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REVIEW

Advancements in Polymer-Based Nanocarriers for Controlled Release of Nitric Oxide: Clinical Applications and Future Prospects

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Abstract: The clinical management of bone defects presents a significant challenge in regenerative medicine due to the limited self-repair capacity of bone tissue and inadequate vascularization. Nitric oxide, a gaseous signaling molecule, has garnered attention as a potent modulator of bone remodeling, exhibiting pro-osteogenic, pro-angiogenic, and anti-inflammatory properties. However, its therapeutic application is limited by its short half-life, high reactivity, and dose-dependent biphasic effects. Advanced polymer-based nanoformulations have been developed to address these challenges and enable controlled and localized NO delivery to bone tissue. This review explores role of NO in bone repair mechanisms and the limitations of conventional delivery systems. Significant focus is given to innovative polymeric platforms, such as dendrimers, micelles, nanogels, and hybrid composites, which offer precise control over release kinetics, high encapsulation efficiency, and targeted delivery. Additionally, integrating NO delivery within nanoengineered scaffolds and coatings for orthopedic implants is explored as a promising strategy to enhance osteointegration and reduce the risk of post-surgical infections. Preclinical studies demonstrate promising osteogenic effects yet face significant challenges including cytotoxicity at elevated NO concentrations along with non-standardized evaluation protocols and scalability limitations. Future perspectives point to the potential of stimuli-responsive systems, co-delivery approaches, and personalized strategies utilizing additive manufacturing technologies. This review consolidates the latest advancements in the field, underscoring the significant potential of polymer-based NO nanoformulations to revolutionize bone tissue engineering.

Keywords: nitric oxide, polymeric nano formulations, bone regeneration, controlled release, nanotechnology and osteogenesis

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Introduction

According to global meta-analysis, prevalence of osteoporosis was estimated at 18.3% across all age groups, with significantly higher rates in women (23.1%) compared to men (11.7%), indicating a large at-risk population for bone degeneration.[1] Regulatory databases reported 87 alloplastic bone grafts have been approved in United States since 1996; 10 products in Japan since 2004; and 36 products in Korea since 1980. These approved products underline widespread clinical demand for bone grafting procedures and the limitations of existing methods, thereby reinforcing the need for advanced and more effective regenerative strategies.[2] Despite notable progress in therapeutic strategies, conventional methods including autografts, allografts and synthetic bone substitutes continue to exhibit critical limitations.[3,4] Autografts, regarded as the clinical gold standard, necessitate a secondary surgical procedure and are associated with donor site morbidity and restricted tissue availability.[5–7] Allografts pose risks related to immune rejection and potential

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disease transmission. Synthetic substitutes, while osteoconductive, fail to provide the bioactivity required for effective tissue integration.[8] Growth factor-based approaches, including bone morphogenetic proteins and platelet-rich plasma, demonstrate therapeutic potential but are constrained by high cost, lack of controlled delivery and adverse outcomes linked to excessive or dysregulated growth factor release.[9,10]

Bone regeneration relies on effective vascularization to support nutrient transport, oxygen supply and molecular signaling essential for tissue formation.[11,12] Nitric oxide (NO), a gaseous signaling molecule, plays a key role in promoting vascularization and osteogenesis. It enhances osteoblast differentiation, stimulates angiogenesis and regulates inflammation, contributing significantly to bone repair.[13,14] Despite its therapeutic potential, NO application is limited by its short half-life, high reactivity and difficulties in achieving controlled, sustained release at the target site. Addressing these limitations requires advanced delivery strategies.[15] Nanotechnology, particularly polymer-based systems, offers promising solutions. Polymers with tunable properties enable the design of controlled-release platforms that maintain NO bioactivity and prolong its local presence.[16,17] Polymeric nanocarriers also facilitate targeted delivery, improved molecular stability and combination therapies with agents such as growth factors or antimicrobials.[18–20]

A key factor in NO-based therapy is the regulation of its release profile, which directly impacts therapeutic outcomes, especially in osteogenic differentiation.[21] Rapid or uncontrolled release may result in cytotoxic levels and early degradation, while sustained release maintains effective NO concentrations at the injury site.[22,] This prolonged exposure enhances osteoblast activity, promotes extracellular matrix mineralization and supports proper bone remodeling.[23] Polymeric carriers offer a promising approach for controlled delivery due to their customizable structural and chemical properties. These materials can be engineered to modulate degradation rates and release kinetics, and to respond to specific physiological conditions.[24–26] Their biocompatibility and functional versatility make them suitable for biomedical applications, allowing for the design of systems that stabilize NO and enable its targeted, sustained release in a biologically active form.[27]

At the nanoscale, polymer-based systems further benefit from increased surface area, enhanced cellular uptake, and improved interaction with the biological microenvironment.[28,29] Nanoformulations can more effectively mimic the architecture of native extracellular matrix, supporting cell adhesion, proliferation, and differentiation.[30] Furthermore, nanoscale delivery systems facilitate deeper tissue penetration and greater bioavailability, enhancing the therapeutic potential of NO in bone regeneration.[31] The integration of polymeric encapsulation techniques with nanoscale engineering and the inherent bioactivity of NO presents a synergistic platform for addressing the multifaceted requirements of bone tissue repair.[32–35] Despite growing interest in this approach, a comprehensive evaluation of recent advances in polymer-based NO nanoformulations for bone regeneration remains lacking in the current literature. Previous studies have not addressed aspects of NO signaling with polymer-based nanocarriers with special focus on their convergence in context of bone tissue engineering. This review offers recent technological advances, mechanistic insights, and translational considerations in polymer-mediated NO delivery for bone regeneration and aims to analyze recent developments, identifying technological innovations, and highlighting emerging trends that are shaping the future of NO-mediated bone tissue engineering strategies.

Methodology

This review aims to explore the effectiveness of biomaterial scaffolds combined with NO delivery systems for bone regeneration in tissue defects. A comprehensive literature search was conducted using databases like PubMed and Google Scholar to identify studies published between 2010 and 2025 that investigated NO delivery for bone repair. Inclusion criteria focused on studies examining osteoblast differentiation, osteoclast inhibition, and vascularization outcomes in in-vitro, in-vivo, or clinical settings. Studies were assessed for methodological quality using standard tools.

Physiological Role of NO in Bone Biology

NO is a small, lipophilic, diatomic free radical endogenously synthesized in mammalian cells by nitric oxide synthase (NOS) enzymes, which convert L-arginine to L-citrulline.[36,37] Its ability to diffuse across cell membranes enables it to participate in diverse physiological processes, including vasodilation, angiogenesis, immune defense, and tissue regeneration. In skeletal tissue, NO plays a pivotal regulatory role in bone remodeling and repair by influencing the activity of various cell types such as osteoblasts, osteoclasts, endothelial cells, and immune cells.[38,39] Three NOS isoforms mediate NO production: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). While eNOS and nNOS are constitutively expressed and generate low, sustained levels of NO involved in homeostatic signaling,[40–43] iNOS is activated under inflammatory conditions and produces higher NO concentrations associated with cytotoxic effects Figure 1.

NO plays a pivotal, concentration-dependent role in bone metabolism, affecting both osteoblasts and osteoclasts.[14,] At low concentrations, NO promotes osteogenesis by enhancing osteoblast proliferation, differentiation, and mineralization through the activation of key transcription factors such as runt-related transcription factor 2 (Runx2) and osterix.[45–47] These effects are primarily mediated via the activation of soluble guanylate cyclase (sGC) and the subsequent increase in cyclic guanosine monophosphate (cGMP) levels, which trigger downstream signaling pathways involved in bone formation and repair.[48] NO also facilitates the expression of alkaline phosphatase, a marker of osteoblastic activity, contributing to functional bone development.[49,50] Additionally, NO plays a critical role in maintaining bone mass by modulating osteoclastogenesis. It inhibits the differentiation of osteoclast precursors through the receptor activator of nuclear factor kappa B ligand (RANKL) pathway, reducing osteoclast formation and activity.[51–53] This dual function of NO stimulating bone formation via osteoblast activation while suppressing bone resorption by osteoclasts ensures the balance necessary for skeletal homeostasis. Beyond its effects on bone cells, NO is a potent mediator of angiogenesis.[54–56] It stimulates endothelial cell proliferation, migration, and the formation of capillary-like structures, essential for the development of a functional vascular network within healing bone tissue.[57] Proper vascularization is crucial for effective bone regeneration, ensuring the delivery of nutrients and oxygen to the site of injury, while also facilitating the recruitment of osteoprogenitor cells required for tissue repair.[11,58–60] NO therefore functions as a molecular link between bone formation and neovascularization, both of which must occur in a coordinated manner for effective tissue regeneration Figure 2.

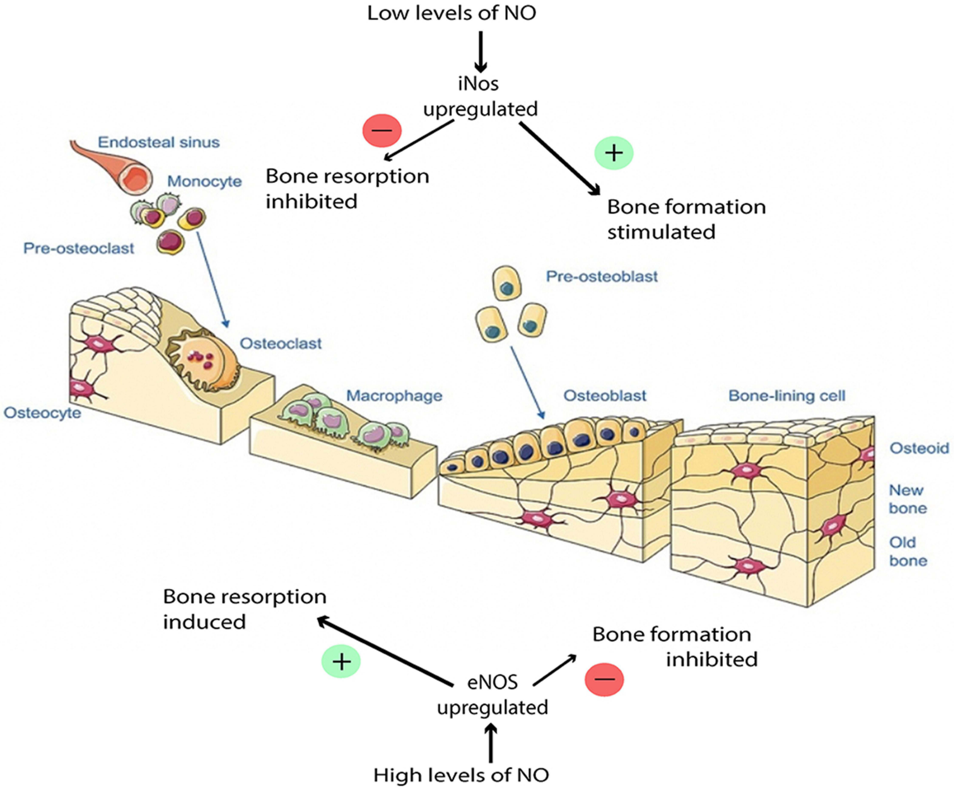

Figure 1 Nitric oxide as key regulatory molecule in bone remodeling by exerting dose-dependent effects on osteoblasts. At physiologically low concentrations, it promotes osteoblast proliferation and differentiation, thereby facilitating bone formation. Conversely at elevated NO levels trigger apoptosis in osteoblasts, contributing to the natural bone resorption process. Dual functionality underscores precise NO regulation in maintaining bone homeostasis and design of targeted NO-based therapeutic strategies for bone regeneration. Reproduced from Anastasio AT, Paniagua A, Diamond C, et al. Nanomaterial nitric oxide delivery in traumatic orthopedic regenerative medicine. Frontiers in Bioengineering and Biotechnology. 2021;8:592008.[44] licensed under CC BY 4.0. The figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by-sa/3.0/legalcode); https://smart.servier.com.

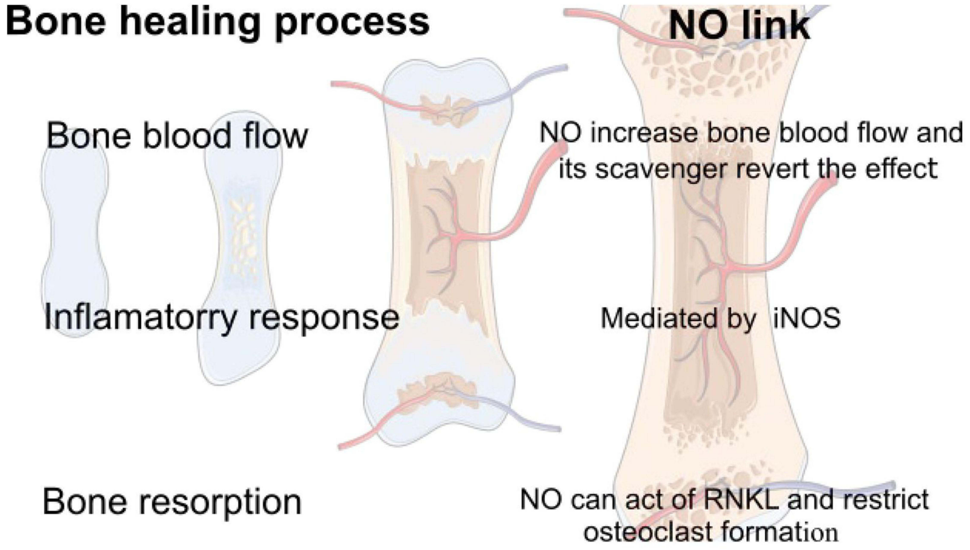

Figure 2 Nitric oxide's role in coordinating both bone formation and neovascularization, processes essential for effective bone regeneration. As signaling molecule, it stimulates osteoblast activity, promoting new bone synthesis, simultaneously enhancing growth of new blood vessels. This dual function ensures regenerating bone tissue receives adequate nutrients and oxygen, facilitating efficient healing and restoration of bone integrity. Reproduced from Nascimento MH, T. Pelegrino M, C. Pieretti J, et al. How can nitric oxide help osteogenesis? AIMS Molecular Science. 2020;7(1):29–48.[61] licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0).

In addition to its osteogenic and angiogenic functions, NO plays a central role in regulating the inflammatory response during the early phases of bone healing.[44,62] At physiologically relevant concentrations, it supports the resolution of inflammation by modulating cytokine production, inhibiting the adhesion and infiltration of leukocytes, and limiting the extent of tissue damage.[63,64] This immunomodulatory effect contributes to the creation of a regenerative microenvironment that favors tissue repair rather than chronic inflammation.[65] Taken together, the multifunctional biological activities of NO highlight its potential as a therapeutic agent in bone tissue engineering.[44,66] However, due to its short biological half-life, chemical instability, and localized activity, effective delivery strategies are essential to achieve sustained and controlled NO signaling within bone defects.[22,44,67] While polymeric nanoformulations hold significant promise for overcoming the challenges in bone regeneration, their successful application still faces numerous hurdles. These challenges, ranging from stability issues to controlled release, are crucial to address for clinical success.

Challenges in NO Delivery for Bone Regeneration

NO therapy for bone regeneration presents a unique pharmacological challenge due to the molecule's paradoxical behaviour in biological systems. While NO demonstrates well-documented osteogenic and angiogenic properties at optimal concentrations, its therapeutic potential is constrained by several intrinsic limitations.[13] The molecule's gaseous nature and small size facilitate rapid diffusion across biological membranes, making containment and targeted delivery particularly difficult. This characteristic, combined with its reactive oxygen species (ROS) scavenging ability, creates a complex dosing paradigm where the molecule's beneficial effects can quickly transition to cytotoxicity.[68,69] The development of effective delivery systems account for NO's concentration-dependent duality in bone metabolism. At physiological levels, NO promotes osteoblast differentiation through the upregulation of key transcription factors while simultaneously inhibiting osteoclast activity.[70] However, these effects are reversed at higher concentrations, creating a narrow therapeutic window that demands precise spatiotemporal control.[39] This challenge is compounded by the dynamic nature of bone healing, which progresses through distinct inflammatory, reparative, and remodelling phases each requiring different NO signaling patterns for optimal tissue regeneration.[71]

Current research focuses on overcoming these barriers through innovative biomaterial approaches. Smart delivery platforms incorporating stimuli-responsive polymers show promise in addressing the temporal aspects of NO delivery, with some formulations capable of detecting and responding to microenvironmental changes in pH, redox potential, or enzyme activity.[72–74] Hybrid systems combining NO donors with structural biomaterials like hydroxyapatite or bioactive glasses attempt to solve the spatial control challenge by providing localized release at the defect site.[33,66] Emerging technologies such as 3D-printed scaffold systems with gradient release profiles aim to mimic the natural healing cascade by delivering differential NO concentrations to distinct tissue zones Figure 3.

However, significant challenges remain in clinical translation. The field must develop standardized protocols for evaluating NO release kinetics and biological effects across different model systems.[76] There is need for delivery platforms that can maintain NO stability during long-term storage while ensuring precise activation upon implantation.[77] Furthermore, the potential for donor molecule degradation products to influence bone regeneration outcomes requires thorough investigation.[78] By optimizing controlled release mechanisms, these therapies can fully leverage the pleiotropic effects of NO.

Polymer-Based Controlled Release Strategies

Polymers serve as the foundation of controlled drug delivery, with their selection dictated by biocompatibility, degradation behavior, and intended release kinetics.[79] Natural polymers, such as chitosan, alginate, and gelatin, are derived from biological sources and offer inherent biocompatibility, making them suitable for applications like wound healing and mucosal delivery.[80,81] However, they may exhibit batch-to-batch variability and occasional immunogenicity. In contrast, synthetic polymers like PLGA, PEG, and PCL provide precise control over mechanical properties and degradation rates, enabling reproducible and scalable drug delivery systems.[82,83] Biodegradable polymers, including PLGA and polycaprolactone, are particularly advantageous as they break down into non-toxic byproducts, eliminating the need for surgical removal.[84,85] Non-biodegradable polymers, such as silicone and ethylene-vinyl acetate, are reserved for long-term implants but carry risks of chronic inflammation and eventual retrieval.[86,87] While PLGA remains most investigated polymer for NO delivery in bone repair, recent studies indicate promising developments using alternative materials. PEG-based nano-particles (mPEG-P) embedded within an injectable thermosensitive hydrogels to deliver NO through photothermal-responsive mechanism, demonstrating enhanced angiogenesis and osteogenesis in bone defect models.[13] Hybrid scaffolds combining inorganic agents like zinc oxide combined with bioactive polymers and ECM components provide anti-inflammation, osteoinduction, and improved healing in osteoporotic bone models.[88] Understanding the

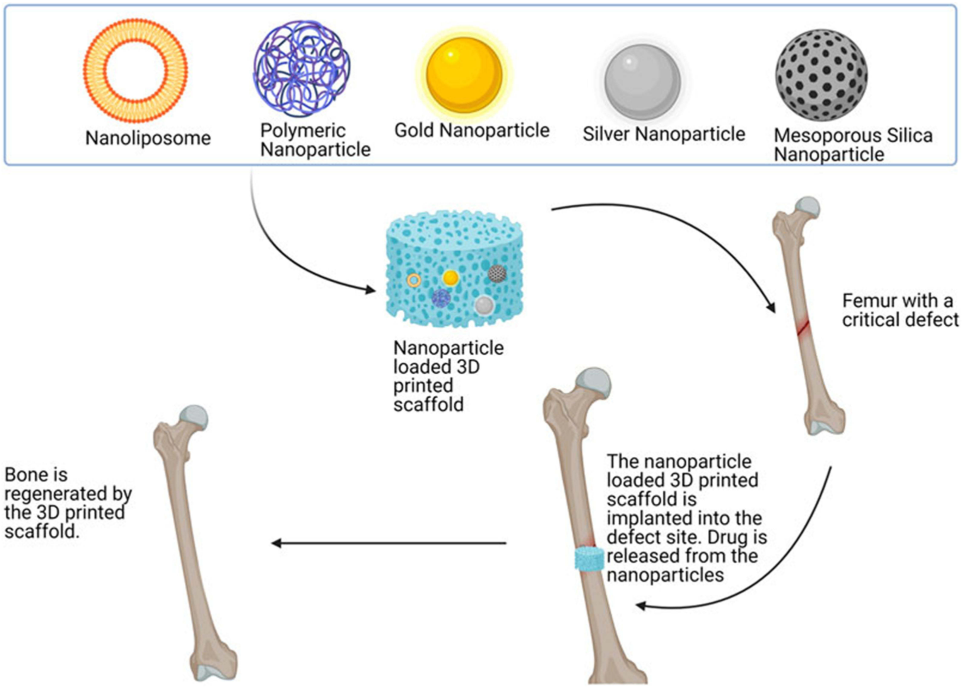

Figure 3 Types of nanocarriers incorporated into 3D printed scaffold for nitric oxide delivery Reproduced from Suleman A, Kondiah PPD, Mabrouk M, et al. The application of 3D-printing and nanotechnology for the targeted treatment of osteosarcoma. Frontiers in Materials. 2021;8:668834.[75] licensed under CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

mechanisms of drug release from polymeric systems is crucial to improving the design of safer and more effective delivery platforms.

Mechanisms of Drug Release from Polymeric Systems

The release of therapeutics from polymeric carriers can be governed by various stimuli-responsive mechanisms (Table 1). Thermally responsive polymers, such as poly(N-isopropylacrylamide) (PNIPAM), undergo phase transitions near body temperature, enabling on-demand drug release in hyperthermia-based cancer therapy.[89,90] Hydrolytic degradation, commonly observed in polyesters like PLGA, results in gradual drug release as the polymer erodes in aqueous environments.[91,92] Enzymatically triggered systems leverage disease-specific enzymes, hyaluronidase in tumor microenvironments, to degrade polymers like hyaluronic acid and release encapsulated drugs.[93] pH-sensitive polymers, including Eudragit and poly(β-amino esters), dissolve selectively in acidic or alkaline conditions, allowing targeted delivery to stomach, intestines, or cancerous tissues.[94] The structural design of polymeric carriers plays a critical role in determining drug release profiles. Matrix systems, where the drug is uniformly dispersed within the polymer, typically exhibit an initial burst release followed by sustained diffusion-controlled release.[95,96]

These systems are widely used in PLGA microspheres for long-acting injectable formulations. In contrast, reservoir systems feature a drug core encapsulated by a rate-controlling polymer membrane, facilitating zero-order release kinetics ideal for hormonal therapies like contraceptive implants.[97,98] While matrix systems are simpler to fabricate, reservoir systems offer more consistent drug release over extended periods.[99] The localization of drugs within polymeric carriers significantly influences release dynamics.[100] Surface loading techniques, such as adsorption or layer-by-layer assembly,

Table 1 Overview of Various Polymer Types Used in Nitric Oxide Delivery Systems Highlighting the Biodegradability, Release Mechanisms, Advantages, and Limitations of Both Natural and Synthetic Polymers Utilized for NO Release in Tissue Engineering Applications

Polymer Type	Natural/ Synthetic	Biodegradability	NO Release Mechanism	Advantages	Limitations	References
Chitosan	Natural	Yes	pH-triggered	Biocompatible, mild release	Poor mechanical strength	[103]
PLGA	Synthetic	Yes	Hydrolytic	Tunable degradation, scalable	Acidic byproducts	[104]
PCL	Synthetic	Yes	Hydrolytic	Slow degradation, biocompatible	Slow release rate	[105]
PVA	Synthetic	Yes	Diffusion-controlled	Hydrophilic, easy processing	Limited mechanical strength	[106]
Alginate	Natural	Yes	Ionic crosslinking	Gel-forming, biocompatible	Low mechanical strength	[107]
Dextran	Natural	Yes	Enzymatic degradation	Biocompatible, hydrophilic	Limited mechanical properties	[108]
Collagen	Natural	Yes	Enzymatic degradation	Biocompatible, promotes cell adhesion	Prone to denaturation	[105]
Gelatin	Natural	Yes	Thermal gelation	Biocompatible, promotes cell adhesion	Poor mechanical strength	[109]
Hyaluronic Acid	Natural	Yes	Enzymatic degradation	Biocompatible, promotes cell migration	Limited mechanical strength	[110]
Polyurethane	Synthetic	Yes	Hydrolytic or enzymatic	Elastic, tunable properties	Potential toxicity	[111]

enable rapid initial drug release, which is beneficial for applications requiring immediate therapeutic effects, such as antimicrobial coatings.[101] Conversely, core loading strategies, where drugs are entrapped within the polymer matrix, provide prolonged release with minimal burst effects, making them suitable for sustained anticancer drug delivery.[102]

Polymeric nanocarriers intended for NO delivery are synthesized through various strategies including incorporation of NO donors during carrier formation or affix them afterward via surface modifications. Encapsulation during fabrication often employs emulsion-based techniques such as oil-in-water (O/W) or water-in-oil-in-water (W/O/W) methods where the NO donor is mixed with polymer solution prior to droplet formation, followed by solvent evaporation under stirring, sonication, or homogenization to yield NO-loaded nanoparticles. Alternatively, post loading includes conjugation of donors (S-nitrosothiols, diazeniumdiolates) onto functional groups of polymer scaffold, or blending of donor doped inorganic nanoparticles into polymer matrices. Polymer molecular weight, donor to polymer ratio, surfactant type, and polymer concentration influence entrapment efficiency, loading capacity, release rate, and stability of these nanoparticles.[112,113]

Due to highly reactive and transient nature of NO, confirming its successful loading within delivery systems requires highly sensitive and selective analytical techniques. Among widely adopted methods are chemiluminescence analysis, which provides quantitative assessment of NO release flux in real time, electrochemical sensors, capable of detecting NO via electrooxidation or electroreduction with modifications to enhance selectivity; and the Griess assay, which allows indirect quantification through nitrite measurement in oxygenated environments. In addition, fluorometric approaches employing diaminofluorescein (DAF) and diaminoanthraquinone (DAA)-based probes facilitate in vitro and in vivo imaging of NO release by exploiting photoinduced electron transfer (PET) quenching mechanisms. These methodologies are routinely utilized not only to verify NO encapsulation within nanocarriers but also to monitor its release kinetics, stability, and bioavailability, which are critical parameters in evaluating therapeutic performance of NO-delivery platforms.[15]

Recent advancements in polymer-based drug delivery emphasize hybrid systems that combine the biocompatibility of natural polymers with the tunability of synthetic ones.[114,115] Stimuli-responsive platforms, including light-, redox-, and ultrasound-triggered systems, are gaining traction for precision medicine applications. Furthermore, 3D printing technology is enabling the fabrication of personalized drug-loaded implants with complex geometries, paving the way for patient-specific therapies.[116]

Nanotechnology Approaches for NO Delivery

NO is crucial in various physiological processes, including vasodilation, immune response, and wound healing.[37] However, its gaseous nature and short half-life pose significant delivery challenges. Nanotechnology offers promising solutions by enabling controlled NO release through diverse nanocarriers and hybrid systems.[117,118] Nanoscale delivery systems nanospheres, nanogels, dendrimers, and micelles have been extensively explored for NO storage and controlled release.[119] Nanospheres, typically composed of polymers like PLGA or silica, encapsulate NO donors (S-nitrosothiols, N-diazeniumdiolates) within their core, providing sustained release kinetics.[120,121] Nanogels, with their hydrophilic and highly tunable networks, allow for high NO payloads and stimuli-responsive release, particularly in response to pH or enzymatic triggers.[119] Dendrimers, with their well-defined branched structures, enable precise NO donor conjugation at terminal functional groups, facilitating high loading efficiency and targeted delivery.[122,123] Polymeric micelles, formed from amphiphilic block copolymers, can solubilize hydrophobic NO donors in their core while maintaining biocompatibility, making them suitable for systemic applications.[124,125]

Recent studies have demonstrated the efficacy of these nanocarriers in enhancing NO bioavailability while minimizing off-target effects. To further improve NO delivery efficiency, hybrid nanosystems combining polymers with inorganic materials have been developed. Polymer-ceramic composites, such as mesoporous silica nanoparticles coated with NO-releasing polymers, leverage the high surface area of ceramics for increased NO donor loading while utilizing polymers for controlled release.[126] Polymer-metal hybrids, including gold or silver nanoparticles functionalized with NO-donating ligands, exploit the plasmonic properties of metals for light-triggered NO release, enabling spatiotemporal control.[77] These hybrid systems not only enhance NO stability but also integrate additional functionalities of imaging capabilities or synergistic therapeutic effects.[127] For instance, NO-releasing polymer-coated iron oxide nanoparticles have been explored for combined NO delivery and magnetic resonance imaging (MRI), highlighting their potential in theranostic applications.[128] Recent studies have also highlighted the potential of NO-releasing nanocomposites in combination therapies, where NO synergizes with chemotherapy, photodynamic therapy, or immunotherapy to enhance treatment

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efficacy.[128] These advancements underscore the versatility of nanotechnology in overcoming the challenges associated with NO delivery and expanding its therapeutic applications.[129]

Beyond NO-release systems, recent developments provide critical insights which reinforce the importance of material diversity in bone regeneration. Studies on PCL/bioactive glass or PCL/Fe_3O_4@ZIF-8 nanocomposites fabricated via 3D printing have demonstrated enhanced osteogenic size or differentiation of stem cells, antibacterial activity, and significant in vivo bone formation in infected defect models.[130] GelMA, PCL nanofibers and bioactive glass have yielded higher bone volume/tissue volume (BV/TV) metrics in rat cranial defects relative to controls.[131] Membranes of PCL/LAP nanosilicate blends exhibit favorable immunomodulation (M2 macrophage polarization), osteogenesis, and in vivo repair efficacy in periodontal and calvarial defect models.[132]

Polymer-NO Nanoformulations in Preclinical Bone Regeneration Models

The therapeutic potential of NO-releasing polymer nanoformulations for bone regeneration has been rigorously demonstrated across multiple preclinical models.[77] Recent advances in controlled delivery systems have enabled precise modulation of osteogenic responses through tailored NO release kinetics and scaffold integration strategies.[133]

In vitro Findings: Cellular Responses and Osteogenic Potential

NO is a gaseous signaling molecule known for its multifaceted biological functions, including regulation of inflammation, vascular tone, and bone remodeling. Due to its ultra-short half-life and high reactivity, delivering NO in a controlled and sustained manner poses a significant challenge. In typical experimental studies, PLGA (poly(lactic-co-glycolic acid)) nanoparticles are used as carriers for NO donors like BPEI/NONOates.[134] These are incorporated through nanoprecipitation or emulsion techniques, ensuring encapsulation efficiency and controlled release. In vitro cell culture experiments are conducted on pre-osteoblasts and macrophages to assess cytocompatibility, osteogenic differentiation (via alkaline phosphatase activity, calcium deposition), and polarization responses using qPCR and immunocytochemistry.[134,135] In vitro studies have consistently shown that polymeric NO delivery systems do not exhibit significant cytotoxicity at therapeutic concentrations.[15] On the contrary, NO-releasing PLGA nanoparticles promote proliferation and migration of osteoprogenitor cells. Moreover, these formulations influence macrophage polarization, favoring an M2-like, anti-inflammatory phenotype.[12,14] This shift supports a regenerative microenvironment conducive to bone formation.

Enhanced proliferation and osteogenic differentiation in MC3T3-E1 pre-osteoblasts cultured with NO-releasing polymeric scaffolds.[136] The scaffolds also induced VEGF expression and angiogenesis-related signaling, key factors in osteogenesis.[69,137] Controlled release of NO has demonstrated a significant impact on osteoblast differentiation markers such as alkaline phosphatase (ALP), osteocalcin, and Runx2.[88,138] Studies have shown elevated ALP activity and matrix mineralization following treatment with NO-loaded nanoparticles. A study using PLGA-based NO nanocarriers observed sustained NO release over several days, with a concomitant increase in calcium deposition and osteogenic gene expression.[88,139,140] These findings underline the role of NO not only as an anti-inflammatory agent but also as a direct promoter of osteogenic pathways.[141] Emerging in vitro studies on hybrid NO delivery systems integrating ZnO within polymeric scaffolds have demonstrated valuable outcomes in bone tissue engineering. These systems enhance endothelial cell migration and upregulate VEGF, promote osteogenic differentiation under co-culture conditions, and offer advancement in functionally integrated approaches for bone regeneration.[88]

In vivo Studies: Animal Models of Bone Repair

The translational potential of these systems has been validated in various animal models of bone regeneration (Table 2). A particularly compelling study in rat critical-sized calvarial defects, achieving greater bone volume compared to controls by micro-CT analysis.[142] Histological evaluation revealed complete bridging of 5-mm defects by week 12, accompanied by a 3.2-fold increase in neovascularization as demonstrated through CD31 immunohistochemistry.[143] For more complex regeneration scenarios requiring structural support, an advanced composite system combining poly(ε-caprolactone) nanofibers with NO-donating dendrimers.[144] In a rabbit femoral condyle model, this approach achieved defect filling compared to in controls through histomorphometric analysis, with restored mechanical strength reaching of native bone.[145] Molecular analysis revealed significant upregulation of both VEGF and BMP-2, suggesting the system's ability to simultaneously promote angiogenesis and osteogenesis.[146]

Table 2 Overview of Clinically Evaluated Polymer-Based Drug Delivery Systems for Bone Regeneration

Product	Key Component	Clinical Application	Efficacy	Limitations	References
OP-1	rhBMP-7 + collagen + CMC	Tibial fractures, nonunions, spine	Accelerated healing (6.3 vs 9.0 months in tibial fractures)	FDA approved; proven safety and effectiveness	[147]
INFUSE® Bone Graft	rhBMP-2 (1.5 mg/mL) + ACS	Spinal fusion (ACDF)	Higher fusion rates across all rhBMP-2 dose levels	Increased risk of dysphagia and wound infections	[148]
INFUSE® Bone Graft	rhBMP-2 (1.5 mg/mL) + ACS	Alveolar ridge augmentation after tooth extraction	Bone formation doubled compared to placebo	Dose-dependent response observed	[149]
INFUSE® Bone Graft	rhBMP-2 (1.5 mg/mL) + ACS	Open tibial fractures	Slight 12% improvement at 13 weeks; no difference at 20 weeks	Comparable to soft-tissue management over time	[150]
INFUSE® Bone Graft	rhBMP-2 (0.5–1.5 mg/mL) + ACS	MRONJ (medication-related osteonecrosis of the jaw)	Complete bone regeneration in mandibular defects within 3 months	Studied at both low and high rhBMP-2 doses	[151]
InductOS®	rhBMP-2 formulation	Orthopedic and dental bone defects (general use)	Similar clinical applications as INFUSE®	Marketed mainly in Europe	[150]
AUGMENT®	rhPDGF-BB + β-TCP-collagen	Ankle and hindfoot fusions	Fusion time reduced (14.3 vs 19.7 weeks); 91% success rate	Superior to autograft in terms of recovery and comfort	[152]
AUGMENT®	rhPDGF-BB + β-TCP-collagen	Arthrodesis of ankle/ hindfoot	Outcomes equivalent to autograft with less pain and morbidity	Supported by RCT and cohort studies	[153]

Bioinspired scaffold of PLGA/magnesium-modified hydroxyapatite/extracellular matrix composite, further functionalized with ZnO/ALN/BMP-2 nanoparticles exhibits robust repair in osteoporotic rat calvarial defects. NO release was confirmed by NO analyzer and DAF-FM fluorescence imaging. Compared to controls, treated group shown higher bone volume/tissue volume (BV/TV), bone mineral density (BMD), vascularization (VV/TV), and elevated expression of angiogenic and osteogenic genes (ALP, RUNX2, OCN). These in vivo findings illustrate integrating NO donors into multifunctional scaffold can restore bone (and vascular) architecture under osteoporotic conditions.[88]

Scaffold and Implant Integration

The successful integration of scaffolds and implants into host bone tissue is a critical determinant of the overall efficacy of bone regeneration therapies. NO-releasing polymer-based nanoformulations, when incorporated into scaffold systems (Figure 4), can significantly enhance the biological performance and integration of implants.[121] Scaffold integration begins with the appropriate selection of biomaterials. Biocompatible materials such as titanium, hydroxyapatite, poly(lactic-co-glycolic acid) (PLGA), and bioactive ceramics have shown excellent results in facilitating osteointegration.[154] The architecture of the scaffold—particularly its porosity and surface topology plays a key role in supporting cellular attachment, proliferation, and vascular infiltration.[155] Ideal scaffolds mimic the extracellular matrix (ECM) and allow for nutrient diffusion and waste removal while providing mechanical support.[156] Surface modification of scaffolds with bioactive molecules like growth factors (BMP-2), peptides (RGD sequences), or NO donors enhances their biofunctionality. NO release has been shown to stimulate osteoblast differentiation and angiogenesis while also modulating local immune responses. This bioactivity fosters a favorable microenvironment at the implant site, accelerating tissue regeneration and reducing the risk of fibrous tissue formation.[156] Mechanical interlocking between the scaffold and host bone is essential for

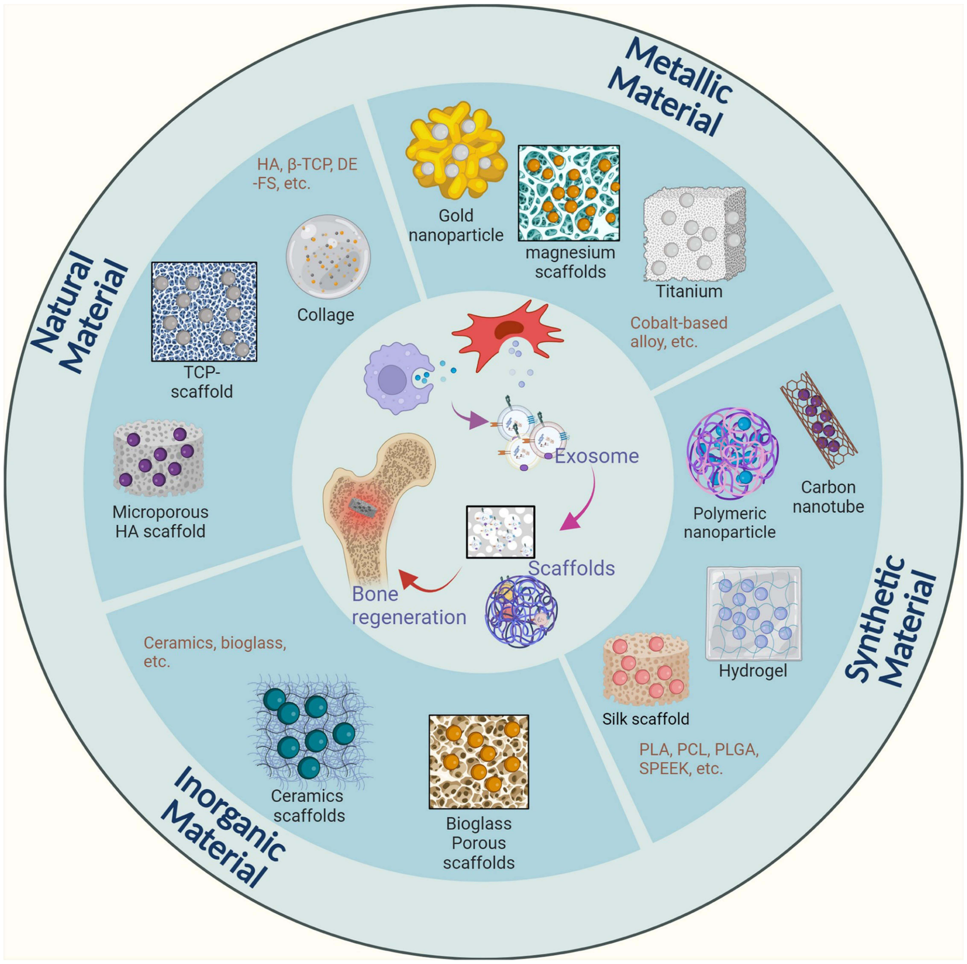

Figure 4 Exosome-based scaffolds combined with NO delivery offer bone regeneration without relying on transplanted cells. Exosomes are incorporated into scaffolds that release nitric oxide in a controlled manner to encourages new bone growth and the formation of blood vessels. This strategy not only promotes healing through enhanced cellular communication and angiogenesis but also helps regulate inflammation. Reproduced with permission from Deng L, Liu Y, Wu Q, et al. Exosomes to exosome-functionalized scaffolds: a novel approach to stimulate bone regeneration. Stem Cell Research & Therapy. 15(1):407. 2024, Springer Nature.[159]

long-term implant stability. (Figure 4) This can be achieved by designing scaffolds with interconnected porosity and appropriate roughness, enabling deep bone ingrowth and tight integration. Studies have demonstrated that scaffolds with pore sizes between 100–400 μm are optimal for promoting osteoconduction and capillary infiltration.[157,158]

Limitations, Toxicity & Regulatory Concerns

The clinical translation of polymer-based NO delivery systems is hindered by multiple limitations related to toxicity, material degradation, and regulatory inconsistency. Though PLGA is FDA-approved, its acidic degradation byproducts can significantly lower local pH, triggering pro-inflammatory responses and impairing osteoblast function.[160] Research indicates that such acidic environments may promote M1 macrophage polarization and increased neutrophil infiltration.[161] Similarly, chitosan degradation into glucosamine oligomers has been shown to activate TLR4-mediated inflammatory pathways when concentrations exceed physiological thresholds, raising concerns for chronic inflammation.[162] In addition, NO itself has a narrow therapeutic window; sustained delivery beyond optimal levels can result in mitochondrial dysfunction, DNA damage, and apoptosis of osteogenic cells.[163] High-output NO donors have been associated with the formation of peroxynitrite ($ONOO^-$). These cytotoxic species aggravates oxidative stress and has been linked to increased bone resorption in diabetic models.[164] Compounding these issues are manufacturing and regulatory barriers. Residual catalysts from synthesis, such as tin in polycaprolactone systems, have been observed to accumulate in hepatic tissue, necessitating stringent purification.[165] Moreover, variability in release kinetics, poor reproducibility, and inconsistent preclinical testing protocols remain significant challenges. Lack of standardized protocols International Organization for Standardization (ISO) or American Society for Testing and Materials (ASTM) protocols and limited Good Laboratory Practice (GLP)-compliant toxicity evaluations further hinder progress.[166]

Future Directions and Emerging Trends

The next generation of NO-releasing polymer systems is moving towards advanced material designs, multimodal delivery strategies, and precision manufacturing to address current limitations in bone regeneration. Emerging smart nanoresponsive platforms are focusing on stimuli-triggered NO release, enabling spatiotemporal control (Table 3). For instance, poly(β-amino ester)-based nanogels exhibit a significant increase in NO release under acidic conditions (pH 6.0), which is ideal for targeting infected or inflammatory bone defects.[167] Similarly, light-responsive systems incorporating upconversion nanoparticles (UCNPs) allow near-infrared (NIR) light-triggered NO liberation with rapid response times, facilitating non-invasive, on-demand therapy.[72] Enzyme-cleavable linkers, such as matrix metalloproteinase (MMP)-sensitive peptides, further enhance specificity.[168] Recent studies have shown that these systems can achieve higher NO release in osteoporotic bone compared to healthy tissue due to elevated protease activity.

Table 3 Emerging Strategies for Nitric Oxide-Based Polymeric Delivery Systems in Bone Tissue Engineering. This Table Summarizes Recent Innovations Including Hybrid Platforms, Smart Scaffolds, and Personalized Systems, Highlighting Their Design Approach, Therapeutic Advantages, and Translational Challenges

Strategy	Description	Advantages	Challenges	References
Hydrogel-Nanoparticle Hybrid Systems	Integration of nanoparticles like GO, nHAp, or cerium oxide into hydrogels for controlled NO release.	Enhanced mechanical properties, sustained NO release, and improved osteogenic activity.	Complex fabrication processes and potential cytotoxicity of nanoparticles.	[169]
3D/4D Printed Smart Scaffolds	Utilization of shape memory polymers (SMPs) in 3D/4D printing to create scaffolds that respond to environmental stimuli.	Dynamic adaptation to defect sites, improved cell infiltration, and localized NO release.	Limited long-term stability and scalability of SMP-based scaffolds.	[170]
Dual Therapy Systems	Combination of NO with growth factors or antibiotics in a single delivery system.	Synergistic effects enhancing bone regeneration and preventing infection.	Challenges in co-delivery and stability of multiple agents.	[171]
3D Bioprinted Scaffolds with NO Release	Fabrication of scaffolds using 3D bioprinting techniques to incorporate NO-releasing materials.	Precise control over scaffold architecture and NO release kinetics.	High production costs and technical complexities.	[66]
Smart Responsive Delivery Systems	Development of NO delivery systems responsive to pH, temperature, or enzymatic activity.	Targeted and controlled NO release at defect sites.	Design complexity and potential for incomplete release profiles.	[172]
Personalized 3D-Printed NO Scaffolds	Customization of scaffolds based on patient-specific defect geometries using 3D printing.	Enhanced fit and function, leading to improved healing outcomes.	Time-consuming design processes and regulatory hurdles.	[173]
NO-Loaded Nanocomposite Scaffolds	Incorporation of NO donors into nanocomposite materials for sustained release.	Improved mechanical strength and prolonged NO release.	Potential for burst release and inconsistent dosing.	[174]
Vascularization-Enhancing NO Delivery	Use of NO delivery systems to promote angiogenesis in bone defects.	Improved blood supply and nutrient delivery to healing tissues.	Risk of excessive angiogenesis leading to abnormal tissue formation.	[175]

Conclusion

Polymer-based NO delivery systems represent a transformative frontier in bone regeneration, bridging the gap between advanced biomaterials engineering and targeted therapeutic intervention. These systems have demonstrated compelling preclinical efficacy by promoting osteogenesis, enhancing angiogenesis, and mitigating infection through localized, sustained NO release. By integrating NO with osteoinductive cues and responsive release mechanisms, these platforms offer precise spatiotemporal control aligned with the dynamic requirements of bone healing. However, their translation into clinical practice demands progress on multiple fronts. Critical challenges include manufacturing scalability, ensuring batch-to-batch consistency, and the development of standardized regulatory frameworks tailored to combination products. Furthermore, long-term safety, immunological compatibility, and efficacy must be rigorously validated in large-animal and disease-specific models. Recent advancements in additive manufacturing, AI-driven formulation design, and real-time biosensing are accelerating the evolution of these systems toward patient-specific, precision-guided therapies. With the convergence of material innovation, computational modeling, and personalized medicine, polymer-NO platforms are well-positioned to redefine the therapeutic landscape of bone repair. Their success will hinge not only on scientific innovation but also on regulatory clarity, interdisciplinary collaboration, and sustained clinical investment. If these align, the next decade could witness the transition of polymer-based NO delivery systems from bench to bedside, offering a sophisticated, responsive, and highly effective alternative to conventional bone grafting techniques.

Data Sharing Statement

This is a review article, and all relevant information is provided in the article.

Ethical Approval and Consent to Participate

This is a review paper and does not involve direct research on humans or animals.

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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. All the authors listed meet the criteria for authorship as per the ICMJE guidelines, read the final manuscript and agree to publish this work.

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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