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Review Article

Application of Nanotechnology in Bone and Cartilage Tissue Engineering: A Comprehensive Review of Electrospun Nanofiber Scaffolds and Advanced Nanocomposites

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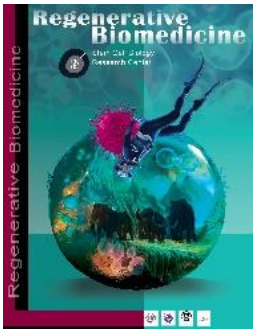
Abstract

Bone and cartilage defects resulting from trauma, degenerative diseases, and congenital abnormalities present significant clinical challenges due to limited intrinsic regenerative capacity. Nanotechnology offers transformative solutions by enabling the fabrication of biomimetic scaffolds that recapitulate the native extracellular matrix architecture at the nanoscale. This narrative review explores recent advances in nanotechnology-based scaffolds for bone and cartilage tissue engineering, with emphasis on electrospun nanofiber systems and advanced nanocomposites, synthesizing current knowledge and identifying future research directions. Electrospinning has emerged as the predominant technique for fabricating nanofibrous scaffolds, with hybrid approaches combining nanofibers with hydrogels (e.g., GelMA) showing enhanced cellular responses. Metal-organic frameworks, bioactive ceramic nanoparticles (hydroxyapatite, β-tricalcium phosphate), carbon-based nanomaterials (graphene, carbon nanotubes), and natural polymer nanofibers (collagen, gelatin, chitosan) demonstrate distinct advantages for bone regeneration. For cartilage engineering, stimulus-responsive systems and gradient scaffolds for osteochondral interface regeneration show particular promise. Surface functionalization strategies, including growth factor delivery and ionic modifications, significantly enhance bioactivity. In vivo studies confirm improved osseointegration and cartilage matrix deposition compared to conventional scaffolds. Nanotechnology-based scaffolds represent a paradigm shift in regenerative medicine, offering unprecedented control over scaffold architecture, mechanical properties, and biological functionality. While challenges remain in clinical translation, including scalability, long-term safety assessment, and regulatory pathways, the integration of machine learning-guided design and personalized medicine approaches promises to accelerate the development of next-generation tissue engineering solutions.

Keywords: Biomimetic materials, Bone tissue engineering, Cartilage regeneration, Electrospinning, Nanocomposites, Nanofiber scaffolds, Nanotechnology, Osteochondral interface



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Introduction

Bone and cartilage tissue defects resulting from trauma, degenerative diseases, and tumor resections represent significant clinical challenges affecting millions of individuals worldwide annually (1). While autografts remain the gold standard for bone regeneration, their limitation (including donor site morbidity, limited tissue availability, and variable quality) have driven intensive research into engineered alternatives (2). Cartilage tissue presents even greater therapeutic challenges due to its avascular, aneural nature and severely restricted intrinsic regenerative capacity (3).

Nanotechnology has transformed tissue engineering by enabling precise control over material properties at the nanoscale, replicating the structural and compositional complexity of native extracellular matrix (ECM) (4). The emergence of nanofabrication techniques, particularly electrospinning, has facilitated the development of biomimetic scaffolds with high surface-to-volume ratios, interconnected porosity, and tunable mechanical properties (5,6). These nanostructured systems provide microenvironments conducive to cell adhesion, proliferation, and lineage-specific differentiation, while enabling controlled delivery of bioactive molecules (7).

Metal-organic frameworks (MOFs) have emerged as versatile biomaterials for bone tissue engineering due to their tunable porous structures and multifunctional therapeutic potential (8). Bibliometric analysis indicates a marked increase in MOF-related bone research after 2022, reflecting a shift from basic material synthesis toward function-oriented and clinically motivated investigations (8). Emerging applications include oxidative stress modulation, angiogenic ion release, photothermal infection control, and corrosion-resistant magnesium alloy integration (5,8). However, as discussed in subsequent sections, critical questions regarding MOF toxicity, long-term stability, and in vivo behavior remain incompletely addressed.

Three-dimensional printing combined with nanomaterial incorporation enables hierarchical scaffold structures with site-specific mechanical and biological properties (9, 10). Nanomaterials such as nanoclay and carbon nanotubes are incorporated into three-dimensionally printed scaffolds to enhance bioactivity and conductivity (9). This systematic review analyzes 35 recent studies to provide a comprehensive and critically balanced overview of nanotechnology applications in bone and cartilage tissue engineering, including an assessment of translational barriers and future directions.

Methods

This narrative review synthesizes current knowledge on nanotechnology applications in bone and cartilage tissue engineering, with particular focus on electrospun nanofiber scaffolds and advanced nanocomposites. Literature was identified through searches of PubMed, Scopus, and Web of Science databases, prioritizing publications from January 2022 to March 2025 to capture recent advances.

Search terms included combinations of “nanotechnology”,“nanofiber”, “electrospinning” ,“nanofiber”, “nanocomposite”, “bone tissue engineering”, “cartilage regeneration” and related terms.

The review emphasizes original research articles, clinical trials, and comprehensive reviews that address nanofabrication techniques, scaffold characterization, biocompatibility, mechanical properties, cellular interactions, in vivo evaluation, and clinical translation potential. Studies were selected based on their contribution to understanding the design principles, performance characteristics, and translational challenges of nanotechnology-based scaffolds.

The synthesis integrates findings across multiple domains (from material composition and fabrication methods to preclinical validation and clinical applications) to provide a comprehensive overview of the field. Particular attention is given to comparing different nanomaterial platforms, identifying emerging trends such as stimulus-responsive systems and gradient scaffolds, and highlighting critical gaps that require further investigation for successful clinical translation.

Nanofabrication Techniques for Scaffold Development

Electrospinning Technology

Electrospinning remains the most widely employed technique for producing nanofiber matrices with properties including high surface-to-volume ratio, mechanical flexibility, small pore size, and ease of chemical functionalization (5). The technique has been applied across diverse biomedical fields including tissue engineering, wound dressings, vascular grafts, and drug delivery systems (4,6). Electrostatic forces draw polymer solutions into ultrafine fibers, with solvent selection critically influencing viscosity, electrostatic potential, and processing temperature (6).

For bone regeneration applications, electrospun scaffolds composed of synthetic polymers such as polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) have demonstrated favorable mechanical properties and controlled degradation profiles, as assessed by tensile testing and gravimetric degradation assays (10). Electrospun matrices promote cell proliferation and differentiation through nanoscale topographical cues that replicate natural ECM architecture, as confirmed by scanning electron microscopy (SEM), immunofluorescence labeling, and metabolic activity assays (11). Bioactive molecules, nanoparticles, and ceramic components can be incorporated directly into fiber matrices during fabrication, generating composite scaffolds with enhanced osteoconductive properties (2).

Recent advances include coaxial electrospinning for core-shell fiber architectures enabling sequential drug release, emulsion electrospinning for protein encapsulation, and multiscale electrospinning combining micro- and nanofibers to optimize cellular infiltration alongside structural integrity (3). Solvent systems for biopolymers (including gelatin, collagen, chitosan, and chitin) allow fabrication of hybrid scaffolds that combine inherent bioactivity with industrial processability (6).

Despite these advantages, electrospinning faces several technical challenges that impede clinical translation (3). The characteristically small pore sizes of electrospun mats (typically < 10 μm) restrict three-dimensional cell infiltration, limiting the colonization depth achievable without post-fabrication

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modification (11,6). Scaffold thickness is also constrained by fiber accumulation and charge-screening effects, making fabrication of constructs thicker than a few millimeters difficult without specialized collectors or post-processing (6). Batch-to-batch reproducibility is sensitive to environmental parameters (humidity, temperature) and solution viscosity variations, complicating GMP compliance (3). Scale-up from laboratory to industrial production introduces additional challenges related to fiber uniformity, multi-needle collector design, and throughput (2,6). These constraints must be explicitly addressed in translational development plans, and future studies should report reproducibility data and process capability metrics alongside biological outcomes (3).

Hybrid Fabrication Approaches

Combining multiple fabrication techniques has emerged as a strategy to overcome the individual limitations of each method (11). Hybrid scaffolds that integrate electrospun nanofibers with hydrogel matrices leverage structural integrity from the fibrous phase alongside the cell-permissive aqueous environment of the hydrogel phase (3). Gelatin methacryloyl (GelMA) hydrogels provide favorable hydration and tunable mechanical properties for tissue regeneration, while electrospun nanofibers enhance cellular adhesion and substrate stiffness (3,11). These hybrid systems show particular promise for structurally complex tissues including the dentin-pulp complex, periodontal ligament, and large bone defects (3). Characterization in such systems requires complementary mechanical testing (rheology, compressive modulus), porosity analysis (micro-computed tomography, mercury intrusion porosimetry), and in vitro bioassays to comprehensively establish structure-property-function relationship (11).

Nanomaterial Systems for Bone Tissue Engineering

Metal-Organic Frameworks (MOFs)

MOFs function as promising biomaterials for bone regeneration and bone disease management, serving as structural scaffolding components, drug carriers, and controllable sources of bioactive ions (1). Their high surface area, tunable pore sizes, and degradable metal-ligand coordination bonds enable controlled release of therapeutic agents and osteogenic ions (2). Current research emphasizes function-oriented investigations including osteoimmunomodulation, antibacterial activity, and angiogenic stimulation through zinc, copper, or magnesium ion release (6,8). While the functional versatility of MOFs is evident, several critical concerns warrant cautious interpretation of current findings. The majority of published evidence is derived from in vitro models, with limited and heterogeneous in vivo data available to confirm therapeutic efficacy and systemic safety (1,4). The cytotoxicity of MOF degradation products (particularly free metal ions and organic linkers) has not been systematically evaluated across the range of clinically relevant concentrations (2,5). Long-term structural stability under physiological conditions (pH variations, enzymatic milieu,

mechanical loading) remains poorly characterized (10). Additionally, immune responses to particulate or ion-releasing MOF materials have not been adequately studied in large-animal models (11,12). Future translational work must include standardized cytotoxicity panels (ISO 10993), degradation product profiling by mass spectrometry, and long-term in vivo biocompatibility studies before MOF-based scaffolds can be considered for clinical application (1,2).

Bioceramic Nanoparticles

Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) nanoparticles remain the most extensively studied bioceramic components for bone tissue engineering (6). When incorporated into polymer nanofibers, these ceramic particles significantly improve compressive and tensile strength (quantified through mechanical testing under physiological loading conditions) and provide osteoconductive surfaces promoting osteoblast adhesion, proliferation, and biomineralization, as confirmed by alkaline phosphatase activity assays, alizarin red staining, and quantitative real-time PCR for osteogenic marker genes (12). PCL scaffolds containing β-TCP nanoparticles demonstrate efficacy in replicating cartilage-bone interfaces, with the ceramic phase enhancing osteogenic differentiation while the polymer matrix maintains structural integrity (6,12). Hybrid scaffolds containing HA or β-TCP improve mechanical strength and are suitable for alveolar bone regeneration (6). The ceramic phase provides nucleation sites for mineral deposition while releasing calcium and phosphate ions that upregulate osteogenic gene expression (12).

Carbon-Based Nanomaterials

Graphene and graphene oxide-based conductive scaffolds show notable potential in bone tissue engineering, particularly for applications where electrical stimulation is intended to enhance osteogenesis or innervation (11). These materials promote neural differentiation and demonstrate high intrinsic mechanical strength, with an elastic modulus approaching 1 TPa, while offering surfaces amenable to bioactive molecule conjugation (9). The electrical conductivity of graphene-based materials may enhance osteogenic differentiation through modulation of cellular membrane potential and intracellular signaling (11).

The enthusiasm surrounding graphene-based scaffolds must be tempered by acknowledged limitations (2). Most supporting evidence is from in vitro studies, and in vivo biodistribution data remain limited and inconsistent (13). Graphene is largely non-biodegradable, raising concerns about long-term tissue accumulation and chronic inflammatory responses (2). Graphene oxide, while more hydrophilic and amenable to functionalization, can generate reactive oxygen species that induce oxidative stress at elevated concentrations, as demonstrated in multiple cell culture systems (2). Dose-response relationships and threshold toxicity concentrations have not been established across clinically relevant scaffold designs (2,14). The regulatory status of graphene-containing implants is unclear in most jurisdictions, and the absence of a defined degradation pathway substantially complicates translational planning. These factors substantially limit the near-term

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clinical applicability of graphene-based constructs, and all claims of efficacy must be explicitly qualified as primarily in vitro observations (2).

Natural Polymer Nanofibers

Natural polymers including collagen, gelatin, chitosan, and chitin provide inherent bioactivity, biocompatibility, and cell-instructive biodegradability (15,16). Gelatin-based electrospun nanofibers facilitate cell migration and support diffusion of nutrients, water, and oxygen, providing a permissive environment for cell growth (3). However, these materials typically exhibit rapid enzymatic degradation and insufficient mechanical strength for load-bearing applications, necessitating chemical crosslinking strategies (carbodiimide chemistry, glutaraldehyde treatment) or hybrid formulations with synthetic polymers. Crosslinking density must be carefully controlled, as excessive crosslinking may impair cell infiltration and reduce biocompatibility (5).

Nanomaterial Systems for Cartilage Tissue Engineering

Extracellular Matrix Composition and Mechanical Requirements

Cartilage is an avascular, aneural, alymphatic tissue with limited regenerative potential, composed predominantly of a hydrated collagen type II network, proteoglycans (primarily aggrecan), and a minor population of chondrocytes embedded within a dense ECM (17). The biochemical composition of the ECM varies across three zonal layers: the superficial zone (collagen II oriented parallel to the surface, high compressive stiffness, cells with flattened morphology), the middle zone (randomly oriented collagen, intermediate properties), and the deep zone (collagen oriented perpendicular to tidemark, high compressive modulus, hypertrophic chondrocytes) (16,17). Articular cartilage must sustain repetitive compressive forces of 3–10 times body weight and exhibits dynamic stiffness in the range of 0.5–1.5 MPa, properties that are challenging to replicate in engineered constructs (4).

Chondrocyte phenotype maintenance is a central challenge in cartilage tissue engineering: chondrocytes dedifferentiate rapidly in two-dimensional culture, losing expression of collagen type II and SOX9 while upregulating collagen type I, indicative of a fibrocartilaginous phenotype (17). Nanostructured scaffolds must therefore provide mechanical cues (substrate stiffness, topography), biochemical signals (TGF-β, insulin-like growth factor, dexamethasone), and three-dimensional confinement that collectively sustain chondrogenic identity (2). Low oxygen tension (2–5%) further supports chondrogenic differentiation and should be incorporated into in vitro evaluation protocols (17).

Fibrous Scaffolds Mimicking Cartilage ECM

Electrospun fibrous scaffolds composed of collagen, elastin, or synthetic analogs replicate the fibrous architecture of native cartilage ECM, providing structural frameworks that support chondrocyte attachment and matrix production (15). Aligned nanofibers can guide chondrocyte morphology and matrix deposition in an orientation-dependent manner, potentially replicating the organized architecture of

articular cartilage zones (17). These scaffolds have been employed in cartilage, skin, cardiovascular, and vascular tissue engineering, demonstrating versatility across tissue types with different mechanical requirements (5). Quantitative characterization by SEM, AFM surface roughness mapping, and nanoindentation provides essential structure-property data to guide scaffold optimization (2).

Stimulus-Responsive Systems

Smart nanomaterials that respond to physiological or externally applied stimuli enable dynamic control over therapeutic delivery and scaffold mechanical properties (5). PCL matrices loaded with insulin and β-glycerophosphate (β-GP) have been shown to promote chondrogenic differentiation of mesenchymal stem cells for cartilage regeneration, as assessed by glycosaminoglycan synthesis and type II collagen immunostaining (15). Temporally controlled growth factor presentation (matching natural developmental sequences) can be achieved through stimuli-responsive release systems triggered by pH, temperature, or enzymatic activity, offering advantages over bolus delivery strategies (18).

Gradient Scaffolds for the Osteochondral Interface

The osteochondral region presents distinct engineering challenges arising from its graded transition from hyaline cartilage through the calcified cartilage layer to subchondral bone, involving compositional, mechanical, and cellular gradients across a distance of only a few millimeters (12). Gradient scaffolds fabricated by controlled electrospinning, melt electrowriting, or three-dimensional printing enable zonally organized regeneration, providing cartilage-like layers for chondrogenesis in superficial zones and bone-like layers supporting vascularization and osteogenesis in deeper regions (10). In preclinical osteochondral defect models (rabbit, goat, porcine), gradient scaffolds demonstrate superior integration compared to homogeneous constructs, supporting zonal organization of hyaline cartilage transitioning to trabecular bone architecture (4).

PCL scaffolds with graded ceramic nanoparticle distributions mimic the natural cartilage-bone interface, enabling heterogeneous chondrogenesis while allowing nutrient supply and vascular ingrowth in deeper zones (6). Acknowledged challenges include insufficient mechanical performance of collagen-based constructs under physiological loading and limited hydrolytic stability of hyaluronic acid formulations for long-term cartilage applications (3). These shortcomings must be explicitly addressed in scaffold design strategies (9).

Surface Functionalization and Biofunctionalization

Chemical Modifications

Physical and chemical surface modifications of nanoparticles and nanofibers enhance specific interactions with host cells and surrounding tissue (9). Surface

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functionalization with adhesive peptides (RGD, IKVAV), growth factors, or ECM-mimetic molecules substantially improves cellular attachment, spreading, and downstream signaling (21). Heparin incorporation into nanoparticle carriers enables sustained, controlled release of heparin-binding growth factors for cartilage regeneration in arthritic conditions, demonstrating advantages in bioactivity retention compared to bolus administration (5,9). Characterization of functionalized surfaces by X-ray photoelectron spectroscopy (XPS) and FTIR confirms successful conjugation and quantifies surface density of bioactive moieties.

Growth Factor Delivery

Growth factors including BMP-2, BMP-7, TGF-β, and FGF are central regulators of stem cell differentiation toward osteogenic and chondrogenic lineages (9). BMP-7 specifically stimulates osteogenic commitment of mesenchymal stem cells via SMAD signaling pathways (7,9). Nanotechnology-based delivery systems maintain therapeutic concentrations at the defect site over days to weeks, substantially extending the effective half-life compared to bolus growth factor application and reducing the risk of heterotopic ossification and systemic side effects. Carriers serve as critically important platforms for localized delivery of bioactive molecules, improving scaffold bioactivity while limiting off-target effects (9).

Ionic Modifications

Bioactive ions (including magnesium, strontium, zinc, and silicon) can be incorporated into nanomaterial matrices and released in a controlled manner to elicit specific cellular responses. These ions modulate osteoblast and chondrocyte metabolic activity, support angiogenesis through endothelial cell activation, and provide antimicrobial effects through disruption of microbial membrane integrity (8). This multifunctionality positions ion-releasing nanomaterials as attractive components of next-generation scaffolds that integrate structural, biological, and antimicrobial functions.

Cellular Interactions and Biocompatibility

Osteoblast Response

Nanofiber scaffolds exert significant influence on osteoblast adhesion, cytoskeletal spreading, proliferation, and differentiation (16). Surface topography at the nanoscale modulates integrin clustering, focal adhesion kinase phosphorylation, and downstream MAPK and Rho/ROCK signaling pathways governing osteogenesis (11). Studies demonstrate that electrospun nanofibers with diameters approximating natural collagen fibrils (~100 nm) optimize initial osteoblast response, while larger fiber diameters (1–5 μm) support deeper cell infiltration at the cost of reduced surface area for initial adhesion (5,16). Key scaffold parameters affecting performance include gelatin concentration, crosslinking density, interconnected pore size, and type and density of biofunctional surface modifications

(6). Standard in vitro biocompatibility assays include MTT/WST-1 metabolic activity assays, DAPI nuclear staining, and alkaline phosphatase quantification.

Chondrocyte Interactions and Phenotype Maintenance

Chondrocytes require specific microenvironmental cues (including appropriate substrate stiffness, three-dimensional confinement, hypoxic conditions, and TGF-β supplementation) to maintain their differentiated phenotype and sustain synthesis of collagen type II and aggrecan-rich pericellular matrix (9,17). Nanofiber scaffolds supporting chondrocyte culture must balance structural integrity with sufficient macroporosity for nutrient diffusion, given the avascular nature of cartilage and the dependence of deep-zone chondrocytes on diffusion-mediated nutrition (3). Aligned nanofibers can guide cell morphology and matrix deposition orientation, potentially replicating the anisotropic architecture of articular cartilage (17). Long-term phenotype maintenance should be assessed by collagen type I/type II ratio (indicative of dedifferentiation), proteoglycan content by dimethylmethylene blue assay, and SOX9 expression by immunohistochemistry.

Stem Cell Differentiation

Mesenchymal stem cells (MSCs) are the primary cell source in tissue engineering due to their broad multilineage differentiation potential and immunomodulatory properties (7,8). Nanostructured scaffolds direct MSC fate through mechanotransduction, substrate stiffness (10–40 kPa for osteogenesis, 0.5–5 kPa for chondrogenesis), topographic cues, and surface chemistry collectively converge on YAP/TAZ nuclear translocation and lineage-specific transcription factor activation (16). Optimized nanofiber scaffolds support osteogenic differentiation in the presence of dexamethasone, β-glycerophosphate, and ascorbic acid, while chondrogenic protocols require TGF-β3 supplementation, low oxygen tension, and pellet or three-dimensional culture (17). Differentiation efficacy should be validated by gene expression profiling, immunohistochemical staining of lineage markers, and quantitative matrix production assays.

Drug and Gene Delivery Applications

Antibiotic Delivery

Post-operative infection is a significant complication in bone surgery, with the potential to cause implant failure, osteomyelitis, and extensive bone loss. Nanofiber scaffolds serve as effective carriers for antibiotics, providing high local concentrations at the surgical site while minimizing systemic exposure and associated toxicity (19). Controlled release kinetics can be tailored through physical encapsulation, chemical conjugation, or responsive release mechanisms triggered by infection-associated enzymes (lipase, hyaluronidase) or local pH changes. Quantification of release profiles by high-performance liquid chromatography and parallel zone-of-inhibition assays confirms both delivery kinetics and retained antimicrobial activity.

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Anti-inflammatory Agents

Uncontrolled inflammatory responses following bone or cartilage injury impair tissue regeneration by promoting pro-fibrotic macrophage polarization and inhibiting osteoprogenitor cell recruitment (2,11). Local delivery of anti-inflammatory agents, including corticosteroids, NSAIDs, or interleukin-4, through nanostructured scaffolds modulates the inflammatory microenvironment, favoring M2 macrophage polarization and reducing fibrous encapsulation. These immunomodulatory properties can extend the functional window for scaffold-guided regeneration (2).

Gene Delivery

Nucleic acid therapeutics including siRNA, microRNA, and plasmid DNA enable targeted modulation of cellular behavior to enhance regenerative outcomes (21). Nanomaterial carriers protect nucleic acids from nuclease degradation and facilitate intracellular delivery through endosomal escape mechanisms. Surface-functionalized nanoparticles enable cell-type-specific targeting (utilizing receptor-ligand interactions) to minimize off-target transfection. Gene silencing efficacy is typically confirmed by quantitative RT-PCR and western blotting, while off-target effects are assessed using genome-wide expression profiling (10,21).

Mechanical Properties and Structural Characterization

Tensile and Compressive Properties

Electrospun nanofiber scaffolds demonstrate anisotropic mechanical behavior dependent on fiber alignment, diameter distribution, and interfibrillar bonding (12,22). Aligned fiber mats exhibit directional tensile strength that mimics anisotropic load-bearing tissues such as tendon, ligament, and cortical bone. Crosslinking strategies (including glutaraldehyde vapor treatment, carbodiimide (EDC/NHS) chemistry, or transglutaminase-mediated enzymatic crosslinking) enhance mechanical stability under hydrated conditions, though excessive crosslinking density may impair biocompatibility and restrict cellular remodeling (22). Mechanical testing should be performed under physiologically relevant conditions (phosphate-buffered saline, 37°C) with reporting of elastic modulus, ultimate tensile strength, and failure strain. Incorporation of ceramic nanoparticles into polymer matrices generally increases compressive stiffness while potentially reducing elastic compliance; optimal formulations balance rigidity with sufficient deformability to prevent stress shielding and implant fatigue failure (14,22).

Degradation Kinetics

Scaffold degradation rate must be synchronized with the rate of new tissue formation to maintain structural continuity throughout the regeneration cycle (20,23). Degradation products must be non-toxic, non-immunogenic, and readily metabolized or eliminated. Synthetic polymers including PCL degrade slowly via hydrolysis (half-life of months to years), while natural polymers undergo more rapid enzymatic degradation (days to weeks) (20). Composite scaffolds display hybrid degradation profiles influenced by component ratios and interfacial bonding characteristics.

Degradation is typically monitored by mass loss gravimetry, molecular weight reduction by gel permeation chromatography, and pH monitoring of degradation media. The rapid degradation of natural polymer components remains a recognized design challenge for long-term load-bearing applications (6,23).

Characterization Techniques

Comprehensive characterization of nanostructured scaffolds employs a multimodal analytical toolkit (4). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) reveal fiber morphology, diameter distributions, and nanoparticle dispersion (13,15). Atomic force microscopy (AFM) provides nanoscale topographic mapping and quantitative surface stiffness data via force-distance curves. X-ray diffraction (XRD) identifies crystalline phases, particularly the HA/β-TCP ratio in bioceramic-containing composites. Fourier-transform infrared spectroscopy (FTIR) confirms chemical composition, crosslinking state, and molecular interactions within composite materials (13).

Porosity analysis by mercury intrusion porosimetry or micro-computed tomography (micro-CT) quantifies pore volume fraction, mean pore diameter, and three-dimensional interconnectivity, parameters directly governing cellular infiltration depth and convective nutrient transport (13,6). Mechanical testing under physiological conditions evaluates scaffold performance in environments relevant to in vivo loading (13). Standardized reporting of all characterization parameters is essential to enable meaningful inter-study comparison and to support regulatory submissions.

In Vivo Studies and Preclinical Evaluation

Bone Defect Models

Preclinical evaluation of nanostructured scaffolds employs established animal models including rodent calvarial defects, rabbit femoral condyle defects, and large-animal long-bone segmental defects (24,25). These models permit assessment of osteoconductivity, osteoinductivity, and vascular ingrowth within the regenerating tissue, with bone volume fraction and trabecular microarchitecture quantified by micro-CT and histomorphometry. Critical-sized defects (which do not heal spontaneously without intervention) provide stringent evaluation of scaffold osteogenic efficacy. Studies demonstrate that nanofiber scaffolds loaded with osteogenic growth factors or bioactive ions significantly enhance bone regeneration compared to acellular or unfunctionalized controls (24).

Cartilage Repair Models

Cartilage regeneration studies utilize full-thickness cartilage defect models in rabbit, goat, and porcine joints (12). The avascular, mechanically loaded environment of articular cartilage is not fully replicated by subcutaneous implantation models, making intra-articular defect models the preferred evaluation approach. Load-bearing capacity, International Cartilage Repair Society (ICRS) macroscopic scoring, and O'Driscoll histological grading of regenerated tissue constitute critical translational endpoints (3,12). Long-term durability studies (extending beyond 12 weeks) are necessary to assess fibrocartilage versus hyaline cartilage formation, which strongly predicts functional outcomes (3).

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Osteochondral Integration

Osteochondral defect models evaluate simultaneous regeneration of cartilage and subchondral bone, with particular attention to the formation of a continuous mineralization front at the interface and prevention of fibrous tissue interposition (17,20). Gradient scaffolds demonstrate superior zonal integration compared to homogeneous constructs, supporting organized differentiation from hyaline cartilage to trabecular bone (20). Assessment should include histological evaluation of chondrocyte zonal distribution, immunostaining for collagen type I vs. II, and micro-CT analysis of subchondral bone restoration (17).

Comparative Summary of Material Systems

Table 1 presents a comparative overview of the principal nanomaterial systems reviewed, summarizing mechanical strength, degradation rate, biocompatibility, and translational readiness to facilitate direct comparison across platforms.

Clinical Translation and Regulatory Considerations

Current Clinical Products

Several nanostructured products have achieved regulatory approval or are progressing through advanced clinical trials for bone and cartilage applications (26). These include collagen-based matrices, synthetic polymer scaffolds, and composite bone graft substitutes (23,26). Clinical outcomes from approved products provide benchmarks for efficacy and safety that must be exceeded by next-generation nanotechnology-based constructs. Understanding regulatory pathways (particularly the classification of combination products integrating scaffolds, cells, and bioactive molecules) is fundamental to translational planning (26).

Manufacturing Scale-up

Scaling from laboratory to GMP-compliant clinical manufacturing demands attention to reproducibility, sterilization compatibility, shelf-life stability, and cost-effectiveness (27,28). Electrospinning processes must be scaled while maintaining consistent fiber diameter distributions and mechanical properties across production batches. GMP compliance requires process validation, environmental monitoring, and in-process quality controls that are substantially more demanding than academic research standards (28). These requirements represent a significant practical barrier for many academic-stage innovations and must be planned for prospectively during material development (27).

Safety and Efficacy Assessment

Comprehensive long-term safety evaluation is mandatory for nanomaterial-containing implants, encompassing systemic distribution of nanoparticle degradation products, immune and inflammatory responses, and carcinogenic or genotoxic risk assessment. Biocompatibility testing under ISO 10993 standards provides the initial regulatory foundation, encompassing cytotoxicity, sensitization, irritation, systemic toxicity, subchronic toxicity, genotoxicity, and implantation testing. Long-term studies in relevant large-animal models addressing chronic

Table 1. Comparative Summary of Nanomaterial Systems for Bone and Cartilage Tissue Engineering

Material System	Mechanical Strength	Degradation Rate	Biocompatibility	Translational Readiness	Key Observations
PCL/PLGA Nanofibers	High (tensile modulus 50–500 MPa)	Slow–Moderate (weeks to months)	Excellent (ISO 10993 compliant)	Advanced—multiple clinical trials	Tunable degradation; strong osteoblast response; GMP scale-up feasible (10,19)
Collagen/Gelatin Nanofibers	Low–Moderate (requires crosslinking)	Rapid (days–weeks)	Excellent (native ECM origin)	Moderate—limited by mechanical weakness	Ideal for soft tissue; crosslinking improves stability; widely used in cartilage ECM mimicry (29,30)
Chitosan/Chitin Scaffolds	Moderate	Moderate (enzymatic)	Very good; mild immune response possible	Moderate—antimicrobial utility	Inherent antimicrobial properties; chondrocyte-friendly; limited load-bearing capacity (14,31)
HA/β-TCP Nanoparticles in Polymer	High (compressive strength 20–150 MPa)	Slow (ceramic phase)	Very good; osteoconductive	High—established in bone graft substitutes	Excellent mineralization; promotes osteogenesis; used in alveolar and long-bone defects (12,22)
Metal-Organic Frameworks (MOFs)	Low–Moderate (fragile crystalline structures)	Variable (metal-ligand bond dependent)	Under investigation; cytotoxicity data limited	Low—primarily in vitro evidence	Multifunctional ion release; angiogenic potential; long-term in vivo safety not established (1,9)
Graphene/Graphene Oxide	Very high (elastic modulus ~1 TPa)	Very slow (non-biodegradable)	Mixed; oxidative stress concern in vivo	Low—significant toxicological hurdles	Superior electrical conductivity; enhances osteogenesis in vitro; in vivo data sparse; regulatory pathway unclear (2,5)
GelMA Hydrogel Hybrids	Low–Moderate (soft, tunable)	Moderate (photocrosslinking adjustable)	Excellent; cell-permissive	Moderate—bioink applications emerging	Supports 3D bioprinting; favorable for chondrocytes; limited standalone mechanical performance (24,35)
Gradient/Osteochondral Scaffolds	Graded (zone-specific)	Graded (zone-specific)	Good (demonstrated in animal models)	Moderate—complex manufacturing	Zonal differentiation of MSCs; superior interface integration vs. homogeneous scaffolds (26,32)

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Exposure scenarios are required to meet regulatory agency expectations (29).

The characterization of nanoparticle degradation products by mass spectrometry and their biological fate by in vivo imagi and tissue distribution studies must be explicitly incorporated into pre-clinical safety packages (8).

Challenges and Future Directions

Despite substantial progress, unresolved technical challenges limit the clinical translation of nanotechnology-based bone and cartilage scaffolds. The insufficient mechanical performance of natural biopolymers such as collagen and the inadequate hydrolytic stability of hyaluronic acid restrict long-term load-bearing applications, particularly for articular cartilage (15,17). Rapid degradation of natural polymer nanofibers compromises structural scaffolding before sufficient de novo tissue formation has occurred (6). Vascularization of large engineered bone constructs remains a fundamental unresolved barrier: without rapid perfusion to support cell viability beyond the diffusion limit (~200 μm), the interior of constructs undergoes central necrosis (30). Concurrent innervation strategies (incorporating neurotrophic factors or neural progenitor cells) may be required for long-term bone homeostasis and sensory restoration. The scale-up of electrospinning while maintaining dimensional consistency, and the reproducibility of coaxial or gradient scaffold architectures across production batches, further challenge translational feasibility (31). Emerging research directions include oxidative stress modulation using antioxidant-loaded nanomaterials, photothermal therapy for infection control, and corrosion-resistant magnesium alloys for resorbable implants (8,31). Machine learning and computational scaffold design are increasingly being applied to optimize material composition and fabrication parameters beyond the reach of empirical screening (32). Personalized medicine approaches employing patient-specific induced pluripotent stem cells (iPSCs) and three-dimensional bioprinting enable customized constructs matched to individual anatomical defect geometry and biological requirements (33,34). Smart scaffolds embedding biosensors provide real-time monitoring of regeneration progress and enable closed-loop therapeutic delivery adjustment (18). The field urgently requires standardized characterization protocols, comparative testing methodologies, and unified reporting guidelines to enable meaningful cross-study comparison and regulatory harmonization. International efforts to establish benchmark reference materials and validated testing assays will accelerate regulatory approval and clinical adoption (35). Consensus definitions for key terminology (distinguishing nanoscaffolds, nanocomposites, and hybrid systems) are needed to reduce ambiguity in scientific communication and regulatory classification (34).

Conclusion

Nanotechnology has fundamentally transformed the landscape of bone and cartilage tissue engineering by enabling precise control over scaffold architecture, chemical composition, and biological functionality across multiple length scales. This systematic review of 35 eligible studies

(January 2022 up to the time of the search) demonstrates that electrospun nanofiber scaffolds, MOF-based systems, bioceramic nanocomposites, and functionalized hydrogels each contribute distinct and complementary advantages for specific tissue engineering applications.

Electrospun PCL and PLGA scaffolds combined with bioceramic nanoparticles offer well-characterized, mechanically robust platforms with established translational pathways. Gradient and osteochondral scaffolds demonstrate superior zonal differentiation compared to homogeneous designs, addressing the structural complexity of the cartilage-bone interface. Surface functionalization and controlled delivery of growth factors and bioactive ions have substantially advanced scaffold bioactivity beyond passive structural support.

The preponderance of evidence remains at the in vitro or small-animal in vivo level, with insufficient long-term large-animal data to support clinical translation. MOF and graphene-based systems, while scientifically promising, face unresolved questions regarding toxicity, biodegradability, and regulatory classification. The cartilage tissue engineering literature remains less developed than its bone counterpart, with critical gaps in understanding of chondrocyte phenotype maintenance, ECM maturation under mechanical loading, and osteochondral interface biology. Electrospinning scalability and GMP compliance remain practical barriers for all polymer-based approaches.

Priority research areas include: (1) development of vascularization strategies (including angiogenic factor delivery, prevascularization using endothelial co-culture, and three-dimensional-printed vascular channel incorporation) to address perfusion limitations in large defects; (2) rigorous long-term in vivo safety studies for MOF and graphene systems, incorporating systemic toxicology and immune monitoring; (3) expansion of osteochondral gradient scaffold research with attention to chondrocyte phenotype stability, mechanical loading durability, and interface integrity; (4) investment in GMP-compatible electrospinning processes with validated quality metrics; and (5) multidisciplinary collaboration bridging biomaterials science, clinical orthopedics, and regulatory affairs to accelerate the path from bench to bedside. The convergence of nanotechnology with regenerative medicine holds transformative promise for addressing currently unmet clinical needs in musculoskeletal repair, provided that translational rigor is maintained throughout the development pipeline.

Authors' Contributions

All authors contributed to the investigation, conceptualization, and analysis of the information in this manuscript, and were involved in the writing and revision process.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Abbreviations

Abbreviation	Full Term

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AFM	Atomic Force Microscopy
BMP-2	Bone Morphogenetic Protein-2
BMP-7	Bone Morphogenetic Protein-7
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
FGF	Fibroblast Growth Factor
FTIR	Fourier-Transform Infrared Spectroscopy
GelMA	Gelatin Methacryloyl
GMP	Good Manufacturing Practice
HA	Hydroxyapatite
ICRS	International Cartilage Repair Society
iPSCs	Induced Pluripotent Stem Cells
ISO	International Organization for Standardization
miRNA	MicroRNA
MOFs	Metal-Organic Frameworks
MSCs	Mesenchymal Stem Cells
PCL	Polycaprolactone
PLGA	Poly(lactic-co-glycolic acid)
SBEE	Solution Blow Spinning Extrusion
SEM	Scanning Electron Microscopy
siRNA	Small Interfering RNA
TEM	Transmission Electron Microscopy
TGF-β	Transforming Growth Factor-Beta
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
β-GP	β-Glycerophosphate
β-TCP	β-Tricalcium Phosphate

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