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REVIEW

Engineering the Bone Microenvironment with Nanocomposite Scaffolds: From Mechanistic Insights to Clinical Translation

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Abstract

Purpose of Review. Bone regeneration is governed by the coordinated interaction of mechanical, electrical, biochemical, vascular, and immune signals within the bone microenvironment. This review examines how nanocomposite scaffolds can be engineered to reproduce selected regulatory functions of the bone microenvironment through the integration of piezoelectric, electroconductive, and biopolymeric components. It also discusses the contribution of biomimetic three-dimensional (3D) models, nanotoxicology, and regulatory science to scaffold development and clinical translation. **Recent Findings**. Current evidence indicates that regenerative performance emerges from the integration of complementary physicochemical functions rather than from the properties of individual materials. Piezoelectric, conductive, and biopolymeric components can modulate electromechanical cues and cell–matrix interactions, although their individual biological contributions remain difficult to isolate. Evidence for osteogenic activity comes predominantly from preclinical systems; vascular integration, disease-specific responses, and durable repair are less established. Biomimetic 3D models, including hydrogels, multicellular spheroids, bioprinted constructs, and microphysiological systems, offer controlled settings for mechanistic investigation, but their clinical predictive validity remains to be established. **Summary.** The main challenge is to determine which scaffold properties reproduce relevant bone microenvironment functions under physiological and pathological conditions. Future research should relate scaffold physicochemical properties to biological function using reproducible

preclinical frameworks that integrate material characterization, mechanistic cell biology, biomimetic models, nanotoxicology, and regulatory considerations.

Keywords: bone microenvironment; nanocomposite scaffolds; microenvironment engineering; mechanotransduction; osteoimmunomodulation; biomimetic models

Introduction

Bone regeneration is regulated by the local microenvironment, where mechanical loading, extracellular matrix (ECM) organization, vascular architecture, immune signaling, and biochemical gradients operate as an integrated network that determines how cells respond to injury and coordinate tissue repair [1–3]. Consequently, regenerative strategies targeting individual molecular pathways cannot fully reproduce the physicochemical conditions required for physiological bone healing. This perspective shifts bone tissue engineering from material-centered design toward microenvironment engineering.

The ECM provides the structural and functional framework for this regulatory system. Composed primarily of type I collagen and carbonated hydroxyapatite, its hierarchical organization provides mechanical support while coupling physical deformation to cellular signaling through collagen piezoelectricity [4,5]. Adhesive interactions, soluble mediators, and matrix architecture further shape these electromechanical signals, which regulate osteogenesis across spatial and temporal scales.

Bone remodeling depends on continuous communication among osteocytes, osteoblasts, osteoclasts, endothelial cells, and immune cells. Osteocytes detect mechanical strain and fluid shear stress within the lacunocanalicular network, initiating signaling pathways that coordinate bone formation, resorption, angiogenesis, and matrix turnover [6–8].

These interactions change during repair. The early hematoma creates a transient hypoxic and inflammatory compartment, whereas tissue stabilization, vascular invasion, progenitor recruitment, and matrix mineralization develop over overlapping phases [9,10]. Their spatial distribution is also heterogeneous: well-perfused defect margins and relatively hypoxic regions experience different oxygen gradients, cell populations, and mechanical constraints [1,8,9]. A stimulus that favors early cell recruitment may not have the same effect during callus mineralization or remodeling. Scaffold studies should therefore distinguish the location and timing of biological responses rather than interpret a single osteogenic endpoint as evidence of coordinated repair.

Antiresorptive and bone-forming agents modulate selected aspects of bone metabolism, while growth factor delivery targets specific osteogenic pathways [11–14]. These approaches do

not independently restore the mechanical, vascular, and immune environment of an injured site, motivating biomaterial strategies directed at complementary microenvironmental functions.

Scaffold development has therefore shifted toward constructs that combine electromechanical activity with ECM-like interfaces. Piezoelectric ceramics, conductive nanomaterials, and biopolymers can be integrated into three-dimensional (3D) constructs, while biomimetic cultures allow controlled investigation of their biological responses and safety.

This review assesses the mechanisms, experimental evidence, and translational limitations of nanocomposite scaffolds designed to modulate the bone microenvironment, including the contribution of biomimetic models and nanotoxicological assessment.

Physical Cues and Cellular Signaling in the Bone Microenvironment

Mechanical loading regulates bone adaptation through matrix deformation, fluid transport, and cell mechanotransduction [7,15]. The ECM provides structural support and mediates physical signaling, providing a reference for scaffold development.

Mechanical deformation of collagen fibers generates local electrical potentials through intrinsic piezoelectricity, whereas nanoscale hydroxyapatite contributes to charge-related phenomena under specific structural conditions [16]. At the same time, fluid flow within the lacunocanalicular network generates streaming potentials that reinforce mechanically induced signaling [17]. Together, these processes establish an electrochemical microenvironment in which matrix architecture and mechanical loading regulate cellular behavior.

Osteocytes are the principal mechanosensors of bone, although osteoblasts and mesenchymal stromal cells also contribute to mechanotransduction. Among the molecular mediators involved, PIEZO1 has emerged as a central regulator by coupling mechanical cues to intracellular Ca^{2+} signaling. PIEZO1-mediated signaling interacts with Yes-associated protein (YAP), transcriptional coactivator with PDZ-binding motif (TAZ), Wnt/β-catenin, and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathways. These pathways contribute to bone remodeling, including regulation of the osteoprotegerin (OPG)/receptor activator of nuclear factor κB ligand (RANKL) axis [7]. However, the effects of these pathways depend on cell type and differentiation stage. Hu et al. demonstrated that PIEZO1 deficiency in late osteoblasts and osteocytes reduces YAP activity, increases Wnt/β-catenin signaling, and impairs osteocyte

maturation and dendritic network formation. The study further identified the YAP–CCN1/2–Src signaling axis as a regulator of osteocyte dendrite development [18]. PIEZO1 therefore links mechanical inputs to coordinated bone remodeling and provides a mechanistic basis for scaffolds designed to reproduce physiological electromechanical signaling. However, the relative contribution of PIEZO1-dependent signaling, integrin activation, and matrix-mediated mechanotransduction remains incompletely understood because these pathways are tightly interconnected and frequently activated simultaneously, particularly within microenvironments.

Mechanical loading also modulates biochemical signaling within the bone microenvironment through mechanosensitive pathways and matrix-dependent regulation of growth factor activity [1,15]. In particular, mechanical forces can activate latent transforming growth factor-β (TGF-β) and influence Wnt/β-catenin signaling, both of which contribute to the regulation of bone remodeling [16].

Vascular and osteogenic responses interact, but their spatial and temporal relationships depend on the skeletal site and mode of repair. Endothelial–osteogenic communication involves vascular endothelial growth factor (VEGF), Notch, and angiocrine signaling, with mechanosensitive pathways contributing to vascular regulation [7,8]. In long-bone fractures, vascular ingrowth supports callus maturation and endochondral ossification [9,19]. In contrast, Bixel et al. demonstrated that vascular sprouting preceded the collective invasion of periosteum-derived osteoprogenitors during calvarial bone regeneration in mice [20]. These differences highlight the importance of considering the skeletal site and mode of repair when interpreting experimental findings, particularly when extrapolating results from calvarial defects to load-bearing long-bone injuries.

Scaffold design must account for the timing and location of mechanical, biochemical, vascular, and immune signals, and their magnitude. The following sections distinguish experimentally demonstrated material effects from mechanisms inferred from cellular markers or physicochemical measurements.

Immune Regulation and Osteoimmunomodulation in the Bone Microenvironment

Immune cells participate in bone remodeling through reciprocal communication with skeletal cells rather than acting solely as responders to injury. This concept, known as osteoimmunology, recognizes that the immune and skeletal systems share the bone marrow microenvironment, common regulatory mediators, and interconnected cellular networks that coordinate immune function and bone remodeling [21].

Bone injury rapidly initiates an inflammatory response characterized by the release of cytokines, bone morphogenetic proteins (BMPs), and tumor necrosis factor-α (TNF-α), leading to the recruitment of innate immune cells [9]. This early inflammatory phase establishes the molecular conditions required for tissue repair. As healing progresses, adaptive immune cells influence osteoblast differentiation, osteoclast activity, angiogenesis, and matrix remodeling according to the evolving inflammatory milieu [10,21]. Successful regeneration therefore depends on the spatial and temporal coordination of inflammatory responses rather than on suppressing inflammation itself.

Macrophages influence debris clearance, osteoprogenitor recruitment, vascular remodeling, and inflammatory resolution. The M1-like/M2-like classification describes experimental activation states but does not fully represent the heterogeneous macrophage populations present during bone repair [22]. Early inflammatory activity is required for tissue repair, whereas persistent proinflammatory signaling may hinder later osteogenesis and promote fibrosis [10]. Adaptive immune cells further refine these responses. Type 1 helper T (Th1) lymphocytes sustain inflammatory signaling through cytokines such as interferon-γ (IFN-γ) and TNF-α, whereas forkhead box P3 $(FoxP3)^{+}$ regulatory T cells suppress excessive immune activation, inhibit osteoclastogenesis, and establish a permissive environment for osteogenesis [21].

Following implantation, scaffolds first interact with plasma proteins and immune cells before contacting osteogenic cells, making the early host response a major determinant of long-term regenerative outcomes. Protein adsorption initiates the foreign body response, whereas material composition, surface chemistry, nanotopography, pore architecture, wettability, surface charge, and nanoparticle properties collectively influence immune-cell activation and subsequent osseointegration [23,24]. Although these physicochemical parameters act in concert, their individual contributions to immune regulation remain incompletely understood, limiting the development of predictive design criteria for immunomodulatory biomaterials.

Accordingly, osteoimmunomodulation should be regarded as a fundamental design principle rather than a secondary property of bone scaffolds. Immune regulation complements mechanical and electrical signaling as a functional axis of bone microenvironment engineering, extending scaffold design beyond structural replacement toward active regulation of tissue repair. Studies of piezoelectric biomaterials and immunomodulatory scaffolds, including hydrogels, have reported changes in macrophage-associated markers, cytokine expression, and osteogenic responses [4,21]. These scaffolds aim to modulate endogenous immune responses associated with vascularization, osteogenesis, and ECM remodeling.

A single measurement of M1-like or M2-like markers cannot establish regenerative osteoimmunomodulation. Scaffold studies should instead consider cytokine trajectories, immune–stromal interactions, vascular organization, and bone formation at successive stages of repair. This distinction becomes particularly important in aging and chronic inflammatory disease.

Bone Regeneration in Pathological Microenvironments

In osteoporosis, deterioration of trabecular architecture and altered bone remodeling change the mechanical environment of implanted scaffolds and may affect host-cell responses. Osteocyte signaling, osteoblast–osteoclast balance, and the availability of progenitor cells vary according to disease etiology and treatment history [7,11–13]. Osteogenic responses observed in healthy cultures may therefore differ from those occurring in osteoporotic defects. Scaffold evaluation should consider the mechanical demands of the defect, integration with compromised host bone, and cellular differentiation markers [25].

Aging affects bone repair through progenitor senescence, altered immune–stromal communication, and impaired revascularization [26,27]. In aged mice, macrophage-derived grancalcin promoted skeletal progenitor senescence and delayed fracture healing [26]. This finding implicates macrophage signaling in age-related impairment of bone repair. Whether targeting this pathway improves scaffold integration in humans remains unknown. Responses to mechanical and electrical stimulation should therefore be investigated using age-defined primary cells and appropriate animal models. Aging and osteoporosis should be investigated separately because they affect the bone microenvironment differently.

Chronic inflammation and impaired perfusion further complicate bone repair. Diabetes is associated with oxidative stress, altered matrix glycation, persistent immune activation, and microvascular dysfunction, which can compromise the regenerative microenvironment [28]. A meta-analysis reported higher odds of delayed union or nonunion among patients with diabetes (odds ratio 1.61; 95% confidence interval 1.44–1.81), although heterogeneity limits predictions for individual patients [29]. Under these conditions, acellular scaffolds depend on the recruitment and activity of endogenous cells, which may already be impaired. Cell-seeded constructs face additional limitations related to cell survival and nutrient transport. The ability of either strategy to improve regeneration must therefore be evaluated in disease-specific experimental models.

These pathological conditions affect scaffold–host interactions and the interpretation of regenerative endpoints. Mineralization observed in healthy preosteoblast cultures does not establish durable bone repair in aged, inflamed, or poorly perfused tissues. Disease-associated changes in the bone microenvironment should therefore inform scaffold design and preclinical evaluation.

Piezoelectric Nanomaterials and Scaffolds

Piezoelectric scaffolds are designed to reproduce the endogenous electromechanical coupling involved in physiological bone remodeling. These materials convert mechanical deformation into localized electrical signals, allowing mechanical loading to stimulate cellular responses without an external electrical power source (**Figure 1A**). Their biological performance depends on the magnitude, spatial distribution, and duration of the electrical signals generated under physiological loading conditions.

The piezoelectric coefficient (d_{33}) describes the electrical response of a material to mechanical loading, although its biological relevance depends on how effectively the generated charges influence surrounding cells [5]. Barium titanate ($BaTiO_3$) is among the most extensively investigated piezoelectric biomaterials because of its ferroelectric properties, chemical stability, and capacity to maintain surface polarization [4,30]. At the nanoscale, phase instability and surface disorder may compromise its ferroelectric performance. Nanoparticle dispersion, aggregation state, orientation, and interfacial interactions within the scaffold matrix also influence electromechanical behavior [31]. Structured coatings, composite formulations, and controlled

polarization strategies have been investigated to preserve piezoelectric performance and mechanical integrity. Potassium sodium niobate (KNN) and zinc oxide (ZnO) are lead-free piezoelectric materials investigated for bone regeneration. ZnO-containing composites have also demonstrated antibacterial activity [4]. Nanoparticle composition and physicochemical properties further influence cellular responses in bone tissue engineering [32].

Piezoelectric materials can influence cellular responses through electrical stimulation and changes at the material–cell interface. Surface polarization affects protein adsorption, ionic organization, and early cell adhesion. Mechanically generated electrical signals can promote Ca^{2+} influx and activate integrin–focal adhesion kinase (FAK)–extracellular signal-regulated kinase (ERK), BMP/Smad, and Wnt/β-catenin signaling pathways associated with runt-related transcription factor 2 (RUNX2)-mediated osteogenic differentiation [4,33]. Piezoelectric stimulation has also been associated with changes in macrophage polarization and inflammatory cytokine production, suggesting interactions between electromechanical signaling and immune regulation. However, the contributions of electrical stimulation, protein adsorption, immune modulation, and scaffold architecture remain difficult to distinguish. These processes are closely interconnected and are rarely investigated under independently controlled conditions.

Scaffold architecture also influences biological performance. Gelatin methacryloyl (GelMA) has been investigated as a hydrogel matrix for nanoparticle-containing scaffolds in bone tissue engineering [32]. Poly(vinylidene fluoride) (PVDF) exhibits piezoelectric properties when processed to favor polar crystalline phases, although its hydrophobicity and limited biodegradability remain challenges for biomedical applications [33]. Electrospinning enables the fabrication of fibrous piezoelectric scaffolds, with fiber morphology and orientation influencing their electromechanical properties [33]. Li et al. used digital light processing (DLP) 3D printing to fabricate porous $BaTiO_3$/β-TCP scaffolds with a defined architecture [34]. These fabrication methods provide different levels of control over scaffold organization, which can influence the distribution and persistence of electrical signals within the regenerative microenvironment.

Osteogenic responses reported for piezoelectric scaffolds vary across experimental systems, limiting direct comparison among studies [4,32,33]. Li et al. evaluated 3D-printed $BaTiO_3$/β-TCP scaffolds and observed an increase in d_{33} accompanied by reduced compressive strength as the $BaTiO_3$ fraction increased [34]. Bhaskar et al. investigated MC3T3-E1 preosteoblasts cultured on PVDF/$BaTiO_3$ composites containing multi-walled carbon nanotubes

(MWCNTs) under externally applied electrical stimulation [35]. Their experimental design does not allow the effects of intrinsic piezoelectricity to be distinguished from those of external stimulation or concurrent changes in conductivity and surface roughness. Increased alkaline phosphatase activity and mineral deposition demonstrate osteogenic responses under the tested conditions, but do not establish the mechanisms responsible for these effects. Composition-matched controls and direct measurements of electrical output in hydrated scaffolds under defined mechanical loading are needed to determine the specific contribution of piezoelectricity to biological performance.

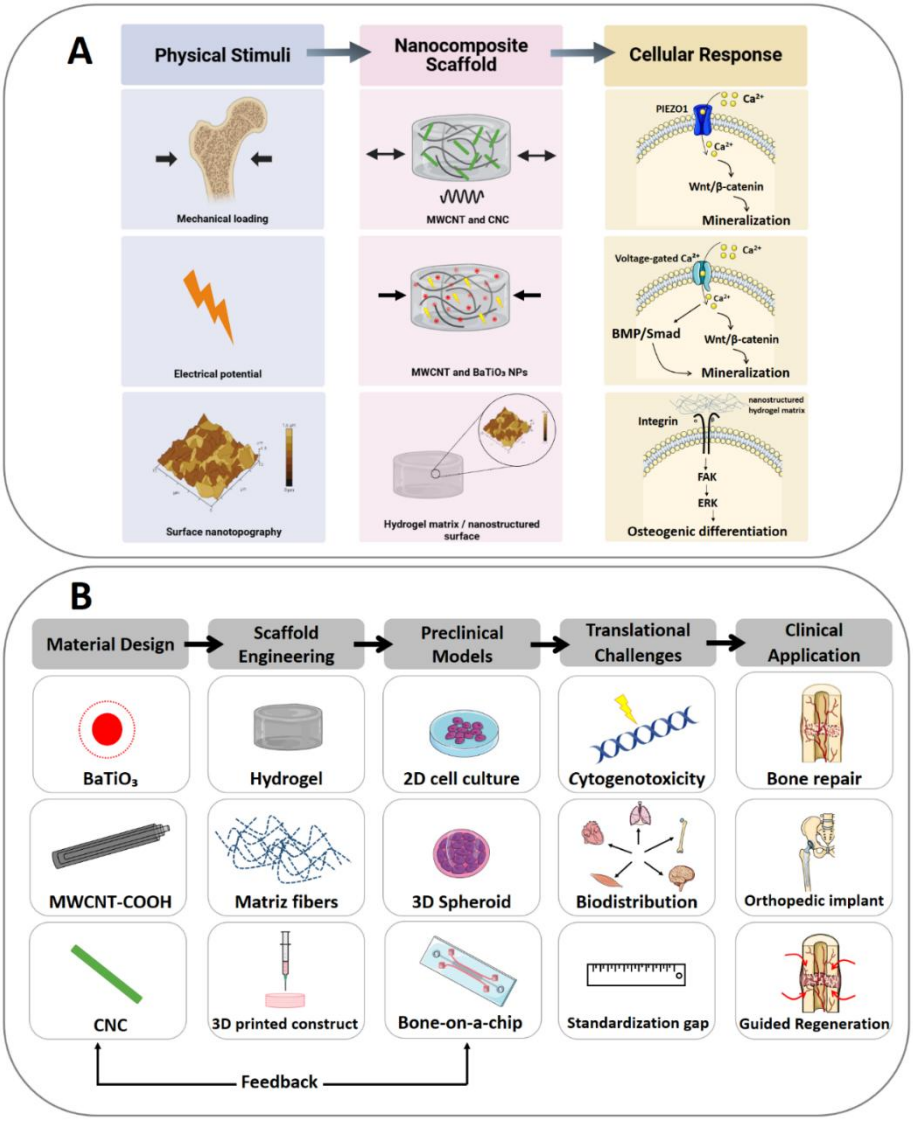


Figure 1. Conceptual framework for bone microenvironment engineering with nanocomposite scaffolds. (A) Schematic representation of the proposed contributions of piezoelectric, electroconductive,

and biopolymeric components to physicochemical signaling, cell–material interactions, and osteogenic responses. The pathways illustrated are based on findings from different experimental systems and have not necessarily been validated together within a single scaffold formulation. **(B)** Proposed translational framework linking material design and scaffold engineering to biomimetic preclinical models, nanotoxicological evaluation, and potential clinical application. The feedback loop illustrates iterative refinement of scaffold design based on mechanistic and preclinical evidence.

Electroconductive Carbon-Based Nanomaterials

Conductive phases may redistribute electrical potentials within electroactive composites, but their effect on signal propagation in hydrated, ionically conductive scaffolds is rarely measured directly. MWCNT networks simultaneously modify stiffness, surface roughness, and protein adsorption. Improved osteogenic responses after adding conductive fillers therefore cannot be attributed to long-range electrical communication without direct electrical measurements and controls that separate conductivity from accompanying structural changes.

Carbon nanotubes (CNTs) are among the most extensively investigated conductive nanomaterials for bone tissue engineering. MWCNTs, in particular, combine high electrical conductivity with mechanical robustness and readily form interconnected conductive networks at relatively low filler concentrations [36]. Their biological activity, however, depends less on conductivity itself than on the way conductive interfaces influence cell–material interactions.

Carboxylation, hydroxylation, and amination can improve CNT dispersion and modify cell–material interactions, including protein adsorption and integrin-mediated adhesion, although their biological effects depend on surface chemistry and scaffold composition [36]. In a separate study, Esmaeili et al. reported improved hydrophilicity, calcium deposition, and osteogenic markers in MG-63 cells cultured on electrospun polyhydroxybutyrate (PHB)/zein scaffolds containing MWCNTs. However, these findings do not isolate the effects of CNT surface functionalization [37]. Carboxylate groups also promote hydroxyapatite nucleation, directly linking surface chemistry to biomineralization [38]. These findings indicate that CNT surface chemistry and scaffold composition influence cell–material interactions and osteogenic responses, although the relative contributions of electrical conductivity, surface chemistry, and matrix properties have not been established independently.

Conductive nanomaterials also participate in immune regulation within the regenerative microenvironment. Nitrogen-doped CNTs modulate macrophage activation and cytokine production, influencing subsequent osteoblast and osteoclast activity during bone remodeling [39]. These findings suggest that CNT-containing scaffolds may influence immune responses through mechanisms beyond charge transport.

Graphene oxide (GO) and reduced graphene oxide (rGO) share a two-dimensional (2D) architecture but differ in conductivity, oxidation state, and interfacial chemistry. The higher oxygen content of GO disrupts electrical transport relative to more extensively reduced graphene materials. Osteogenic responses involving PI3K/Akt and Wnt/β-catenin have been reported for graphene-derived materials, but these effects cannot be assigned to conductivity independently of surface chemistry [40]. Unlike interconnected CNT networks, graphene-derived sheets exhibit electrical behavior that depends on their reduction state, orientation, and dispersion within the matrix. Whether these architectural differences translate into distinct biological responses remains uncertain because electrical conductivity, nanotopography, surface chemistry, and scaffold architecture are rarely examined independently.

Conductive carbon nanomaterials are commonly incorporated into electrospun fibers or hydrogel matrices, where they improve charge distribution while reinforcing scaffold architecture and promoting osteogenic differentiation [36,41]. Conductive substrates have also been associated with endothelial differentiation and angiogenic signaling, suggesting that electrical communication contributes to the coordinated regulation of vascularization and bone regeneration [42].

Despite these advantages, biological performance remains strongly influenced by concentration, aspect ratio, purity, dispersion state, and surface functionalization. Inadequate processing may increase oxidative stress and inflammatory activation, offsetting regenerative benefits. Physicochemical characterization under relevant exposure conditions, standardized fabrication protocols, and long-term safety assessment therefore remain essential for establishing reproducible structure–property–function relationships and supporting clinical translation.

Biopolymeric Nanostructures: Cellulose Nanocrystals and Beyond

Electromechanical signals are interpreted by cells through the ECM, which functions as an active regulator rather than a passive structural support. Its nanoscale architecture, adhesive ligands, mineralization sites, and viscoelastic properties continuously influence mechanotransduction, cytoskeletal organization, and osteogenic commitment. Biopolymeric nanostructures are therefore essential components of bone microenvironment engineering because they define the physicochemical context in which mechanical, electrical, and biochemical signals are integrated.

Nanocellulose illustrates this concept. Cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial nanocellulose (BNC) differ in morphology and supramolecular organization but share high mechanical strength and abundant hydroxyl groups suitable for chemical modification [43,44]. Beyond reinforcing scaffold mechanics, these materials regulate protein adsorption, cell adhesion, and ECM organization, shaping how cells perceive and respond to their surrounding microenvironment.

Each nanocellulose format contributes distinct structural functions. CNCs increase stiffness and mechanical reinforcement, CNFs form hydrated fibrillar networks that improve viscoelasticity, whereas BNC generates highly pure nanofibrous membranes and hydrogels suitable for cell encapsulation and soft tissue processing [45]. Biological performance therefore depends less on the specific nanocellulose type than on matching its structural organization to the intended regenerative application.

Surface chemistry further modulates these interactions. Hydroxyl, carboxyl, aldehyde, and sulfate groups influence hydrophilicity, protein adsorption, ionic interactions, and mineral nucleation, whereas functionalization strategies, including 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation, peptide conjugation, and phosphonate modification, alter surface charge, ligand presentation, and degradation behavior [43,46,47]. Carboxylated and sulfated nanocellulose materials also promote hydroxyapatite nucleation through electrostatic interactions with Ca^{2+} ions, directly linking surface chemistry to biomineralization.

Nanoscale organization is equally important. Fibrillar architecture regulates integrin clustering, focal adhesion assembly, cytoskeletal organization, and downstream mechanotransduction, while adsorption of ECM proteins amplifies osteogenic signaling. Reports of osteogenesis in nanocellulose-containing scaffolds without exogenous growth factors suggest that stiffness, topography, and interface chemistry act together to regulate tissue formation [46].

At the same time, the relative contribution of matrix mechanics, surface chemistry, and nanoscale architecture remains difficult to resolve because these parameters are commonly modified simultaneously.

Differences in experimental design further complicate comparisons of nanocellulose bioactivity. The nanofiber/chitosan scaffold investigated by Zanette et al. combines more than one matrix component, whereas Rashad et al. evaluated nanocellulose bearing different functional groups using rat mesenchymal stromal cells [46,47]. These studies address distinct material variables and biological contexts. Their findings cannot establish a general ranking of cellulose formats or demonstrate that osteogenic markers predict vascular integration and long-term repair. Comparisons should control cell source, scaffold composition, and the physical conditions under which differentiation was assessed.

Biopolymers provide distinct biological interfaces: chitosan contributes amine-rich chemistry, collagen preserves cell-adhesive motifs, silk fibroin offers adjustable mechanics and degradation, and alginate permits hydrogel fabrication but may require biofunctionalization [44,45]. Their selection should follow the required cell–matrix interactions rather than assume functional equivalence.

Combined with electroactive phases, biopolymeric matrices support material dispersion and mineralization while defining the interface perceived by cells. Their function depends on component compatibility and processing, not simply the presence of conductive or piezoelectric fillers.

Batch variability, sterilization, dispersion, and interfacial compatibility remain obstacles to reproducible biopolymeric scaffolds. More fundamentally, material processing must preserve the matrix properties through which cells interpret electromechanical cues.

Nanocomposite Scaffolds: Integrating Functions to Reconstruct the Bone Microenvironment

Nanocomposite scaffolds combine complementary physicochemical functions to address the interactions among mechanical loading, matrix organization, vascular responses, and immune signaling (**Table 1**). Their intended advantage is functional integration, which requires direct evidence rather than inference from the behavior of isolated components.

Piezoelectric ceramics generate localized electrical potentials during mechanical loading but exhibit limited charge propagation. Electroconductive carbon nanomaterials facilitate charge transport without intrinsically coupling mechanics to electricity, whereas biopolymeric matrices recreate ECM architecture and biochemical interfaces but generally lack electroactivity [5,30,36]. Integrating these materials allows complementary functions to operate simultaneously, potentially combining functions that individual material classes provide separately.

Scaffold performance cannot be predicted from composition alone. Material compatibility, nanoparticle dispersion, interfacial interactions, degradation kinetics, and architectural organization collectively determine how cells perceive and respond to implanted constructs [33,35]. Biological responses arise from the interplay among these material properties rather than from the contribution of any individual component. Scaffold design therefore focuses on reconstructing biological functions instead of simply combining functional materials.

The proposed coupling of piezoelectric particles and conductive fillers illustrates both the design rationale and the evidential gap. $BaTiO_3$ can generate polarization under deformation, and MWCNT networks can increase composite conductivity. Whether these properties yield spatially propagated, cell-relevant electrical signals under physiological loading has not been established for every scaffold formulation [35]. Electrical effects must therefore be distinguished from changes in matrix mechanics, surface chemistry, and local cell adhesion.

Studies of $BaTiO_3$-containing composites report osteogenic responses, but differ in polymer or ceramic matrix, filler fraction, stimulation regimen, and experimental cell model [33,35]. Increased alkaline phosphatase activity in MC3T3-E1 cells represents an early differentiation endpoint, not proof of vascular integration or mechanical restoration of a bone defect. Comparisons with single-component controls may remain confounded when filler addition simultaneously alters porosity, stiffness, and protein adsorption. Factorial experiments that independently vary piezoelectric and conductive components, while matching architecture and applied loading, are needed to determine whether their effects are additive, interactive, or absent.

Biopolymeric matrices further strengthen this integration by recreating the extracellular environment in which electromechanical signals are interpreted. Collagen, GelMA, and related hydrogels provide cell-adhesion motifs, hierarchical organization, nutrient transport, and compatibility with additive manufacturing while supporting homogeneous distribution of electroactive components [30]. Printable bioinks also enable precise control of scaffold geometry,

porosity, and composition, allowing material organization to be tailored to both defect morphology and biological function [48].

Scaffold design must balance electromechanical properties, degradation, architecture, manufacturability, and biological compatibility within the intended clinical setting. Increasing one property without measuring its effect on the others may compromise performance.

Table 1. Functional comparison of representative nanomaterials used for bone microenvironment engineering.

Nanomaterial	Primary Function in the Bone Microenvironment	Representative Experimental Models	Major Biological Mechanisms	Current Limitation	Reference
BaTiO3 (Piezoelectric)	Electromechanical stimulation	MC3T3-E1; hBMSCs; MSCs	Ca^{2+} influx; BMP/Smad; Wnt/β-catenin; integrin–FAK–ERK	Charge propagation; polarization stability	[4,30,33]
MWCNT-COOH (Electroconductive)	Electrical signal propagation	MC3T3-E1; hBMSCs; MG-63	Integrin–FAK–ERK; PI3K/Akt; cytoskeletal mechanotransduction	Dispersion; long-term biocompatibility	[36,37,41]
Graphene/rGO (Electroconductive)	Conductive biointerface modulation	MSCs; osteoblasts	PI3K/Akt; Wnt/β-catenin; integrin-mediated mechanotransduction	Limited structure–property–function understanding	[40]
Cellulose nanocrystals (Biopolymeric)	Extracellular matrix regulation	hBMSCs; SHED; primary osteoblasts	Integrin-mediated adhesion; focal adhesion signaling; Ca^{2+}-dependent osteogenesis	Source variability; degradation control	[43,46,47]

Table 1 abbreviations: hBMSCs = human bone marrow-derived mesenchymal stromal cells. MSCs = mesenchymal stromal cells. SHED = stem cells from human exfoliated deciduous teeth. MWCNT-COOH = carboxylated multi-walled carbon nanotubes.

Biomimetic 3D Models as Mechanistic and Preclinical Tools

Reconstructing the bone microenvironment requires experimental systems capable of reproducing the spatial and physicochemical complexity that regulates cell behavior in vivo. Conventional 2D cultures remain valuable for mechanistic studies but cannot reproduce the structural, mechanical, and biochemical context encountered by cells within living tissues. They also lack vascular perfusion and immune dynamics needed to infer tissue-level repair from isolated cellular endpoints. Biomimetic 3D models can reproduce selected structural and physicochemical features that are absent from conventional 2D cultures, providing a more representative setting for investigating cell–material interactions and tissue regeneration [49].

Hydrogels are among the most versatile 3D platforms because they permit independent control of stiffness, porosity, degradability, ligand density, and viscoelasticity, parameters that directly regulate mechanotransduction and osteogenic differentiation [50]. Zauchner et al. demonstrated that matrix metalloproteinase-degradable microporous poly(ethylene glycol) (PEG) hydrogels support the spreading of human mesenchymal stromal cells and osteoblasts, the formation of interconnected 3D cell networks, and ECMx remodeling [51]. However, these findings do not establish vascular infiltration or the regenerative performance of these hydrogels following implantation. Integration with microfluidic platforms further introduces controlled nutrient gradients, interstitial flow, and shear stress, extending the physiological relevance of these systems [52].

Spheroid models complement hydrogels by emphasizing multicellular organization rather than extracellular architecture. Gradients of oxygen, metabolites, and signaling molecules develop spontaneously within spheroids, reproducing conditions found in poorly vascularized regions of healing bone and often enhancing osteogenic differentiation compared with monolayer cultures [49]. Embedding spheroids within hydrogels combines multicellular communication with controlled matrix mechanics, enabling simultaneous investigation of cell–cell and cell–matrix interactions.

These characteristics are particularly relevant for evaluating nanocomposite scaffolds. 3D matrices introduce diffusion barriers, local nanoparticle retention, and concentration gradients that strongly influence transport, bioavailability, and biological responses but are largely absent from conventional 2D cultures. Hydrogel- and spheroid-based systems therefore offer more

physiologically relevant platforms for assessing nanoparticle penetration, spatial distribution, long-term cellular responses, and dose-dependent effects while revealing how the engineered microenvironment influences nanomaterial behavior.

Bioprinting further expands these capabilities by enabling precise spatial organization of cells, biomaterials, and nanomaterials within predefined architectures. Constructs incorporating osteogenic and electroactive components reproduce aspects of cortical and trabecular organization while supporting long-term cell viability and differentiation [53,54]. Control over porosity, stiffness, biochemical composition, and perfusable vascular compartments also makes it possible to investigate how local microenvironmental heterogeneity influences scaffold performance.

An important advantage of biomimetic 3D models is the ability to manipulate individual components of the bone microenvironment under controlled conditions. This experimental flexibility allows scaffold architecture, material properties, and physicochemical cues to be investigated systematically, providing mechanistic insight rather than simply comparing scaffold performance.

Such control is particularly valuable for establishing predictive structure–property–function relationships under physiologically relevant conditions. By linking material design with microenvironmental regulation and biological outcome, biomimetic models help bridge the gap between material engineering and translational prediction (**Table 2**).

These models should not be treated as interchangeable. Microporous hydrogels support human bone-cell network formation and matrix remodeling [51], whereas spheroids emphasize multicellular interactions and diffusion-dependent gradients [49]. Bone-on-chip platforms incorporate controlled mechanical loading but impose different constraints on cell maturation and throughput [52]. Variation in species, donor age, osteogenic supplementation, oxygenation, loading, and endpoint timing can explain divergent results without implying that one scaffold chemistry is intrinsically superior. Reporting these parameters is essential for meaningful comparison.

Differences in cell sources, hydrogel composition, and analytical methods limit reproducibility across 3D models [49]. Standardization of matrix processing, quantitative imaging, and outcome reporting is necessary before such models can be used consistently to compare scaffold performance or improve preclinical prediction [51].

Table 2. Functional comparison of biomimetic 3D models for investigating the bone microenvironment.

Model	Primary Microenvironment Feature Reproduced	Primary Research Application	Major Limitation	Reference
Osteoblastic spheroids	Cell-cell communication and physiological oxygen/nutrient gradients	Osteogenesis; nanoparticle penetration; cell-cell interactions	Lack of vascularization; limited size	[49,54]
Hydrogel-based 3D cultures	ECM mechanics, cell-matrix interactions, and mechanotransduction	Cell-material interactions; scaffold evaluation	Batch variability; limited structural complexity	[30,51]
Bioprinted scaffold	Tissue architecture and spatial heterogeneity	Patient-specific scaffold design; spatial organization of cells and biomaterials	Cost; printing resolution; bioink limitations	[53,54]
Co-culture models	Osteoblast–endothelial communication and osteocyte–osteoblast–osteoclast interactions	Osteogenic–vascular crosstalk; multicellular bone remodeling	Experimental complexity; reproducibility	[8,52]
Microfluidic bone-on-chip systems	Dynamic perfusion, vascular interfaces, and biochemical gradients	Mechanistic studies; nanotoxicology; drug testing	Technical complexity; low throughput	[55,56]

Nanotoxicological Considerations

Nanomaterial properties that influence regenerative performance also affect toxicological responses. Particle size, surface chemistry, morphology, and aggregation govern protein adsorption, cellular uptake, inflammatory signaling, and persistence. Scaffold development therefore requires biological safety and regenerative efficacy to be evaluated under related exposure conditions.

This relationship becomes particularly important when interpreting conventional cytotoxicity assays. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) release assays are useful screening methods but assess only selected aspects of cellular responses. Nanoparticles may adsorb assay reagents, interfere with optical detection, or catalyze redox reactions, leading to misleading experimental outcomes [57]. A more informative evaluation combines cytotoxicity, oxidative stress, inflammatory signaling, and genotoxicity endpoints within the same experimental strategy.

Genotoxicity is especially relevant because long-term bone regeneration depends on preserving genomic stability during osteogenic differentiation. The comet assay and micronucleus (MN) assay remain the principal methods for detecting DNA strand breaks and chromosomal damage, respectively [58]. These alterations arise through interconnected mechanisms involving oxidative stress, mitochondrial dysfunction, inflammatory signaling, and disruption of DNA repair pathways [58,59].

Interpreting nanomaterial-induced biological responses also requires consideration of concentration-dependent effects. Biological responses observed at lower concentrations may not persist at higher exposure levels, where increased toxicity has been reported [59,60]. Nominal mass concentration alone therefore does not adequately describe biological exposure. Delivered dose, particle number, and available surface area represent complementary exposure metrics and should be interpreted alongside characterization of particle size, morphology, aggregation state, surface chemistry, and functionalization [59,60]. Integrating these variables into quantitative relationships between scaffold properties and biological responses will improve interpretation of both regenerative performance and toxicological outcomes.

Long-term biodistribution remains another important source of uncertainty. Tissue distribution, clearance, and persistence depend on hydrodynamic size, aggregation, dissolution,

protein adsorption, and the biological context [59–61]. The fate of particles released from degrading scaffolds cannot be inferred directly from studies of freely dispersed nanoparticles. Chronic retention, degradation products, and cumulative biological effects remain insufficiently characterized for many nanomaterials incorporated into regenerative scaffolds. Evidence from $BaTiO_3$ nanoparticles, MWCNTs, and CNCs likewise indicates that biological responses are governed primarily by physicochemical attributes, including particle size, surface chemistry, functionalization, and aggregation state, rather than by material class alone [44,58,60,61]. Material composition by itself is therefore an unreliable predictor of biological performance.

Regulatory Gaps and Standardization Challenges

Advances in bone microenvironment engineering have outpaced the regulatory frameworks available to evaluate nanomaterial-enabled regenerative products. Most current standards were developed for conventional bulk materials and do not fully address the complexity introduced by nanoscale systems. Material attributes such as aggregation, protein corona formation, dissolution kinetics, surface reactivity, and intracellular localization strongly influence biological behavior but remain inconsistently incorporated into regulatory testing strategies [58].

This limitation is reflected in the lack of harmonized approaches for nanotoxicological assessment. Differences in dose metrics, dispersion protocols, exposure conditions, sterilization procedures, and endpoint selection reduce reproducibility and complicate comparison across studies. Exposure itself may be reported as mass concentration, particle number, or available surface area, each describing a different aspect of cell–material interactions [59]. Such variability also hinders efforts to establish quantitative relationships between material properties and biological responses that can support scaffold optimization and regulatory decision-making.

The challenge extends beyond methodological standardization. Bone regeneration is a dynamic process in which scaffold degradation, particle release, chronic inflammation, immune regulation, vascular remodeling, and ECM turnover occur simultaneously. Current testing strategies rarely capture these interconnected events, limiting their ability to predict long-term biological performance and clinical outcomes.

These limitations have stimulated the development of New Approach Methodologies (NAMs) and integrated assessment strategies that combine advanced in vitro models, high-content

imaging, omics-based measurements, and computational approaches [57,58]. In parallel, safer-by-design strategies seek to reduce nanomaterial hazards by modifying physicochemical properties during material development [59]. Collectively, these approaches generate mechanistic evidence linking material characteristics to biological responses across multiple levels of biological organization. Their broader implementation in regulatory practice, however, still depends on harmonized protocols, interlaboratory reproducibility, and validated acceptance criteria.

Within bone microenvironment engineering, standardization serves a broader purpose than improving experimental consistency. Reproducible approaches to material characterization, biological evaluation, and data reporting are necessary to determine how specific scaffold features regulate osteogenesis, immune modulation, angiogenesis, and long-term safety. International reference materials, minimum reporting standards, and tiered testing strategies would facilitate meaningful comparison across laboratories while strengthening the evidence base required for regulatory evaluation.

Translational Gaps and Future Perspectives

Although major advances have been made in the development of nanocomposite scaffolds, their clinical translation remains limited. Physicochemical properties measured under controlled experimental conditions do not necessarily predict the biological responses that emerge within the bone microenvironment, where mechanical loading, immune regulation, vascular remodeling, and ECM turnover occur simultaneously. Consequently, conventional in vitro and biomimetic 3D models reproduce only selected features of human bone repair [49,51]. These limitations complicate the translation of experimental scaffold performance to clinically complex bone defects [25].

A distinction is necessary between evidence for a biological mechanism and evidence for a therapeutic scaffold effect. PIEZO1-mediated mechanotransduction, inflammatory regulation, and the importance of vascular supply are supported by mechanistic studies [7,9,10]. In contrast, the proposal that a particular $BaTiO_3$/MWCNT formulation restores physiological electrical signaling, or that in vitro macrophage markers predict clinical osseointegration, remains insufficiently validated. The $BaTiO_3$ scaffold studies discussed in this review include experiments using MC3T3-E1 preosteoblasts [34,35], whereas other mechanistic evidence derives from mouse

models of calvarial regeneration and age-related fracture healing [20,26]. These experimental approaches address different biological questions and cannot independently establish scaffold performance in human load-bearing defects.

Bridging this gap requires experimental systems that more faithfully reproduce the interactions governing tissue regeneration. Variability in cell source, differentiation state, scaffold fabrication, sterilization, and culture conditions continues to limit reproducibility, whereas many commonly used assays remain focused on short-term viability or early osteogenic markers. Chronic inflammation, nanoparticle release, scaffold degradation, and cumulative cytogenotoxicity therefore remain insufficiently represented during preclinical evaluation. Biomimetic 3D models and organotypic systems reproduce several of these processes under controlled conditions but have not yet been routinely incorporated into standardized testing strategies.

Translation-oriented studies should compare healthy and compromised hosts, including age-defined, osteoporotic, chronically inflamed, or poorly perfused models. Primary human cells from multiple donors and disease-matched co-cultures may reveal responses masked by immortalized cell lines. *In vivo* assessments should extend beyond early bone volume to perfusion, host immune responses, functional mechanical strength, scaffold degradation, and long-term particle retention. Findings from calvarial regeneration models [20] should not be directly extrapolated to load-bearing defects, which impose additional mechanical and clinical constraints [25].

Nanomaterial-specific risk assessment remains necessary because particle aggregation, protein corona formation, and degradation products may alter biological exposure [57,58]. NAMs, including advanced in vitro testing and computational analyses, can complement conventional evidence but require validation before routine regulatory application.

Scaffold engineering, biological evaluation, and nanotoxicology often proceed as separate stages. Iterative experiments that use biological outcomes to refine the material formulation provide a more informative development strategy (**Figure 1B**).

A research priority is to establish quantitative structure–property–function relationships linking material characteristics to biological outcome. Particle size, stiffness, electrical conductivity, polarization state, degradation profile, surface chemistry, and scaffold architecture collectively influence cell behavior, immune modulation, angiogenesis, and ECM remodeling, yet

their individual and combined contributions remain incompletely understood [59,60]. Biomimetic experimental models, quantitative material characterization, and computational analysis provide complementary tools for investigating these interactions under physiologically relevant conditions.

The same mechanisms inform the choice between cell-based and acellular approaches. Acellular scaffolds depend on recruitment and activity of endogenous cells, which may be limited in aging or ischemic defects. Cell-seeded constructs introduce an osteogenic or vascular cell population but add concerns about donor variability, survival after implantation, nutrient transport, and manufacturing consistency. Neither strategy is universally preferable. The choice should account for defect mechanics, vascular access, and host pathology [25,27,50].

Translation to clinical production requires scalable manufacturing, reproducible scaffold properties, and quality control across production batches [25]. Nanomaterial-specific safety evaluation requires physicochemical characterization and toxicological testing appropriate to the intended exposure [58,59]. Translational evaluation should inform scaffold design throughout development.

Clinical translation requires evidence that complementary scaffold functions improve repair under the conditions in which the material will be implanted. Disease-matched models, quantitative characterization under physiological loading, and long-term comparisons with clinically relevant controls are necessary to test this premise. Greater scaffold complexity should not be assumed to produce greater regenerative benefit.

Conclusions

Bone regeneration is regulated by coordinated mechanical, electrical, biochemical, vascular, and immune interactions within the bone microenvironment. In this context, nanocomposite scaffolds are designed to provide structural support and modulate physicochemical cues involved in tissue repair.

The evidence reviewed here indicates that scaffold performance arises from the integration of architecture, surface chemistry, electromechanical behavior, and ECM interactions rather than from any individual material property. This perspective shifts scaffold engineering from optimizing isolated materials toward reconstructing the functional organization of the bone microenvironment.

Despite substantial progress, clinical translation remains limited by the predictive capacity of current preclinical models, incomplete understanding of long-term host responses, and insufficient integration of material characterization, mechanistic biology, nanotoxicology, and regulatory science.

Future research should separate mechanical, electrical, immune, and matrix-mediated effects in comparative experiments and determine whether their integration improves repair in pathological bone microenvironments. Human disease-relevant models and long-term studies in load-bearing defects are needed to test functional benefit, degradation, and safety. The goal is to identify measurable scaffold properties that predict durable tissue regeneration, not to assume that increasing material complexity improves clinical outcomes.

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  Provides the conceptual framework for interpreting bone regeneration as an integrated process governed by coordinated cellular communication and biomechanical and biochemical signaling within the bone microenvironment.

- Hu YJ, Wu X, Wang F, et al. Piezo1-mediated mechanotransduction controls osteocyte maturation and dendrite development via a YAP-CCN-Src signaling axis. Nat Commun. 2025;16:10859. https://doi.org/10.1038/s41467-025-65636-9

  Provides direct mechanistic evidence that PIEZO1-mediated mechanotransduction regulates osteocyte maturation through the YAP–CCN–Src signaling axis, linking mechanical loading to bone remodeling.

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  Provides experimental evidence that synthetic microporous hydrogels recreate key features of the bone microenvironment, supporting the use of biomimetic 3D platforms for mechanistic studies and predictive preclinical evaluation of bone tissue engineering strategies.

- Li S, Shan Y, Chen J, et al. Piezoelectricity promotes 3D-printed BTO/β-TCP composite scaffolds with excellent osteogenic performance. ACS Appl Bio Mater. 2025;8(3):2204–2214. https://doi.org/10.1021/acsabm.4c01754

  Provides experimental evidence that 3D-printed $BaTiO_3$/β-TCP piezoelectric scaffolds enhance osteogenesis, illustrating how electromechanical properties can be rationally integrated into scaffold design.

- Bhaskar N, Kachappilly MC, Bhushan V, et al. Electrical field stimulated modulation of cell fate of pre-osteoblasts on PVDF/BT/MWCNT based electroactive biomaterials. J Biomed Mater Res A. 2023;111:340–353. https://doi.org/10.1002/jbm.a.37472

  Demonstrates that electroactive PVDF/$BaTiO_3$/MWCNT composite scaffolds enhance pre-osteoblast proliferation, migration, and osteogenic differentiation under electrical stimulation, illustrating the potential of multifunctional electroactive nanocomposites for bone tissue engineering.

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Summarizes the current challenges in nanomaterial genotoxicity assessment, highlighting the importance of physicochemical characterization, adapted OECD Test Guidelines, assay interference controls, and New Approach Methodologies for predictive nanosafety evaluation.

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