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Bone organoids and microphysiological systems for functional and safety assessment of bone bioinks

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Abstract

Three-dimensional bone bioprinting combines living cells, hydrogels, mineral phases, bioactive molecules, and stabilization methods to guide tissue formation. Bioink studies still rely mainly on printability and short-term cell viability. These measures can miss delayed injury and defects in matrix maturation, osteocyte differentiation, vascular organization, remodeling, and immune–bone signaling. This narrative review examines how bone organoids and microphysiological systems can extend bioink testing from acute cytocompatibility to tissue function and functional safety. We define bioink as a process-defined test article that changes during printing, stabilization, culture, and degradation. We use a four-class evidence taxonomy and distinguish self-organized bone organoids from organotypic bioprinted constructs, engineered bone microtissues, and organoid-like constructs. Representative studies are compared by material, cells, culture time, quantitative endpoints, findings, and limitations. We then propose a tiered framework that links material characterization and process-matched cytotoxicity tests to organotypic function, challenge–recovery studies, and separate predictive validation. Published studies provide mainly pairwise or platform-specific evidence, and no identified study tested osteogenesis, vascular function, inflammation, resorption, and marrow support in one validated bioink-assessment system. We therefore distinguish those findings from an author-proposed factorial framework for future cross-domain studies. Direct evidence remains uneven, especially for exposure-based reference organoids and long-term osteoimmune assessment. The framework should therefore be treated as a testable decision strategy rather than an established regulatory method. When the context of use, exposure controls, and reference benchmarks are defined in advance, these models may help distinguish bioinks that preserve short-term survival from those that support coordinated bone function.

Keywords: Three-dimensional bioprinting; Bioink; Bone organoid; Bone-on-a-chip; Microphysiological system; Osteoimmunology; Biocompatibility; Functional safety

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1. Introduction

Bone regeneration is not a single osteogenic event. It is a temporally coordinated sequence in which inflammatory initiation and resolution, progenitor recruitment, angiogenesis, matrix organization, mineralization, mechanoadaptation, and osteoclast–

osteoblast remodeling must occur in a compatible order.[1] Critical-sized defects disrupt several of these processes simultaneously. This is why a material that increases an early osteogenic marker can still fail to restore durable bone: the relevant unit of performance is the coupled tissue response, not the isolated activity of one cell population.

Three-dimensional (3D) bioprinting offers unusually direct control over the spatial placement of cells, hydrogels, mineral components, and signaling molecules. But the same control can create a misleading equivalence between geometric fidelity and biological success. A printed lattice with intact filaments may still impose injurious stresses during deposition, restrict transport after crosslinking, or produce an extracellular environment that arrests maturation. ASTM F3659-24 appropriately treats bioink performance across pre-printing, printing, and post-printing stages, including rheology, crosslinking, degradation, cell health, and biological function.[2] The practical implication is that neither a rheological measurement nor a viability image, considered alone, sufficiently describes the final test article. [3-10]

Modern bone bioinks are often designed to be bioactive. Bone morphogenetic protein-2-loaded nanoparticles, hydroxyapatite-containing hybrid networks, mechanically conditioned constructs, and cellular spheroid-based inks can influence differentiation, mineral formation, or vascular organization.[11-14] These examples illustrate both the promise and the assessment problem. A bioactive component that accelerates one desirable pathway may alter immune behavior, remodeling, or dose distribution at a later stage. In other cases, a formulation that is modest in a short assay may become effective after cell-mediated remodeling. Bioink assessment must examine changes over time and multiple mechanisms.[15-19]

Fabrication history contributes to the biological state of the construct. Extrusion exposes cells to pressure, shear, extensional deformation, and residence-time-dependent stress; the resulting injury can range from transient membrane disruption to apoptosis or altered phenotype.[20,21] Photocrosslinking adds light dose, photoinitiator concentration, radical generation, and conversion efficiency to the exposure profile. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate with 405-nm light, for example, has shown condition-dependent cytotoxicity that cannot be inferred from testing the photoinitiator or light in isolation.[22] Post-printing exposures then arise from unreacted species, ionic exchange, particles, incorporated drugs, and degradation products.[22-26]

Standard cytocompatibility assays help identify clearly harmful formulations. Live/dead staining, membrane-integrity assays, metabolic activity, apoptosis, morphology, proliferation, and standardized direct-contact or extract tests can efficiently eliminate poor candidates.[27,28] But the absence of acute cell death does not show that bone-specific function is preserved. A hydrogel may support high viability while suppressing osteocyte maturation, blunting endothelial organization, altering macrophage resolution, or uncoupling resorption from formation. In this review, we use the term functional safety for the absence of material- or process-related effects that preserve apparent survival but compromise a tissue-relevant function. The term is operational, not a new regulatory category.

Immune effects make this distinction important. Bioactive cations and osteoimmunomodulatory bioinks can change macrophage signaling, osteogenesis, and defect repair.[25,29,30] Immune-instructive bone organoids made with plant-derived composite bioinks further suggest that an ink can be designed to shape immune homeostasis during tissue maturation.[31] These findings should not be reduced to the binary language of M1 and M2 phenotypes; macrophage states are more heterogeneous and time-dependent. The main point is that the immune response is part of bioink function, not just a complication after implantation.

Bone organoids and microphysiological systems (MPS) provide a route toward this wider assessment. Bone-organoid consensus work has emphasized self-organization, structural and functional attributes, and the need for clearer quality criteria.[32] Bioprinted periosteum-like organotypic constructs, mechanically matured osteocyte constructs, vascularized tissues, and perfused bone-development or remodeling chips reproduce distinct components of skeletal physiology.[13,14,33-36] Microfluidic production can also improve organoid uniformity and throughput.[37] The field still risks substituting one superficial label for another: calling a cell aggregate an organoid does not make it more predictive, just as calling a hydrogel cytocompatible does not establish its long-term suitability.

Another review of 3D cell models for bone-regenerative materials found that organotypic systems can add useful evidence, but endpoints and validation methods remain inconsistent.[38] This review adds four analyses outside the scope of the previous article. We examine risks introduced by printing and stabilization, define the bioink as a test article whose identity changes over time, map model attributes to specific assessment questions, and connect functional readouts to explicit decision rules. We also grade the evidence behind each use. A model-building study shows that a platform can reproduce a function. It does not, by itself, show that the platform can identify a bioink hazard.

We conducted a focused narrative search of PubMed and checked the reference lists and publisher records of relevant primary studies and reviews through September 20, 2026. Search terms combined bioink or bioprinting with bone organoid, bone-on-a-chip, bone marrow-on-a-chip, microphysiological system, osteocyte, vascularization, macrophage, osteoclast, remodeling, cytotoxicity, crosslinking, degradation, and functional safety. We prioritized primary studies that reported a defined material or process, cell source, culture or exposure window, control, and tissue-relevant endpoint. Standards and reviews were used for terminology, test principles, and regulatory context. Non-bone organoid studies were included only when they provided a transferable method for exposure control, assay qualification, or evidence interpretation.

Evidence was assigned to four classes, and the same terms are used throughout this review. Direct bioink evidence tests a cell-bearing formulation or printing condition and measures a tissue outcome. Model-capability evidence shows that an organoid, organotypic construct, engineered microtissue, or MPS can reproduce a relevant structure, response, or cell interaction without directly comparing candidate bioinks. Mechanistic/pharmacologic evidence identifies a pathway, perturbation, or response that supports endpoint selection or causal interpretation; this class includes relevant animal and non-bioprinted studies. Author-proposed evidence comprises the configurations, tiering rules, interaction tests, and decision criteria synthesized in this review and still requires prospective validation. Functional domains such as osteogenesis, vascular function, inflammation, resorption, and marrow support are outcomes, not additional classes of evidence. This distinction is important because a sophisticated model does not provide direct bioink evidence unless the candidate formulation or process is part of the comparison.

2. Bioink identity, lifecycle hazards, and limits of conventional testing

2.1. Bioinks as complex test articles: Definitions, contexts of use, and assessment boundaries

2.1.1. Defining the bioink in three-dimensional bone bioprinting

A widely used definition describes a bioink as a formulation containing living cells and suitable for processing by an automated biofabrication technology; this distinction separates bioinks from acellular biomaterial inks.[39] That definition remains valuable, but it is not sufficient for biological assessment. In a bone-bioprinting study, the cells interact not with an abstract formulation but with a succession of material states: the premixed suspension, the pressurized ink in the cartridge and nozzle, the deposited filament, the newly stabilized network, and the maturing or degrading construct. Each state can differ in oxygen tension, stress history, crosslink density, diffusivity, interfacial chemistry, and soluble-species concentration.[3-5,23,29]

We define a bone bioink as a process-defined test article. Its identity includes the material and its source; the cell source, state, and density; device-contact materials; printing and environmental conditions; stabilization chemistry and energy input; and changes during culture or use. This definition is needed because the same polymer concentration does not always create the same biological exposure. A smaller nozzle, a higher light dose, or a longer diffusion distance can produce a different test article.

Printability frameworks correctly connect rheology to filament formation and shape retention.[40,41] But printability is a manufacturing attribute, not a biological endpoint. The often-repeated objective of balancing resolution and viability is itself incomplete, because shear conditions can preserve most cells while altering a vulnerable subpopulation or producing delayed effects.[42] Cell loading can also change rheology and resolution, meaning that an apparent improvement in printability may reflect a change in the biological fraction of the ink instead of an intrinsically superior material.[43] The term printable should always be linked to the process window and the biological state that remains after printing.[4,5,23-25,34]

Rheological descriptors are most informative when connected to exposure mechanisms. Yield stress, shear thinning, thixotropic recovery, viscoelastic moduli, and gelation kinetics influence extrusion pressure, residence time, post-deposition recovery, and cell redistribution.[35,44-46] No single parameter serves as a universal printability index. A rheologically favorable ink can still be biologically unfavorable if its recovery is achieved by interactions that restrict cell spreading or if high post-print stiffness inhibits remodeling. Qualification should distinguish manufacturing adequacy from biological adequacy while retaining the causal link between them.[23-26,47-49]

2.1.2. Cytocompatibility, biocompatibility, biofunctionality, and functional safety

The four terms are related but not interchangeable. Cytocompatibility concerns the response of cells under specified test conditions, commonly survival, metabolic activity, morphology, or proliferation. Biocompatibility is broader and, under a risk-management approach, is evaluated for a finished device or material based on its nature, duration of contact, and the hazards relevant to that use.[50] A positive live/dead result is evidence for one cytocompatibility endpoint; it is not a general claim of biocompatibility.

Biofunctionality describes the capacity of a bioink to support or direct an intended biological function. We find it useful to distinguish permissive from instructive biofunctionality. A permissive matrix allows cells to execute an expected program without material-imposed obstruction; an instructive matrix actively changes the program through mechanics, ligand presentation, mineral phases, soluble cues, or degradation. Matrix dimensionality and stiffness can alter osteogenic differentiation, and hydrogel biophysical properties can change co-culture-mediated osteogenesis.[51,52] Nanoengineered inks can be intentionally osteoinductive.[53] These are not simply stronger versions of cytocompatibility; they concern different biological questions.

Functional safety asks whether the candidate bioink causes unintended loss, distortion, or temporal uncoupling of a relevant tissue function. The concept includes failure without widespread death. For example, matrix stiffness can alter osteoclast fate, sustained calcium release can reprogram macrophage signaling, and calcium-phosphate materials can induce macrophage-derived osteogenic factors.[54-56] Whether these effects are beneficial depends on timing, magnitude, and context. A formulation that suppresses osteoclastogenesis may be attractive in one disease model but problematic for a regenerative construct that must remodel. Functional safety is inseparable from context of use.

A measured functional change can have three meanings. Expected modulation is an intended and bounded response, such as reversible suppression of sclerostin by PTH(1-34) in a responsive osteocyte model.[57] Functional insufficiency means a candidate fails to reach an efficacy or maturation target, but this alone does not establish biological injury. Adverse functional injury is an unintended loss, distortion, or delay that is large enough, persistent enough, or dose-related enough to matter for the intended use. Interpretation should consider the effect relative to a reference range, its duration and reversibility, the exposure–response relation, and whether it compromises the product or model purpose. The same change can be useful in a disease model and unacceptable in an implant intended to support repair.

These terms are related, but they should not be placed on one scale from toxic to bioactive. Bioink performance has several dimensions. A formulation can preserve cell survival but impair function. It can slow cell growth and still permit recovery. It can also promote osteogenesis while causing an unwanted inflammatory response. Each conclusion should state the domain that was measured and the domains that were not tested.

2.1.3. Contexts of use for bioink assessment

At least three contexts of use should be separated. The first is regenerative implantation, where the bioink is intended to remain at a defect site and interact with host tissue. The second is fabrication of an *in vitro* tissue model, where the ink should faithfully represent normal or diseased biology. The third is production of a screening platform, where reproducibility, throughput, and assay compatibility may be more important than direct implantability. A bioink optimized for one context can be unsuitable for another. An osteoinductive matrix may be desirable for repair but confound a model intended to compare patient-intrinsic osteogenic capacity.

Cellular configuration is equally important. In a construct-based test, the candidate bioink forms the organoid or tissue and is evaluated as the complete material–cell–process system. Co-culture printing of macrophages and mesenchymal stromal cells illustrates how this configuration can reveal reciprocal osteoimmune effects.[58] In an exposure-based test, a standardized reference organoid receives extracts, released components, degradation products, or conditioned medium. This configuration improves comparability and helps separate soluble hazard from matrix-mediated effects. It can also reduce material-driven changes in tissue architecture, but it will miss contact, mechanics, and local gradients.

Matrix type can change organoid phenotype and treatment response. This has been shown in patient-derived osteosarcoma organoids.[59] Construct-based models reproduce the final material more closely, but they make causal analysis harder. Exposure-based models isolate the material effect more clearly, but they do not reproduce the whole construct. For decisions with high consequences, using both can be useful. A reference tissue can identify a soluble effect, and the candidate construct can show whether that effect still occurs in the final matrix.

Perfused MPS add controlled flow, repeated sampling, compartmentalization, and defined gradients. Prevascularized bone organoids, vascularized bioprinted constructs, and dental pulp-derived vascularized organoids demonstrate several routes to vascular complexity.[60-62] These features also introduce sources of variation, including device absorption, bubbles, flow changes, and operator effects. A study should use perfusion or separate compartments only when testing the biological mechanism.

2.1.4. Assessment boundaries

This review focuses on cell-containing bioinks for 3D bone bioprinting and on *in vitro* models used to evaluate their function and biological safety. We included acellular

scaffolds, non-bioprinted bone models, and non-bone organoid methods only when they clarified a material exposure, a functional endpoint, or a control strategy that can be transferred to bioink testing. Osteoimmune mechanism studies were included when they connected a material cue to an immune mediator and a downstream skeletal response. These studies are treated as indirect evidence and are not presented as proof that a bioink assessment method has been validated. We did not catalog every hydrogel chemistry, printer, or animal model.

Acellular and non-cell-laden printed materials remain useful when they identify a mechanism or design principle relevant to bioink assessment. For example, a 3D-printed tissue-specific nanospike-based adhesive was designed for timed antitumor treatment and bone-tissue regeneration after osteosarcoma resection.[63] This study broadens the material design space for bone healing, but it is classified here as mechanistic/pharmacologic evidence rather than direct bioink evidence because the printed article was not evaluated as a cell-bearing bioink.

This framework does not treat organoids or MPS as replacements for all *in vivo* evidence. Their current role is to test mechanisms, compare candidates under controlled conditions, and find failures that standard tests miss. Qualification of a model should be tied to a stated context of use, consistent with the logic used for medical-device development tools.[64] Spatially controlled delivery of growth factors in bioprinted bone models further illustrates why a test must reflect where and when an active cue is presented, not just its nominal concentration.[65] Figure 1 applies these limits by treating the bioink as a test article that changes during processing and culture. Its identity includes the nominal formulation, cell handling, deposition exposure, stabilization, transport, degradation, and intended use. This lifecycle view follows the workflow in ASTM F3659-24 and extends assessment from printability and acute cytocompatibility to long-term tissue function.

2.2. Lifecycle-derived hazards and failure modes

2.2.1. Raw materials, provenance, and batch variability

Raw-material risk begins before printing. Polymer molecular-weight distribution, degree of functionalization, residual catalysts, endotoxin, ionic composition, protein content, particle size, and sterilization history can change both processing and cell response. Natural materials and decellularized extracellular matrix (dECM) are particularly information-rich but also compositionally variable. Manufacturing studies of dECM hydrogels show that tissue source, decellularization, digestion, neutralization, and storage affect biochemical retention and gel behavior.[66] The biological attraction of dECM is inseparable from the need for lot-level characterization.[9,10,15-17]

The influential demonstration of tissue-specific dECM bioinks established that native matrix cues can improve tissue-relevant behavior.[67] Subsequent work showed that detergent choice changes the performance of dECM-derived bioinks, while rapid volumetric printing has introduced additional demands on optical properties

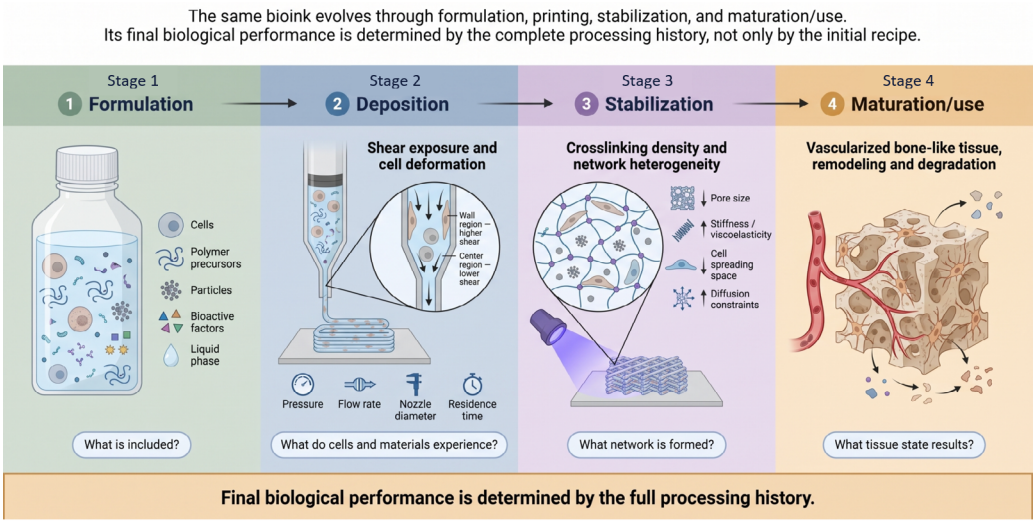

Figure 1. A bioink as a process-defined and time-evolving test article. Composition, cells, device settings, stabilization, transport, and degradation jointly determine biological exposure. The same nominal formulation can produce different tissue outcomes when the process window or construct geometry changes. The arrows beside pore size, stiffness, and diffusion show common but formulation-dependent changes and should not be read as universal directions. Deposition and stabilization may also overlap, as in in-nozzle or light-assisted printing.[2,22,39,42] Created with BioRender. Minglu Hao (2026). https://BioRender.com/c1ef3un.

and photochemistry.[68,69] We consider statements such as "dECM-based" too imprecise for comparative safety assessment. The test article must be linked to source tissue, donor pooling, decellularization metrics, residual DNA, key matrix proteins, endotoxin, digestion conditions, and batch acceptance criteria.[4,24-26,47,70-74]

Cell components also require provenance control. Donor age, sex, disease, isolation method, passage, cryopreservation, expansion medium, and pre-print phenotype can affect stress tolerance and differentiation. A material should not be called cytocompatible because one cell lot shows high survival. At minimum, process optimization should use a stable reference lot, while confirmatory functional testing should include biologically relevant donor diversity. Otherwise, technical reproducibility and biological heterogeneity are impossible to separate.

Sterilization and storage should be treated as changes to raw-material quality attributes. Filtration, irradiation, ethylene oxide exposure, freezing, and repeated thawing can change polymer size, protein activity, particle dispersion, or gelation. The relevant comparison is therefore the material before and after the selected treatment. A cell-laden final construct usually cannot be terminally sterilized without destroying the product, so aseptic manufacture and process controls must be distinguished from sterilization of the acellular raw material.

2.2.2. Printing-induced biomechanical injury

Extrusion injury is frequently summarized by nozzle diameter or pressure, but neither variable alone defines cell exposure. Stress depends on ink rheology, nozzle geometry, flow profile, wall slip, cell concentration, residence time, temperature, and how pressure is generated.[20,40,42,44] A short print may produce a tolerable average viability while selectively depleting larger cells or inducing sublethal membrane and cytoskeletal injury. The appropriate comparison is a matched unprinted ink that has undergone the same handling, temperature, and waiting time, along with printed conditions spanning the intended process window.[10,15,19,48,49,75]

Immediate membrane integrity should be complemented by delayed endpoints. Calcium supplementation can reduce shear-related damage, showing that the surrounding bioink modifies the cell's mechanical response.[21] But an intervention that improves acute survival may also change crosslinking, signaling, or mineralization. We recommend a challenge–recovery design: measure damage soon after printing, allow a defined recovery interval, and then test phenotype and function. This distinguishes reversible process stress from injury that redirects tissue maturation.[75-79]

Most quantitative evidence in this area concerns extrusion. The hazard profile changes with the printing method. Droplet systems add nozzle actuation, transient pressure, and limits on particle size and viscosity. Light-based systems reduce nozzle shear but add prolonged optical exposure, photoinitiator chemistry, light scattering, and spatially variable conversion. Aspiration-assisted placement mainly stresses preformed spheroids during pickup and release. These processes should not inherit extrusion-specific acceptance limits without a measured link to exposure.

2.2.3. Stabilization chemistry and energy input

Photocrosslinking creates a composite exposure involving initiator, light, oxygen, radicals, and conversion kinetics. Testing each factor separately can identify the mechanism, but safety interpretation must include their combination in the actual construct.[22] Optical attenuation makes this especially important in thick or particle-containing bone inks: surface regions may receive excessive light while deeper regions remain under-crosslinked and retain mobile species. A single nominal exposure time is not a spatial dose map.[24,48,49,76-78]

Recent work on antioxidative strategies for photocrosslinked bone constructs reinforces that oxidative stress may alter osteogenic behavior even when gross viability remains acceptable.[80] Crosslinking should be evaluated through a factorial design that separates dark initiator controls, light-only controls, fully formulated unprinted controls, and printed/crosslinked constructs. Conversion, swelling, extractables, and mechanical stability should be measured alongside biological endpoints. Ionic and enzymatic stabilization require analogous controls for osmolarity, pH, counter-ions, enzyme residues, and diffusion-limited crosslinking.

Crosslinking is not a one-time manufacturing event. It sets the starting network in which cells must spread, produce matrix, and remodel the construct. A strongly crosslinked construct can pass an acute assay but later restrict these processes. The aim is enough early stability followed by changes that permit tissue development.

Ionic and enzymatic stabilization require the same exposure logic as photocrosslinking. Bulk ion concentrations do not capture the local ionic environment within a filament, while enzyme dosage alone does not reflect the extent of conversion under diffusion-limited conditions. Conversion, residual reactant, local stiffness, and release into medium should be measured together, as they can affect cell spreading or later matrix deposition. A dark control or light-only control identifies one source of

injury, but neither replaces a process-matched control that combines material, cells, and the complete stabilization step.[76,78,80]

2.2.4. Transport limitation, degradation, and released species

Construct dimensions, pore architecture, cell density, oxygen consumption, and medium exchange determine whether a viable rim conceals a compromised core. When the intended construct is large, limited diffusion is a design problem, not just a culture artifact, and the test should detect it. But it should not be confused with intrinsic chemical toxicity. Spatial viability, oxygen-sensitive reporters, histology, and sectioned functional readouts can distinguish a transport gradient from a uniformly distributed hazard.[70,79,81,82]

Degradation changes mechanics and generates a moving chemical exposure. Polymer fragments, residual initiator, proteins, ions, nanoparticles, incorporated drugs, and adsorbed manufacturing residues may appear at different times. Sample preparation and extraction conditions should be justified, as emphasized by ISO 10993-12, while chemical characterization and toxicological risk assessment are addressed by ISO 10993-18 and ISO 10993-17.[75,83-85] Exhaustive extracts are useful for hazard identification, but they may not represent the dose and kinetics experienced by cells in culture or by tissue after implantation. Physiologically relevant release studies should accompany worst-case extraction.[16,17,71-74,76,86]

Three diagnostic questions should be separated. Transport failure is suggested when the construct core improves after reducing thickness or increasing perfusion without changing chemistry. Structural failure is suggested when mechanics or geometry deteriorates before a change in released species. Release-mediated injury is supported when a measured analyte reproduces the effect in a reference tissue, and the effect tracks delivered dose. Material-only constructs, size-matched controls, and time-resolved release measurements are needed because these mechanisms can occur together.[83-85]

Bioink-related hazards can be classified according to the stage at which they occur. Intrinsic hazards come from known components. Process hazards arise during printing, sterilization, or crosslinking. Later hazards result from transport limits, degradation, or cell-mediated remodeling. This grouping links each measurement to a likely cause. Table 1 lists the main uncertainties at each stage, the failures that acute viability tests may miss, and suitable models or readouts. It is a guide for selecting tests, not a checklist for every bioink.

2.3. Limitations of conventional bioink testing in detecting functional failure

2.3.1. Scope and limitations of acute cytotoxicity assessment

Acute cytotoxicity answers an essential but limited question: under the specified conditions, does the test article cause sufficient injury to produce a measurable loss of membrane integrity, metabolism, cell number, or morphology? ISO 10993-5 provides a structured basis for such testing.[27] The result is most useful as an early gate. It is less useful when translated into broad claims such as "safe," "biocompatible," or "suitable for bone regeneration." Those conclusions require evidence from additional biological domains.[25,26,75-78]

Viability assays answer a narrow question. A formulation can change lineage commitment, extracellular matrix organization, contractility, mechanosensitivity, cytokine responses, or remodeling before cell number reduces. Metabolic activity can also rise because of stress or proliferation without showing tissue maturation. Reports should use endpoint-specific language: the candidate did not reduce a defined viability measure at a stated dose, time, and test condition.[84,86-90]

2.3.2. Assay interference and spatial averaging in three-dimensional

Three-dimensional matrices can interfere with fluorescent penetration, light collection, dye binding, enzymatic reactions, and product diffusion. Mineral particles may scatter light or adsorb proteins; colored plant or tissue-derived materials may alter optical readouts; reducing compounds can perturb redox-based metabolic assays. The Organisation for Economic Co-operation and Development *Guidance Document on Good In Vitro Method Practices* emphasizes the characterization of test-system and test-item interactions, appropriate controls, and reproducible execution.[91] For bioinks, matrix-only blanks, spiked recovery controls, and orthogonal assays are not optional details when the material is optically or chemically active.

A mineral-containing bioink provides a simple example. The material-only construct should define optical and calcium-staining background at every relevant time point. A known amount of fluorophore, protein, or metabolite should be added to the blank matrix to test recovery. Mineral accumulation should then be confirmed with an orthogonal method, such as time-lapse micro-computed tomography, Raman analysis, or histology that separates preloaded particles from new matrix. A candidate should not be called more osteogenic when the difference can be explained by higher mineral loading or assay recovery.

Table 1. Lifecycle hazards and model strategies for bioink assessment

Lifecycle stage	Risk and causal mechanism	Failure missed by acute viability	Mechanism-linked readout	Diagnostic control
Raw materials	Identity, impurities, endotoxin, or lot variation changes ligand content, gelation, or immune stimulus	Different maturation or immune behavior between nominally identical lots	Composition plus lot-matched organotypic function	Supplier/lot comparison; material-only control; reference cell lot
Mixing and holding	Settling, aggregation, hypoxia, osmotic stress, or temperature drift creates a nonuniform dose	Spatial heterogeneity and loss of potency during a print run	Mapping across cartridge position and print time	Time-matched unprinted control; top/middle/bottom samples
Extrusion	Pressure, shear, extensional strain, and residence time injure cells or select resistant subpopulations	Delayed apoptosis, phenotype drift, or loss of a vulnerable compartment	Printed versus handled controls; recovery and differentiation trajectory[20,21,42]	Same cells and hold time without nozzle passage
Crosslinking	Light–initiator interaction, radicals, incomplete conversion, or ionic/enzyme residues alter cells and network	Oxidative stress or depth-dependent maturation failure	Local dose, conversion, residuals, and spatial function[22,76,80]	Dark, light-only, initiator-only, and full process controls
Early culture	Stiffness, confinement, and diffusion limits create spatially unequal oxygen and nutrients	Viable rim with hypoxic or nonfunctional core	Sectioned imaging, oxygen mapping, and spatial protein or transcript analysis	Thickness/geometry control; perfused versus static comparison
Degradation and release	Fragments, ions, particles, or drugs create time-dependent exposure while mechanics decline	Persistent inflammation, vascular injury, or false resorption	Measured release plus dose-matched reference tissue or microphysiological systems[83-85]	Material-only degradation; analyte recovery; medium and storage control
Maturation and use	Matrix remains non-remodelable or mechanically inadequate despite survival	Mineral signal without osteocyte function, perfusion, or controlled remodeling	Long-term loading or perfusion with direct functional readouts[13,34,36]	Reference matrix; positive functional stimulus; preloaded-mineral baseline

Bulk measurements also average across distinct microenvironments. A high mean viability can coexist with a necrotic core, a damaged filament boundary, or a depleted endothelial compartment. Spatial sampling should be planned according to the printing path and expected transport field. Confocal depth limitations should not be mistaken for absence of signal; sectioning or tissue clearing may be required. When an assay cannot access the full construct, the manuscript should report the accessible volume instead of implying whole-construct coverage.

2.3.3. Monoculture and early-marker bias

Mesenchymal stromal cells and osteoblast-lineage cells are sensible starting systems, but they cannot reveal all relevant bioink effects. Endothelial cells, macrophages, osteoclast precursors, and marrow progenitors differ in stress tolerance, matrix requirements, and exposure routes. A bioink that appears favorable in an osteogenic monoculture may suppress endothelial network formation or induce persistent inflammatory signaling. The converse is also possible: immune-derived signals may rescue or amplify osteogenesis.[29,56,58,92-95]

Direct co-culture can also change the conclusion reached for the same material. In dual-channel printing, macrophages were placed in a gelatin methacryloyl/hyaluronic acid phase while stromal cells were printed in an alginate/graphene-oxide phase. The study linked early macrophage polarization with later osteogenic and repair outcomes.[58] This design provides more information than a mesenchymal stem cell monoculture because it preserves soluble crosstalk, but using separate inks also makes attribution harder. A useful comparison therefore includes each cell-containing phase alone, the combined construct, and a process-matched material control.

Early markers can also be overinterpreted. Runt-related transcription factor 2, alkaline phosphatase, or calcium staining may indicate entry into an osteogenic program, but they do not establish mature osteocyte function, organized lamellar matrix, mechanosensitivity, or balanced remodeling. We recommend that at least one endpoint in a claimed functional domain be a direct or integrative

function instead of only a transcript. For mineralization, this might include mineral apposition or quantified matrix distribution; for vascularization, perfusable continuity instead of endothelial-marker expression alone; for remodeling, coupled formation and resorption instead of tartrate-resistant acid phosphatase (TRAP) staining alone.

2.3.4. The need for temporal challenge–recovery designs

Static endpoint comparisons compress a dynamic response into a single number. A pro-inflammatory phase may be appropriate if it resolves, while a modest early deficit may be acceptable if function recovers. MPS studies of bone-marrow toxicity have shown the value of repeated sampling and recovery after injury.[96] For bioinks, a similar design can separate transient fabrication stress from persistent functional damage.[92,97-102]

Challenge selection should follow the likely failure mode and begin only after the model reaches a defined baseline. Mechanical loading is useful only when the construct has a load-bearing matrix and the local strain can be estimated. An inflammatory challenge should use a magnitude and duration that produce a reversible response in the reference model. Repeated extract or ion exposure should match a plausible release window. Recovery should be reported as the fraction of baseline or reference function regained within a prespecified interval. Area under the curve can summarize total response, but it can hide a harmful peak or an incorrect sequence. Therefore, peak change, time to peak, slope of recovery, and residual deficit should be shown with the trajectory. Table 2 compares conventional assays with the additional information from organotypic constructs and MPS; these models extend, rather than replace, acute cytotoxicity tests.[13,34-36,57,96,103-105]

Table 2. Conventional bioink assays and the additional information obtained from organotypic constructs and microphysiological systems

Biological question	Conventional assay	Information provided	Unresolved uncertainty	Organotypic/MPS endpoint	Added decision information
Acute survival	Live/dead, membrane integrity, apoptosis, metabolic activity	Cell injury or bulk activity at a defined dose and time	Regional or lineage-selective loss; delayed failure; assay interference	Spatial, lineage-resolved survival during maturation, challenge, and recovery	Separates an acute gate from later functional loss; requires matrix blanks and orthogonal checks[13,96,103]
Osteocyte maturation	RUNX2, ALP, calcium stain	Entry into an osteogenic program and mineral-associated signal	Preloaded mineral and early markers do not establish mature function	Mineral apposition/density, DMP1, SOST, lacunar–canalicular organization, loading response	Bioprinted models reveal cell-density- and loading-dependent effects over 56–70 d[13,103]
Vascular function	Endothelial markers or static tube morphology	Endothelial identity and network-like organization	No proof of lumen continuity, perfusion, permeability, or flow response	Tracer perfusion, permeability, vascular invasion, and angiocrine support	Developmental bone MPS can resolve perfusion and maturation-dependent invasion[34]
Osteoimmune response	Terminal cytokines or macrophage markers	Selected inflammatory mediators or states	No resolution trajectory or downstream tissue consequence	Time-resolved cytokines plus endothelial, osteogenic, or resorption outcomes	Distinguishes transient activation from persistent functional injury[58,106-111]
Osteoclast activity and remodeling	TRAP staining or osteoclast count	Differentiation or abundance	No resorption rate or coupling to bone formation	Resorption pits/products and time-linked formation–resorption measures	Perfused bone models can test interaction, although current quantitative coupling remains limited[35,105]
Marrow support	Bulk viability or selected lineage markers	General cell survival or marker expression	Selective lineage loss and recovery may be missed	Lineage output, vascular niche support, and recovery after repeated exposure	Vascularized marrow MPS resolve lineage-specific toxicity and recovery[96]

Abbreviations: ALP: Alkaline phosphatase; DMP1: Dentin matrix acidic phosphoprotein 1; MPS: Microphysiological systems; RUNX2: Runt-related transcription factor 2; SOST: Sclerostin; TRAP: Tartrate-resistant acid phosphatase.

3. Bone organoids, organotypic bioprinted constructs, and microphysiological systems for functional assessment

3.1. Model selection and evidence boundaries

3.1.1. Minimum criteria, strict model labels, and evidence classes

A skeletal function beyond nonspecific aggregation and survival is a minimum entry criterion, but it does not fully define an organoid. Bone organoid is reserved for a multicellular system that shows reproducible self-organization and at least one tissue-level skeletal structure or function that is not fully imposed by an external template. A 3D aggregate or small engineered system with a limited skeletal phenotype is termed an engineered bone microtissue. A partially self-organizing system in which mature function or autonomous architecture remains incomplete is termed an organoid-like construct. A printed tissue whose geometry and maturation are mainly prescribed by fabrication is termed an organotypic bioprinted construct. Current organoid concepts support this distinction between self-organization and externally imposed architecture.[12,13,32,112-118]

Model description should use independent attributes rather than treating labels as a hierarchy. The first attribute is material participation: the candidate bioink forms the tissue, contacts a reference tissue, or supplies an extract or released species. The second is culture condition: static, mechanically loaded, or perfused. The third is tissue composition: osteogenic, osteocyte-rich, vascularized, osteoimmune, remodeling, or marrow. The fourth is demonstrated function. The strict model label and the evidence class are reported separately. An organotypic bioprinted construct can be perfused, and a self-organized reference organoid can be placed in an MPS. Table 3 uses these attributes to define assessment configurations without using bone organoid as a broad synonym for any printed tissue.

Model selection should begin with the decision. A soluble degradation product may be tested more clearly in a mature reference organoid. Matrix support for osteocyte maturation requires an organotypic bioprinted construct made from the candidate bioink. Flow-dependent endothelial or marrow behavior may require a perfused MPS. Figure 2 shows three configurations that can be combined. The evidence label remains separate: any configuration provides direct bioink evidence only when it tests the candidate formulation or process; otherwise, it provides model-capability, mechanistic/pharmacologic, or author-proposed evidence.

3.1.2. Organotypic bioprinted constructs and engineered bone microtissues

Organotypic bioprinted constructs show that the time needed to detect a useful difference depends on the function. Hydroxyapatite hybrid bioinks can support large self-mineralizing tissues, but preloaded mineral makes material-only baselines essential.[12] In extrusion-bioprinted graphene-oxide composite constructs, loading from day 1 was compared with delayed or absent loading. Osteocyte markers, lacunar–canalicular features, matrix maturation, and higher mineral density emerged by day 56.[13] In a related study, increasing the initial cell density from 5 to

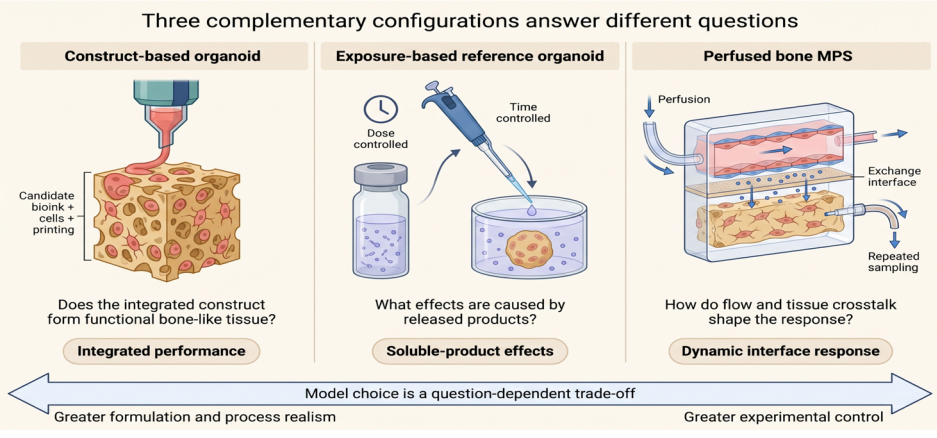

Figure 2. Complementary configurations for bioink assessment. Organotypic bioprinted constructs test the integrated material–cell–process system but make causal attribution difficult. Exposure-based self-organized reference organoids isolate soluble or released hazards but miss contact, mechanics, and local gradients. Perfused microphysiological systems (MPS) add controlled flow, gradients, compartmentalized crosstalk, and repeated sampling but introduce device absorption, flow variation, bubbles, and lower throughput. These are model configurations, not evidence classes, and the appropriate configuration depends on the decision question.[12,34,36,96] Created with BioRender. Minglu Hao (2026). https://BioRender.com/4peknci.

Table 3. Assessment configurations using strict model labels and one evidence taxonomy

Configuration and strict label	Material participation	Culture	Tissue composition	Demonstrated function	Evidence class	Main limit
Candidate-bioink organotypic bioprinted construct	Candidate forms the printed tissue	Static or mechanically loaded	Use-specific; may include vascular or immune cells	Matrix maturation, osteocyte response, vascular organization, or immune effect	Direct bioink	Attribution among chemistry, mechanics, transport, and process
Engineered bone microtissue or organoid-like construct	Candidate may form or contact the tissue	Usually static; may be loaded	Limited skeletal phenotype or partial organization	Defined phenotype without full self-organization	Direct bioink or model capability, depending on comparison	Label must not imply autonomous organoid development
Self-organized reference bone organoid	Standard tissue receives extract, released species, or contact exposure	Static or placed in a microphysiological system	Self-organized skeletal tissue	Reproducible tissue-level skeletal structure or function	Usually author-proposed for bioink safety; model capability when platform alone is shown	Misses construct mechanics, contact, and local gradients
Perfused bone MPS	Candidate tissue, upstream release source, or measured extract	Controlled perfusion	Osteogenic ± vascular/ remodeling compartments	Transport, invasion, mechanical response, or repeated sampling	Direct bioink if candidate is compared; otherwise model capability	Device absorption, flow failure, and medium compatibility
Bone marrow microphysiological systems	Measured extract or released species; candidate contact when justified	Perfused and compartmentalized	Vascular niche and marrow lineages	Lineage output and recovery	Usual model capability for bioink assessment	Requires a plausible marrow exposure and stable lineage baselines

10 million cells/mL shifted peak mineral formation from days 49–56 to days 28–35 and increased mineral density and stiffness by day 70, although both groups retained high early viability.[103] A 2026 dual-bioink piezoelectric system produced compartment-specific cartilage and bone tissues; because its geometry and maturation cues were externally prescribed, it is classified here as an organotypic bioprinted osteochondral construct rather than a self-organized bone organoid.[119] These studies show why a 1–2-week viability assay cannot predict late matrix performance.

The main limitation of organotypic bioprinted constructs is attribution. If one bioink produces less mineralization, the cause may be toxicity, altered stiffness, different ligand density, reduced cell spreading, impaired transport, or a measurement artifact caused by pre-existing mineral. Candidate constructs should therefore be compared with a biologically permissive reference matrix, an unprinted handling control, and a process-matched control. Material-only mineral baselines are required when calcium phosphates are present.

Organotypic bioprinted constructs can also test vascular and immune organization. Granular prevascularized spheroid constructs supported post-print sprouting and osteogenic–angiogenic organization, while dual-channel constructs placed macrophages and stromal cells in different material phases to study osteoimmune crosstalk.[14,58] These are direct bioink studies, but their evidence is formulation-specific. *In vivo* vascularization or defect repair does not show that the pre-implant construct had autonomous perfusion, and macrophage marker shifts do not establish a resolved immune trajectory. The pre-implant tissue state, host response, and final repair outcome should be reported separately.

3.1.3. Exposure-based reference organoids

Exposure-based reference organoids are a plausible way to compare raw-material lots, photochemical by-products, ions, particles, and degradation media without rebuilding

the tissue for every formulation. However, direct evidence for their use in bone–bioink safety assessment remains limited. References commonly used to support this configuration mainly describe bone-organoid construction or characterization rather than tests of bioink extracts.[112,116,117] We therefore treat this section as a proposed application direction, not an established method.

The reference tissue can alter the exposure it is meant to measure. Its matrix may adsorb proteins or particles, buffer pH and ions, or protect cells by slowing diffusion. Extract preparation can also exaggerate or dilute exposure, and labile species may disappear during filtration or storage.[83-85] Studies should report extraction pH, osmolality, protein content, particle stability, storage, and the delivered concentration where it can be measured. A matrix-free cell control can show whether the reference organoid is buffering the exposure. A positive control must change the functional domain being evaluated.

A paired design can separate soluble and construct-dependent effects. If an extract suppresses endothelial organization in a qualified reference tissue and the same effect appears in the candidate construct, a released species is plausible. If the construct fails but the extract does not, mechanics, contact, spatial dose, or transport becomes more likely. If neither configuration has adequate control separation, the result is a model failure rather than evidence that the candidate is safe. This triangulation needs direct validation with bioinks of known composition before it can support broad safety prediction.

3.1.4. Perfused bone and bone-marrow microphysiological systems

Perfused bone MPS and marrow MPS answer different questions. Bone development and remodeling chips can control vascular invasion, precursor migration, mechanical exposure, or communication between osteogenic and osteoclastic compartments.[34-36] Their value for bioinks lies in controlled time and transport, not in miniature anatomy. A candidate can form part of the tissue, sit upstream as a release source, or be delivered as a measured extract. Each placement creates a different exposure.

Marrow MPS are relevant when released components can plausibly reach marrow or when the printed construct contacts a marrow cavity. A vascularized marrow chip reproduced lineage-specific toxicities and recovery after drug exposure, and later marrow chips added immune-mediated liabilities during biologic exposure.[96,120] These are model-capability studies, not direct bioink tests. Medium compatibility, device absorption, delivered dose, and lineage-specific baseline stability must be established before a bioink-related change can be interpreted.

Perfusion adds technical failure modes. Flow rate, bubbles, scaffold loading, channel coating, device absorption, sampling loss, donor effects, and incompatible media can dominate the signal. Experimental-design studies in marrow MPS show that repeated measures and deliberate allocation of technical factors can improve power.[121] Acceptance criteria should include flow stability, recovery of a dosed analyte, tissue-formation success, and rules for failed devices. Detailed metadata and continued performance checks are part of model qualification.[122,123]

Table 3 separates the strict model label from material participation, culture condition, demonstrated function, and evidence class. Table 4 then compares representative studies with the quantitative parameters that can be extracted without converting unlike assays into a common scale. A value reported as not directly comparable marks a reporting or design limit, not evidence that platforms are equivalent.

Table 4. Quantitative comparison of representative studies and their evidence

Study and strict model label	Evidence class	Material/cells and exposure design	Duration and quantitative parameters	Functional result	Interpretation and limit
Wang *et al.*;[12] organotypic bioprinted bone construct	Direct bioink	Hydroxyapatite hybrid bioink with bone-forming cells	Large constructs; cross-study-comparable mineral values were not reported in the present synthesis	Self-mineralizing bone-like tissue	Preloaded mineral requires acellular baselines
Zhang *et al.*;[13] organotypic bioprinted osteocyte construct	Direct bioink	Graphene-oxide composite; loading from day 1 or 21 versus no loading	56 d; DMP1 increased about 20-fold and 7.2-fold; SOST increased about 7.8-fold and 4.7-fold for early and delayed loading versus no loading	Mechanical loading promoted osteocyte maturation	Study-specific fold changes are not universal acceptance limits

(*Cont;d...*)

Table 4. (*Continued*)

Study and strict model label	Evidence class	Material/cells and exposure design	Duration and quantitative parameters	Functional result	Interpretation and limit
Fang *et al.*;[14] organotypic prevascular spheroid construct	Direct bioink	Granular prevascularized spheroids after printing	Post-print sprouting; a directly comparable vascular-length or perfusion metric was not reported here	Osteogenic–angiogenic organization	Prevascular organization is not autonomous perfusion
Yu *et al.*;[58] organotypic osteoimmune bioprinted construct	Direct bioink	Macrophages and stromal cells in separate printed material phases	Early culture plus later animal repair; no common quantitative coupling metric	Macrophage state was linked to osteogenic and repair outcomes	Formulation-specific and partly host-dependent
de Leeuw *et al.*;[103] organotypic bioprinted bone construct	Direct bioink	5 versus 10×10^6 cells/mL; cyclic loading at 1% strain, 5 Hz, 5 min, five times/week	70 d; mineral density 176.9 ± 21.42 versus 230.8 ± 15 mg HA/cm^3; stiffness 0.85 ± 0.65 versus 3.83 ± 2.01 N/mm	Higher cell density accelerated mineralization and increased late stiffness	Shows a late difference despite high early viability
Whelan *et al.*[34] developmental bone-organoid MPS	Model capability	Matured bone analogues integrated with a vascularized perfusion system	Up to 28 d maturation plus 3–7 d in the MPS	Dextran perfusion and maturation-dependent vascular invasion	No candidate bioink comparison
Vis *et al.*;[35] perfused scaffold-free bone MPS	Model capability	Human osteogenic and osteoclastic co-culture	35 d; secreted TRAP and CTX group differences were not significant	Osteoblast-lineage and multinucleated TRAP-positive cells were maintained	Supports interaction studies but not yet a validated coupling assay
Knowles *et al.*;[57] engineered osteocyte microtissue	Mechanistic/pharmacologic	Primary human osteocyte model exposed to PTH(1-34)	About 12 weeks maturation; 2–3 d exposure and 7 d washout	Reversible sclerostin suppression without increased LDH	Demonstrates responsiveness, not bioink safety
Chou *et al.*;[96] vascularized marrow MPS	Model capability	Repeated clinically relevant exposures with recovery sampling	Repeated exposure and recovery; endpoints were lineage-specific rather than a single common effect size	Lineage-selective toxicity and recovery	Direct bioink relevance requires matched dosimetry
Park *et al.*;[104] secretome-mixing chip	Mechanistic/pharmacologic	Defined osteoblast: osteoclast conditioned-medium ratios	Strongest osteogenic response at 75:25	Nonlinear crosstalk changed osteogenic readouts	Does not reproduce concurrent resorption and formation
Wang *et al.*;[119] organotypic bioprinted osteochondral construct	Direct bioink	ChSMA/BTO/TGF-β and GelMA/BTO/nHA compartment-specific bioinks	Compartment-specific outcomes; no single cross-platform effect size	Spatial cartilage and bone formation with piezoelectric stimulation	Repair performance does not qualify as an assessment assay
Merife *et al.*;[124] BMU-chip	Model capability	Osteocytes, osteoblasts, and sequential osteoclast phase under flow stimulation	Up to 31 d; daily 15-min flow stimulation	Longitudinal viability, expression, morphology, and Ca^{2+} signaling	Murine cell lines and no candidate bioink comparison

Abbreviations: ALP: Alkaline phosphatase; BMU: Bone multicellular unit; BTO: Barium titanate; ChSMA: Chondroitin sulfate methacrylate; CTX: C-terminal telopeptide of type I collagen; DMP1: Dentin matrix acidic phosphoprotein 1; GelMA: Gelatin methacryloyl; HA: Hydroxyapatite; LDH: Lactate dehydrogenase; MPS: Microphysiological systems; nHA: Nanohydroxyapatite; SOST: Sclerostin; TGF-β: Transforming growth factor beta; TRAP: Tartrate-resistant acid phosphatase.

Representative studies are assigned to the same four evidence classes defined in Section 1: direct bioink, model-capability, mechanistic/pharmacologic, and author-proposed evidence. Figure 3 and Table 4 use these exact labels. The classification prevents platform sophistication or regenerative benefit from being mistaken for direct evaluation of a candidate bioink.

3.2. Functional endpoint domains

Functional assessment should follow biological function and likely failure mechanisms rather than a long marker list. Figure 4 is a conceptual synthesis of five interacting domains: osteogenesis and osteocyte maturation, vascular function, inflammation and immune resolution, resorption and remodeling, and marrow or progenitor support. These domains are outcomes, not evidence classes. The arrows define hypotheses and do not show that the interactions were tested in every cited platform. Authors should state which relationships the model tests and which remain outside its scope. High overall viability can still coexist with failure in any one domain.

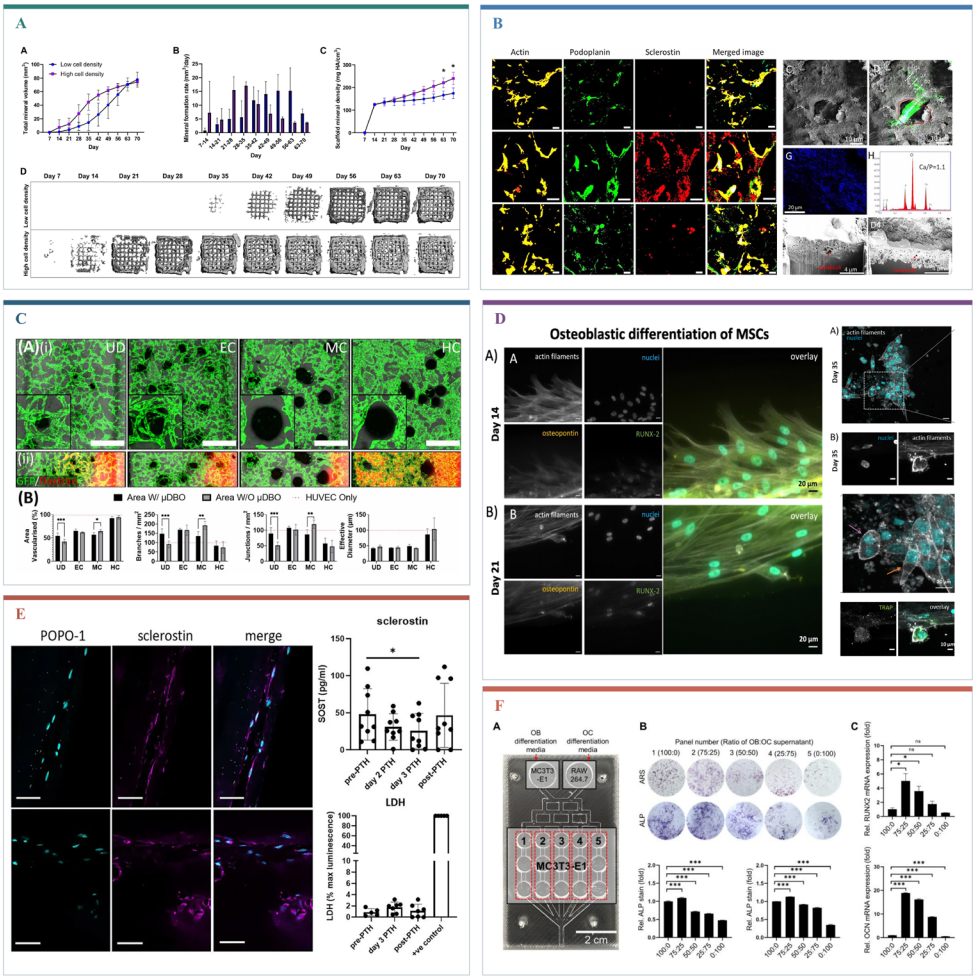

Figure 3. Representative experimental readouts classified using the evidence taxonomy defined in Section 1. (A) Direct bioink evidence: Higher initial cell density accelerated mineralization and increased scaffold mineral density in dynamically loaded organotypic bioprinted constructs. Reprinted from de Leeuw *et al.*[103] (B) Model-capability evidence: Long-term mechanical loading promoted osteocyte-marker expression and lacunar–canalicular features by day 56. Scale bars: 4 µm, 10 µm, 20 µm. Reprinted from Zhang *et al.*[13] (C) Model-capability evidence: A developmental bone MPS formed perfusable endothelial networks whose invasion and morphology depended on the maturation state of the bone microtissues. Scale bar: 1 mm. Reprinted from Whelan *et al.*[34] (D) Model-capability evidence: A 35-day perfused human co-culture supported osteoblast-lineage and multinucleated TRAP-positive osteoclast-like phenotypes, although changes in secreted TRAP and CTX were not statistically significant. Scale bars: 10 µm, 20 µm. Reprinted from Vis *et al.*[35] (E) Mechanistic/pharmacologic evidence: PTH(1-34) reversibly suppressed sclerostin secretion without increasing LDH release. Scale bar: 50 µm. Reprinted from Knowles *et al.*[57] (F) Mechanistic/pharmacologic evidence: defined mixtures of osteoblast- and osteoclast-conditioned media altered osteogenesis, with the strongest response observed at an osteoblast (OB): osteoclast (OC) ratio of 75:25. Reprinted from Park *et al.*[104]
Abbreviations: CTX: C-terminal telopeptide of type I collagen; LDH: Lactate dehydrogenase; TRAP: Tartrate-resistant acid phosphatase.

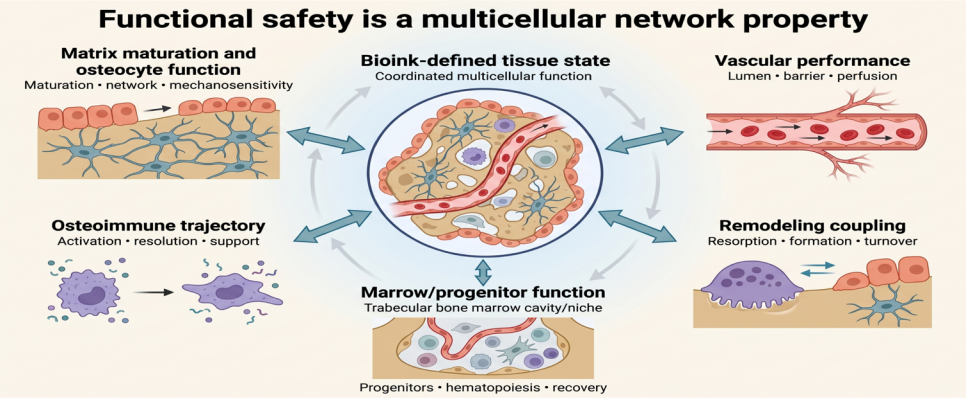

Figure 4. Functional safety as a multicellular network property. Bioink composition, processing, exposure, and time can influence five functional domains: osteogenesis and osteocyte maturation, vascular function, inflammation and immune resolution, resorption and remodeling, and marrow or progenitor support. The arrows represent testable hypotheses, not interactions already demonstrated by every model. Individual systems need only contain the domains required by their context of use, and an absent domain should be reported as a limitation. High viability does not preclude divergence of one or more functions.[36,125-128] Created with BioRender. Minglu Hao (2026). https://BioRender.com/6x1k7nu.

3.2.1. Matrix maturation, mineralization, and osteocyte function

Matrix maturation should be separated into new matrix production, organization, mineral apposition, and osteoblast-to-osteocyte transition. Collagen quantity does not show fiber organization. Calcium staining cannot distinguish ink mineral from cell-derived deposition. Serial micro-computed tomography can resolve mineral kinetics, but its threshold must be anchored to a material-only baseline. Raman or compositional imaging can test mineral quality, while histology or second-harmonic imaging can test collagen organization. These measurements are complementary and should not be collapsed into a single osteogenesis score.[13,57,81,97,98,103,129,130]

Maturity markers and functional response should also be separated. Dentin matrix acidic phosphoprotein 1, podoplanin, and sclerostin, along with dendritic morphology, support osteocyte differentiation, but a measurable response to loading or parathyroid hormone tests a higher level of function. In the loaded bioprinted model, key osteocyte and lacunar–canalicular features were reported at day 56.[13] In primary human organotypic cultures, sclerostin secretion reached a relative plateau around 12 weeks, and fibroblast growth factor 23 increased at a later time, with marked donor variation.[57] These timelines make mature osteocyte assays better suited to focused late-stage questions than rapid formulation screens. Local strain must be measured or modeled because actuator displacement alone does not define the stimulus experienced by cells.

3.2.2. Vascular function and osteogenic–angiogenic coupling

Vascular evidence has four levels. An endothelial network shows organization. Lumen formation shows a possible transport path. Tracer movement shows perfusability under the tested condition. Host vessel entry after implantation shows integration with the animal vasculature. These outcomes are related but not interchangeable. Specialized bone endothelial states and angiocrine signaling link vessels with osteogenesis, so human umbilical vein endothelial cell networks may provide a robust engineering test while still failing to reproduce bone microvascular identity.[125,126,131-137]

A vascular endpoint panel should therefore combine continuity and lumen measurements with permeability or tracer perfusion and a downstream osteogenic readout. Conditioned-medium transfer or separated compartments can test whether endothelial signals support osteogenic cells. If true flow through a lumen is not shown, the accurate term is prevascular organization. If only host vessels are seen after implantation, the result supports host-driven vascular integration, not autonomous *in vitro* vascular function.

3.2.3. Osteoimmune trajectory

Osteoimmune assessment should follow a time sequence. A practical minimal design measures an early inflammatory phase, an expected transition phase, and a later resolution or persistence phase. At each point, cytokines and cell-state markers should be linked to a downstream osteogenic or endothelial effect. A terminal M1/M2 ratio cannot show whether an initially useful response resolved. Macrophages are required for fracture healing and regulate osteoblast behavior, but the desired trajectory depends on the product and disease context.[127,128,138-143]

Direct bioink studies remain fewer than mechanistic biomaterial studies. Dual-channel printing linked

macrophage and stromal-cell compartments, while a 2026 plant-based bioink maintained an M2-associated state for more than 28 days together with osteogenic and matrix outcomes.[31,58] These studies support longitudinal, multicellular assessment, but they do not remove the need to measure dose, resolution, and alternative macrophage states. Useful readouts include cytokines, phagocytic function, inflammasome activation when mechanistically relevant, and the effect of conditioned medium on osteogenic or endothelial cells. Positive and adverse immune controls are needed to interpret direction.

We also caution against treating immune modulation as a property intrinsic to a material independent of dose. Particle size, agglomeration, protein corona, degradation rate, and local concentration determine the encountered stimulus. A material described as osteoimmunomodulatory should be accompanied by exposure characterization and evidence that the response remains favorable across the intended process and release window.

Figure 5 summarizes indirect evidence that can guide osteoimmune endpoints. Figure 5A is an *in vitro* material–macrophage study, Figure 5B–E uses conditioned medium, extracellular vesicles, or pathway perturbation to test cell communication, and Figure 5F combines an animal injury time course with computational analysis. None directly validates a bone–bioink safety assay. Their value is to show which causal steps a bioink study would need to test.

The evidence increases from phenotype description to transfer of a secreted signal, pathway perturbation, and time-resolved response. Even this sequence does not establish that a candidate bioink is beneficial unless the exposure and downstream bone function are measured in the intended construct. Animal injury trajectories also provide timing hypotheses rather than direct *in vitro* acceptance limits.

3.2.4. Osteoclastogenesis and remodeling coupling

Osteoclast assessment should distinguish differentiation, actual substrate resorption, and dynamic coupling with formation. Multinucleation, actin rings, and TRAP support differentiation. Pit area, collagen-degradation products, or loss of a defined mineralized substrate provide stronger evidence of resorption. In mineral-containing bioinks, material dissolution can mimic resorption and should be measured in acellular constructs under the same medium and flow. Exogenous receptor activator of nuclear factor kappa-B ligand, macrophage colony-stimulating factor, osteogenic supplements, or platelet lysate can also dominate the response, so their concentration and timing should be part of the exposure definition. [35,54,105,140,141,144]

Dynamic coupling requires time-linked evidence that a change in resorption is followed by a compatible formation response. Vis *et al.*[35] maintained osteoblast-lineage tissue and osteoclast-like cells for 35 days; however, differences in TRAP and C-terminal telopeptide of type I collagen levels were not statistically significant, potentially reflecting limitations related to sample dilution and assay sensitivity discussed by the authors. It is therefore a platform for studying interaction rather than a validated coupling assay. Park *et al.*[104] showed that defined osteoblast-to-osteoclast supernatant ratios changed osteogenesis, but the experiment did not measure concurrent resorption. Stronger evidence for coupling requires repeated formation and resorption measures in the same system, with material-dissolution controls.

3.2.5. Marrow and progenitor function

Marrow endpoints should be tied to a plausible exposure route. Local contact with an open marrow cavity, sustained release into perfusate, or measured systemic availability can justify testing. The possibility that a material releases ions or particles is not enough. The delivered concentration, duration, and protein-bound or particulate state should be estimated. Useful readouts include maintenance of progenitor pools, lineage-specific output, stromal support, barrier function, and recovery after exposure. A bulk viability result can miss selective loss of neutrophil, erythroid, megakaryocyte, or stem/progenitor output.[96,120,145-153]

A marrow MPS is useful only when a plausible marrow exposure could affect the decision. Across all five domains, the available evidence is partial and mostly pairwise. Table 5 therefore separates representative published findings and their limits from author-proposed perturbations and analyses. This distinction prevents model capability or correlated terminal markers from being mistaken for a validated interaction assay.

4. Context-of-use qualification, experimental design, and validation

4.1. A tiered, context-of-use qualification framework

4.1.1. Tier 0: Define identity, exposure, and decision

Tier 0 defines the test article, intended construct, relevant exposure, cell population, and decision. It also records the material composition, impurities, rheology, mechanics, crosslinking, degradation, and extractable or leachable profiles needed to assess the proposed hazard.[2,83-85,139] Clear test-article information is required before an organoid result can be interpreted.[3-5,23,71-74]

A complete context-of-use statement names the product, decision, exposure, population, and endpoint.

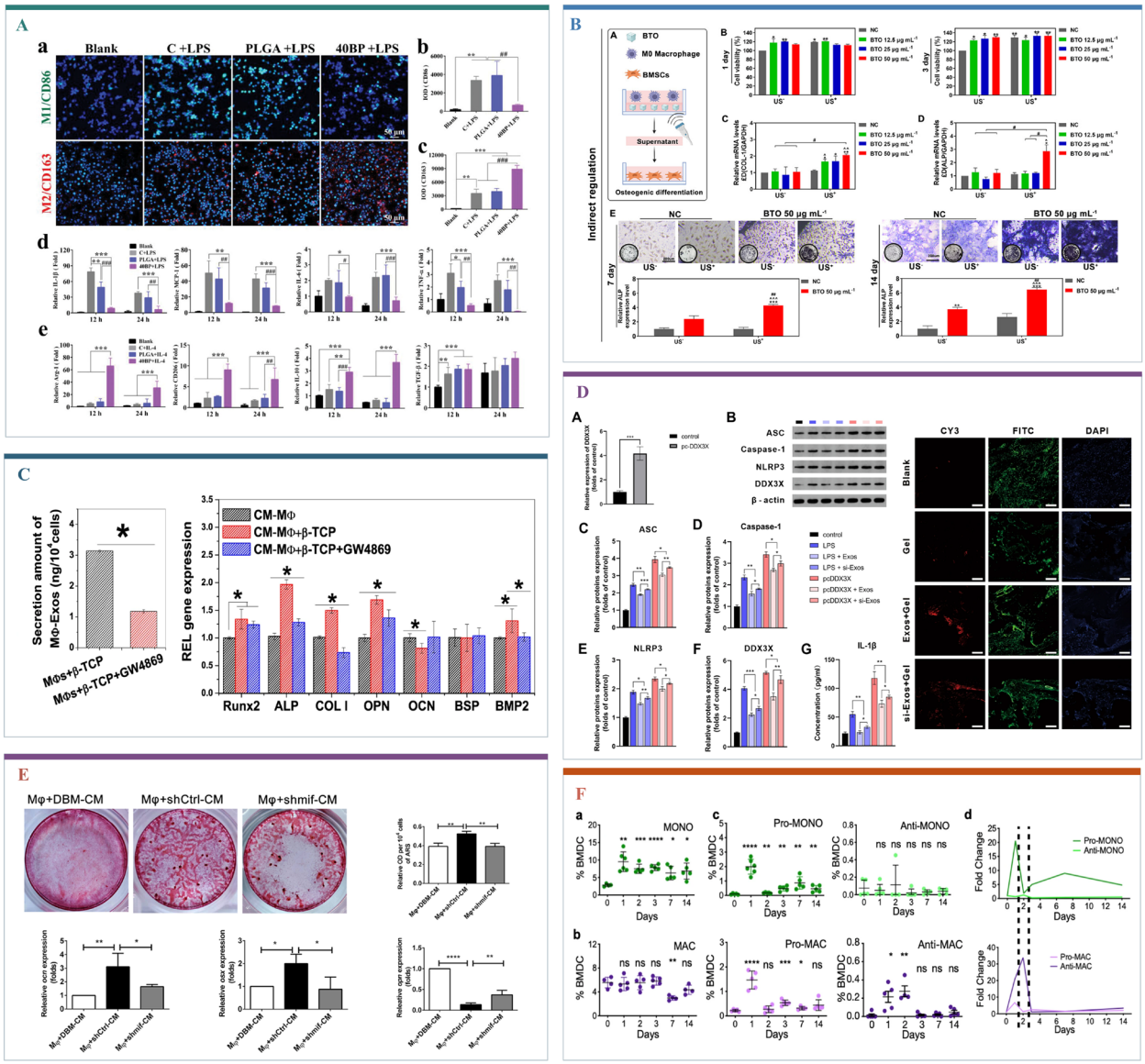

Figure 5. Related mechanistic studies that inform osteoimmune endpoint selection without directly qualifying bioinks. (A) Under inflammatory challenge, PLGA/black-phosphorus scaffolds altered CD86/CD163 staining and inflammatory- and M2-associated gene expression in macrophages. Scale bar: 50 μm. Reprinted from Long *et al.*[106] (B) Under ultrasonic loading, macrophage-conditioned medium generated by $BaTiO_3$ exposure produced a stronger osteogenic response than direct material exposure, supporting an immune-mediated component of piezoelectric-stimulation-induced osteogenesis. Reprinted from Sui *et al.*[107] (C) Pharmacological inhibition of exosome release weakened β-TCP-induced macrophage-to-BMSC osteogenic signaling. Reprinted from Sun *et al.*[108] (D) Endothelial exosomal NEAT1 suppressed DDX3X/NLRP3 inflammasome signaling and altered CD86/CD206 macrophage polarization in a hydrogel-treated calvarial-defect model. Scale bar: 100 μm. Reprinted from Chen *et al.*[109] (E) MIF knockdown reduced mineralization and osteogenic gene responses in BMSCs exposed to conditioned medium from macrophages cultured with MSC-derived ECM-modified scaffolds. Reprinted from Deng *et al.*[110] (F) Animal tibial injury produced distinct temporal waves of pro- and anti-inflammatory monocytes and macrophages, supporting time-resolved rather than terminal immune assessment. Reprinted from Lo *et al.*[111]

Abbreviations: β-TCP: Beta-tricalcium phosphate; BMSC: Bone marrow-derived mesenchymal stem cell; DDX3X: DEAD-box helicase 3 X-linked; ECM: Extracellular matrix; MIF: Macrophage migration inhibitory factor; MSC: Mesenchymal stem cell; NEAT1: Nuclear paraspeckle assembly transcript 1; NLRP3: NLR family pyrin domain containing 3; PLGA: Poly(lactic-co-glycolic acid).

For example: "This assay will rank three photocrosslinked mineral-containing bone bioinks for advancement by testing whether the final printed construct preserves osteogenic matrix maturation and endothelial network

Table 5. Published evidence, remaining gaps, and author-proposed tests for cross-domain interactions

Cross-domain question	Representative published evidence and current finding	Remaining gap	Author-proposed perturbation	Prespecified analysis
Osteogenesis/ osteocyte ↔ vascular function	Whelan *et al.*[34] showed maturation-dependent vascular invasion in a developmental bone MPS; Fang *et al.*[14] demonstrated post-print vascular sprouting in a prevascularized construct	The evidence establishes model capability or regenerative organization, not a candidate-versus-reference bioink comparison or causal vascular effect	Compare candidate and reference bioinks under perfused and static culture; add endothelial inhibition or rescue	Test bioink × perfusion × time for DMP1/SOST, mineral apposition, network continuity, perfusability, and permeability
Inflammation ↔ osteogenic and vascular function	Yu *et al.*[58] linked macrophage state to osteogenic and repair outcomes in a bioprinted construct; related transfer and pathway studies support immune-mediated downstream effects[106-111]	Many studies use terminal markers, conditioned medium, or animal repair. Few quantify challenge and recovery during a direct candidate-bioink comparison	Apply a transient inflammatory challenge, followed by cytokine neutralization or receptor blockade and recovery sampling	Test bioink × challenge × time for cytokine resolution, vascular permeability, and osteogenic recovery
Resorption ↔ formation and osteocyte function	Vis *et al.*[35] maintained both lineages, but secreted TRAP and CTX changes were not significant; Park *et al.*[104] altered osteogenesis with conditioned-medium ratios without concurrent resorption; the BMU-chip models sequential phases[124]	No study provides quantitative formation–resorption coupling for a candidate bioink, and material dissolution can confound resorption readouts	Inhibit or rescue osteoclast function and include acellular dissolution controls	Use repeated formation and resorption measures with time-lagged mixed-effects analysis
Vascular/immune niche ↔ marrow support	A vascularized marrow MPS resolved lineage-specific toxicity and recovery after repeated exposure[96]	The platform has not been tested with measured bone-bioink exposure or a coupled skeletal compartment	Perturb endothelium or macrophages and expose the marrow module to measured extracts or released species, with recovery sampling	Test exposure × perturbation × time for progenitor survival, lineage output, and recovery
Integrated five-domain response	No identified study simultaneously tests osteogenesis, vascular function, inflammation, resorption, and marrow support in a validated candidate-bioink assay[34,35,58,96,104,124]	Current evidence is pairwise and platform-specific, with no validated integrated model or decision rule	Use a staged 2 × 2 × 2 core design (candidate/reference × perfused/static × transient challenge/vehicle) plus targeted inhibition or rescue modules	Use hierarchical mixed-effects models with donor, material lot, print run, and device as random effects; use mediation only after temporal order and directionality are established

Abbreviations: BMU: Bone multicellular unit; CTX: C-terminal telopeptide of type I collagen; DMP1: Dentin matrix acidic phosphoprotein 1; MPS: Microphysiological system; SOST: Sclerostin; TRAP: Tartrate-resistant acid phosphatase.

function during a 28-day *in vitro* use period." The statement does not claim implant safety. Acceptance criteria and the rule for discordant domains should be set before testing.

4.1.2. Tier 1: Acute and process-specific hazard screening

Tier 1 combines standardized cytotoxicity with process-matched controls. Direct-contact and extract tests identify overt chemical hazards, while printed-versus-unprinted comparisons identify fabrication effects.[27,91] A single positive result should first be checked for assay interference and reproducibility. A short-lived process response can proceed to a prespecified recovery test if acute injury remains below the disqualifying limit. Clear dose-related cell loss that repeats with an orthogonal assay should trigger reformulation before organotypic testing.

Passing Tier 1 means only that no disqualifying acute or process-specific hazard was detected under the defined conditions. It does not establish functional equivalence. We recommend explicitly documenting this boundary in internal reports and manuscripts because the word safe tends to expand beyond the evidence that generated it.

4.1.3. Tier 2: Organotypic function

Tier 2 selects the least complex model that reproduces the intended function. A bone-forming implant needs cell-derived matrix and mineral above material background, plus a late osteocyte or mechanical-response endpoint when that claim is made. A prevascularized implant needs network continuity and a transport-related endpoint. An immune-instructive ink needs an early-to-late immune trajectory linked to an osteogenic or endothelial consequence. A screening bioink used only to build a disease model may instead need phenotypic neutrality and assay reproducibility. Candidate-bioink and exposure-based configurations can be combined when they resolve different causes of failure.

Decision rules should distinguish biological failure from model failure. If positive and negative controls do not separate, the assay is uninformative. If a candidate effect is accompanied by major construct-to-construct variance, technical sources should be resolved before biological claims are made. If a difference occurs only in a single marker without coherent functional evidence, it should be treated as hypothesis-generating instead of decisive.

4.1.4. Tier 3: Advanced dynamic testing and separate predictive validation

Advanced dynamic testing asks whether function is maintained or recovered after a relevant challenge. The challenge may involve repeated exposure to release, inflammation, hypoxia, or loading. The model should have a stable baseline, a reference response, and a defined recovery interval. Perfusion is useful when exposure kinetics or repeated sampling matters.[96,121] Completing a long-term challenge–recovery study does not validate prediction. It establishes a dynamic response within that model and exposure.

Predictive validation is a separate task. It requires a panel of reference materials, compounds, or processes with justified expected outcomes, prespecified rules, and an independent or blinded test set. Concordance with animal or clinical evidence can help when the comparator measures the same mechanism. A model may be qualified to rank formulations within a defined material and process domain without predicting every implant outcome. Unknown or disputed reference labels should not be used to calculate accuracy; they can be studied prospectively after the decision rule is locked.

4.1.5. Evidence integration and decision gates

Evidence should be assessed by domain rather than collapsed into one unweighted score. The framework uses three transition gates. Gate A asks whether identity and exposure are defined well enough to test. Gate B asks whether acute and process-specific hazards are acceptable. Gate C asks whether the use-relevant function is preserved. Tier 3 then supports a context-specific conclusion about dynamic response or, in a separate validation study, prediction. A program can stop after any tier when the question is resolved. It does not need to advance to Tier 3.

Three outcomes should be distinguished. Candidate failure occurs when controls perform as expected, and the candidate crosses a prespecified adverse or performance limit. Model failure occurs when acceptance criteria, control separation, delivery, or technical stability fail. Evidence is insufficient when the model is valid, but the effect is imprecise, isolated to one marker, or discordant without a decision rule. If mineralization improves while endothelial regression or persistent inflammation worsens, the domains should not be averaged. The context of use determines which failure is critical. Figure 6 shows the three gates, feedback paths, and the option to stop when further complexity will not reduce decision-relevant uncertainty.

4.1.6. Worked example: A hypothetical mineral-particle photocrosslinked bone bioink

This example is hypothetical and does not report experimental results. The candidate contained mesenchymal stem cells, mineral particles, gelatin methacryloyl, and a photoinitiator and was printed for implantation into a vascularized bone defect. Tier 0 records particle identity and loading; polymer and initiator lots; nozzle and hold time; light wavelength and local dose; conversion; construct dimensions; degradation; and measured release. The decision is whether one formulation should advance to a long-term implant program. Material-only mineral baselines and a nonmineralized process-matched reference are defined before testing.[22,78,80,83-85]

Tier 1 compares printed and unprinted cells, light-only, initiator-only, mineral-only, and complete-process conditions. Live/dead imaging was paired with an orthogonal cell number or apoptosis measure and spiked recovery because particles may scatter light or adsorb reagents. A repeatable dose-related loss after interference correction was a candidate failure. Poor recovery of the assay spike or failure of the positive control was a model failure. A small acute change that returns to the reference range within the prespecified interval was recorded as transient process stress rather than proof of safety.

Tier 2 measures cell-derived collagen organization, new mineral above the acellular baseline, and an osteocyte maturation or loading-response endpoint. Because the intended site requires vascular integration, endothelial

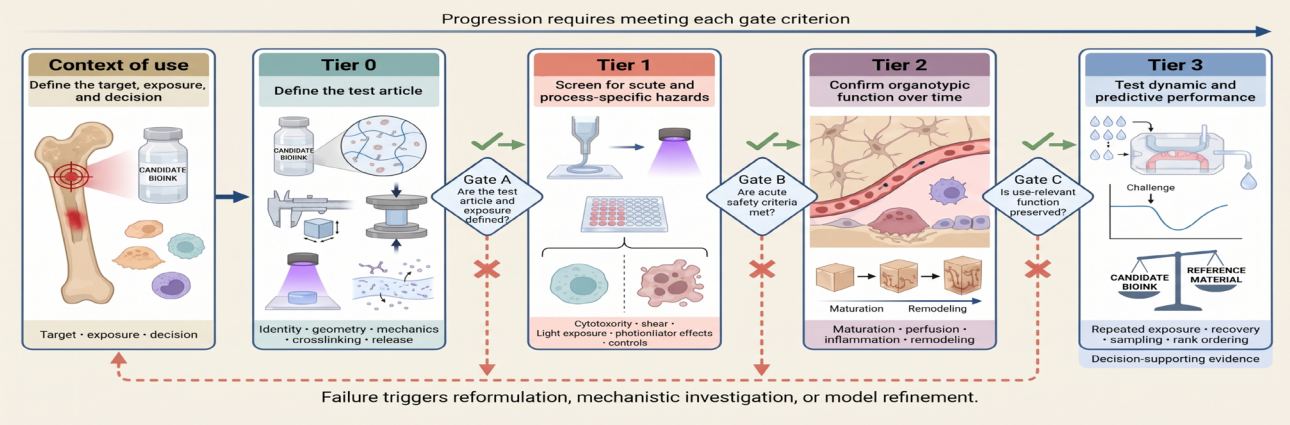

Figure 6. Tiered, context-of-use qualification framework. Tier 0 defines identity, exposure, and the decision. Tier 1 screens acute and process-specific hazards. Tier 2 tests the minimum organotypic function required by the intended use. Gate A, Gate B, and Gate C govern transitions between these stages. Tier 3 is optional and addresses a defined dynamic-response question or a separate predictive-validation program. Testing may stop at the lowest tier that resolves the decision. Failure can trigger reformulation, mechanistic investigation, or model refinement rather than automatic progression.[2,64,85,123] Created with BioRender. Minglu Hao (2026). https://BioRender.com/m4n43of.

continuity and tracer transport were added. If osteogenic measures improve but vascular function falls beyond the adverse reference range, the formulation does not pass by averaging the results. The critical vascular deficit triggers reformulation or a targeted mechanism study. If there are only changes in mineral staining, while micro-computed tomography and matrix measures do not show any changes, the evidence is insufficient.

Tier 3 is used only if a remaining decision depends on repeated release, inflammatory challenge, recovery, or prediction. A repeated-ion challenge can test whether vascular and osteogenic function recover after washout. Predictive validation would require several independent reference formulations with known outcomes and a locked ranking rule. One long perfusion experiment cannot establish prediction. The candidate advances only when the required domains pass, the model meets acceptance criteria, and uncertainty is small enough for the stated decision.

4.2. Experimental design, quality attributes, and validation

4.2.1. Exposure matching and dosimetry

Printing exposure and release exposure should be modeled separately. Pressure, shear, residence time, and local light dose occur during fabrication. Ions, particles, residuals, and degradation products reach tissue later and follow different kinetics. For extracts, the extraction ratio, medium, temperature, duration, agitation, filtration, and storage determine composition. Filtration can remove particles or protein-bound species. Storage can destroy labile products. In perfusion, tubing and device absorption can lower delivered concentration while continuous flow changes residence time. Nominal concentration is therefore not a sufficient description of the dose.

Whenever possible, at least one analyte or physical variable should be measured to anchor the exposure model. This may be photoinitiator conversion, released calcium, particle concentration, oxygen, local strain, or perfusate concentration. Worst-case extraction helps identify hazards, while physiologically plausible release tests show whether the exposure is relevant.[75,83-85]

4.2.2. Controls, benchmarks, and assay acceptance

Controls serve two different purposes. A capability control shows that the model can respond through the claimed domain, such as parathyroid hormone suppression of osteocyte sclerostin or a defined inflammatory stimulus followed by recovery. An adverse-reference control shows that the assay can detect a harmful direction or persistence. Material blanks, cell-only or reference-matrix controls, handling controls, process controls, and vehicle controls address attribution. A positive cytotoxic control does not validate an osteocyte, vascular, immune, or remodeling assay.

Assay acceptance criteria should include tissue-formation success, baseline range, control separation, and technical performance. For MPS, flow stability, bubble exclusion, sampling recovery, and device integrity should be recorded. Failed devices should be handled by predefined rules instead of excluded after outcome inspection. The *Guidance Document on Good In Vitro Method Practices* and fit-for-purpose MPS recommendations provide useful principles for such quality systems.[91,123,154,155]

4.2.3. Reproducibility, donor diversity, and statistical design

Replication occurs at several levels. One example is a design with two material lots, three donors, two independent print runs per donor–lot pair, and several constructs within each run. The donor–lot–run combination, not each image field, carries independent biological and manufacturing information. Constructs and fields are nested observations. Mixed models or other hierarchical analyses should reflect this structure and repeated sampling. Marrow–MPS work shows that deliberate allocation of technical factors can increase sensitivity to true effects.[121]

Donor diversity should be introduced after the assay is technically stable. Otherwise, donor effects and process noise are inseparable. A staged strategy uses a reference donor or cell lot for method development, followed by a prespecified donor panel for generalization. Reproducibility metrics should distinguish absolute agreement from concordant trends, and metadata should capture material lot, cell lot, printer, operator, device, time, and analysis pipeline.[122]

Throughput should be judged by usable, quality-controlled tissues instead of nominal wells per plate. Microfluidic generation of large numbers of uniform vascularized and mineralized organoids is promising.[37] But uniform size does not guarantee uniform function. Acceptance criteria and randomization remain necessary even in high-throughput formats.

4.2.4. Published evidence and an author-proposed framework for cross-domain interactions

Published studies currently support selected cross-domain links rather than a comprehensive interaction test. In the developmental bone MPS reported by Whelan *et al.*,[34] the maturation state of bone microtissues altered vascular invasion and network morphology, providing model-capability evidence for osteogenic–vascular crosstalk. Yu *et al.*[58] linked macrophage state to osteogenic and repair outcomes in an osteoimmune bioprinted construct, although part of the evidence depended on subsequent host responses.

Evidence for formation–resorption coupling is also incomplete. Vis *et al.*[35] maintained osteoblast-lineage tissue and multinucleated TRAP-positive osteoclast-like cells in a perfused model, but secreted TRAP and CTX did not differ significantly between groups. Park *et al.*[104] showed that defined osteoblast and osteoclast conditioned-medium ratios altered osteogenesis, but concurrent resorption was not measured. Vascularized marrow MPS can resolve lineage-specific toxicity and recovery, but these systems have not been used to compare candidate bone bioinks.[96] The bone multicellular unit-chip further shows that sequential bone–cell phases and mechanical stimulation can be studied longitudinally, although it does not incorporate all five domains or directly assess candidate bioinks.[124] Taken together, the evidence remains pairwise and platform-specific. No identified study has tested all five domains as an integrated response to a defined candidate bioink.

Based on these gaps, we propose, as author-proposed evidence, an illustrative 2 × 2 × 2 longitudinal framework comprising candidate versus reference bioink, perfused versus static culture, and transient inflammatory challenge versus vehicle. Sampling before challenge, during the response, and after a predefined recovery period would measure mineral apposition or dentin matrix acidic phosphoprotein 1/sclerostin response, vascular continuity and permeability, cytokine-resolution kinetics, resorption activity, and lineage-support output. This factorial component tests whether vascular transport and inflammatory challenge modify bioink-dependent response and recovery. It does not by itself establish formation–resorption coupling or marrow-niche interaction. Those questions require additional targeted experiments, such as osteoclast inhibition or rescue followed by osteogenic measurements, cytokine neutralization followed by vascular and skeletal readouts, and endothelial or macrophage perturbation followed by progenitor-lineage analysis.

The core factorial analysis should use a mixed-effects model with fixed effects for bioink, perfusion, inflammatory challenge, time, and prespecified interaction terms. Donor, material lot, print run, and device should be random effects when applicable. The bioink × challenge × time term tests whether inflammatory response and recovery depend on the formulation, whereas the bioink × perfusion × time term tests whether perfusion modifies the formulation response. These terms identify effect modification; they do not by themselves prove causal interaction between biological domains. Directionality requires the targeted inhibition, neutralization, or rescue experiments listed in Table 5. Mediation or path analysis is appropriate only when temporal order and perturbation support the proposed causal sequence. Effect sizes and confidence intervals should be reported with multiplicity control. Correlations among terminal markers alone are not evidence of biological interaction.

4.2.5. Validation and predictive performance

Validation has four layers. Reliability asks whether the same sample gives a stable result across constructs, runs, operators, devices, and laboratories. Domain sensitivity

asks whether a relevant positive and negative control changes the claimed function. Ranking ability asks whether the model orders candidates consistently within a defined formulation space. Predictive accuracy asks whether a locked rule agrees with independent outcomes. These layers need different evidence and should not be described with one general statement that the model is validated.[155]

Sensitivity and specificity are useful only when the reference labels are credible, and the decision is binary. Formulation studies may instead use rank agreement, effect-size agreement, or trajectory similarity. Independent samples should cover the intended material and process domain and should be analyzed after the rule is fixed. Samples with unknown or disputed outcomes can support prospective learning but should not be counted as correct or incorrect predictions. The Food and Drug Administration Medical Device Development Tools framework is relevant because it ties evidence to a defined context of use, but use of that logic does not constitute formal qualification.[64] Table 6 lists information that is required for all studies and additions that apply only to specific models.

5. Translation and research priorities

5.1. Translational and regulatory considerations

The regulatory object is generally the finished product used in a defined clinical context, not the bioink formulation in isolation. A cell-laden printed construct may involve medical-device, biologic, tissue-engineered, or combination-product considerations depending on jurisdiction and mode of action. ISO 10993 principles

Table 6. Minimum and model-specific reporting requirements for organoid/microphysiological systems bioink studies

Element	Minimum information for all studies	Additional information when applicable	Why it matters
Test article	Required: composition, supplier/lot, functionalization, particles/proteins, cells, storage, and raw-material sterilization	Report aseptic process simulation and release specifications for living products when relevant	Defines what was tested
Process window	Required: printer/nozzle, pressure or flow, speed, temperature, dwell time, support bath, and crosslinking dose[2,40]	Add local light dose, conversion, aspiration settings, or droplet actuation only when applicable	Links fabrication to exposure
Construct	Required: geometry, dimensions, cell density/distribution, culture medium, and duration	Add porosity, perfusable channels, loading regime, or compartment dimensions when applicable	Defines transport and tissue context
Exposure	Required: dose, timing, repetition, and washout[83-85]	For extracts/releases: preparation, filtration, storage, measured analyte, device recovery, and flow residence time	Separates hazard identification from delivered dose
Model identity	Required: cell sources, donor metadata, passage, and demonstrated functional attributes	Add vascular, immune, marrow, or remodeling compartments only when they support the question	Prevents label-based claims
Controls	Required: material blank, handling/process control, reference matrix, and domain-specific capability control	Add adverse-reference, dose-recovery, or dissolution control when relevant	Enables attribution and assay acceptance
Endpoints	Required: time points, normalization, spatial coverage, and at least one direct function for each claim	Add orthogonal assay when the matrix can interfere or when a high-consequence decision is made	Limits interference and overinterpretation
Design and analysis	Required: experimental unit, nesting, randomization, blinding, exclusions, and analysis plan[121]	Add repeated-measures and mixed-effects structure for perfused or longitudinal studies	Protects against pseudoreplication
Reproducibility	Required: independent runs and acceptance criteria[122,123]	Add lots, donors, operators, devices, and interlaboratory transfer according to context	Separates technical variation from biological variation
Applicability statement	Required: mark each item as applicable, not applicable with reason, or not measured	State the claim that cannot be made when a model-specific item is absent	Avoids treating the checklist as a universal fixed protocol

can inform material characterization and biological risk management, but they do not by themselves establish safety of a living, actively remodeling construct.[50,83-85] The proposed framework should be understood as a development strategy that organizes evidence and identifies gaps.

Organoid and MPS data are most useful when they answer a question that standard tests or animal models handle poorly, such as human-specific immune signaling, lineage-selective marrow effects, repeated low-level exposure, or patient-cell interaction. Formal Medical Device Development Tools qualification is a specific Food and Drug Administration conclusion for a defined context of use. Applying the same logic in research does not mean that a model is qualified.[64] The 2026 Food and Drug Administration New Approach Methodologies document is a draft, nonbinding guidance from the Center for Drug Evaluation and Research for drug development. It offers useful principles for validation and reporting but does not establish regulatory acceptance of bioinks or living printed implants.[156] Collaborative MPS programs likewise support method development and reference data rather than automatic product approval.[155,157,158]

Translation also requires manufacturing consistency and a potency strategy. A change in polymer supplier, dECM process, printer, nozzle, crosslinking source, construct scale, storage, shipping, or thawing can alter the test article. Living products also require aseptic manufacture, defined release criteria, and evidence that function is retained through the proposed handling window. Bridging studies should follow the affected risk. When a change alters a critical material attribute or exposure mechanism, repeating viability alone is unlikely to be adequate. A targeted Tier 0–2 panel can test comparability without claiming that the full product has been approved or qualified.

Clinical relevance does not require the most complex cell model. A simpler model can be more useful when its performance is stable, its response is known, and its exposure is well defined. Regulatory confidence depends on a clear scope, reliable performance, and reference outcomes. A complex model cannot replace these requirements.

5.2. Outlook and research priorities

The first priority is to establish reference bioinks, adverse controls, and shared assay acceptance criteria. A reference item does not need to be an ideal clinical product. It should produce a stable response across runs and show whether the assay can detect a meaningful functional difference.[3,5,23,47,75]

The second priority is cross-batch and cross-laboratory evidence. Process variables, delivered exposure, and tissue outcome should be measured in the same study so that a change in rheology, light dose, oxygen transport, or degradation can be linked to a mechanism. Transfer studies should begin with simple endpoints and predefined acceptance ranges before adding donor diversity or complex chips.

The third priority is direct evidence for osteoimmune, vascular, remodeling, and marrow-relevant bioink effects. Current studies more often show model capability or regenerative benefit than prospective identification of an adverse bioink. Recent work adds compartment-specific piezoelectric osteochondral constructs and a mechanically stimulated bone multicellular unit-chip, but these studies still illustrate direct regenerative evidence or model capability rather than a validated safety assay.[119,124] Direct studies should compare candidate formulations, process-matched controls, and downstream bone function rather than stop at macrophage markers or early osteogenic transcripts.[31,36,58,105,138,139,142,144,154]

Only after these foundations are stable should the field emphasize higher throughput, added compartments, or predictive analysis. Automation can improve consistency when source metadata, failed-device rules, image sampling, and analysis pipelines are controlled.[37,122,123] Machine learning may detect complex response patterns, but it cannot correct biased reference labels or poorly defined test articles.

Generalizability remains a separate question. Donor sex, age, disease, and anatomical source can change baseline behavior and bioink response. These variables should be added after technical performance is stable, then tested with a prespecified donor panel that distinguishes biological variation from process noise.

6. Conclusion

Bioink assessment should not stop when printed cells remain alive. The main evidence gap is not the absence of complex models. It is the limited number of direct, controlled studies that connect a defined bioink exposure to a later tissue function and a decision rule.

Bone organoids, organotypic bioprinted constructs, engineered bone microtissues, organoid-like constructs, and MPS reproduce selected aspects of matrix maturation, osteocyte response, vascular behavior, immune signaling, remodeling, and marrow function. Most studies, however, establish model capability, mechanistic/pharmacologic response, or regenerative performance. Exposure-based self-organized reference organoids, cross-domain interaction tests, and predictive safety use remain proposed applications that need direct validation.

The proposed framework links model complexity to a defined decision, uses one evidence taxonomy, and separates candidate failure, model failure, and insufficient evidence. Its immediate value is to improve study design and claim boundaries. Prospective factorial studies, quantitative benchmarks, reference bioinks, independent validation sets, and cross-laboratory transfer are needed before these models can be said to predict bioink safety.

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Conflict of interest

The authors declare they have no competing interests.

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AI tools statement

During the preparation of this manuscript, the authors used ChatGPT (GPT-6.2, OpenAI) solely for language polishing, including improvements to grammar, clarity, and readability. The tool was not used for study design, data collection, data analysis, interpretation of results, or generation of scientific conclusions. All AI-assisted revisions were carefully reviewed and edited by the authors, who take full responsibility for the final content of the manuscript.

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