of attention because of the qualities that come from being so small. Polymeric NP are useful as drug carriers because they can be used for controlled release, protect biologically active chemicals and drugs from the environment, and make them more bioavailable and effective.[46] They can be different in size, shape, surface properties, crystallinity, and dispersion state, as well as in physical qualities like composition and concentration. Usually, more than one way is used to test these properties to fully describe the NPs. Some of the most

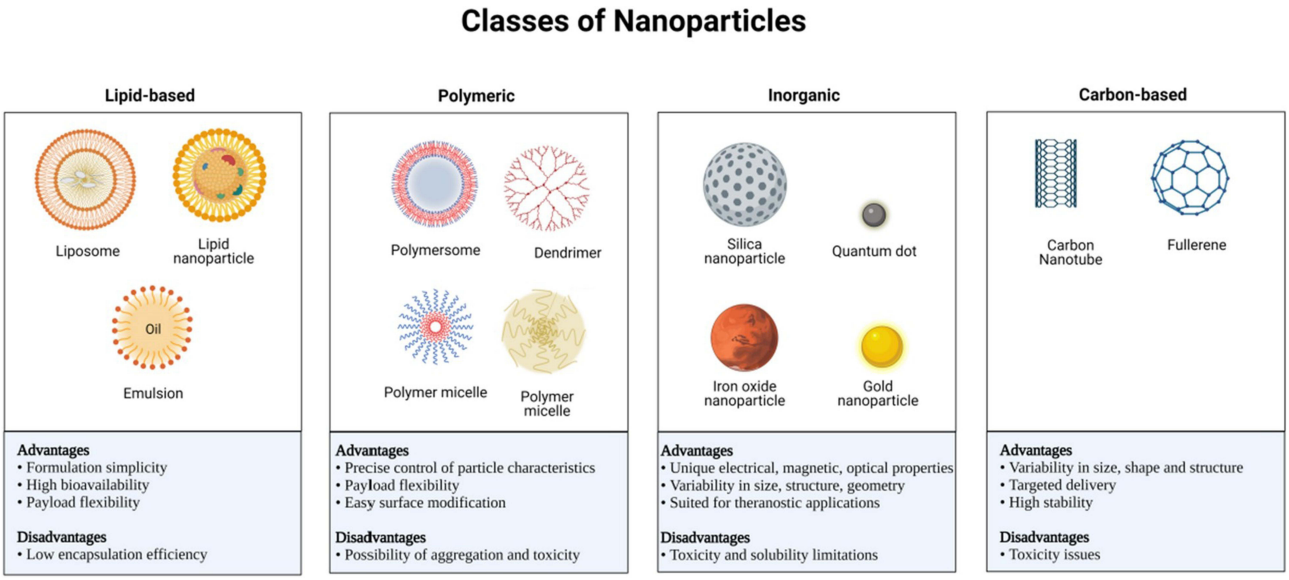

Figure 2 NPs have been categorized according to the properties, shape or size. Here, the advantages and limitations of some major classes of NPs are presented for each class. Adapted from reference[45] under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

popular ones are electron microscopy, dynamic light scattering (DLS) or photon correlation spectroscopy (PCS), near-infrared spectroscopy, electrophoresis, and chromatography.[47] In general, polymeric NPs that are made in different ways may have average sizes of 100 to 300 nm. The size distribution should only have one main shape, and the polydispersity should be as low as possible, ideally almost zero. It is also possible to get particles with sizes of 60 to 70 nm or even less than 50 nm.[48] There are several ways to measure the size of NP. The most popular are dynamic (DLS) and static (SLS) light scattering, but TEM, SEM, and AFM are also often used.[49] Polymeric NP can be designed to selectively target bone tissue, hence improving the effectiveness of medicinal medicines. NP altered with bisphosphonates such as alendronate exhibit a strong affinity for bone tissue, enhancing localized therapeutic efficacy and reducing systemic adverse effects. The structure of polymeric NP facilitates the regulated release of pharmaceuticals, sustaining therapeutic concentrations for prolonged durations. Maintaining stable medication levels is essential for controlling OP, since it aids in preserving bone density and preventing fractures.[19]

Lipid-Based Nanocarriers

Lipid based nano carriers or LNPs are under current consideration as potential therapy for the management of OP, a clinical condition that is associated by low bone mass and increased fracture risk. Of these nanocarriers including liposomes; solid lipid NP (SLNs); and nanostructured lipid carriers (NLCs), the following advantages in drug delivery systems can be discussed: biocompatibility, encapsulation of both hydrophilic and lipophilic medicines, and potential for targeted delivery. Cationic lipid NP (LNPs) can be synthesized to have specificity for the bone tissue to enhance therapeutic efficacy with minimum systemic toxicities. For example, SIM/ASP 6-LNPs for example, contain bone targeting ligand moieties which increases the uptake of simvastatin in bone tissue for promotion of osteoblast differentiation and mineralization. Animal studies show that these LNPs increase the density of bone more so than when simvastatin was administered aurally or used in its usual format.[50] Many OP medicines have a low bioavailability when administered by the oral route. These pharmaceuticals may be incorporated within LNPs to provide protection from degradation and improve absorptive ability. Lipid NP have been used in the improvement in the solubility and stability of the lipophilic drugs including the simvastatin indicated to have different impacts on bone in normal doses. The structure of lipid NP promotes controlled delivery of the encapsulated pharmaceuticals necessary for maintaining a constant therapeutic level within the body. This aspect is particularly useful in the management of OP because stable levels of medications will help maintain bone mass and prevent fractures.[51] Researchers aim to develop lipid nanocarriers specifically designed to target bone tissue in the context of bone diseases. Song et al and Ferreira et al have respectively

developed pamidronate-conjugated liposomes and alendronate salt-conjugated PEGylated liposomes, demonstrating that the former exhibited an increased affinity for bone tissue, while the latter displayed bone-targeting capability. At present, investigations are being conducted regarding the application of liposomes in the treatment of OP. A recent investigation employed modified liposomes for the delivery of antagomir-148a, a miRNA modulator that targets and suppresses the osteoclastogenic miR-148a. The findings indicated that these modified liposomes predominantly localized in bone tissue and effectively downregulated miR-148a expression in osteoclasts, thereby inhibiting bone resorption in murine models of OP.[52–54]

Metal and Hybrid Nanocarriers

Currently, Metal nanocarriers, and Metal NP (MNPs), are beginning to attract attention for various biological applications including drug delivery, diagnostics and therapeutic purposes. MNPs can easily be conjugated with various biomolecules such as antibodies or nucleic acids including targeting specific cell or tissue. All these changes can be made through covalent bonding, electrostatic functions, as well as hydrogen connections, making it possible to give everyone an individualized therapeutic regimen. They also have a large surface area to volume ratio and a unique stability profile, and flexible for drug loading. This characteristic is critical in furthering the pharmacokinetics of drugs meaning efficient delivery of the drug to the intended areas within the body, but minimal effects on other areas generally causing side effects.[55] Gold NP have been of interest because they are biocompatible and possess unique optical properties. These factors can promote osteoblast differentiation and inhibit osteoclast formation, thus can be useful in the regulation of skeletal homeostasis. According to scientific evidence, it has been found that the possibility of AuNPs may enhance the cellular osteogenesis process without affecting the adaptogenic differentiation; therefore, AuNPs may provide effective treatment options for OP. It enhanced the therapeutic efficacy and the reduction of side effects that act systematically on the body.[56] When both calcium carbonate NP and silver NP were used, a significant improvement in the osteogenic potential was observed. When used as composites in experimental models, both hydroxyapatite and bioactive glass have been found to exhibit better bone-forming capabilities than each of the individual materials. The enhancement of mechanical properties of the carrier through incorporation of AgNPs increases its bone formative capability more than conventional options, making it a very viable solution for treatment of defects associated with OP. Recent advancements have unveiled cell membrane-coated NP as an innovative strategy for treating OP. The NP utilizes the inherent targeting abilities of cell membranes to improve biocompatibility and the effectiveness of drug delivery. Coating NP with membranes sourced from cells, like osteoblasts or stem cells, enables the precise delivery of therapeutic agents straight to bone tissue. This approach shows potential for minimizing the systemic side effects typically linked to conventional OP therapies while enhancing treatment results.[57] Xu et al (2016) show the effective integration of silver and strontium into HA/chitosan scaffolds, leading to a notable decrease in bacterial presence and indicating a combined role of promoting bone growth and providing antibacterial properties. The extensive use of AgNPs in different biomedical areas is outlined, highlighting their function in wound dressings, drug delivery systems, and as coatings for medical devices, primarily due to their antimicrobial characteristics.[58] A study explores how AgNPs influence cellular processes, focusing on their effects in wound healing and bone regeneration by analyzing their interactions with human mesenchymal stem cells (HMSCs) and their contribution to osteoblast differentiation.[59] The document highlights the importance of weighing the advantages of AgNPs alongside their possible cytotoxic effects, which vary based on concentration and duration of exposure. Recent studies, such as those conducted by Akturk et al (2020) and Kumar Saini et al (2019), investigate the creation of nanocomposite materials that integrate AgNPs for scaffold applications. The goal is to improve antibacterial properties while maintaining cell viability and support for bone tissue engineering.[60,61] Hasan et al (2018) and Wang et al (2015) provide additional support for the effectiveness of these silver-doped materials in demonstrating considerable antimicrobial activity, thus playing a crucial role in the ongoing endeavor to reduce the risks associated with implant-related infections and improve outcomes in bone regeneration.[62,63] Copper (Cu) has been recognized as an important trace element for the human body, vital for various biochemical and physiological functions, such as the production of Cu-proteins with enzymatic roles, regulation of bone metabolism, and affecting the balance of the nervous system.[64,65] The increasing interest in CuNPs arises from their improved physical properties, enabling lower dosages, and their acknowledged promise as antibacterial agents and therapies for OP.[66]

CuNPs demonstrate effectiveness in bone mineralization, promoting the adhesion and proliferation of osteoblast cells, as well as the production of bone connective tissue.[67,68]

Mechanism of Action in OP Treatment

Bone-Targeted Delivery Strategies

Typically, adult bone consists of 50–70% mineral content, 20–40% organic material, and 5–10% water. The mineral component of bone is hydroxyapatite (Ca10(PO4)6(OH)2), characterized by its nanometer scale and low crystallinity, accounting for 99% of the total Ca2+ present in the body. The primary component of the organic matrix is type I collagen. Collagen and HA may serve as potential target sites for bone-seeking. The interaction between anionic ligands, including phosphate- and carboxylate-rich compounds, and calcium ions reveals that numerous compound molecules exhibit significant potential for targeting bone minerals. Additionally, to attain targeting of bone, it has been reported that nucleic acids, polymers, nanomaterials, and cells can be conjugated with these ligands,[69] as illustrated in Figure 3.

Mesenchymal stem cells (MSCs) are stromal cells that can divide and self-renew. They can also differentiate into different types of cells, making them a possible target in the bone. Additionally, integrin molecules like integrin α1-α6 have been recognized on the surfaces of MSCs, opening avenues for the targeting of stem cells. For example, a synthesized peptidomimetic ligand (LLP2A) targeting integrin α4β1 demonstrated a strong binding efficiency for MSCs. Alongside LLP2A, Apt19S, a nucleic acid aptamer, was evaluated for its ability to target pluripotent stem cells. Hu et al developed a bilayer scaffold adorned with MSC-specific aptamer aimed at knee repair. The aptamer-bilayer scaffold demonstrated a specific binding affinity for MSCs, effectively recruiting them to the bilayer scaffold to facilitate the repair of the osteochondral defect.[71] Osteoblasts, originating from MSCs, have the capability to form bones by producing the bone matrix and mineralizing it. Specific aptamers for osteoblasts, like the CH6 aptamer, were chosen for their ability to bind to osteoblasts through the cell-SELEX process. CH6 functionalized lipids have the potential to

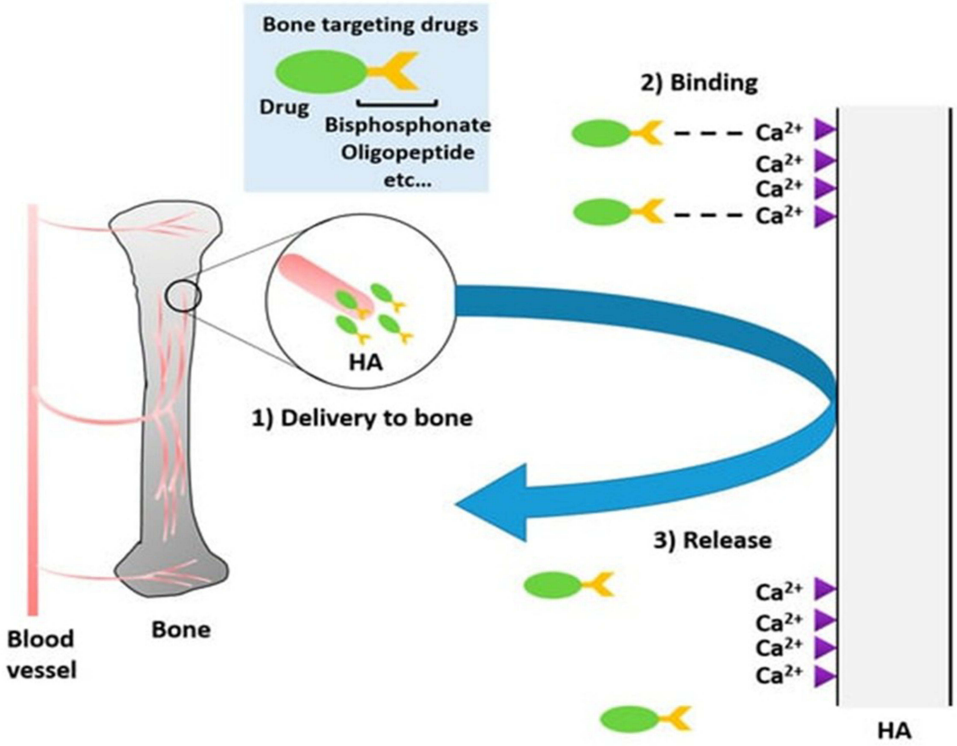

Figure 3 The delivery scheme for bone-specific drugs. Adapted from reference[70] under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

promote bone formation, enhance bone microstructure, and facilitate bone recovery. Additionally, it has been reported that polypeptides (AspSerSer)6 exhibit a strong affinity for the skeleton and show potential for the targeted transport of Sema3A plasmids to osteoblasts.[72] Osteocytes arise from MSCs through the differentiation of osteoblasts, characterized by distinct expression markers such as CD105, Stro1, CD29, and CD166. Furthermore, the uniquely expressed markers such as dentin matrix protein 1 (DMP1) and sclerostin positioned osteocytes as a promising target area within bone tissue. Zoledronic acid (ZA) has been identified as a compound that specifically targets osteocytes and cancer cells. ZA-anchored bone-targeting Gd-UCNP nanocarriers were created to precisely target osteocytes in the fight against early bone metastasis, leading to a reduction in RANKL expression in osteocytes and sclerostin.[73]

Inhibition of Bone Resorption and Promotion of Bone Formation

Bisphosphonates represent a key category of drugs utilized to inhibit the process of bone resorption. The effectiveness of these nonbiodegradable pyrophosphate analogues in addressing disorders linked to excessive bone loss, including OP and Paget's disease, is noteworthy. Bisphosphonates function by attaching hydroxyapatite to bone, resulting in the apoptosis or dysfunction of osteoclasts when they try to resorb the bone surface coated with bisphosphonates. For example, 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (AHPrBP) has been recognized as a strong inhibitor of bone resorption in vivo, showing considerable effectiveness in decreasing osteoclast activity and encouraging osteoclast degeneration when used on contaminated surfaces. It has also been established that the effectiveness of bisphosphonates depends on their chemical structure as well as the situation in which they are administered. This study shows that AHPrBP boasts of a higher potency as evaluated against Cl2MBP or other bisphosphonates under in vivo situation where the specific interaction of the drug to the bone surface is critical. The strength of interaction and the absorption rates of bisphosphonates defines their efficiency in treatment.[74] One of the possible approaches to diminish the rate of bone resorption are based on blocking the Cathepsin K (CatK) – extracellular matrix proteases enzyme which has significant importance for the osteoclasts. The studies also show that the suppression of CatK activity leads to reduced bone resorption by osteoclasts and has no negative impact on osteoblast function. Antibodies like the odanacatib can demonstrate the rate of bone resorption by interfering with the capacity of the osteoclast to degrade collagen in bone matrix. This targeted approach not only prevents the loss of bone mass but fewer side effects than are normally associated with traditional bisphosphonates treatments.[75] Besides the inhibition of bone resorption, it is important to produce new bones to maintain the structural framework of the skeleton. There is one strategy aimed at the increase in the rate of osteoblasts – the cells that are responsible for bone deposition. Of the signaling pathways, Wnt/β-catenin and NF-kB have been claimed to play an essential role in the control of osteoblast activity. Through modification of these pathways, it is possible to further encourage differentiation and activity of osteoblasts that precipitate increased new bone formation.

For instance, calcination a hormone known for its anti-resorptive properties has been found to indirectly promote osteoblastic activity. This enhances the production of Wnt10b which plays a crucial role in enhancing the production of osteoblasts. From the multifunctional activity of calcitonin, it becomes possible to conclude about its significance as a therapeutic approach to OP. Further, molecules like Xanthohumol (XN) have emerged as possible candidates for increasing the effectiveness of the inhibition of osteoclastogenesis besides encouraging osteoblast activity. XN inhibits crucial signaling processes implicated in osteoclast differentiation in addition to augmenting the markers associated with osteoblastic activity; therefore, XN aspired to be a comprehensive solution because it addresses issues such as postmenopausal OPOP.[76]

Recent Advances in Enzyme Immobilized Nanocarriers

Innovations in Enzyme-Nanocarrier Research for Bone Health

Calcium-based NP in drug delivery are under investigation so is the potential of NP to control release of calcium within the body. These NP are able to home in on bone matrix sequestra, increase the solubility of the therapeutic agent and decrease the side effects on the rest of the body particularly in treatments that employ bisphosphonates.[77] Titanium nanotubes can be deemed to possess high efficiency applicability in bone tissue engineering, due to the high degree of osseointegration. Targeting bone tissue, enhancing the bioavailability of therapeutic agents and reducing systemic side

effects, especially in treatments that utilize bisphosphonates.[78] Experimental investigations indicate that significant enhanced bone regeneration can be observed. NP Mesoporous bioactive glass scaffolds are used since they have good adhesion properties on the bone tissue as well as possess drug release properties. Its special structure helps the absorption of therapeutic molecules, and improved osteoblast activity is likely to yield improved healing outcomes in orthopedics applications.[79] The advancement in nanocarriers for calcitonin, hormone that lacks the property of bone resorption, has been made recently. These carriers enhance the bone forming activity of this hormone and inhibit osteoclast activity to make them useful in the treatment of OP.[80] Bioactive glasses are developed to enhance bone forming capabilities and promote tissue repair. The method of conversion of silica ions for boron ions to improve the MSCs viability and differentiation for better efficiency in the context of bone repair. A review of sci-entific literature has also gone further to explore the ability of carbon nanotubes to improve tissue healing and promote bone formation through interface with osteoblasts and osteoclasts. Due to these differences, MgO NP can effectively be used as vehicles for medications and growth factors to improve comprehensive treatment strategies. MgO NP are being integrated with orthopedic implants to support hydroxyapatite on antimicrobial aspects. This approach also helps in preventing infections and enhancing the mechanical properties of the implants thus enhancing integration with bone tissue.[81] TiO2 nanotubes represent a notable development in bone regeneration owing to their distinctive characteristics and interactions with osteocytes. The fabrication of these nanotubes occurs via an electrochemical anodization technique, enabling perfect control over their nanoscale characteristics. This control is essential as the nanostructure of TiO2 can be modified to improve osseointegration, the process by which bone cells adhere directly to the substance without intervening fibrous tissue.[82] The role of TiO2 nanotubes in bone regeneration is linked to their capacity to replicate the natural extracellular matrix, which is essential for facilitating cell adhesion and promoting growth in bone tissue. Research indicates that the nanoscale size and surface structure of these nanotubes enhance the attachment and growth of osteoblasts, which are the cells that facilitate new bone development. The increased surface area of the nanotubes promotes protein adsorption, facilitating cell attachment and spreading.[83,84] An extensive biosilicification approach has been established to produce nanosilica-collagen (nSC) scaffolds sourced from porcine demineralized cancellous bone (DCB). The scaffolds feature a porous architecture that closely mimics native bone, complemented by a consistent nanosilica coating. The surface roughness and silicon content of the silica coating have a considerable impact on the osteoinductivity of these scaffolds. This novel method guarantees consistent and thorough coverage of both internal and external surfaces with a nanosilica coating across the scaffold, thereby improving its mechanical characteristics and osteoinductive capabilities. nSC scaffolds have shown efficacy in addressing critical-sized bone defects in animal models, eliminating the requirement for external cells or growth factors. The topographic and chemical cues of the scaffolds trigger various signaling pathways that are associated with the recruitment of MSCs and the process of bone regeneration. This innovative, one-step implantation method, which is devoid of cells and growth factors, demonstrates significant promise for clinical application in the treatment of extensive bone defects.[85,86] Nanoclays play a crucial role in promoting bone regeneration by providing mechanical support, enhancing bioactivity, and enabling the targeted release of drugs and growth factors. Enhancing the mechanical properties of hydrogels and scaffolds through the incorporation of nanoclays renders these materials more effective for the support of bone tissue. The liberation of ions like magnesium, sodium, lithium, and silicon from nano clays facilitates osteogenic differentiation and the formation of bone tissue. Furthermore, nanoclays possess the ability to sequester and release therapeutic molecules, which is crucial for ensuring controlled and sustained delivery that is vital for effective bone healing.[87–89]

Dual Function Carriers Combining Drug and Enzyme Delivery

Recent trends reveal that drug and enzyme carriers, functioning as dual-function carriers, have potential in the modulation of bone health, particularly OP, as illustrated in Figure 4. Moreover, they are also a unique way of delivering drugs and drug molecules especially in a particular context of enhancing therapeutic value against diseases such as cancer. These carriers involve specific chemical entities such as bisphosphonates (BPs) infamous for their affinity to hydroxyapatite-a constitute of bone. This targeting ability enhances the accumulation of therapeutic agents in bone tissue and decrease the side effects on the body as a whole.[90] The design of these carriers enables a controlled release which can be programmed to deliver medication over small periods of time or in response to specific stimuli and so improve

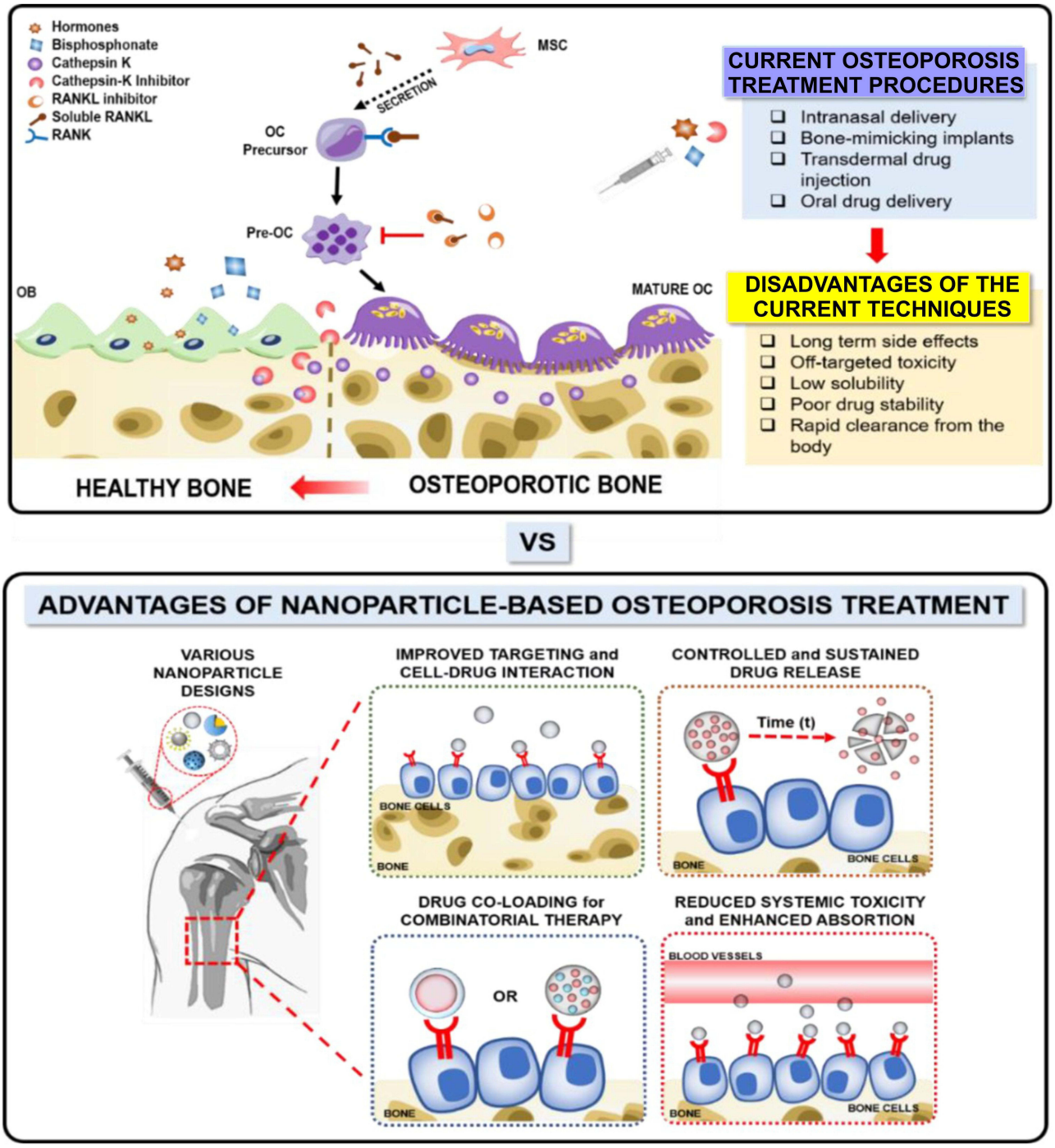

Figure 4 Overview of the advantages and disadvantages of both the conventional and nanoscale-based treatment in handling OP. Other drugs that can be used in osteoporosis treatment include bisphosphates and hormones, which have methods of treatment like oral and intranasal administration, but have several drawbacks related to the long-term use of the drug with such problems as low solubility of the drug and its instability. Considering these shortcomings, the possible solution can be the use of a nano-based DDS system. Nano-based DDSs provide various advantages like controlled and sustained drug release, improved targeting, and reduced systemic toxicity. Adapted from reference[21] under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).
Abbreviations: MSC: MSCs, Mesenchymal stem cells; OB, Osteoblast; OC, Osteoclast.

therapeutic effectiveness. Calcium-Bisphosphonate NP, NP combines bisphosphonate with calcium to form a stable drug delivery vehicle that enhances the circulation time of bisphosphonates and enhances their specificity to bone.[91] Several research conducted in recent years have demonstrated that silica NP are capable of successfully delivering medications such as salmon calcitonin, which encourages osteoblast development and improves bone mineralization.[91] Some studies

performed in the last years have proved that silica NP are able to effectively deliver drugs, which promotes osteoblastic formation and enhances bone mineralization, as mentioned in Table 1.

Superoxide dismutase (SOD) encapsulated in solid lipid nanoparticles has demonstrated high post-formulation activity retention, along with improved wound healing, enhanced angiogenesis, and reduced inflammatory cell infiltration in animal models. Conversely, polymersome-based SOD systems, though exhibiting slightly lower initial activity, provide extended retention within joint cavities and are effective in suppressing oxidative stress and cartilage degradation in osteoarthritic conditions.[100] Alkaline phosphatase (ALP), when delivered through hydrogels embedded with strontium-substituted hydroxyapatite, has been associated with enhanced calcium deposition and upregulation of osteogenic markers, indicating superior bone-regenerative potential. In contrast, ALP immobilized on chitosan membranes enriched with magnesium ions supports sustained enzymatic stability and provides antimicrobial functionality, which is particularly beneficial during early-stage tissue healing. Matrix metalloproteinase (MMP)-responsive hydrogels incorporating cleavable peptide linkers enable site-specific release under inflammatory conditions, facilitating M2 macrophage polarization and promoting bone regeneration outcomes typically not observed with passive-release systems.[103]

Because bone loss and bone formation occur simultaneously, the carriers should have the potential to deliver anti-resorptive medicines including BPs as well as anabolic agents including growth factors or peptides at the same time. For instance, conjugation of alendronate with estrogen-filled NP has been proposed and shown to be useful in decreasing bone loss in OP, while reducing the side effects that are bound to estrogen therapy.[105] It may be feasible to immobilize enzymes such as alkaline phosphatase on to these carriers for the purpose of stimulating osteoblast activity as well as improving mineralization in osteoporotic conditions. As stated in other researches, the application of enzyme-loaded NP could have a positive impact to increase comparable parameters associated with bone density and structure in animal models of OP.[106]

Inorganic Nanozymes in Bone Regeneration

Recent studies highlight several inorganic nanozymes for example Hollow Prussian blue nanozymes (HPBZs) that mimic enzyme for managing osteoporosis.[107] These nanozymes are synthesized through hydrothermal, template-free method and have shown strong potential in counteracting bone degeneration. In cell-based studies, HPBZs were able to lower intracellular oxidative stress by scavenging reactive oxygen species (ROS), which in turn reduced osteoclast formation. Mechanistic insights suggest that these effects are linked to suppression of key inflammatory signaling pathways, specifically MAPK and NF-κB. In mouse model of postmenopausal osteoporosis, HPBZ treatment helped preserve bone mass and restore healthier bone microenvironment, indicating their ability to disrupt the cycle of inflammation-driven bone resorption. These findings highlight HPBZs as dual-action agent, offering both antioxidant and anti-resorptive benefits, and suggest their broader potential in treating bone diseases.[107]

Studies using osteoblastic precursor cells have reported enhanced mineral deposition and upregulation of osteogenic markers such as Runx2, osteocalcin, and collagen type I following CeO_2 nanoparticle treatment. These effects are believed to be associated with the material's capacity to maintain redox balance by scavenging excessive ROS, which are commonly elevated in osteoporotic conditions. Additionally, when incorporated into biomaterial coatings, CeO_2 nanozymes have demonstrated protective effects on bone marrow-derived stem cells exposed to oxidative stress, restoring their osteogenic differentiation capacity.[108]

3D-printed scaffold composed of poly-L-lactide (PLLA) and embedded MnO_2 nanoparticles was employed to treat refractory bone defects. Within the ROS-rich defect microenvironment, MnO_2 functions in decomposing hydrogen peroxide (H_2O_2) to generate oxygen (O_2) while simultaneously lowering ROS levels. This dual action alleviates local hypoxia and oxidative damage. As the scaffold degrades, it gradually releases Mn^2+ ions, which are known to exert immunomodulatory and osteogenic effects. Indeed, the scaffold was shown to shift macrophage polarization toward the anti-inflammatory M2 phenotype, thereby improving the immune milieu around the injury site. In human bone marrow mesenchymal stem cells (hBMSCs), the scaffold triggered upregulation of osteogenic differentiation via activation of the TGF-β/Smad signaling pathway, supporting new bone formation in vivo.[109,110] Comparative analysis of various inorganic nanozymes and their therapeutic mechanisms in orthopaedic disorders is presented in Table 2.

Table 1 Overview of Enzyme-Immobilized Synthetic Nanocarriers to Enhance Effectiveness in the Management of Osteoporosis. The Table Presents the Enzymatic Devices Employed, the Synthetic Nanocarrier Materials, Their Manner of Action to Support Bone Health, Superiorities Over Traditional Strategies, and Current Research Sources for Their Usage

Enzyme	Nanocarrier Material	Mechanism of Action	Advantages	Research Reference
Alkaline Phosphatase (ALP)	Mesoporous silica nanoparticles (MSNs)	Enhance bone mineralization by hydrolyzing phosphate-containing substrates for calcium phosphate formation.	High biocompatibility, targeted delivery to bone tissues, controlled enzyme release.	[92]
	Zoledronic acid-modified strontium-hydroxyapatite nanoparticles in CMC/OD hydrogel	Incorporated into injectable nanocomposite hydrogel (physical entrapment and chemical bonding of HAp NPs)	Enhanced ALP activity and calcium deposition in vitro; improved rheological and mechanical properties; tablepotential for orthopedic/craniofacial bone regeneration	[93]
	ALP immobilized on chitosan (CHI) membranes with Mg^{2}+ ions	Inclusion/surface immobilization on polymeric membrane	Improved cellular viability; favorable biocompatibility; antimicrobial and hemocompatibility; supports ALP activity on bone-relevant materials	[94]
Cathepsin K Inhibitors	Polymeric micelles (PEG-PLGA)	Inhibits osteoclast-mediated bone resorption by neutralizing Cathepsin K activity.	Effective osteoclast inhibition, reduced off-target effects, enhanced stability.	[95]
	Small molecule inhibitors (optimized oxazole compound)	Delivered as free drug, but with improved selectivity and in vivo activity (not immobilized)	In vivo in dogs: reduced urinary C-telopeptide of collagen type I; good selectivity vs other cathepsins; supports antiresorptive role with less off-target activity	[96]
β-Glycerophosphatase	Metal-organic frameworks (MOFs)	Stimulates hydroxyapatite formation to enhance bone regeneration.	High enzyme loading capacity, sustained release, and osteoinductive properties.	[97]
Collagenase	Gold nanoparticles (AuNPs)	Degrades damaged collagen to stimulate new bone matrix synthesis.	Precise enzyme activity control, anti-inflammatory properties, and easy surface functionalization.	[98]
Superoxide Dismutase (SOD)	Lipid-based nanoparticles	Reduces oxidative stress, preventing bone loss due to ROS-induced osteoclast activation.	Protecting bone cells from oxidative damage promotes osteoblast differentiation, excellent biocompatibility.	[99]
	Solid lipid nanoparticles (SLNs) loaded with SOD	Encapsulation/entrapment in lipid matrix; solid lipid nanoparticle delivery	~90% initial enzyme activity retained; in vivo burn model (rats) showed better wound healing, enhanced angiogenesis and reduced inflammatory cells compared to free SOD or blank nanoparticles	[100]
	Porous polymersomes loaded with SOD targeting synovium (joint)	Encapsulation + surface modification; intra-articular injection	Prolonged retention in synovium; reduced ROS and catabolic factors; in vivo OA model: attenuated initiation and progression of osteoarthritis	[101]
Matrix Metalloproteinase (MMP) Inhibitors	Dendrimers (PAMAM)	Prevents excessive matrix degradation, promoting bone structural integrity.	High targeting efficiency, reduced systemic toxicity, and potential for surface modification.	[102]
	PEG hydrogel with MMP-cleavable peptide ("PEG-pp-PS network")	Encapsulation of phosphatidylserine; MMP-responsive release (triggered by MMPs)	In vivo: injectable hydrogel filled bone defect; reduces inflammation, promotes macrophage polarization to M2, enhances osteogenic differentiation leading to better bone regeneration	[103]
Carbonic Anhydrase	Hydrogel-based nanoparticles	Regulates pH in the bone microenvironment to optimize mineralization.	Enhances enzyme stability, efficient delivery, and local pH control for bone formation.	[104]

Table 2 Summary of Recent Inorganic Nanozymes and Their Enzyme-Mimetic Mechanisms in Orthopedic Disorders

Nanozyme/Nanomaterial	Enzyme-Like Activity	Target	Mechanism of Action	Outcomes	Ref
Prussian Blue Nanozymes (HPBZs)	Peroxidase, catalase	Osteoporosis (OP)	Scavenges ROS, inhibits MAPK & NF-κB signaling	Reduced osteoclastogenesis, preserved bone in OVX mice	[107]
CeO_2 Nanoparticles	Superoxide dismutase (SOD), catalase	Osteoporosis, bone tissue engineering	Neutralizes ROS, promotes osteoblast differentiation	Increased ALP, mineralization, reduced oxidative damage	[108]
SPIO based Nanozymes	Peroxidase-like	Osteoporosis (OP)	ROS scavenging, NF-κB inhibition, repolarizes macrophages M1→M2	Decreased bone loss, increased osteoblast activity and M2 macrophages	[111]
Prussian Blue + Mn Nanozymes	Multi-enzyme mimicry	Osteoarthritis (OA)	Neutralizes ROS, modulates chondrocyte apoptosis, preserves ECM	Reduced cartilage degradation, decreased inflammation	[112]
CeO_2 NPs in Intervertebral Disc Degeneration	SOD, catalase	Intervertebral Disc Degeneration (IVD)	Maintains redox balance, reduces nucleus pulposus (NP) cell apoptosis	Less disc degeneration, increased cell viability	[113]
SPIO@15HA Core-Shell Nanocomposites	Hydroxyapatite + SPIO	OP (OVX mice)	Suppresses osteoclasts; promotes MSC osteogenesis via TGF-β, PI3K-AKT	Improved bone microstructure, prevented bone loss	[114]
Hollow MnO_2/β-TCP Scaffold	Catalase-like	Bone tissue engineering	Reduces ROS, promotes ALP activity, matrix mineralization	Better osteogenic differentiation, improved bone formation	[115]
CeO_2 NPs/CeO_2 Nanocomposite Bioactive Material	SOD, catalase	Oxidative stress models (MG-63 cells)	Antioxidant activity, upregulates bone differentiation genes	Increased mineralization, ALP, bone markers	[116]
Mesoporous Bioactive Glass (MBG) Scaffold	Indirect ROS reduction	Osteoporosis (rat model)	Reduces ROS in MSCs via cAMP/PKA, enhances osteogenic and angiogenic signaling	Improved bone regeneration and vascularization	[117]

Recent evidence highlights the broad applicability of nanozymes in orthopaedics, particularly in osteoarthritis (OA), osteoporosis (OP), and intervertebral disc degeneration (IVD).[118] In OA models, ultrasmall Prussian blue nanozymes have shown to alleviate joint inflammation by scavenging ROS and promoting M2 macrophage polarization, thereby preserving cartilage integrity and reducing matrix degradation.[11,119] Superparamagnetic iron oxide (SPIO)-based nanozymes have shown dual functionality in oxidative stress regulation and immune modulation in osteoporosis. A 2024 study introduced SPIO:Eu@PLGA nanospheres, where europium-doped SPIO particles were encapsulated in poly(lactic-co-glycolic acid) to improve stability and biocompatibility. They enhanced osteoblast differentiation and matrix mineralization while simultaneously inhibiting osteoclastogenesis.[120] Metal-phenolic network (MPN) nanoplatform combining kaempferol and Mn^{2}+ ions, with BMP-2 immobilization in a hyaluronic acid hydrogel, exhibited pH-responsive release, reduced oxidative stress, and demonstrated significant bone mass regeneration in senile osteoporotic defect models.[121] A list of

Challenges in Development

Biocompatibility and Toxicity

The primary challenge is to ensure that the NP are safe for use within the human body and that they are biocompatible with existing tissues. There is a possibility that certain NP could be toxic or cause immunological reactions, both of which could be detrimental to the health of the patient. When trying to reduce off-target effects while still achieving accurate targeting NP to bone tissue, it can be very challenging. The process of ensuring that NP will correctly release their payload at the appropriate location without causing damage to healthy tissue is extremely challenging or even impossible. There is a possibility that the behavior and effectiveness of NP can be modified by their size and surface qualities.[122] Recent in vivo investigations demonstrated accumulation of NP in liver, spleen, and kidneys over 120 days, with a 39% reduction in liver load but increases in spleen (≈53%) and kidneys (≈150%), indicating that even "biocompatible" coatings may not prevent long-term organ accumulation.[123]

Targeted Delivery Efficiency

The effectiveness of targeting is greatly influenced by variables such blood circulation duration, nanoparticle size, and surface changes. These nanocarriers' therapeutic potential could be lowered in the absence of effective targeting because of off-target effects and decreased bioavailability at the site of action.[124] Traditional oral pharmacotherapies frequently exhibit inadequate bioavailability attributable to their chemical characteristics and the physiological obstacles they face. Oral medicines may experience substantial first-pass metabolism, greatly diminishing the quantity of active drug that enters systemic circulation and subsequently the bone. Moreover, gastrointestinal adverse effects may further restrict patient adherence, complicating therapeutic protocols.[125] For example, AuNPs larger than 50 nm in osteoporosis models have been shown to be trapped by reticuloendothelial system, reducing bone targeting and increasing accumulation in hepatic and renal tissues.[126]

Other Challenges

For optimal therapeutic outcomes, it is crucial to ensure that therapeutic agents are efficiently encapsulated within NP and that their release profiles are carefully controlled. From a technical perspective, attaining suitable medication loading and release properties can pose challenges. The transition from experimental or pre-clinical studies to clinical trials and subsequent clinical application is a lengthy and intricate journey. Obtaining regulatory approval, conducting safety evaluations, and addressing large-scale production considerations can pose considerable challenges. The expense associated with producing NP could hinder their broader application. To enhance the accessibility of these medicines, it is essential to identify manufacturing methods that are cost-effective and capable of being scaled up. OP patients exhibit differences in bone health, genetic factors, and responses to therapy. Customizing nanoparticle-based treatments to meet the specific needs of each patient presents significant challenges. It is crucial to guarantee the enduring safety of therapies that utilize NP.[127] This involves keeping an eye on possible negative impacts that might not be obvious right away. Recent studies report nanoparticles often accumulate in organs such as the liver and spleen and interfere with

metabolism which pose long-term safety risks.[128] Over time, it is possible that therapies utilizing NP for OP could develop drug resistance or tolerance. The application of NP in healthcare raises important considerations regarding informed consent, patient rights, and various ethical and legal matters. Moreover, regulatory agencies highlight current lack of standardized physicochemical characterization and harmonized safety guidelines for nanomedicines, which limits its clinical translation and approval processes.[129] It is essential for experts in nanotechnology, pharmacy, medicine, and regulatory affairs to work together to ensure the successful creation and application of nanoparticle-based therapies. While achieving successful interdisciplinary collaboration can be challenging, it is essential for success. The public's perception and acceptance of nanoparticle-based medicines could play a significant role in their adoption. Some polymeric nanomaterials exhibit persistent organ retention in vivo, necessitating long-term post-market surveillance to monitor possible adverse effects.[128] It is essential to maintain the trust of patients and the public in the effectiveness and safety of these medications.[130,131]

Clinical Application of Bone Remodeling-Targeted Therapy

Bone tissue features a surface-mineralized extracellular matrix predominantly composed of hydroxyapatite (HAP) and facilitates several processes, including ion exchange, crystal formation, dissolution, and the incorporation of foreign molecules on its surface. Consequently, this mineralized element provides a means for targeting bone. Research demonstrates that the crystal size of hydroxyapatite in the bone tissue of osteoporotic patients is greater, facilitating the targeted administration of medications to the bone surface.[132] Advancements in the understanding of bone remodeling biology indicate that aberrant bone remodeling may play a role in the onset or advancement of arthritis and bone cancers, whereas reinstating normal bone remodeling could enhance their treatment or prevention. Furthermore, innovative therapeutic medicines demonstrating potential in enhancing bone remodeling regulation have been utilized in clinical studies for bone metabolic disorders.[133,134]

Recent preclinical investigations have underscored the importance of the gut-bone axis. Numerous trials have been undertaken to assess the impact of probiotic supplementation in postmenopausal women with OP.[135] The correlation between statins and a reduced incidence of OP has prompted several trials. Despite the therapeutic efficacy of stem cell homing demonstrated in various preclinical studies, only two trials have been conducted: one involving the intravenous infusion of fucosylated autologous bone marrow stem cells (BMSCs) in patients with established OP and low-impact fractures, and another involving the intravenous infusion of LLP2A-Ale to enhance BMSC homing in patients with glucocorticoid-induced osteopenia.[135,136] Nevertheless, the outcomes of these trials have not been published. The restricted advancement in stem cell therapy trials can be ascribed to the complexities of cell preparation, the potential for cancer and thrombosis, and their inadequate delivery efficacy in vivo.[137,138] The absence of effective consolidation therapy following standard chemotherapy has sparked interest in the possibility of examining whether targeting bone remodeling can produce supplementary benefits in osteosarcoma therapy. This is particularly evident in the approval of denosumab for giant-cell tumor of bone (GCT). In contrast to GCT and OP, the osteosarcoma microenvironment is heterogeneous, characterized by a predominantly osteoblastic, osteolytic, or mixed lytic/proliferative skeleton change.[139] An interim analysis of a current Phase 3 trial indicated a modest elevation in recurrence and metastatic rates produced by bisphosphonates.[140]

Future Directions

AI Driven Nanocarrier Optimization

High-quality, comprehensive datasets are crucial for the effective training of AI algorithms. In the realm of OP treatment, there frequently exists a deficiency of comprehensive datasets that encompass a variety of biological responses to different drug formulations. The limited availability of data can result in overfitting, causing models to excel on training datasets while struggling with new, unseen data. This discrepancy ultimately undermines their predictive accuracy and dependability in practical scenarios. Creating optimization strategies for AI-driven nanocarriers in laboratory environments does not ensure that they can be scaled for use in clinical settings. Moving from experimentation to production also brings questions of scale with it: how to guarantee the quality of the product or how

to meet legislative requirements, or how to run the production sustainably at a lower cost. There is a need to ensure the designed nanocarriers can be synthesized at large scale as to ensure their efficient use in the management of OP.[21] Drugs in the human body are affected by multiple factors which are interrelated and as a result affects the effectiveness of drugs. To this, it could be said that it is high time that AI models introduced factors such as individual patient variability, genetic differences, as well as constant fluctuations of the bone remodeling processes. Capturing these interactions is not easy, and as a result, the AI algorithms must be sophisticated to accommodate complex biological data. In order to obtain the best nanocarrier optimization, artificial systems should employ the multi-omics data that is the genomics, proteomics, and metabolomics for OP. However, assembling these diverse types of data into a single analytical schematic proves to be extremely computationally challenging. The integration process must ensure that the resulting models are robust and can clearly predict results from various biological inputs as we shall infer from the literature.[141,142] Developing optimization protocols for AI-controlled nanocarriers in one's bench work does not guarantee applicability in clinical work. There are several challenges including scale up of manufacturing, for example issues to do with consistency, compliance with regulatory body rules and regulations as well as combating high production costs when the manufacturing has gone up from pilot scale to large scale manufacturing. Lastly, the NSs used must ensure that optimised nanocarriers may be manufactured effectively for large scale use in the OP treatment. Taking into consideration the innovations of artificial intelligence in the healthcare sector certain ethical questions come into account such as confidentiality of patients and their information. Since many AI systems require patients' records for training purposes, patient information security is vital with special regard to ethical issues. Moreover, there are apprehensions regarding bias in AI algorithms that may result in unequal treatment outcomes across various patient groups.[143] Multimodality platforms could represent a valuable avenue for further investigation. The application of CT assessment for OP is thoroughly documented in existing literature, with multiple tools demonstrating outstanding diagnostic efficacy.[144,145] In addition, MRI tools might be helpful: Using a brief lumbar mDIXON sequence for opportunistic screening, Zhao et al developed a fully automated radiomic screening pipeline for OP. This pipeline could be carried out in as little as sixteen seconds.[146] Deep learning (DL), a branch of machine learning (ML), emulates the architecture of neural networks. It utilizes artificial neural networks featuring several hidden layers to address complex issues. The concealed layers facilitate the system's ongoing learning and enhancement of performance through the integration of fresh information. In contrast to conventional ML, which necessitates human feature extraction from input photos, deep learning techniques autonomously learn features from input images through multilayer neural networks, such as convolutional neural networks (CNNs). This method enables deep learning systems to both associate image features with outputs and acquire the features independently. Instances of deep learning outputs encompass picture classifications, object localization, and pixel labeling. Deep learning has led to the emergence of radiomics, which involves extracting numerous quantitative aspects from medical images, such as CT scans, to reveal concealed patterns through computational analysis. In the field of bone health, radiomics with deep learning methodologies can identify imaging features associated with significant pathological and histological attributes of bone trabeculae.[147,148] CT exams conducted for other purposes offer a distinctive opportunity for incidental OP screening without incurring extra costs, time delays, or radiation exposure for patients.[149–151] Numerous investigations have demonstrated that data from a solitary L1 vertebral body on CT exhibits a strong correlation with T-scores derived from DEXA.[152,153]

Conclusion

Enzyme-immobilized synthetic nanocarriers represent an advancement in therapeutic management of osteoporosis. Emerging studies highlight their potential to enhance bone-targeted delivery, improve drug bioavailability, and reduce systemic side effects compared to conventional therapies. These nanocarriers have also shown bone regeneration and support sustained therapeutic action. Their flexibility in delivering a wide range of agents from antiresorptive drugs to genetic materials further supports their relevance in addressing multifaceted nature of osteoporosis. However, despite these advances, several critical challenges, including long-term biosafety, immune compatibility, and large-scale reproducibility of these systems require thorough investigation before clinical translation. Additionally, regulatory landscape for nano-based therapeutics is evolving and poses significant limitations to approval and commercialization. Future

research prioritize well-designed preclinical studies, scalable preparation methods, and multidisciplinary collaborations to bridge this gap.

Data Sharing Statement

Not Applicable. This is a review article and all relevant information is provided in the article.

Ethical Approval and Consent to Participate

Not Applicable. This is a review paper and do not involve direct research on humans or animals.

Consent for Publication

"Not applicable" as this manuscript does not contain data from any individual person.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by Health Commission of Chengdu (No. 2024071), and Horizontal Project (No. 2023HX059).

Disclosure

The Authors declare that they have no competing interests financial or non-financial or any other interests that might be perceived to influence the results and/or discussion reported in this paper.

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