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Clinical translation of 3D-bioprinted orthopedic grafts: Design specifications, evaluation domains, and regulatory considerations for bone, cartilage, and osteochondral repair

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Clinical translation of 3D-bioprinted orthopedic grafts: Design specifications, evaluation domains, and regulatory considerations for bone, cartilage, and osteochondral repair

Running title: Clinical translation of 3D-bioprinted orthopedic grafts

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Abstract

Three-dimensional (3D) bioprinting has advanced rapidly, but clinical translation of orthopedic grafts remains constrained by a mismatch between printable constructs and indication-specific failure modes. Bone, cartilage, and osteochondral defects impose distinct requirements: vascularized remodeling and load sharing for bone, phenotypic stability and low-friction durability for cartilage, and mechanically integrated compartmental organization for osteochondral repair. This narrative review synthesizes recent evidence on 3D bioprinted orthopedic grafts with a translational focus on design specifications, validation endpoints, manufacturing control, and regulatory readiness. Rather than treating bioinks, printing platforms, and cell sources as isolated technologies, the review organizes them according to clinical function: structural support, biological instruction, process control, maturation, immune compatibility, and product qualification. Current evidence indicates that many constructs remain validated mainly by early biological markers, representative imaging, or short-term animal outcomes, whereas clinically relevant tests of fatigue, wear, degradation-coupled mechanics, host-graft integration, vascular perfusion, immune response, sterility, dimensional tolerance, and release criteria are less consistently reported. Near-term translation appears most plausible for acellular or minimally cellular reinforced constructs, patient-specific but quality-testable bone scaffolds, and selected intraoperative applications with clear surgical utility. More complex living grafts that combine vascular, immune-instructive, and multi-tissue functions will require stronger preclinical models, standardized reporting, and product-specific regulatory strategies. Translation in orthopedic bioprinting should begin with the clinical failure mode and proceed toward measurable release specifications, not with printability alone.

Keywords: 3D bioprinting; Orthopedic regeneration; Bone defect; Cartilage repair; Osteochondral interface; Vascularization; In situ bioprinting; Regulatory science

1. Introduction

The habit of treating all orthopedic tissue repair as one biofabrication problem ignores how differently bone, cartilage, and osteochondral defects actually fail. Bone, cartilage, and osteochondral defects fail through distinct mechanisms. Segmental bone defects fail because the construct cannot establish mechanical continuity with host bone, cannot sustain vascular ingrowth, and cannot remodel into a load-sharing structure [1-3]. Cartilage fails differently: the regenerated tissue turns fibrocartilaginous, loses its lubricated surface, and deforms when loaded [4-6]. Osteochondral repair fails when neither compartment matures as part of the other — when cartilage, subchondral bone, and their shared interface do not develop as a coordinated load-bearing unit [7-9]. Printable and cytocompatible is not clinically useful.

Cartilage bioprinting, osteochondral scaffold design, and interface engineering have each been reviewed at length [10-15]. Machine learning-assisted process optimization, stem-cell programming, and regulatory strategy have also received dedicated treatment [16-18]. However, few reviews have explicitly used clinical failure modes as the starting point for organizing the design specifications and validation evidence required before a construct enters a translational development program. To our knowledge, few reviews have integrated indication-specific failure modes, endpoint-stratified evaluation considerations, and jurisdiction-dependent regulatory considerations into a single translational framework for orthopedic bioprinting (Figure 1).

Failure modes define the design problem. Bioinks, platforms, cells, and maturation protocols are selected by function, not by chemistry or novelty. Translational evaluation domains should be indication-specific and anchored to clinically relevant failure modes. Manufacturing and regulatory constraints enter at the design stage, not after the construct is built.

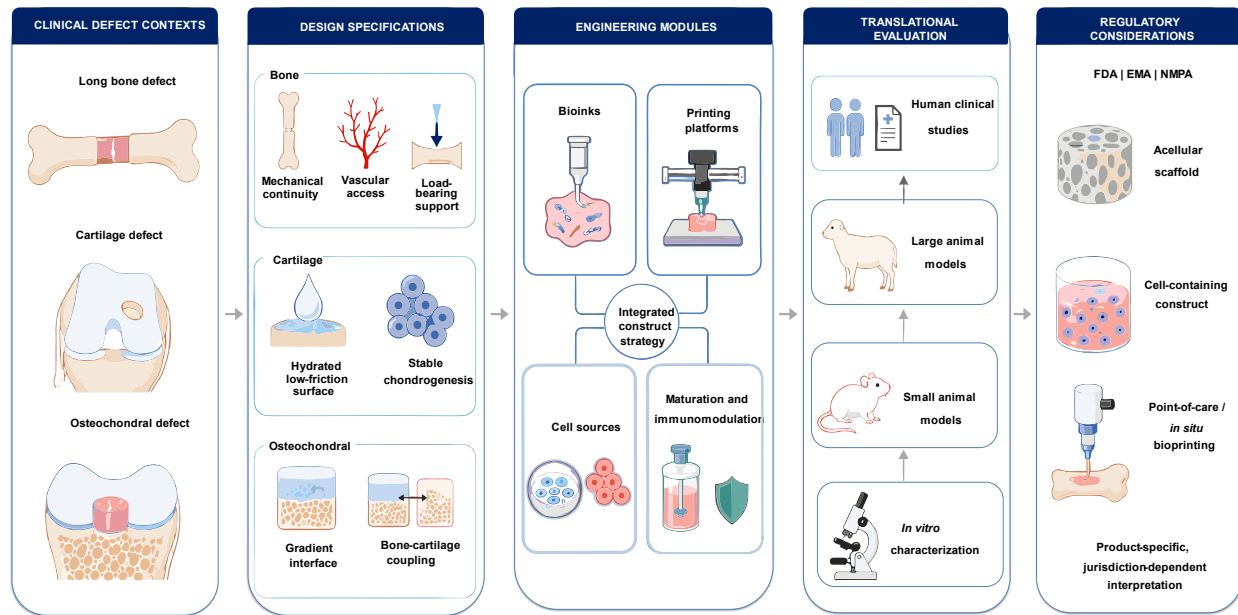


Figure 1. Translational framework for 3D bioprinted orthopedic grafts. The schematic summarizes the progression from orthopedic defect contexts to graft design requirements, engineering modules, translational evaluation, and regulatory considerations. Different clinical defects, including long bone, cartilage, and osteochondral defects, require distinct mechanical, biological, and interfacial design features. These requirements are integrated through bioinks, printing platforms, cell sources, maturation strategies, and immune modulation, followed by staged evaluation from in vitro testing to animal models and human clinical studies. Regulatory pathways remain product-specific and jurisdiction-dependent across the FDA, EMA, and NMPA. **Abbreviations:** FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; NMPA, National Medical Products Administration.

2. Scope and Narrative Review Approach

This article was designed as a narrative review with a translational focus. PubMed, Web of Science, and Scopus were searched, with the final search completed in April 2026, using combinations of terms related to 3D bioprinting, orthopedics, bone, cartilage, osteochondral repair, bioink, vascularization, immunomodulation, mechanical testing, manufacturing, quality control, and regulatory pathways. Literature published from 2020 to 2026 was prioritized to capture recent advances, while earlier foundational studies were retained when they defined clinically relevant failure modes, benchmark mechanical requirements, regulatory principles, or established preclinical models relevant to orthopedic translation.

Eligible sources included primary in vitro studies, animal studies, early clinical reports, systematic or scoping reviews, and regulatory or standards-related publications. Different evidence types were used for different purposes: primary experimental studies were used to identify

technical strategies and measurable endpoints; animal and clinical studies were used to assess translational relevance; reviews were used to summarize established gaps and areas of consensus; and regulatory or standards-related documents were used to contextualize manufacturing, quality control, and development requirements. Conference abstracts, non-peer-reviewed preprints, and studies without clear relevance to orthopedic repair, tissue reconstruction, or translational validation were excluded.

Because this was not a systematic review, no formal PRISMA screening, quantitative synthesis, or risk-of-bias assessment was performed. Instead, the selected literature was synthesized thematically to distinguish evidence-derived observations from consensus-informed recommendations and author-proposed framework elements.

The scope deliberately compresses topics that are already well covered elsewhere, including detailed cartilage zonal biology, exhaustive lists of hydrogel chemistries, and modality-by-modality descriptions of extrusion, inkjet, stereolithography, digital light processing (DLP), and 4D printing. Those topics are included only when they change the translational decision being made (Table 1).

Table 1. Key studies on 3D bioprinting for bone repair and reconstruction, organized chronologically.

Study	Year	Study Design	Material Bioink	/ Printing Platform	Key Findings	Indication
Rouwkema & Khademhosseini [19]	2016	Review	Prevascularized constructs, sacrificial templates	Extrusion, inkjet	Systematic analysis of vascularization strategies; anastomosis must occur within days in critical-size defects — this is where most vascularization approaches fail, not in vitro but in vivo post-implantation.	Vascularized bone/tissue constructs
Baino et al. [20]	2018	Review	Bioactive (45S5 derivatives)	glass and / extrusion / Robocasting / SLA	Bioactive glasses dissolve to release Si, Ca, and P ions that activate HIF-1alpha and VEGF transcription; angiogenic and osteogenic signaling are coupled, making bioactive glass uniquely suited for vascularized bone repair.	Bone and craniofacial repair

Nandi et al. [21]	2018	In vivo (rabbit tibia)	beta-TCP printed)	(3D Binder jetting / robocasting	beta-TCP scaffolds with interconnected 500 um pores supported new bone formation at 8 weeks; surface chemistry (Mg and Sr substitution) significantly influenced osteoblast attachment and resorption rate.	Trabecular bone defect repair
Nauth et al. [22]	2018	Expert consensus / review	N/A	N/A	Defined diagnostic and management thresholds for critical-size bone defects; >2 cm segmental defects rarely heal without reconstruction -- establishes the clinical indication floor for bioprinted bone grafts.	Critical-size bone defect (definition)
Pobloth et al. [23]	2018	In vivo (sheep, critical-size tibial defect)	Titanium alloy mesh (Ti6Al4V)	Selective laser melting (SLM)	Mechanobiologically optimized titanium mesh scaffolds with patient-specific load-adaptive architecture achieved greater bone regeneration than standard-geometry controls in sheep at 12 weeks.	Large segmental bone defect (femur/tibia)
Reznikov et al. [2]	2018	Basic science	N/A	N/A	Revealed fractal-like hierarchical bone organization from nanoscale collagen fibril arrays to lamellar structures; foundational for biomimetic scaffold architecture design.	Bone scaffold design principles
Diloksumpan al. [24]	2020	In vitro proof-of-concept	/ PCL (structural) + GelMA hydrogel (biological)	Multi-scale extrusion + inkjet hybrid	Multi-scale printing enabled reinforced interfaces; construct stiffness and cell viability were simultaneously tuned, demonstrating hybrid manufacturing feasibility.	Osteochondral/bone interface
Sparks et al. [25]	2020	Preclinical model	Acellular scaffolds	Various	Established	^a Critical-size bone defect (translational

		development (sheep)	(multiple materials)			standardized large animal critical-size load-bearing bone defect protocol; vascular access failure -- not scaffold architecture -- is the primary cause of central necrosis in thick constructs.
Migliorini et al. [1]	2021	Systematic review	N/A (surgical comparator)	N/A		Systematic review of Large bone defects surgical options for (clinical context) large bone defects (trauma, tumor, infection); no current off-the-shelf solution reliably achieves load-sharing vascular remodeling -- defines the unmet clinical need.
Shen et al. [26]	2022	In vivo (rat, critical-size femoral defect)	GelMA + VEGF-loaded nanoparticles + MSCs	+ Extrusion bioprinting		In situ vascularized Critical-size bone constructs with segmental bone sustained VEGF release defect achieved bridging and vascular ingrowth in critical-size rat femoral defects at 8 weeks; absence of vascular connection leads to central necrosis.
Toh et al. [27]	2022	Clinical pilot study	PCL burr-hole scaffold	Osteoplug 3D-printed scaffold		126-patient pilot: better Cranial burr-hole cosmetic outcomes with cover comparable safety; evidence limited to non-load-bearing cranial burr-hole covers.
Xu et al. [28]	2022	In vitro + In vivo (rabbit)	Porous alloy (Ti6Al4V)	titanium Selective laser melting (SLM)	Optimized pore architecture (600 um)	Segmental bone defects enhances osteogenic cell adhesion, proliferation, and in vivo bone ingrowth in rabbit femoral condyle model.
Yazdanpanah et al.	2022	Review	PCL, HAp, beta-	Extrusion,	Comprehensive	Large bone defects

al. [29]			TCP, bioactive SLA/DLP, glass, hydrogels inkjet	overview of 3D bioprinted scaffolds for bone; highlights porosity, vascularization, and mechanical competence as critical translational gaps.	
Zhang et al. [30]	2022	Review	Calcium phosphate (HAp, beta-TCP, biphasic CaP)	Extrusion / robocasting / inkjet	Calcium-phosphate biomaterials achieve osteoinductivity through surface ion release and protein adsorption; sintering temperature and phase ratio control degradation and osteogenic potency. Bone defect repair
Gharibshahian et al. [31]	2023	Review	PCL composites (HAp, beta-TCP, collagen)	+ Extrusion-based (FDM/FFF)	Ceramic reinforcement of PCL scaffolds improves osteoconductivity and mechanical properties; degradation rates require precise tuning for each clinical indication. Bone tissue engineering
Loukelis et al. [32]	2023	Review	Nanocomposites (graphene, CNT, nanoclay, nanoHAp)	Extrusion, SLA/DLP	Nanofillers enhance mechanical strength, electrical conductivity, and osteoinductivity; batch-to-batch consistency and nanoparticle cytotoxicity remain key translational barriers. Bone and osteochondral engineering
Raveendran et al. [33]	2023	In vitro / comparative	PCL (frame) + osteoblast-laden GelMA hydrogel	Extrusion hybrid	Culture conditions (static vs. dynamic perfusion, medium composition) significantly altered matrix mineralization and ALP activity of 3D bioprinted osteoblast-laden constructs. Bone graft maturation
Zenobi et al. [34]	2023	Experimental / in vitro	Calcium phosphate ceramics	Extrusion / robocasting	Strut diameter, pore geometry, and print orientation jointly control compressive stiffness and cell- Bone scaffold fabrication

					seeding efficiency; microarchitecture must be co-designed with biology.	
Budharaju et al. [35]	2024	Review	Sacrificial inks (Pluronic, gelatin) within hydrogel matrix	Embedded FRESH-style bioprinting	/ Embedded bioprinting enables vascular channels within cm-scale bone constructs; anastomosis with host vasculature within days post-implantation is identified as the crux of success.	Vascularized bone constructs
Verbist et al. [36]	2024	Retrospective clinical series	Bioceramic (beta-TCP/HAp) 3D-printed scaffolds	Robocasting / extrusion	Patient-specific 3D-printed bioceramic implants for craniomaxillofacial defects achieved satisfactory osseointegration at 12-month follow-up; patient-specific digital workflow is identified as a regulatory requirement.	Craniomaxillofacial bone defects
Son et al. [37]	2025	Review	Vascularizing bioinks: fibrin, GelMA + endothelial cells	Extrusion, coaxial, + light-based	Pre-vascularization, sacrificial templating, and growth-factor release each offer distinct trade-offs for vascularized bone repair; large animal validation remains lacking across all strategies.	Vascularized bone repair
Wang et al. [38]	2025	Review	Multiple (PCL, PLGA, ceramic, hydrogel composites)	Extrusion, SLA/DLP, SLS	Fatigue degradation and host-graft transfer are consistently underreported across scaffold types; mechanical characterization must	Bone engineering scaffolds

move beyond single-
point stiffness
measurements.

Abbreviations: ALP, alkaline phosphatase; beta-TCP, beta-tricalcium phosphate; CaP, calcium phosphate; CNT, carbon nanotube; DLP, digital light processing; FDM, fused deposition modeling; FFF, fused filament fabrication; FRESH, freeform reversible embedding of suspended hydrogels; GelMA, gelatin methacryloyl; HAp, hydroxyapatite; HIF-1alpha, hypoxia-inducible factor 1-alpha; MSC, mesenchymal stem/stromal cell; N/A, not applicable; PCL, polycaprolactone; PLGA, poly(lactic-co-glycolic acid); SLA, stereolithography; SLS, selective laser sintering; SLM, selective laser melting; VEGF, vascular endothelial growth factor.

Table 1 summarizes representative and translationally relevant studies identified through this structured narrative search process, organized chronologically to illustrate how evidence has evolved across materials, platforms, models, and clinical comparators.

3. Clinical Failure Modes as Design Inputs

The clinical numbers put the problem in perspective. An estimated 2.2 million bone-grafting procedures are performed annually worldwide [39]; segmental defects from trauma, tumor resection, and periprosthetic infection account for a disproportionate share of surgical complexity and cost [22]. Articular cartilage lesions appear in more than 60% of knee arthroscopies [40]. Microfracture fills defects with fibrocartilage. Autograft transfer damages donor sites. Autologous chondrocyte implantation is constrained by lesion size and risks delamination under load [41]. Osteochondral defects — requiring simultaneous repair of cartilage and subchondral bone as a coordinated unit — have no durable off-the-shelf solution [9]. The gap matters. Translation-first analysis starts with the failure mode, not the printer (Figure 2).

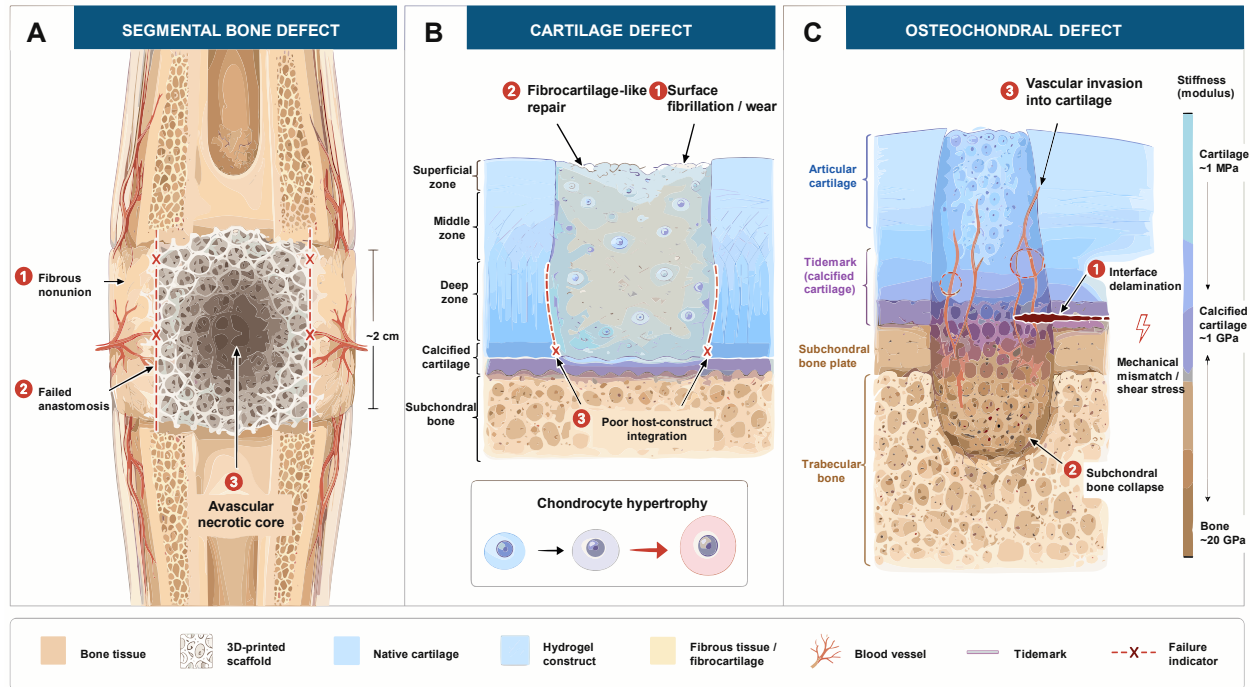


Figure 2. Indication-specific clinical failure modes in 3D bioprinted orthopedic repair. (A) Segmental bone defect: fibrous nonunion at host-graft junctions, failed vascular anastomosis, and avascular necrotic core formation. (B) Cartilage defect: surface fibrillation and wear, fibrocartilage-like repair, chondrocyte hypertrophy, and poor host-construct integration. (C) Osteochondral defect: interface delamination under shear stress, subchondral bone collapse, and pathological vascular invasion across the tidemark. The stiffness gradient bar illustrates the modulus transition from articular cartilage (~1 MPa) through calcified cartilage (~1 GPa) to subchondral bone (~20 GPa) (Table 2).

A large bone defect demands three things at once: hierarchical mechanical organization [2], osteoconductive or osteoinductive signals, and the capacity to remodel into load-sharing host bone [3,42]. Miss any one, and the construct stays a placeholder. Vascular access is non-negotiable: living bone repair depends on angiogenesis and perfusion-mediated remodeling [26,29,43]. Pack a scaffold with the most potent osteogenic cells available. Without vascular access it will still fail. Angiogenesis is not an optimization variable—it is a prerequisite.

Mechanical continuity also requires an architecture-level stiffness match rather than simply a strong base material. For polymer, ceramic, and composite scaffolds, the relevant design variable is the effective modulus of the printed architecture, which depends on porosity, pore geometry, strut or fiber diameter, fiber orientation, and print direction. If the construct is too stiff, stress shielding may reduce load transfer to the regenerating host bone and delay remodeling; if it is too compliant, early collapse, micromotion, or fibrous nonunion may occur. Therefore, load sharing should be evaluated as a time-dependent interaction between scaffold stiffness, degradation, and new tissue formation rather than as a static material property.

Cartilage is a different game entirely. The target is not a chondrogenic mass. It is a hydrated, low-friction surface that holds a stable non-hypertrophic phenotype under repetitive loading. Gene

expression and GAG staining work as early markers [5], but they screen rather than test function. Because cartilage and hydrogel-rich constructs are viscoelastic, functional testing should report not only indentation modulus and dynamic compression but also loading rate, stress relaxation, creep behavior, dynamic modulus, storage and loss moduli, loss tangent, lubrication, wear resistance, type II collagen retention, and lateral integration [6,44]. Lubrication is not incidental. Joint surface protection depends on a thin layer of lubricin and hyaluronan-phospholipid complexes [45-46], and reproducing this boundary-lubrication regime is a minimum requirement for any cartilage substitute intended to survive the joint environment [47].

Osteochondral repair adds a further constraint: the two compartments run incompatible biological programs. Cartilage must stay non-mineralized; bone depends on vascular invasion and mineralized matrix remodeling. Nature solves this through the tidemark and calcified-cartilage zone—a graded mineralization interface offering anisotropic stiffness and a permeability barrier that keeps vessels out of the hyaline layer [48]. Across this submillimeter zone, modulus rises from roughly 1 GPa in calcified cartilage to 20 GPa in subchondral bone, depending on hydration state and measurement site [49]. Gradient scaffolds aim to replicate this architecture [9], but replication on paper rarely predicts function under load. Direct testing of load transfer, delamination resistance, and compartmental stability separates a credible osteochondral design from one that looks plausible on imaging but fails under load [50-52]. For osteochondral interfaces, these tests should be performed under hydrated, cyclic, and shear-relevant conditions because delamination is driven by time-dependent interfacial fatigue rather than by a single static failure event.

Table 2. Clinical failure modes, design implications, and validation endpoints by orthopedic indication.

Indication	Dominant clinical failure mode	Design implication	Key validation endpoint
Bone defect	Fibrous inadequate invasion, and sharing at the junction.	nonunion, Hierarchical vascular early poor load competence, host-graft osteoconductive osteoinductive cues, interconnected architecture for vascular ingrowth.	Compression/fatigue performance, bridging, perfusion or vascular remodeling, and histologic host-graft integration.
Cartilage defect	Fibrocartilage fill, surface wear, poor lateral integration, and phenotype drift under cyclic loading.	Hydrated surface with hypertrophic chondrogenesis and lateral bonding to native cartilage.	low-friction stable non-wear testing, type II collagen/GAG retention, and integration strength.
Osteochondral defect	Interfacial delamination, subchondral collapse, and biological mismatch between cartilage and bone	Spatially graded cartilage-cartilage-bone construct with compartment-specific	Interfacial shear strength, load transfer, zonal matrix distribution, and compartment-specific

Abbreviations: GAG, glycosaminoglycan.

4. Engineering Modules Aligned with Clinical Requirements

4.1 Bioinks as role-allocated systems

The standard way to talk about bioinks is to group them by chemistry—natural polymers, synthetics, dECM, ceramics, nanocomposites [53-55]. That classification is convenient for a textbook but actively misleading for translation. What matters is function in the final construct, not chemical identity. Natural and modified hydrogels deliver ECM-like cues for chondrogenesis [56-58]; synthetic polymers and reinforced ceramic systems provide the mechanical control and degradation tuning that hydrogels alone cannot [24,31,59]. Calcium-phosphate and nanocomposite systems add osteoinductive or reinforcement capacity [30,32]. Bioactive glasses dissolve in physiological fluid, releasing silicate, calcium, phosphate, and trace metals that activate osteogenic and angiogenic transcription — effects that inert ceramics do not replicate [20,60]. Solubilized dECM bioinks carry tissue-specific signals but introduce batch variability as a manufacturing liability [61-62]. Conductive polymers and self-healing hydrogels extend the palette — enabling electrical stimulation of osteogenesis or structural recovery after placement [63-64]. Below, we replace chemical origin with functional role (Figure 3) (Table 3).

Four functional roles organize the discussion. Structural—shape retention, load sharing, dimensional stability. Biological instruction—osteogenesis, chondrogenesis, angiogenesis, immune modulation. Processing control—rheology-defined printability, shear-thinning behavior, yield stress, thixotropic recovery, crosslinking kinetics, and post-print fidelity. Clinical compatibility—sterilization, sourcing, storage, degradation safety, regulatory classification. That last layer is the one that determines whether a promising formulation ever reaches a patient. The other three are necessary; the fourth is decisive.

Printability should be treated as a rheological and process-control problem rather than a qualitative descriptor. For extrusion-based bioinks, relevant parameters include viscosity as a function of shear rate, shear-thinning index, yield stress, thixotropic recovery after extrusion, and storage/loss moduli before and after crosslinking. These parameters determine filament formation, filament collapse, strand fusion, cell exposure to shear stress, and final shape fidelity. Therefore, bioink development should report rheological curves together with printing parameters and cell viability, rather than reporting “printable” as a binary outcome.

Table 3. Role-allocated bioink modules for orthopedic translation.

Functional role	Representative components	Core question	development	Main translational risk
Structural support	PCL, PLGA, ceramic frameworks, fiber reinforcement, reinforced hydrogels.	Does the construct retain shape, transfer load, and degrade without mechanical collapse?	Biologically active but mechanically irrelevant construct.	
Biological instruction	dECM, hyaluronic acid, beta-TCP, HAp, bioactive growth factors, immunomodulatory cues.	Does the signal match the target compartment: osteogenic, angiogenic, or modulatory?	Batch variability, uncontrolled release, or inappropriate compartment signaling.	
Processing control	GelMA, alginate, collagen, gelatin, photoinitiators, shear-thinning additives, nanocomposites.	Does the formulation have a defined rheological window—viscosity, shear-thinning, yield stress, thixotropic recovery, and storage/loss moduli—that supports printing, crosslinking, viability, and post-print fidelity?	Laboratory printability that fails under sterile, automated, or intraoperative constraints.	
Clinical compatibility	GMP-grade raw materials, aseptic cartridges, sterilizable components, closed-system workflows.	Can sourcing, storage, degradation, release testing be qualified for patients?	Regulatory sterility, Laboratory or manufacturing barriers that prevent clinical deployment.	

Abbreviations: dECM: decellularized extracellular matrix; GelMA: gelatin methacryloyl; GMP: good manufacturing practice; HAp: hydroxyapatite; PCL: polycaprolactone; PLGA: poly(lactic-co-glycolic acid); TCP: tricalcium phosphate.

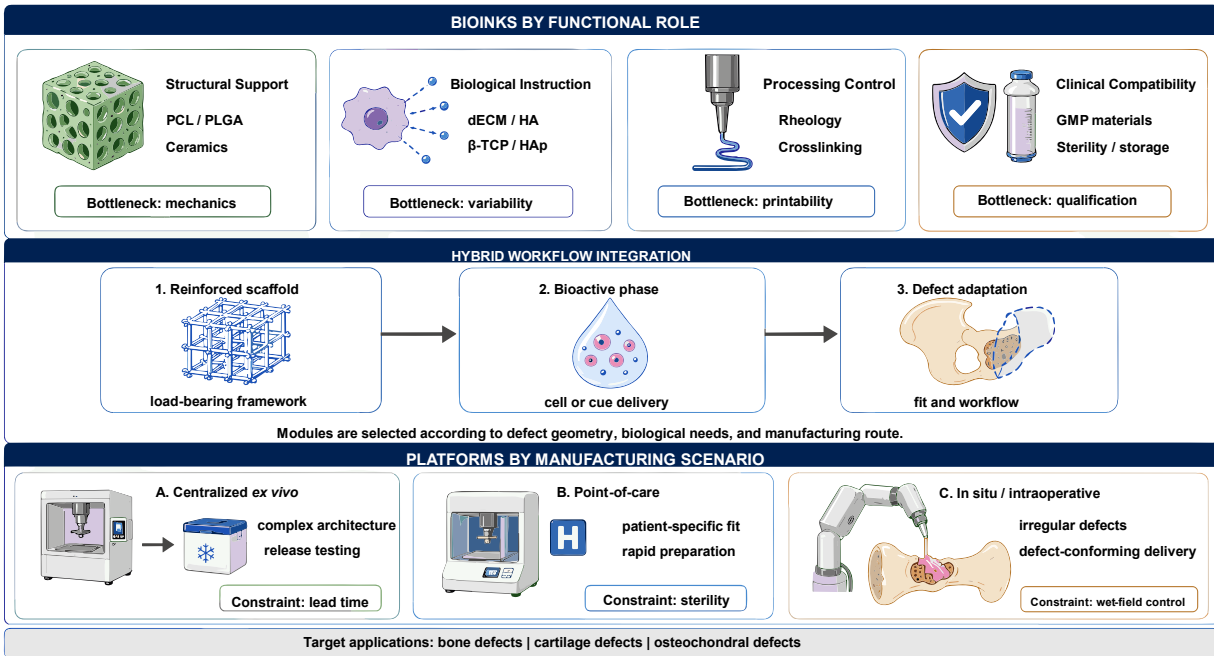


Figure 3. Functional role-based bioink classification and manufacturing scenario selection. Bioinks are grouped by translational function rather than chemical origin, including structural support, biological instruction, processing control, and clinical compatibility. The middle workflow illustrates how reinforced scaffolds, bioactive phases, and defect-specific adaptation can be combined in hybrid construct design. The lower panels summarize representative manufacturing scenarios—centralized ex vivo, point-of-care, and in situ/intraoperative printing—and their main implementation constraints. **Abbreviations:** dECM : decellularized extracellular matrix; GMP : good manufacturing practice; HA : hyaluronic acid; HAp : hydroxyapatite; PCL : polycaprolactone; PLGA : poly(lactic-co-glycolic acid); TCP : tricalcium phosphate.

4.2 Platform selection by manufacturing scenario

Platform choice should follow the manufacturing scenario—not the reverse. Centralized ex vivo production handles complex architecture, prolonged maturation, and rigorous quality testing well [18,36]; porous titanium and musculoskeletal bioprinting workflows demonstrate what structural qualification under controlled conditions looks like in practice [28,65]. Point-of-care production trades that control for geometric adaptability, at the cost of stricter sterility management, documentation requirements, and operator qualification [66-68]. DLP and other light-based platforms improve resolution but add photoinitiation and validation burdens that must be addressed before clinical deployment [69-71]. Two emerging modalities merit attention. Volumetric tomographic bioprinting fabricates centimetre-scale constructs in seconds by superimposing patterned light projections through a rotating photoresin volume—build time decouples from feature density — well suited to acellular structural carriers [72]. Two-photon polymerization achieves sub-micron resolution through nonlinear femtosecond absorption, encoding surface micro-topographies and zonal porosities unmatched by other methods [73]. Robotic and arthroscopically guided in situ platforms close the imaging-to-deposition loop but introduce

ergonomic and sterility constraints requiring formal qualification [74]. In situ printing pushes every requirement to its limit: rapid curing, wet-tissue adhesion, defect-conforming deposition, curing shrinkage control, early mechanical stability, resistance to synovial or bleeding-field dilution, field ergonomics, and intraoperative quality verification must all be satisfied before mechanical claims can be made (Table 4).

Table 4. Printing platform and manufacturing-route selection for orthopedic bioprinting.

Platform or route	Best applications	suited Translational strengths	Key constraints	Critical QC focus
Extrusion or polymer-hydrogel hybrid printing	Large bone scaffolds, osteochondral supports, reinforced hydrogel constructs.	Broad window, geometry, compatibility structural reinforcement.	material Shear stress, limited fine resolution, slow printing for complex architecture.	Nozzle pressure, strand fidelity, pore architecture, fatigue and compression testing.
SLA/DLP light-based printing	High-resolution cartilage or osteochondral constructs.	Fine feature control and smooth surfaces.	Photoinitiator toxicity, light-dose validation, restricted resin chemistry.	Curing dose, residual photoinitiator, dimensional accuracy, post-cure safety.
Volumetric tomographic printing	Centimeter-scale or hydrogel constructs requiring rapid fabrication.	soft Rapid build time with reduced layer-by-layer constraints.	Narrow material library incompletely standardized viability after volumetric exposure.	Optical dose distribution, absorption, uniformity, cell survival.
Two-photon polymerization	Microarchitecture, topography, and zonal interface studies.	Submicron precision and fine control of local structure.	Slow, small volume, high cost, limited clinical scalability.	Microfeature fidelity, reproducibility, and relevance to larger constructs.
Centralized ex vivo manufacturing	Patient-specific grafts requiring maturation and rigorous release testing.	Controlled environment, extended culture, richer quality testing.	Lead time, transport logistics, mismatch between planning intraoperative defect geometry.	Batch record, sterility, and release criteria, stability, transport and validation.
Point-of-care or in situ printing	Irregular defects, cartilage resurfacing, rapid defect filling.	Anatomical conformance reduced handling between fabrication and implantation.	Sterile-field and ergonomics, wet adhesion, bleeding synovial fluid, intraoperative verification.	Aseptic workflow, curing safety, operator qualification, real-time fit, wet adhesion, early construct stiffness, dimensional conformance, and intraoperative mechanical verification.

Abbreviations : DLP, digital light processing; QC, quality control; SLA, stereolithography.

For many indications, hybrid workflows are the most credible near-term strategy. A mechanically reinforced phase can be fabricated and quality-tested *ex vivo*, while a biological or defect-conforming phase is added closer to surgery. This approach assigns high-risk mechanical functions to controlled manufacturing and reserves intraoperative flexibility for geometry or biological delivery.

4.3 Biological programming and maturation

Putting cells into a printed scaffold does not make it a tissue, and pretending otherwise is where many programs go off track. MSCs, primary chondrocytes, and osteogenic populations each present a distinct trade-off — between potency and variability, between biological activity and manufacturing complexity, and between regenerative potential and safety burden [75-77]. Single-cell analyses have begun to resolve the lineage heterogeneity within MSC preparations during osteogenesis [78]; induced pluripotent stem cell (iPSC)-derived cells expand the option set but carry a distinct safety and qualification package [79]. Spheroid culture, oxygen-generating biomaterials, and immunomodulatory scaffolding support maturation in complex constructs, but each extends the characterization burden [80-82]. Cell-free strategies are increasingly viable: MSC-derived extracellular vesicles, including exosomes, deliver secretome-mediated repair signals without a viable cell payload and show chondroprotective activity in preclinical models [83-84]. Senescence is a subtler problem. p16-positive chondrocyte and MSC populations accumulate silently with passage number, impairing matrix production after implantation — a liability requiring tracking and management during manufacturing [85].

Whatever cell source you pick, the question is the same: can the program be qualified, controlled, and linked to a defined clinical function? If the answer is no, biological activity in a dish does not matter.

Maturation protocols must be matched to the target tissue. Bone constructs depend on mineralization, vascular ingrowth, and progressive mechanical loading [37,86]. Sustained matrix deposition without hypertrophic or calcification signals is the cartilage requirement [44,76-77]. Bioreactors, perfusion systems, and oxygen gradients belong in the product-development plan because they influence matrix organization and mechanics [87-88]. Embedded and light-based vascular bioprinting strategies show how maturation and perfusion become linked design variables [35,89]. Osteochondral constructs need spatially separated conditioning programs that do not compromise the interface zone (Figure 4).

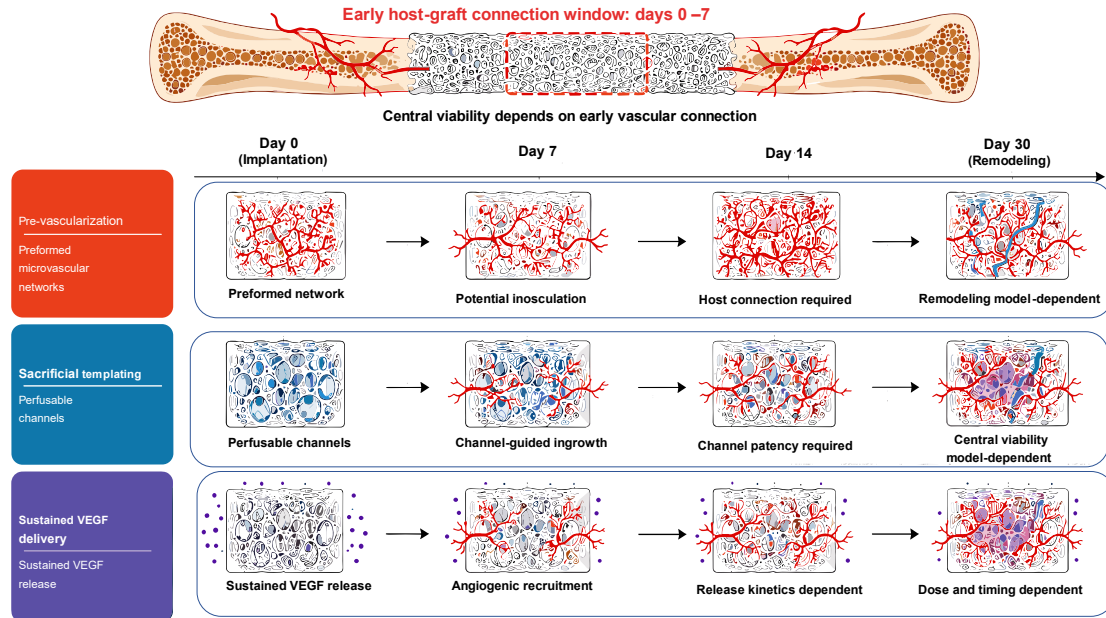


Figure 4. Conceptual vascularization strategies for 3D biprinted bone constructs. The figure summarizes three commonly reported approaches for vascularizing 3D biprinted bone constructs, including pre-vascularization with preformed microvascular networks, sacrificial templating with perfusable channels, and sustained VEGF delivery. Each strategy is illustrated across a postoperative timeline from implantation to remodeling, highlighting key mechanistic dependencies such as host–construct connection, channel patency, and release kinetics. The patterns shown are a conceptual synthesis derived from non-identical preclinical studies and should not be interpreted as direct head-to-head comparisons or performance rankings. Abbreviation: VEGF: vascular endothelial growth factor.

4.3.1 Host immune response: orthopedic bioprinting evidence and biomaterials-derived principles

Scaffold mechanics and cell programming get most of the attention, but the immune microenvironment can also influence integration. Macrophages are major early responders to implanted constructs, and their activation state can influence whether repair proceeds toward constructive remodeling or fibrous encapsulation [82,90]. Bioprinted constructs may allow immunomodulatory cues to be incorporated into scaffold architecture, surface chemistry, and controlled-release profiles, although direct orthopedic bioprinting evidence remains limited. Scaffolds delivering immunomodulatory cytokines in sequence may facilitate the M1-to-M2 transition and enhance vascularization of bone scaffolds [91]. Surface modification with cytokine-mimicking peptides, incorporation of interleukin-4 (IL-4) or interleukin-10 (IL-10) releasing microspheres, and dECM-derived matrices that retain immunomodulatory matrikines may shift the early host response toward constructive remodeling [82,90,92].

Adaptive immunity may also influence scaffold integration, although much of the current evidence is extrapolated from broader musculoskeletal repair and biomaterials studies. FoxP3+

regulatory T cells recruited to a healing musculoskeletal site can coordinate macrophage transition and suppress chronic inflammation in preclinical models [93]. Complement activation should be considered for blood-contacting or highly vascularized constructs, and bioink composition and surface chemistry should be screened when complement-mediated inflammation is relevant to the intended implantation environment [94].

For allogeneic cell-laden constructs, the immune challenge may be more acute. Allogeneic chondrocytes and MSCs should not be assumed to be immunologically inert; donor-recipient human leukocyte antigen mismatch, T-cell activation, and antibody-mediated responses may require immunologic risk assessment, immunomodulatory strategies, or low-immunogenicity cell sourcing [95-96]. iPSC-derived cells could offer immunological advantages once hypoinmunogenic lines are validated, but current complexity and safety requirements limit near-term deployment.

The scaffold material can also generate its own immune response. Degradation products, residual crosslinkers, and endotoxin from biological components should be characterized when relevant to the intended construct. Foreign-body giant-cell formation, fibrous encapsulation, and chronic granulomatous inflammation remain important failure modes for materials with unfavorable surface chemistry or slow resorption; these responses should be evaluated histologically rather than assumed absent [97]. Constructs that perform well in immunodeficient rodents may not reproduce the same integration profile in immunocompetent large animals, where T-cell activation, macrophage responses, and complement deposition may occur simultaneously.

5. Translational Evaluation Domains for Orthopedic Bioprinted Constructs

The main translational bottleneck is not only construct originality but also the selection of evaluation domains that reflect clinically relevant failure modes. Print fidelity, cell viability, and histological staining are necessary first checks, but they do not map directly onto clinical implant failure. Bone constructs fail through insufficient load transfer and weak early mechanics [38,98]. Culture conditions and scaffold microarchitecture shape that potential independently [33-34]. Cartilage constructs fail through surface wear, fibrocartilage replacement, and phenotypic drift [6]. At the interface, osteochondral constructs fail through delamination, compartmental mismatch, and fatigue [9]—failure modes that multizonal scaffold designs should address directly [99], and that large animal joint mechanics expose in ways a bioreactor cannot [100-101]. Meniscal and disc models offer adjacent examples where anisotropy and cyclic loading define translational relevance [102-103]. Studies targeting regulatory acceptance or clinical comparison should embed validated outcome measures from the outset. Validated outcome measures such as KOOS and MOCART should be incorporated when cartilage or osteochondral constructs are intended for clinical comparison, because they provide clinically interpretable functional and imaging endpoints [104-105]. Systematic reviews of existing interventions provide comparator context for judging whether

a bioprinted construct produces clinically meaningful benefit [106] (Figure 5).

Bone constructs require demonstrated biomechanical recovery and vascular remodeling, not merely radiographic fill. For cartilage, histological staining cannot substitute for surface wear testing under articulation. And for osteochondral constructs, favorable compartmental imaging means little if interfacial shear strength and load transfer are never directly measured. Animal model selection should follow the same logic: rat and rabbit models support early screening [21,52,107]. Goat, sheep, and other large joint models are needed when load, joint size, and surgical workflow matter [76-77]. Meniscus and intervertebral disc models add adjacent examples where anisotropy and cyclic loading shape translational relevance (Table 5).

Product-specific release windows should be prospectively defined before implantation studies begin. A clinical product should specify acceptable ranges or decision rules for sterility, identity, potency, cell viability when applicable, construct dimensions, print fidelity, stiffness, degradation, bioactive-factor content, and contamination risk. These windows should be interpreted according to indication, model system, defect geometry, loading environment, and comparator rather than treated as universal numerical thresholds. Without prospectively defined release considerations, a positive animal result remains difficult to compare, reproduce, or translate.

In the revised framework, evaluation items are separated into product-specific release considerations and desirable translational endpoints. Release considerations refer to tests or acceptance windows that should be defined before implantation or formal translational comparison, whereas desirable endpoints strengthen clinical relevance but may depend on indication, model, and product complexity. The evidence basis for each item should also be identified as evidence-derived, consensus- or standard-informed, failure-mode-based inference, or author-proposed recommendation.

Table 5. Indication-specific evaluation domains and release considerations for orthopedic bioprinted constructs.

Evaluation domain	Bone constructs	Cartilage constructs	Osteochondral constructs
Architecture	Interconnected porosity, Smooth vascular access, and load-sharing geometry.	Smooth articulating surface, lateral zone, and matrix-retaining architecture.	Zonal cartilage, calcified cartilage, and bone compartments with a stable interface.
Mechanical evidence	Compression, fatigue, degradation-dependent mechanics, and host-graft load transfer.	Indentation, dynamic compression, lubrication, wear, and shear at the repair margin.	Interfacial shear, fatigue, compartment stiffness gradient, and whole-construct load transfer.
Biological evidence	Osteogenesis, vascular remodeling, mineralization, and immune-compatible integration.	Stable chondrogenesis without hypertrophy or calcification.	Compartment-specific matrix formation with vascular invasion limited to the bone phase.
In vivo evidence	Large-defect model when load sharing and vascular access determine success.	Immunocompetent joint model with cyclic loading and surface assessment.	Large-joint model assessing interface integrity, subchondral remodeling, and cartilage durability.
Product-specific release considerations	Sterility, dimensions, pore architecture, stiffness, degradation, and bioactive cue content.	Sterility, viability when applicable, matrix markers, surface mechanics, and friction/wear behavior.	Compartment identity, interface strength, stiffness gradient, sterility, and dimensional fidelity.
Clinical evaluation focus	Union, load-bearing recovery, revision avoidance, and imaging-confirmed remodeling.	Pain/function improvement, durable surface restoration, and delayed degenerative progression.	Joint function, interface durability, subchondral stability, and long-term graft survival.

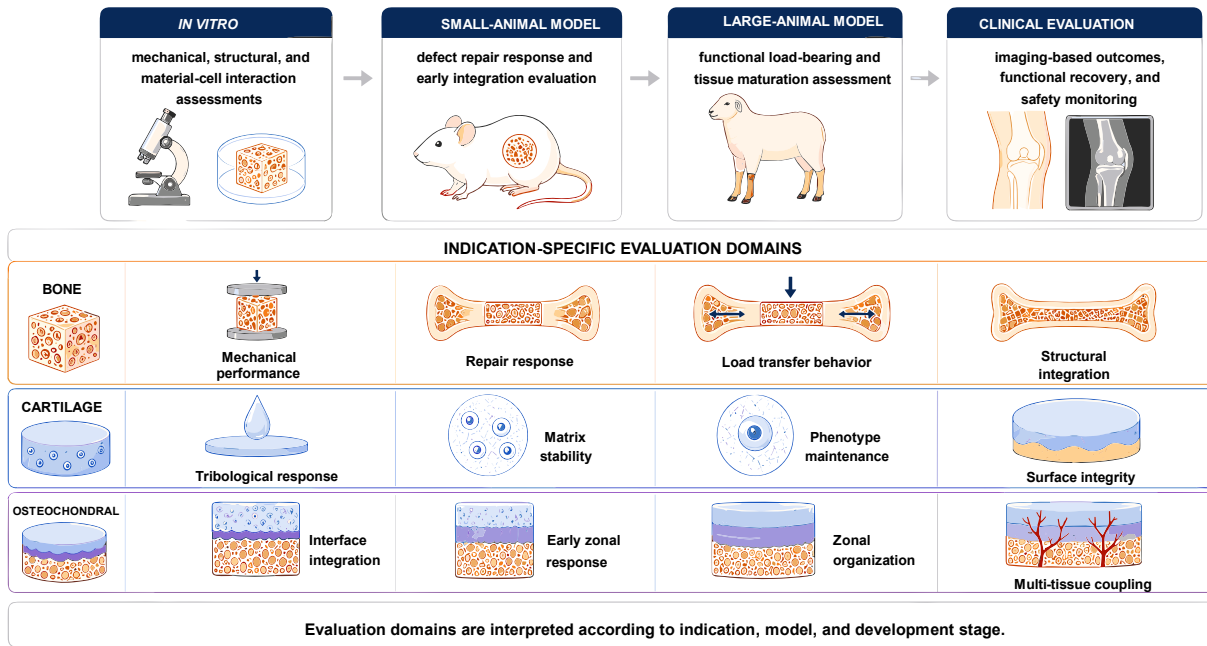


Figure 5. Indication-specific evaluation domains for 3D bioprinted constructs.

The figure outlines a non-threshold-based evaluation framework for 3D bioprinted bone, cartilage, and osteochondral constructs across in vitro, small-animal, large animal, and clinical evaluation stages. Key assessment domains include mechanical and structural behavior for bone constructs, tribological and matrix-related responses for cartilage constructs, and interface, zonal, and multi-tissue integration for osteochondral constructs. The framework is intended to support indication-specific interpretation rather than define universal numerical criteria.

5.1 Engineering validation, metrology, and quantitative release windows

Quantitative release windows should be indication-specific rather than universal. For bone constructs, reporting should include compressive modulus and strength, fatigue behavior, retained mechanical properties after degradation, effective modulus of the printed architecture, and host-graft load transfer. For cartilage constructs, reporting should include equilibrium and dynamic modulus, stress relaxation, creep, storage and loss moduli, friction coefficient, wear depth, and integration strength under hydrated cyclic loading. For osteochondral constructs, interfacial shear strength, stiffness-gradient integrity, delamination resistance, and fatigue under compartment-specific loading should be prioritized. These values should be interpreted against native-tissue ranges, clinically used comparators, defect geometry, and the intended loading environment.

Manufacturing metrology is the operational basis of reproducibility. Printed constructs should

report dimensional tolerances for strand or fiber diameter, pore size, pore interconnectivity, layer height, surface roughness, and overall construct geometry. Post-print dimensional verification may include micro-CT, optical profilometry, high-resolution optical imaging, or coordinate measuring systems, depending on material opacity and construct scale. These measurements should be linked to predefined acceptance windows rather than presented only as representative images.

Degradation should be evaluated together with mechanical integrity. For load-bearing or load-sharing applications, mass loss alone is insufficient; retained modulus, retained strength, fatigue resistance, porosity evolution, molecular weight loss, degradation products, and local pH should be tracked over the remodeling period. The central question is whether the construct maintains sufficient mechanical competence while progressively transferring load to newly formed tissue.

Fatigue testing should be incorporated when constructs are intended to experience cyclic loading. Relevant protocols may include cyclic compression, bending, torsion, or shear, depending on the target indication. Reporting should include stress amplitude, frequency, number of cycles, run-out definition, failure mode, and whether testing was performed after hydration, maturation, or partial degradation. These engineering validation domains and quantitative reporting parameters are summarized in Table 6.

Table 6. Engineering validation domains and recommended quantitative reporting parameters for orthopedic bioprinted constructs.

Construct type	Minimum mechanical tests	Quantitative parameters to report	Acceptance-window logic
Bone scaffold	Compression; fatigue; degradation-conditioned mechanics; host-graft load transfer.	Compressive modulus/strength; effective modulus; retained modulus/strength; fatigue life; porosity-adjusted stiffness.	Benchmark against defect geometry, host-bone range, and intended load-sharing period; avoid stress shielding or early collapse.
Cartilage construct	Indentation; dynamic compression; stress relaxation/creep; friction/wear.	Equilibrium/dynamic modulus; storage/loss moduli; loss tangent; friction coefficient; wear depth; integration strength.	Test hydrated constructs under physiologic loading rates and frequencies and compare with cartilage repair comparators.
Osteochondral construct	Interfacial shear; cyclic delamination; compartment stiffness gradient; fatigue.	Interface shear strength; delamination force; stiffness-gradient retention; compartment-specific mechanics.	Benchmark against joint location, tidemark/interface stability, and cartilage-bone load transfer.
Manufacturing metrology	Dimensional verification using micro-CT, optical profilometry, high-resolution optical imaging, or coordinate measuring systems.	Strand/fiber diameter; pore size and interconnectivity; layer height; surface roughness; overall construct dimensions.	Predefine tolerance windows linked to release criteria rather than relying on representative images.

5.2 Current clinical translation landscape

A handful of 3D-printed or biofabricated constructs have reached clinical testing or device clearance. What they prove depends heavily on the indication. In cranial reconstruction, a clinical pilot study of a 3D-printed bioresorbable PCL burr-hole cover reported improved patient-reported cosmetic outcomes with comparable safety, supporting feasibility for non-load-bearing cranial scaffold applications rather than validating load-bearing orthopedic grafts [27]. In long bone repair, patient-specific titanium scaffolds have been implanted for large segmental femoral and tibial defects; medium-term follow-up supports their role as structural bridging devices, and mechanobiologically optimized mesh architectures confirm that load-adaptive design matters for integration [23]. For cartilage, the regulatory benchmark is matrix-induced autologous chondrocyte implantation (MACI, Vericel), which earned FDA approval in 2016 after beating microfracture at five years in the SUMMIT trial [108]. Earlier randomized data from the TIG/ACT/01/2000 trial likewise documented that structural repair quality and clinical outcome with characterized chondrocyte implantation depend strongly on early intervention, which constrains the eligible patient window [109]. Osteochondral autograft transplantation provides another active comparator, with registry data showing graft survival figures that any bioprinted alternative must improve upon, particularly in lesions larger than 2 cm² [110]. Any cell-laden bioprinted cartilage product entering clinical development will need to define its advantage over these existing options in terms of lesion size, surgical simplicity, off-the-shelf availability, or functional outcome (Table 7).

Table 7. Selected orthopedic bioprinting products, biofabricated constructs, and clinical comparators.

Product or benchmark	Category	Indication	Status	Evidence	role and limitation
Osteoplug hole scaffold [27]	PCL burr- Acellular bioresorbable PCL scaffold.	Cranial cover.	burr-hole Clinical pilot study.	Supports non-load-bearing cranial feasibility; not evidence for load-bearing orthopedic grafts.	
Mechanobiologically optimized titanium mesh [23]	Patient-specific printed implant.	3D- Large structural femoral defects.	segmental or tibial Investigational or early clinical follow-up.	Supports load-adaptive structural design; not a living bioprinted graft.	
MACI/Vericeal [108]	Clinical comparator: autologous chondrocytes on collagen membrane.	Focal knee defects. on	cartilage FDA-approved; supported by 5-year randomized evidence.	Provides a regulatory and functional benchmark for cartilage repair; not a 3D bioprinted construct.	
Osteochondral autograft transfer [110]	Clinical native tissue.	comparator: Focal osteochondral knee lesions.	Established surgical practice.	Benchmark for integrated cartilage-bone repair; limited by donor-site morbidity and graft availability.	
Bioprinting clinical-study landscape [111]	Scoping review of registered observational bioprinting studies.	Broad and indications; repair remains a limited subset.	bioprinting Early clinical research landscape.	Maps clinical-translation immaturity; not a product-specific efficacy source for bone/cartilage repair.	
Candidate bioprinted constructs	Bone, cartilage, or osteochondral concepts.	Focal cartilage, bone graft void, or osteochondral repair.	Preclinical, feasibility, registry-level intent unless product-specific records are cited.	Specific claims require registry identifiers and published outcomes; no phase III comparative evidence yet.	

Abbreviations: CE, Conformance Européenne; ChiCTR, Chinese Clinical Trial Registry; FDA, US Food and Drug Administration; HAP, hydroxyapatite; MACI, matrix-induced autologous chondrocyte implantation; PCL, polycaprolactone.

A 2025 scoping review of clinical bioprinting studies paints a sobering picture: the field is still in early-phase clinical research, nowhere near mature comparative deployment [111]. For bone, cartilage, and osteochondral repair, the public trial landscape should be interpreted as registry-level or feasibility intent unless individual trial records and published outcomes are cited directly. At present, this review did not identify published randomized controlled data with functional endpoints at two or more years for product-specific 3D bioprinted bone or cartilage repair. The gap between registered trials and published results tells its own story: running a controlled study when product complexity, surgical technique, and patient diversity all vary independently is genuinely hard.

5.3 Lessons from failure and negative results

Read the bioprinting literature and you would think nearly everything works. Failure gets published far less often than it should, and that is a problem because failed constructs teach more than successful ones. Constructs that look strong in vitro consistently underperform in vivo: the implanted joint environment imposes synovial fluid, cyclic loading, immune infiltration, and vascular remodeling simultaneously, none of which static bioreactor testing fully captures [25]. Hydrogel cartilage constructs that hold their GAG content and chondrogenic markers through four weeks of culture routinely lose that phenotype after implantation, reverting toward fibrocartilage or hypertrophy once the joint's mechanical and biochemical signals take over [6,112]. Vascularization is the most consistent failure point in bone: capillary networks that look patent in fibrin gels in vitro repeatedly fail to connect with host vasculature in critical-size defects, where anastomosis must happen within days or central necrosis follows [19,25]. These failures make the case for a more demanding evidence framework — one requiring longer follow-up periods, physiologically relevant loading conditions, and immunocompetent large animal models before any move to human translation.

6. Manufacturing, Quality Control, and Regulatory Considerations

A bioprinted graft is not a standalone invention. It is a product system: materials, cells, printer hardware, software, process parameters, post-print culture, packaging, transport, and surgical handling all interact to determine clinical performance. Regulatory considerations may change when a construct becomes cell-containing, drug-releasing, patient-specific, load-bearing, or manufactured at the point of care or in the operating room [16,18,68].

Good manufacturing practice (GMP)-compatible development requires qualified raw materials, environmental controls, calibrated equipment, sterility assurance, batch documentation, operator training, and validated release testing. Patient-specific products pile on additional steps: image acquisition, segmentation, CAD modeling, slicing, toolpath generation, parameter selection. Every step alters the final graft; every step must therefore be traceable.

Quality by design is the organizing framework that fits this field best. Every development program should start with a quality target product profile for the intended indication—because a femoral segmental graft and a focal cartilage plug do not share the same critical quality attributes, and treating them as if they do produces constructs that satisfy neither. Material composition, extrusion pressure, crosslinking dose, culture duration, pore architecture, stiffness, potency, and degradation rate should each be connected, in advance, to the clinical function they are meant to support.

Artificial intelligence (AI) and digital twins are useful only when tied to measurable manufacturing or biomechanical decisions. Machine-learning models can map composition and process parameters to print fidelity, cell viability, and mechanics; digital twins can support patient-specific load simulation. Neither should be presented as a regulatory shortcut before validation

against experimental and clinical data [16,66].

6.1 Jurisdiction-specific regulatory considerations: FDA, EMA, and NMPA

The three major regulatory jurisdictions can handle bioprinted orthopedic constructs differently, and classification should be treated as product-specific rather than assumed from the printing technology alone. In the United States, the primary mode of action, intended use, material composition, cellular component, and drug-release function influence whether device, biologic, HCT/P, or combination-product frameworks are relevant. Acellular scaffolds may be reviewed primarily as medical devices, whereas cell-laden or drug-releasing constructs may trigger additional biologic or combination-product considerations [18,68]. Early FDA interaction can help clarify the lead center and evidence package, but it does not lower the required validation burden (Figure 6).

Europe likewise requires product-specific classification. Cell-containing constructs may fall within the Advanced Therapy Medicinal Product (ATMP) framework under Regulation (EC) 1394/2007 when the definition is met, whereas acellular 3D-printed scaffolds may be governed by the Medical Device Regulation (MDR 2017/745). The Hospital Exemption clause may allow member-state authorization for selected individualized ATMP use in licensed establishments, but it is limited and does not substitute for full marketing authorization [113].

In China, the National Medical Products Administration (NMPA) has issued technical review guidance for 3D-printed medical devices and patient-specific implants [114-115]. However, classification and evidence requirements should be assessed case by case according to composition, biologic activity, intended use, and patient-specific manufacturing workflow. Requirements for local clinical evidence, bridging data, or additional testing should be confirmed through jurisdiction-specific regulatory consultation rather than assumed uniformly. Terminal sterilization remains a high-risk manufacturing step across jurisdictions: gamma irradiation, ethylene oxide, and supercritical-CO₂ can each alter polymer chain length, residual monomer content, or bioactive-factor activity, and each method must be validated against the specific formulation [116-117]. Post-market vigilance should also be incorporated into the development plan because reported failure modes for 3D-printed orthopedic implants, including malposition, fracture, and infection, are relevant design-control considerations for related bioprinted products [118].

Table 8. Product-specific regulatory considerations and quality-control priorities for orthopedic bioprinting.

Product class	Regulatory consideration	Core QC focus	Jurisdictional note	Key uncertainty
Acellular reinforced scaffold	Device-oriented implant assessment may apply.	Sterility; mechanics; degradation; biocompatibility; dimensions.	Pathway depends on composition, intended use, degradation, and biologic or drug components.	Evidence package may be more established for device-like constructs, but classification remains product-specific.
Cell-containing construct	Cell, tissue, biologic, or combination-product considerations may apply.	Cell identity, viability, potency, traceability, tumorigenicity, immune compatibility.	Classification depends on cell source, manipulation, intended use, and device-cell integration.	Cell source, manipulation, potency, tumorigenicity, and immune safety remain major uncertainties.
Drug- or factor-releasing construct	Combination-product assessment may apply.	Dose; release kinetics; local toxicity; degradation products; scaffold interaction.	Lead-center or joint review should be clarified through product-specific regulatory interaction.	Dose, release kinetics, local toxicity, and combination-product assessment require early clarification.
Patient-specific graft	Digital workflow may become part of the product definition.	Imaging; segmentation; CAD/slicing; toolpath; fit; traceability.	Workflow validation and local evidence or bridging needs should be assessed case by case.	Traceability and patient-specific manufacturing control are central uncertainties.
Point-of-care or in situ printed construct	Device-procedure-product coupling may shape evidence expectations.	Aseptic field control; curing safety; adhesion; intraoperative verification.	Early regulator engagement is essential; pathway remains product-jurisdiction-specific.	Sterile-field manufacturing control and operator-dependent variability remain major uncertainties.
Individualized or limited-use pathway	Custom device, IDE, hospital exemption, or investigator-initiated use may be considered in selected contexts.	Case justification; informed consent; registry follow-up; adverse-event capture.	Routes differ by jurisdiction and do not substitute for marketing authorization.	Use is jurisdiction-specific and does not substitute for full marketing authorization.

Abbreviations: ATMP, advanced therapy medicinal product; CAD, computer-aided design; CAT, Committee for Advanced Therapies; CBER, Center for Biologics Evaluation and Research; CDE, Center for Drug Evaluation; EMA, European Medicines Agency; FDA, US Food and Drug Administration; HCT/P, human cells, tissues, and cellular and tissue-based product; IDE, investigational device exemption; MDR, Medical Device Regulation; NMPA, National Medical Products Administration; QC, quality control.

Table 8 integrates product-level regulatory considerations with the quality-control package required for translation. The key point is practical rather than semantic: product classification, intended use, biologic activity, manufacturing site, and patient-specific workflow jointly determine which evidence should be generated, which tests should be validated, and how early developers should engage regulators in the intended market.

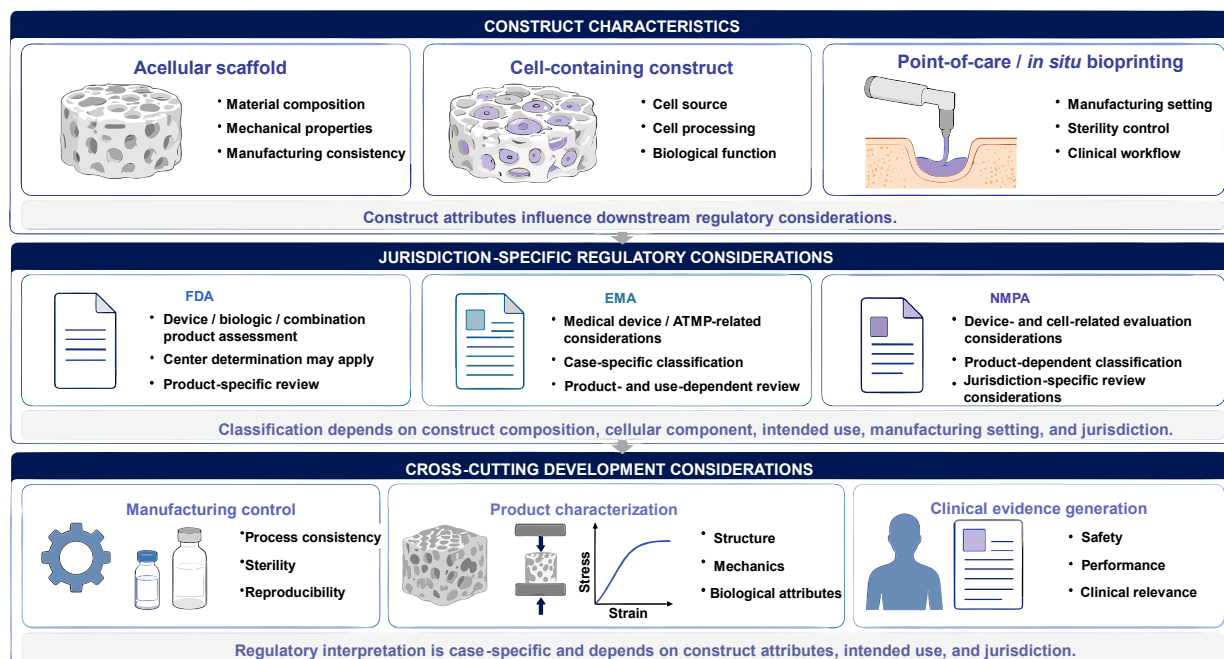


Figure 6. Regulatory considerations for 3D bioprinted orthopedic constructs. The schematic summarizes product-level and jurisdiction-specific regulatory considerations for acellular, cell-containing, and point-of-care or *in situ* bioprinted orthopedic constructs. Construct composition, cellular components, intended use, manufacturing setting, and biological function may influence regulatory interpretation across FDA, EMA, and NMPA frameworks. This figure is intended as a high-level conceptual summary and should not be interpreted as assigning fixed approval pathways, uniform classifications, or approval-difficulty rankings. Abbreviations: ATMP, advanced therapy medicinal product; EMA, European Medicines Agency; FDA, US Food and Drug Administration; NMPA, National Medical Products Administration.

6.2 Manufacturing informatics and AI-enabled quality control

AI and digital modeling are most relevant to orthopedic bioprinting when linked directly to process control, printability prediction, dimensional fidelity, mechanical design, and release testing. Supervised models can map composition and process parameters to print fidelity, gelation kinetics, post-extrusion cell viability, and construct dimensions [16,119-120]. Closed-loop systems may support real-time adjustment of deposition parameters when validated sensor feedback is available, but these tools should be presented as process-control aids rather than regulatory shortcuts. Physics-informed modeling may also support scaffold mechanics and degradation analysis when the underlying assumptions and validation datasets are explicitly reported [121].

Digital twins and finite element models can support patient-specific scaffold design by simulating stress distributions, load transfer, and remodeling trajectories before or after fabrication [122]. Their translational value depends on the quality of input data, the fidelity of material-property assignments, and validation against the same physical system. If AI/ML tools are used to

support regulated product release or device control, their role, update boundaries, validation dataset, and human oversight should be defined prospectively and aligned with applicable regulatory expectations.

7. Roadmap for Future Studies

The field has spent a decade running isolated demonstrations and now has a large preclinical literature to show for it. What it lacks is structured development programs. A single day-0 modulus measurement is not a mechanical characterization — mechanically reinforced hydrogels, fiber-reinforced constructs, and hybrid polymer-hydrogel systems must be challenged under fatigue, wear, degradation, and physiological loading before any functional claim is made [53,123]. On the materials side, batch-to-batch consistency and dECM standardization deserve as much attention as biological activity: raw material variability is one of the most direct barriers to GMP compliance, and it is routinely underreported [124].

Vascularized bone constructs should be judged on perfusion, host vessel connection, oxygenation, and remodeling trajectory. Endothelial staining alone is insufficient to establish functional perfusion [35,37]. The field lacks prospective vascular integration data from large animal models: what exists is mostly cross-sectional histology at a single endpoint, not the contrast-enhanced imaging and terminal perfusion measurements that would track anastomosis over time. Host-graft anastomosis remains a central unresolved requirement for thick or critical-size vascularized constructs. It must occur within days in critical-size defects; when it fails, central necrosis follows, and this is where prior vascularization strategies have most consistently broken down [19,25]. Future comparative work should evaluate pre-vascularization, sacrificial templating, and growth-factor-releasing approaches under equivalent large animal conditions. For cartilage, surface mechanics and phenotype stability under cyclic loading in immunocompetent hosts are the relevant metrics. For osteochondral constructs, interfacial shear strength and compartmental stability belong in the primary endpoint list, not appended as secondary observations.

Immunomodulatory assessment should be incorporated into translational study design when host response is expected to influence integration. Immunocompetent models are preferable for constructs intended for implantation, particularly when allogeneic cells, immunomodulatory cues, or degradation products are involved. Macrophage activation trajectories can be tracked at early time points such as days 3, 7, and 14, with immune findings analyzed against integration outcomes rather than as separate parallel experiments [90-91]. For allogeneic cell-laden constructs, immune tolerance strategies and their durability under physiological conditions require direct experimental validation.

Translation also demands reporting standards. Published studies should include: bioink composition with batch identifiers, cell source and passage number, printing parameters (nozzle diameter, temperature, pressure, speed, layer height), crosslinking conditions, construct dimensions, culture conditions, mechanical testing protocols, animal-model rationale, sample size justification, and follow-up duration. Without these details, the field accumulates results that are

difficult to compare, replicate, or pool in meta-analysis. A field-specific minimum reporting checklist—think CONSORT for clinical trials or ARRIVE for animal studies—would close this gap, consistent with the MIBBI framework [125]. Depositing bioink composition, print parameters, and outcome data in public repositories—aligned with the FAIR (findable, accessible, interoperable, reusable) principles—would make cross-study meta-analyses possible; the infrastructure to perform them does not yet exist [126]. The proposed minimum reporting checklist for future orthopedic bioprinting studies is summarized in Table 9.

Table 9. Proposed minimum reporting checklist for orthopedic bioprinting studies.

Reporting domain	Minimum required elements
Bioink and materials	Full composition, supplier, batch identifier, crosslinking chemistry, degradation profile, and sterilization or aseptic processing method.
Cellular component	Cell source, donor status, passage number, seeding density, viability, phenotype, and immunologic characterization when applicable.
Printing process	Printer type, nozzle or optical parameters, temperature, pressure, speed, layer height, toolpath, print environment, and rheological parameters such as viscosity-shear-rate curves, yield stress, storage/loss moduli, and thixotropic recovery.
Post-processing and maturation	Crosslinking dose, culture medium, maturation time, bioreactor or perfusion conditions, and storage or transport procedure.
Construct characterization	Dimensions, dimensional tolerances, post-print verification method, fidelity, porosity, surface roughness, mechanics, retained mechanical properties after degradation, matrix composition, bioactive-factor release, and sterility testing.
Preclinical model	Species, defect size, immune status, loading environment, sample-size rationale, control group, and follow-up duration.
Outcome measures	Histology, imaging, mechanical testing, integration, vascularization, immune response, function, and adverse events.
Data transparency	Raw or processed print parameters, bioink composition, statistical code when applicable, and repository accession or availability statement.

Note: This checklist is intended to improve reproducibility and comparability across preclinical and translational studies.

Clinical translation may be most defensible when it begins with products of manageable complexity, such as acellular or minimally cellular reinforced scaffolds, patient-specific constructs that remain compatible with quality testing, and selected in situ applications where defect filling and rapid curing provide a clear surgical advantage. Fully vascularized, immune-instructive, multicellular living grafts should be advanced only after stronger evidence and manufacturing packages are established.

8. Conclusion

The clinical value of orthopedic bioprinting will not be determined by printing complexity

alone. It will depend on whether each construct can be matched to a defined failure mode, assigned measurable design specifications, manufactured reproducibly, and evaluated with endpoints that predict function in the target tissue. For bone, this means vascularized remodeling and progressive load sharing; for cartilage, a durable low-friction surface with stable chondrogenic phenotype; and for osteochondral repair, a mechanically competent interface that permits bone and cartilage programs to mature without destabilizing each other.

Future development should therefore shift from demonstration-driven printing to product-oriented translation. Bioinks, printing platforms, cell sources, maturation protocols, and AI-enabled process controls should be selected according to the role they perform in the final graft and tied to predefined release criteria, including architecture, mechanics, degradation, biological integration, host response, sterility, and manufacturing tolerance. The most realistic near-term opportunities are likely to involve acellular or minimally cellular reinforced scaffolds, patient-specific constructs that remain amenable to quality testing, and selected intraoperative approaches with clear surgical utility.

Several boundaries should temper this roadmap. This review is narrative rather than systematic, and the evidence base remains uneven across bone, cartilage, and osteochondral applications. Many proposed development priorities are therefore framework-based and failure-mode-informed rather than derived from direct comparative trials. Regulatory classification will also remain product- and jurisdiction-specific. These limitations do not weaken the translational argument; they define it: orthopedic bioprinting will advance when promising constructs are evaluated as reproducible, risk-matched products rather than as isolated proof-of-principle studies.

List of abbreviations

3D: three-dimensional
AI: artificial intelligence
ATMP: Advanced Therapy Medicinal Product
CAD: computer-aided design
dECM: decellularized extracellular matrix
DLP: digital light processing
ECM: extracellular matrix
EMA: European Medicines Agency
FDA: US Food and Drug Administration
GAG: glycosaminoglycan
GelMA: gelatin methacryloyl
GMP: good manufacturing practice
HA: hyaluronic acid
HAp: hydroxyapatite

iPSC: induced pluripotent stem cell
MSC: mesenchymal stem/stromal cell
NMPA: National Medical Products Administration
PCL: polycaprolactone
PLGA: poly(lactic-co-glycolic acid)
QbD: quality by design

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

Not applicable.

Further disclosure

During the preparation of this manuscript, the authors used AI-assisted tools for language polishing,

grammar checking, formatting refinement, and conceptual discussion of figure organization and visual presentation. AI-assisted tools were not used to generate primary research data, perform statistical analyses, conduct independent literature screening, or draw scientific conclusions. All AI-assisted suggestions, including those related to figure concepts and layout, were critically reviewed, revised, and approved by the authors. The authors take full responsibility for the accuracy, integrity, and final content of the manuscript. All figures and the graphical abstract were created by the authors as schematic illustrations for this review, with AI-assisted discussion used only to help refine figure organization, visual layout, and textual clarity. The scientific content, terminology, and final interpretation of all figures were determined and approved by the authors. No primary experimental images, patient-identifiable images, or unpublished clinical images are included. The figures do not present newly generated experimental data.

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