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Advanced *in situ* bioprinting strategies for musculoskeletal injury repair

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Advanced *in situ* bioprinting strategies for musculoskeletal injury repair

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Abstract

Musculoskeletal injuries often involve irregular geometries, heterogeneous tissue interfaces, and mechanical demands that challenge conventional grafts and prefabricated tissue-engineered constructs. In situ bioprinting seeks to address these constraints by coupling patient-specific imaging and path planning with direct deposition of cells, biomaterials, and bioactive cues within living defects. This review links intraoperative constraints to technology and bioink selection across musculoskeletal injuries, separating direct in vivo printing from enabling studies and distinguishing geometric feasibility, tissue formation, and functional recovery. Extrusion-, inkjet-, light-, laser-, and assembly-based strategies are compared in terms of printability, cellular exposure, spatial control, and compatibility with the operative environment. We further examine collagen, gelatin methacryloyl (GelMA), alginate, hyaluronic acid derivatives, decellularized extracellular matrix, fibrin, cellulose-based materials, self-assembling peptides, mineral phases, and synthetic networks according to their biological and mechanical roles. Direct in vivo studies demonstrate the feasibility of conformal deposition and tissue formation in bone, cartilage, and temporalis muscle defects. Studies of vascularized constructs and tendon-bone interfaces provide additional evidence, largely from fabrication followed by implantation. However, small cohorts, short follow-up, and accessible or relatively low-load models predominate; durable mechanical and functional superiority over standard treatment remains unproven. Translation will require reliable intraoperative imaging and registration, wet-tissue fixation, vascular perfusion, sterile material exchange, closed-loop process control, scalable manufacturing, and clinically relevant large-animal comparisons. In situ bioprinting should therefore be developed as an integrated surgical platform that complements established reconstruction through digital planning, regenerative materials, and precise delivery. Standardized reporting and long-term functional endpoints are essential to establish clinical value.

Keywords: *In situ* bioprinting; Musculoskeletal injury; Bone regeneration; Osteochondral repair; Bioinks; Translational medicine

1. Introduction

Musculoskeletal injuries remain a major source of post-traumatic disability worldwide. Fractures and injuries involving joints, tendons, and skeletal muscle commonly result from falls, road traffic incidents, sports, and occupational hazards, with particularly pronounced treatment gaps in low- and middle-income regions.¹ National inpatient trauma data from China indicate that falls and road traffic incidents account for approximately 40% and 25% of trauma admissions, respectively.² Current treatment options include debridement, reduction and internal fixation; bone grafting or bone substitutes; microfracture or chondrocyte implantation; tendon repair and reconstruction; muscle transfer; and structured rehabilitation. Nevertheless, critical-sized bone defects rarely heal spontaneously,³ the architecture and long-term durability of cartilage repair tissue remain suboptimal,⁴ and scar-mediated tendon healing and volumetric muscle loss frequently fail to restore native function.^{5,6} Donor-site morbidity, limited graft availability, poor fit in complex defects, inadequate integration, and insufficient long-term load-bearing capacity remain barriers to anatomical and functional restoration. These limitations motivate patient-specific approaches that coordinate reconstruction with tissue regeneration.

Three-dimensional (3D) bioprinting has attracted increasing attention as clinical objectives have shifted from defect filling toward patient-specific functional reconstruction. Guided by digital models, the technology permits programmable deposition of living cells, biomaterials, and bioactive components, while multimaterial, zonal, and microchannel designs enable the fabrication of living constructs with spatial heterogeneity.⁷⁻¹⁰ Conventional *ex vivo* bioprinting generally involves imaging-based design, printing, and implantation, with culture and transport requirements varying by workflow. In irregular defects, the true boundaries after debridement may deviate from the preoperative model, complicating geometric fit and fixation of prefabricated constructs; thick constructs also remain constrained by diffusion and vascularization.¹¹⁻¹⁴ *In situ* bioprinting instead seeks to deposit bioinks directly into living defects using handheld or robotic systems, followed by crosslinking or assembly at the treatment site.¹²⁻¹⁴ Although this approach may reduce the need for construct transport and secondary reshaping, it simultaneously transfers imaging, registration, material crosslinking, tissue adhesion, and quality control into a dynamic operative field. Its translational value therefore depends on whether fabrication can reliably accommodate the final defect geometry and operative conditions.

In situ bioprinting could combine patient-specific defect conformity, spatial control of biological cues, and intraoperative delivery (Figure 1). Intraoperative imaging or surface scanning may be used to reconstruct the actual defect after debridement and convert the resulting digital model into a printing path, whereas multimaterial or oriented deposition may assign distinct cells, materials, and signals to different tissue compartments.^{13,14} Current studies encompass computer- or robot-assisted deposition, handheld delivery, and load-bearing cartilage models.¹⁵⁻¹⁹ However, the evidence remains dominated by proof-of-concept experiments, short follow-up periods, and often small animal cohorts and does not yet establish superiority over standard treatment in terms of long-term loading, functional recovery, safety, or clinical outcomes.

We used a structured narrative search strategy to identify English-language studies published from January 1, 2010, through July 31, 2026, in PubMed, Embase, Web of Science Core Collection, and Scopus. Core search strings combined bioprinting-related terms with contextual descriptors, including *in situ*, *in vivo*, intraoperative, handheld, robotic, and point-of-care, and with tissue- or injury-related terms, including bone, cartilage, osteochondral, tendon, enthesis, skeletal muscle, musculoskeletal injury, and related variants. The searches were supplemented with 3D bioprinting, bioink, and the names of specific printing technologies and were adapted to the syntax of each database. Backward reference screening and forward citation tracking were also performed. Included experiments were classified by the location and sequence of fabrication and validation, using the four evidence categories defined below.

The A–D framework classifies individual experiments according to their reported methods, irrespective of the article title or use of the term *in situ*. Category A requires deposition directly into a defect in a living animal; Category B covers printing into explanted tissue or *in vitro* fabrication without subsequent implantation for the reported repair outcome; Category C covers fabrication outside the living defect followed by implantation, including point-of-care manufacture; Category D covers nonbiological phantoms or hardware-only engineering tests. Cell-containing *in vitro* constructs remain Category B. Classification follows the methods, operative sequence and figure descriptions. Animal species, target tissue, cellular versus acellular ink, follow-up and measured outcomes are recorded separately. A paper with several experimental settings can contribute to more than one category, but a repair outcome after implantation does not qualify an *ex vivo* fabrication experiment for Category A. If direct deposition is not

documented, the experiment is not treated as confirmed Category A. Table 1 applies these rules to selected studies; the categories describe evidence setting and are not an ordinal quality score or a technology readiness level (TRL). The reproducibility of the A–D classification was independently assessed by five expert reviewers from the Department of Orthopaedic Surgery, The Second Hospital of Jilin University (Fleiss' $\kappa = 0.973$); detailed procedures, inter-rater reliability analysis, and discrepant-case resolution are provided in the Supplementary File.

This structured narrative review examines in situ bioprinting for the repair of bone, osteochondral, tendon, tendon-bone enthesis, and skeletal muscle injuries; compares the major printing technologies, bioinks, and delivery strategies; and critically evaluates translational barriers involving dynamic imaging and registration, vascularization, tissue-specific mechanics, sterility, quality control, and regulatory pathways. We assess the potential clinical contribution and current limitations of in situ bioprinting relative to conventional reconstruction and *ex vivo* bioprinting and identify the evidence required before clinical adoption in orthopedic practice can be considered. Earlier reviews have established the general principles and surgical implementation of in situ bioprinting,¹²⁻¹⁴ while orthopedic reviews and musculoskeletal biofabrication reviews have addressed bone and cartilage restoration or the use of cellular building blocks.^{20,21} What remains unclear is which surgical, material, and biological requirements have been validated together for a specific musculoskeletal indication. Separate demonstrations of printability, implant performance, or robotic accuracy cannot establish the readiness of the complete procedure. This review links each experimental setting to the additional validation required for intraoperative use. Direct printing in living defects is assessed separately from implantation and engineering validation, so that an advance in material design or geometric accuracy is not taken as evidence of intraoperative therapeutic efficacy. The resulting appraisal identifies the principal gaps between component performance and a validated surgical procedure.

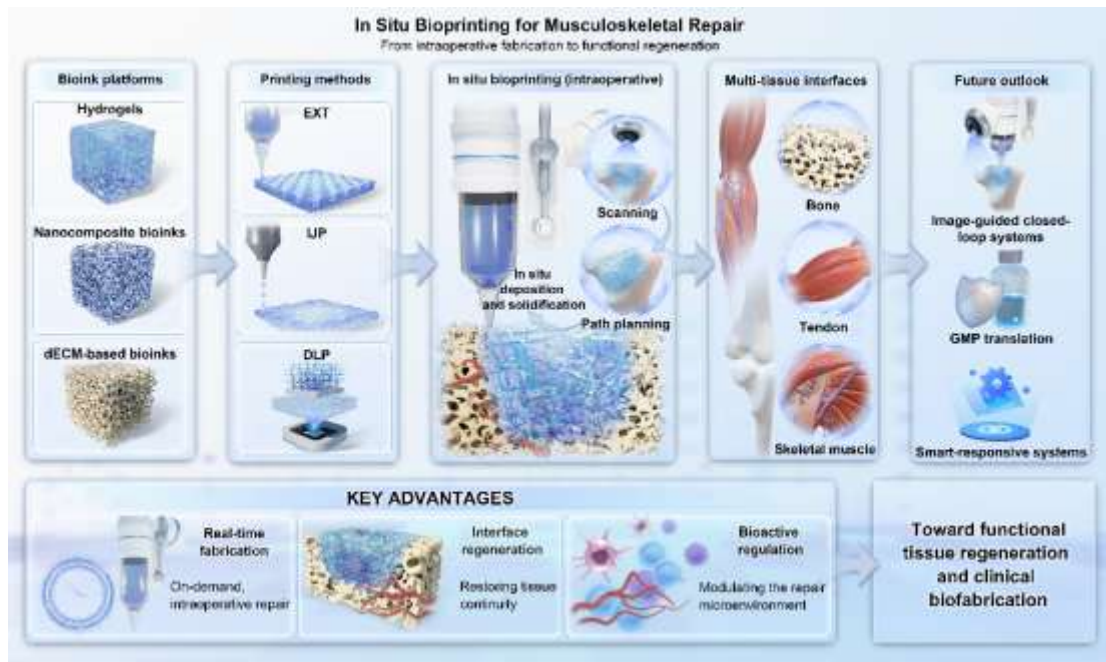


Figure 1. Schematic overview of in situ bioprinting for musculoskeletal repair. Representative bioink platforms include hydrogels, nanocomposite bioinks, and decellularized extracellular matrix (dECM)-based bioinks, while commonly used printing modalities include extrusion-based bioprinting (EXT), inkjet bioprinting (IJP), and digital light processing (DLP). The central panel illustrates the intraoperative workflow of in situ bioprinting, comprising scanning, path planning, and in situ deposition and solidification directly at the defect site. Potential applications span different musculoskeletal tissues and interfaces, including bone, tendon, and skeletal muscle. Potential translational directions include image-guided closed-loop systems, good manufacturing practice (GMP)-compliant workflows, and smart-responsive systems. Key proposed advantages include real-time fabrication, restoration of tissue continuity across heterogeneous interfaces, and spatially controlled bioactive regulation of the repair microenvironment. Together, these developments may support the progression of in situ bioprinting toward functional tissue regeneration and clinical biofabrication, although their long-term regenerative efficacy and clinical feasibility remain to be established. This conceptual figure was created by the authors.

Abbreviations: dECM, decellularized extracellular matrix; DLP, digital light processing; EXT, extrusion-based bioprinting; GMP, good manufacturing practice; IJP, inkjet bioprinting.

2. Bioprinting technologies for intraoperative use

For in situ use, a printing modality must operate within the final defect after debridement, with limited access, variable surface orientation, blood or irrigation fluid, and a finite operative time. The relevant comparison is therefore whether deposition and stabilization can be controlled on living tissue while preserving cells and adjacent structures. The following subsections assess access and positioning, resistance to displacement before fixation, crosslinking exposure, and compatibility with sterile surgical handling. Ex vivo methods are included where they provide a transferable capability, with their untested intraoperative requirements stated explicitly.^{13,14}

2.1. Extrusion-based bioprinting

Extrusion-based bioprinting uses pneumatic, piston-driven, or screw-driven actuation to dispense a continuous bioink filament through a nozzle along a predefined

trajectory. During surgery, delayed pressure responses can cause excess deposition after a stop command, whereas prolonged passage through narrow delivery paths can increase the duration of shear exposure in viscous or particle-containing inks. Reliable start-stop delivery and aseptic cartridge exchange therefore matter alongside the ability to extrude the formulation.²² Compared with inkjet and light-based techniques, extrusion accommodates a broader viscosity range, higher cell densities, and heterogeneous formulations containing microspheres, mineral particles, decellularized matrix, or short fibers. It also more readily supports material zoning and hollow-channel fabrication through multiple nozzles, coaxial nozzles, and sacrificial materials. Accordingly, extrusion remains one of the most widely used bioprinting approaches for bone, cartilage, tendon, and skeletal muscle applications²³ (Figure 2A).

For intraoperative extrusion, the printability window must reconcile stable delivery and cell function with retention on the wet defect during deposition and crosslinking. This window is jointly defined by material rheology, nozzle geometry, actuation mode, and crosslinking kinetics.²⁴ Soft hydrogels with low polymer content or low crosslink density generally favor nutrient diffusion, cell migration, and matrix remodeling, yet their low-viscosity precursors readily spread or collapse after deposition. Increasing polymer concentration, yield stress, or crosslink density improves filament continuity and interlayer stability but can restrict cell spreading, mass transfer, and tissue maturation. Shear-thinning behavior reduces resistance within the nozzle, whereas shape recovery after extrusion also depends on yield stress, the rate of thixotropic recovery, surface tension, and crosslinking kinetics.²² A single viscosity value or an image of a successfully printed construct is therefore insufficient to define printability. Reports should additionally specify nozzle geometry, pressure or flow rate, speed, temperature, and layer height, and quantify filament fidelity and retention on inclined, wet tissue under the intended irrigation conditions. This processing window also determines the mechanical load imposed on cells. At a given flow rate, a smaller nozzle can improve feature resolution but generally requires greater pressure and exposes cells to higher shear stress, potentially compromising stem-cell integrity.²⁵ Immediate post-printing Live/Dead staining primarily reflects intracellular esterase activity and plasma-membrane integrity and cannot exclude sublethal injury, proliferative delay, or lineage drift. Validation should therefore include cell recovery, short-term survival, metabolic activity, proliferation, and lineage-specific phenotypic stability or differentiation. Within this constrained processing window, reversible physical gelation

can transiently stabilize GelMA filaments during deposition, followed by permanent photocrosslinking after printing.²⁶ Alternatively, in situ photocrosslinking during extrusion can directly convert low-viscosity photocrosslinkable precursors into stable filaments.²⁷ For intraoperative use, these strategies require a defined interval between deposition and fixation: body-temperature exposure can reverse temporary gelatin-based stabilization, while rapid photocuring requires unobstructed illumination of each layer. Its acceptable duration should be established by measuring washout, nozzle blockage, and cell function under representative operative conditions.

When a single material cannot provide both a compliant cellular microenvironment and shape retention in free space, extrusion can combine a bioactive hydrogel with a load-bearing framework or support medium. Integrated tissue-organ printing systems co-deposit cell-laden hydrogels, biodegradable thermoplastics, and sacrificial materials to impart macroscopic form, internal channels, and early structural stability to human-scale constructs.¹⁰ Suspension-support approaches such as freeform reversible embedding of suspended hydrogels (FRESH) deposit low-modulus hydrogels into a particulate bath with yield-stress and rapid self-healing behavior, preventing gravitational collapse before crosslinking.²⁸⁻³⁰ These predominantly ex vivo approaches are enabling strategies. A support bath cannot be assumed to be compatible with an exposed surgical defect, and thermoplastic co-deposition requires validation of the temperature experienced by adjacent tissue; external support during printing does not establish load-bearing capacity after implantation. Residual support medium and its removal efficiency, perfusion and washing of enclosed cavities, printing time during scale-up, fatigue at the interface between thermoplastic frameworks and hydrogels, and sterility throughout the process all require independent validation. For load-bearing orthopedic constructs, a pragmatic division of function may assign early geometric and mechanical support to a rigid framework, while the bioink provides cells and bioactive signals.

Early Biopen studies and the subsequent coaxial core-shell design established the feasibility of combining handheld extrusion, cell delivery, and photocrosslinking for cartilage-defect filling.^{17,18} Subsequent studies in load-bearing ovine cartilage defects and porcine segmental tibial defects provided feasibility evidence of handheld and robotic *in vivo* deposition, respectively.^{19,31} These studies primarily demonstrate that deposition in the operative field and short-term repair are feasible; they do not show that hydrogels can independently restore long-term load-bearing function. Handheld

approaches require control of operator-dependent variability in trajectory and layer thickness, whereas robotic systems require validation of image registration, motion compensation, and fail-safe mechanisms. Overall, extrusion appears applicable to macroscopic filling, multimaterial zoning, integrated fabrication of load-bearing frameworks and bioactive hydrogels, and continuous in situ deposition. Its main limitations arise from trade-offs among resolution, mechanical exposure of cells, and printing time, as well as the long-term reliability of soft-hard material interfaces. Intraoperative acceptance criteria should therefore address deposited volume, deviation from the registered defect boundary, time to fixation, retention after irrigation, and safe interruption and restart. These measures would distinguish a reproducible surgical process from a successful static print.

2.2. Inkjet-based bioprinting

In contrast to the continuous filaments produced by extrusion, inkjet-based bioprinting uses discrete droplets as its fundamental manufacturing unit. The most common implementation in tissue engineering is drop-on-demand printing, in which droplets are generated as required by thermal bubbles, piezoelectric pulses, or microvalves. Although microvalve actuation differs from conventional thermal and piezoelectric inkjet mechanisms, it is commonly discussed within the broader inkjet category because it likewise enables programmable droplet metering.³²⁻³⁴ Inkjet printing minimizes material consumption, responds rapidly to start-stop commands, and permits digital mixing of cells, factors, and crosslinking components through multiple printheads. In an operative field, control over individual droplet placement is useful only if cells or factors remain at the intended site; concentration gradients and cell patterns must survive contact with the wet tissue and subsequent fixation.

This addressability, however, depends on a relatively narrow range of jettable material properties that can shift as cells sediment or aggregate. Droplet formation depends on viscosity, surface tension, density, nozzle dimensions, and the actuation waveform; in surgery, these variables must remain stable during cartridge residence and interruptions in delivery.³² Cell-laden bioinks are time-dependent particulate suspensions, and the stable jetting range of a homogeneous Newtonian fluid cannot be assumed to ensure a consistent biological dose. High cell concentrations, aggregation, or particles approaching the nozzle diameter increase the risks of clogging and satellite droplets; during prolonged printing, cell sedimentation can also generate systematic dose differences between early and late droplets. Circulation-assisted ink delivery can

mitigate sedimentation and aggregation,³⁵ but it increases shear exposure and residence time. A stable droplet volume therefore does not guarantee a consistent number of cells or amount of bioactive material per droplet. Optimized thermal inkjet parameters can preserve high immediate cell viability,³⁶ yet transient dedifferentiation-like phenotypes and transcriptional changes have been observed in fibroblasts.³⁷ Piezoelectric and microvalve systems likewise expose cells to pressure pulses, shear, droplet impact, and changes in interfacial tension. Evaluation should encompass cell recovery, acute injury, stress responses, long-term proliferation, and tissue-specific phenotypes and should relate these outcomes to the actuation waveform and printhead status.

Once jettability and cellular safety are controlled, a frequently cited capability of inkjet printing is precise spatial organization of cells and bioactive components. Droplet printing has produced high-resolution multicellular patterns and controlled cell-cell interfaces³⁸ (Figure 2B), and sequential droplet crosslinking has been used to generate complex cell-laden hydrogel structures.³⁹ Effective resolution, however, is not determined by nozzle diameter alone. It also depends on droplet flight deviation, spreading and rebound after impact, substrate wettability, coalescence of adjacent droplets, and diffusion during crosslinking.⁴⁰ On living tissue, blood or irrigation fluid can dilute droplets before crosslinking, while curvature changes the impact angle and deposition footprint. Local bleeding control, a validated working distance, and fixation coordinated with droplet arrival are therefore prerequisites for preserving the intended pattern. Inkjet printing may therefore be more applicable to gradients in cell density, discrete factor libraries, and small tissue interfaces than to the independent fabrication of thick, continuous constructs requiring immediate load bearing.

Musculoskeletal studies suggest that inkjet printing is best suited to localized cell or factor delivery within a mechanically supported construct. Layer-by-layer deposition of human chondrocytes and poly(ethylene glycol) dimethacrylate (PEGDMA) according to defect geometry produced constructs that integrated with surrounding tissue in full-thickness cartilage defects in *ex vivo* bovine osteochondral plugs and generated cartilaginous matrix during culture.¹⁶ In another hybrid-manufacturing study, an electrospun polymer structure provided mechanical support while inkjet-deposited cells provided the cellular component; the resulting construct showed higher mechanical performance than the hydrogel-only control and supported cartilage-like matrix formation *in vitro* and *in vivo*.⁴¹ These studies suggest that, in bone and osteochondral repair, a plausible role for inkjet printing is the position-specific delivery

of cells or signals rather than reconstruction of large load-bearing tissues. Combining inkjet deposition with an extruded framework, a photocured substrate, or a preformed porous scaffold is generally better aligned with material and mechanical constraints than a fully inkjet-printed, full-scale construct. Extension to an in situ setting additionally requires coordinated control of the printhead-to-tissue distance, ejection direction, droplet trajectory, and wet substrate conditions. Open, relatively flat cartilage or bone surfaces with controlled bleeding may be amenable to localized microdosing, whereas deep, vertical, or continuously bleeding defects are vulnerable to droplet displacement, dilution, and loss. Inkjet printing may therefore function as a digital microdosing tool for delivering cells, chemotactic factors, antimicrobial agents, or crosslinkers to specified locations after macroscopic filling. Light-induced fabrication changes the stabilization step by spatially immobilizing a photocurable precursor, which may limit spreading where illumination is accessible. It does not remove the need to control bleeding: blood and deep tissue can attenuate the light, so poor optical access may make photocuring unsuitable as the sole fixation mechanism. Light-based methods therefore require a separate assessment of optical access and exposure, as discussed below.

2.3. Light-based bioprinting

Light-based bioprinting includes point-scanning stereolithography (SLA) and two-photon lithography, layer-wise digital light processing (DLP), and tomographic or volumetric bioprinting based on multiview dose reconstruction.⁴²⁻⁴⁴ These nozzle-free approaches avoid clogging and direct extrusion-induced shear but require bioresins with controllable light absorption, polymerization kinetics, and cytocompatibility.⁴⁵ In SLA and DLP, exposure dose is jointly determined by light intensity and duration; cure depth additionally depends on the critical polymerization energy, absorber concentration, and optical penetration depth.⁴² Pixel size or focal-spot diameter indicates nominal optical resolution; radical diffusion, oxygen inhibition, light scattering, network shrinkage, and removal of uncured resin can cause actual feature dimensions to deviate from design values. DLP is commonly used for the rapid fabrication of continuous curved surfaces, internal channels, and microscale compartments. Two-photon methods offer higher precision but are restricted by field of view, speed, and cost, whereas volumetric photopolymerization places greater demands on light propagation and dose reconstruction throughout the resin volume. For intraoperative use, nominal resolution is secondary to whether light can reach the

complete target volume without excessive exposure of adjacent tissue. Conventional resin-vat immersion and rotation around an optically accessible volume are generally incompatible with an attached musculoskeletal defect; projection onto an exposed surface would require separate validation of dose uniformity on curved and partially shadowed tissue.

Nozzle-free photofabrication reduces direct extrusion-induced shear but introduces exposure to photoinitiators, reactive species, and light. Polymer concentration, degree of functionalization, type and concentration of photoinitiators and absorbers, wavelength, light intensity, exposure time, and oxygen conditions collectively determine both crosslinking and the dose experienced by cells.^{46,47} Increasing exposure or crosslink density generally improves shape fidelity and initial modulus but may increase radical-mediated injury, network shrinkage, and restriction of cell migration. Insufficient dose, conversely, leads to poor interlayer bonding, low conversion, and mechanical instability. Visible light is not intrinsically nontoxic, nor should ultraviolet irradiation be categorically excluded on the basis of wavelength alone. Comparable safety assessment across platforms should, at minimum, specify the spectrum, cumulative dose, concentrations of initiator and absorber, degree of conversion, post-processing, leachables, and post-printing cell function, rather than only short-term viability after exposure. The intraoperative dose should be evaluated at the tissue surface and through the deposited layer under representative blood contamination and optical obstruction. Increasing incident dose to compensate for incomplete curing may expose accessible regions excessively while leaving shadowed regions unstable. Shielding adjacent tissue, limiting layer thickness, and documenting delivered dose are therefore part of the surgical workflow, not merely printer settings.

Light projection can encode spatial heterogeneity in three-dimensional constructs. Prevascularized constructs with complex microarchitecture have been fabricated,⁴⁸ and SLA/DLP has been used to generate branched, interpenetrating, and helical channel networks. Studies of multivascular networks have shown that complex perfusable topologies can be directly fabricated in biocompatible hydrogels.^{49,50} For orthopedics, these findings suggest that cartilage surface curvature, trabecular-like porosity, vascular channels, and osteochondral interfaces may be defined within a single digital model. A geometrically perfusable channel, however, is not equivalent to a mature vessel with an endothelial barrier, antithrombotic function, and capacity for host anastomosis, nor

does it confer long-term load-bearing function. Structural replication and tissue maturation must be evaluated as distinct endpoints.

Multimaterial and gradient fabrication may extend light-based bioprinting from shape replication toward interfacial function, but these strategies remain significant engineering challenges. Conventional single-vat exposure does not readily permit frequent material exchange, and vat changes increase cross-contamination, registration error, and processing time. Composition-programmable DLP has been used to create continuous or discrete gradients in composition, mechanics, and biological signals within a single construct,⁵¹ providing a route to fabricate bone-cartilage and tendon-bone interfaces with more gradual transitions than a simple bilayer. Initial gradients, however, are altered by diffusion of monomers, absorbers, and radicals and are subsequently redistributed during culture, degradation, and cell-mediated remodeling. Studies should therefore report actual composition, local mechanics, regional phenotypes, and temporal interfacial stability. Translation to direct printing additionally requires material switching without contaminating the surgical field, removing uncured components without dislodging the repair, and maintaining registration after each exchange. The cited gradient studies establish fabrication capabilities rather than a validated intraoperative workflow.

To increase speed and construct volume, volumetric bioprinting uses multiview projections to generate a target dose distribution throughout the resin and can fabricate complex living-tissue constructs within seconds.⁵² Heterocellular bone-like tissues have likewise been formed rapidly by tomographic volumetric printing⁵³ (Figure 2C and 2D). High cell density, mineral particles, pigments, bubbles, and opaque tissues, however, alter scattering and absorption; vat scale-up, cell sedimentation, recovery of uncured material, and washing of enclosed cavities also remain incompletely resolved. Light-sheet laser patterning can simultaneously monitor crosslinking and image cells,⁵⁴ but these high-speed optical methods currently remain predominantly *ex vivo*. Their performance in an open, bleeding orthopedic field with obstructed optical access remains unvalidated. Light-based technologies currently appear most applicable to continuous surfaces, microchannels, fine gradients, and bioactive compartments and may be combined with extruded load-bearing frameworks and intraoperative fixation. Quality assessment should jointly address optical dose and conversion, geometric error, residual components, cell function, and long-term mechanics.^{42,45}

2.4. Emerging bioprinting technologies

The approaches in this section act at four different levels of the printing process: laser-induced forward transfer (LIFT) changes the mechanism of cell transfer; spheroid and organoid assembly changes the biological building block; embedded printing changes the mechanical support environment; and handheld or robotic platforms integrate deposition with surgical access and control. This organization distinguishes material transfer, biological assembly, physical support, and operative integration while allowing these approaches to be combined. Laser-assisted bioprinting (LAB) commonly uses LIFT: a pulsed laser irradiates an absorbing layer on a donor ribbon, generating transient pressure that transfers bioink droplets to a receiving surface. The method avoids nozzle clogging, accommodates relatively high viscosity and cell density, and enables microscale cell patterning.⁵⁵ An early murine calvarial study provided proof of concept for computer- and robot-assisted *in vivo* deposition of nanohydroxyapatite.¹⁵ Subsequent *in situ* printing of mesenchymal stromal cells (MSCs) in collagen/nanohydroxyapatite matrices promoted bone regeneration⁵⁶ (Figure 2E and 2F), whereas different geometric patterns of endothelial-cell deposition produced distinct vascularization and bone-formation outcomes.⁵⁷ These findings suggest that spatial positioning may influence biological outcomes. Nevertheless, donor-ribbon preparation, narrow laser-energy windows, droplet splashing, material waste, printable area, and system integration constrain large-volume applications. LAB may therefore be more appropriately considered as a high-precision cell-patterning module used in conjunction with macroscopic filling technologies than as an independent method for full-scale reconstruction of load-bearing bone. Direct use also requires control of donor-to-tissue spacing on an irregular surface, containment of splashing, and aseptic donor-ribbon exchange. The small, exposed calvarial models do not resolve these requirements for deep or moving defects.

Assembly of spheroids, organoids, or tissue strands preserves dense cell–cell contacts that may be disrupted when cells are dispersed in a large hydrogel volume.²⁰ Aspiration-assisted bioprinting uses controlled negative pressure to pick and position spheroids, tissue strands, and even single cells. The reported placement error is approximately 11% of spheroid diameter, and the method has been used to assemble angiogenic and osteogenic spheroids.⁵⁸ Such strategies can achieve high cell density and abundant endogenous extracellular matrix (ECM), but they depend on uniform building-block dimensions, fusion kinetics, and maturation in culture and may undergo contraction, central hypoxia, or incomplete interfacial fusion. The high-throughput

integrated tissue fabrication system for bioprinting (HITS-Bio) uses a digitally controlled multinozzle array to position multiple spheroids in parallel, increasing assembly speed by approximately one order of magnitude while maintaining cell viability above 90%; intraoperative printing has also been demonstrated in rat calvarial defects.⁵⁹ Further scale-up would require attention to batch-to-batch consistency of spheroids, aseptic preparation, immediate vascular supply, and the stability of large constructs.

During assembly, embedded printing provides mechanical support through an external support bath or a highly cellular matrix, preventing soft bioinks from collapsing before crosslinking. FRESH relies on a removable particulate support bath,^{28,29} whereas sacrificial writing into functional tissue (SWIFT) creates perfusable channels within a matrix of organ building blocks.⁶⁰ Direct evidence remains lacking for complete removal of support media from irregular *in vivo* defects, the biological effects of residues, and fixation at soft-hard boundaries. These approaches are therefore currently better suited to *ex vivo* or point-of-care prefabrication.

Integration into the operative environment adds requirements beyond deposition performance. Clinical deployment of *in situ* printing would require a closed loop that integrates sensing, registration, path planning, material exchange, deposition, and crosslinking. The INSIGHT (intelligent *in situ* printing guided by eye-in-hand robot technology) platform uses an eye-in-hand depth camera to identify targets at different positions and orientations and combines extrusion with spraying for continuous deposition at multiple sites.⁶¹ Another robotic platform integrates extrusion, inkjet, valve-jet deposition, automatic tool exchange, and photocrosslinking and has been used to demonstrate continuous multimaterial, multiscale fabrication in a human cranial phantom.⁶² Existing evidence, however, derives primarily from wounds or phantoms. Specular reflection from deep bone surfaces, blood obscuration, subtle limb motion, obstruction by instruments, and sterile tool exchange may all degrade the accuracy achieved with static paths. Handheld devices can be deployed rapidly and preserve the surgeon's tactile judgment, which may favor their use for superficial, visible, and spatially limited defects, although layer thickness, orientation, and dose are more operator dependent. Robotic systems may be considered for large-area, patient-specific defects or applications requiring consistent layer thickness but impose additional burdens involving registration, motion compensation, fail-safe operation, and operating-room space.

Quality control for in situ printing encompasses not only cell source, material lot, and equipment calibration, but also process variables such as sterility boundaries, the delivered dose, photocuring dose, and intraoperative deviation.¹⁴ Technology selection should therefore account for defect accessibility, load-bearing requirements, cell density, tissue-interface dimensions, allowable operative time, and fixation strategy. Hybrid systems can combine load-bearing structures, cell- or factor-laden bioinks, precise patterning, and real-time sensing, assigning different repair functions to different components. Within this division of technological roles, the next section examines how bioinks can reconcile the cellular microenvironment, intraoperative printability, and mechanical stability.

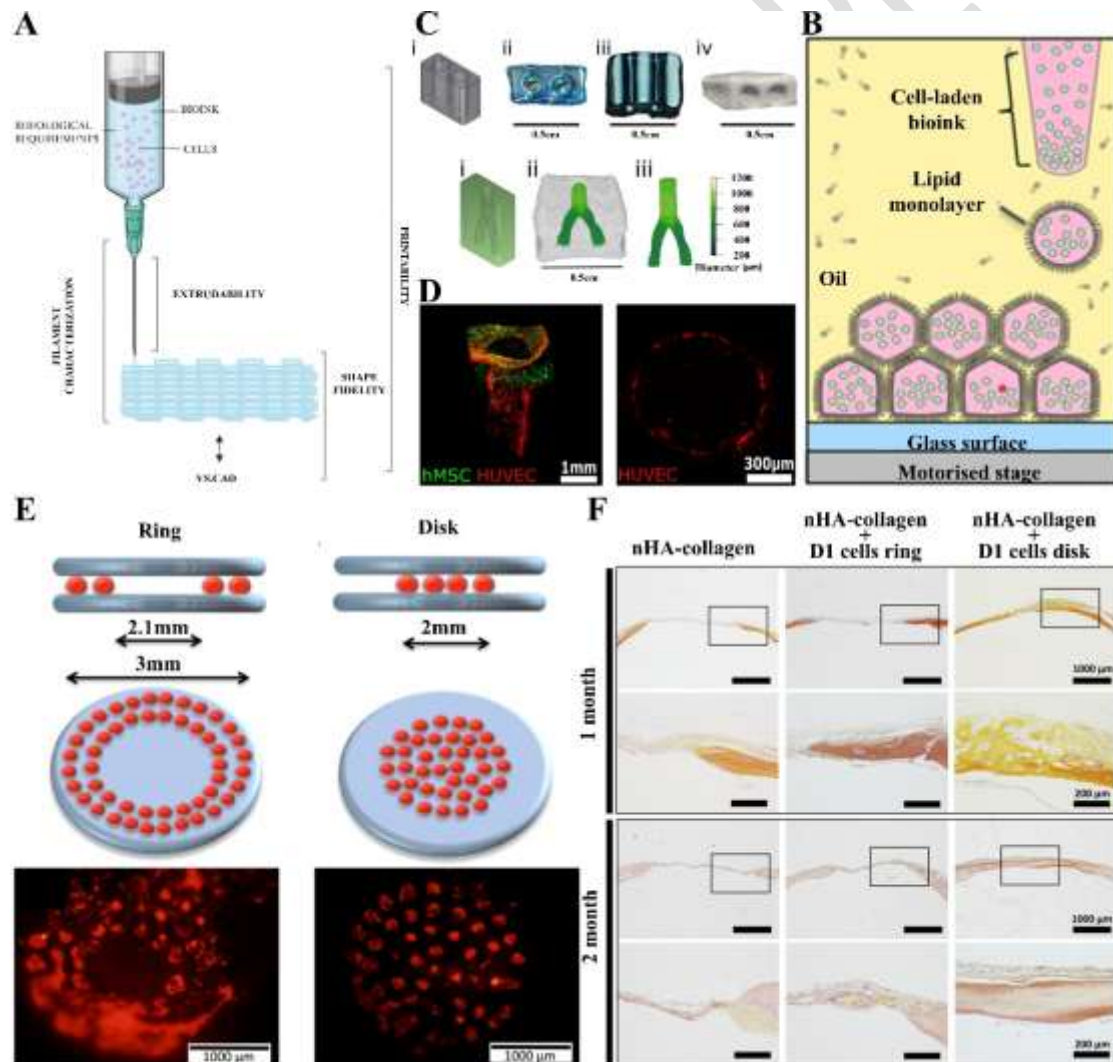


Figure 2. Representative bioprinting technologies and their applications in bone tissue fabrication and regeneration. (A) Extrusion-based bioprinting and the rheological, extrudability, filament-formation, and shape-fidelity requirements that govern bioink printability.²³ (B) Droplet-based printing of cell-laden bioink in oil, in which lipid-coated droplets are positioned by a motorized stage and form droplet-interface bilayers.³⁸ (C) Tomographic volumetric bioprinting rapidly generates complex three-dimensional constructs with internal channels.⁵³ (D) Volumetric bioprinting spatially organizes

human mesenchymal stromal cells and endothelial cells within bone-like matrices.⁵³ (E) Laser-assisted in situ bioprinting patterns mesenchymal stromal cells in ring or disk geometries within a collagen/nHAp matrix.⁵⁶ (F) The resulting spatial cell patterns produce distinct bone-regeneration outcomes in mouse calvarial defects.⁵⁶ Panels A-D illustrate general printability or ex vivo fabrication capabilities that may enable in situ systems; panels E and F represent direct printing in living defects. The former panels do not demonstrate compatibility with an operative field.

Abbreviations: CAD, computer-aided design; hMSC, human mesenchymal stromal cell; HUVEC, human umbilical vein endothelial cell; nHA/nHAp, nanohydroxyapatite.

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Table 1. Direct in vivo printing studies and selected enabling evidence for musculoskeletal repair.

Target / model	Setting / platform	Material / biological component	Evidence / follow-up	Remaining validation need	Ref.
A. Direct printing within living defects					
Calvarial defect / mouse	Direct in vivo / LAB	Acellular nHAp	Layered deposition was feasible; early bone formation was observed at 4 weeks.	Functional benefit versus standard repair.	15
Calvarial defect / mouse	Direct in vivo / LAB	Collagen/nHAp + D1 stromal cells	Cell pattern influenced bone formation at 1–2 months.	Larger defects and physiological loading.	56
Calvarial defect / mouse	Direct in vivo / LAB	Collagen + MSCs, endothelial cells and VEGF	Patterned endothelial delivery improved vascularization and bone formation at 2 months.	Durable perfusion and scale-up.	57
Calvarial defect / rat	Direct intraoperative extrusion	Collagen/chitosan/ β -GP/nHAp + pPDGF-B and pBMP-2	Sequential gene delivery improved bone formation and defect coverage at 6 weeks.	Expression safety and ectopic bone surveillance.	63
Calvarial defect / rat	Direct intraoperative extrusion in both groups	PEI-pBMP-2 ink versus pretransfected BMSC ink	Direct polyplex delivery produced greater bone formation.	Gene-delivery comparison; printing benefit unresolved.	64
Calvarial defect / rat	Direct intraoperative / HITS-Bio	GelMA/HAp composite + engineered hADSC spheroids	High-density spheroid deposition supported early defect closure at 3–6 weeks.	Long-term mechanics and operative scale-up.	59
Cranio-maxillofacial defects / rat and sheep	Direct robotic extrusion	Collagen composite; hADSCs in rats; acellular + rhBMP-2 in sheep	Scale-up to sheep was demonstrated, with repair and mechanical readouts at 12 weeks.	Cellular formulation not tested in sheep.	65
Calvarial defect / rabbit	Direct in vivo extrusion	Alginate/HAp $\pm$ autologous ADSCs	Cell-laden ink increased bone formation at 6 weeks.	Inflammation, encapsulation and long-term mechanics.	66
Segmental tibial defect / pig	Direct robotic extrusion	Acellular alginate/GelMA/PEGDA	Deposition took approximately 12 min; repair assessed at 3 months.	Long-term loading, fatigue and gait.	31
Critical-sized femoral defect / rabbit	Direct handheld structural printing	Acellular PCL/HAp	Conformal deposition and new bone formation at 12 weeks.	Thermal safety and load-bearing durability.	67
Articular-cartilage defect / sheep	Direct handheld / coaxial Biopen	GelMA/HAMA + ovine stromal cells	Early macroscopic and histological repair at 8 weeks.	Long-term wear and joint function.	19

Target / model	Setting / platform	Material / biological component	Evidence / follow-up	Remaining validation need	Ref.
Knee-cartilage defect / rabbit	Direct robotic extrusion	Acellular HAMA / four-arm PEG network	Rapid filling; repair comparable to prefabricated hydrogel at 12 weeks.	Benefit over a composition-matched implant.	68
Temporalis muscle defect / rat	Direct in vivo / Pri-Actor	Collagen + hASCs ± HUVECs	Aligned muscle tissue and reduced fibrosis at 5 weeks.	Limb VML force recovery and reinnervation.	69
B. Enabling studies — ex vivo defects or in vitro validation					
Cartilage / ex vivo bovine plugs	Ex vivo defect / inkjet	PEGDMA + human chondrocytes	Localized deposition, simultaneous curing and cartilage-matrix formation.	Retention and durability in a living joint.	16
Cartilage / benchtop and ex vivo tissue	Tool validation / coaxial Biopen	GelMA/HAMA core-shell ink + hADSCs	Cell viability and early construct stabilization supported handheld delivery.	Direct in vivo joint-function testing.	18
Bone and cartilage / ex vivo animal tissue	Ex vivo defects / scanning-guided extrusion	Acellular alginate/PEGDA or modified HA	Conformal filling linked scanning to deposition.	Bleeding, motion and functional loading.	70
Osteochondral interface / in vitro	Microcapillary extrusion / visible-light curing	Modified alginate/CMC-gelatin + hBMSCs	Continuous gradients supported regional differentiation over 3 weeks.	In vivo fixation and gradient durability.	71
Osteochondral defect / ex vivo sheep	Minimally invasive robotic access	Acellular alginate/PEGDA	Scanning, milling and deposition were integrated through a restricted access path.	Living-tissue safety and functional testing.	72
Cartilage lesion / ex vivo human tissue	Scanning-guided robotic extrusion	Fibrinogen/nanocellulose ± HA + ES-MSCs	Cartilage-like tissue developed during 8-week culture.	Synovial washout and cyclic loading.	73
C. Indirect evidence — fabrication followed by implantation					
Calvarial defect / beagle	Point-of-care printing, then implantation	PCL + autologous bone particles/BMSCs	Host integration evaluated over 9 months.	Direct printing in a living defect.	74
Calvarial defect / rat	Co-printed ex vivo, endothelialized, then implanted	GelMA/BMSCs + sacrificial endothelial-cell ink	Channel endothelialization and bone formation supported a vascular strategy.	Early perfusion and host anastomosis.	75
Calvarial defect / rat	Organoid printing, then implantation	GelMA + prevascularized bone organoids	Vascularized bone formation supported use of cellular building blocks.	Direct intraoperative delivery and batch control.	76

Target / model	Setting / platform	Material / biological component	Evidence / follow-up	Remaining validation need	Ref.
Rotator cuff enthesis / rabbit	Layered printing, then implantation	RGD-alginate/fibrinogen + hUCMSCs and factor-loaded PLGA	Improved fibrocartilage organization and mechanics at 12 weeks.	Direct printing and fixation at the enthesis.	77
D. Engineering validation — nonliving phantoms					
Calvarial defect / cranial phantoms	Robotic registration and multitool workflows	Acellular demonstration materials	Curved-surface deposition and automatic tool exchange were demonstrated.	Living-tissue and sterile-workflow validation.	62,78

Notes: Part A includes direct deposition within a living animal defect and identifies cell-containing bioinks separately from acellular biomaterial or structural inks. Parts B–D contain selected enabling studies: B, ex vivo defect or in vitro validation; C, fabrication followed by implantation; D, phantom engineering validation. These settings describe the location and sequence of fabrication, not study quality. Direct in vivo studies are presented separately; enabling studies illustrate access, stabilization, vascularization, tissue interfaces, and workflow integration. General material-screening studies are discussed in the text rather than exhaustively tabulated. No clinical efficacy is implied by inclusion.

Abbreviations: ADSC/hADSC, (human) adipose-derived stromal cell; hASC, human adipose-derived stem cell; BMSC/hBMSC, (human) bone marrow mesenchymal stromal cell; CMC, carboxymethyl cellulose; dECM, decellularized extracellular matrix; ES-MSC, embryonic stem cell-derived mesenchymal stromal cell; GelMA, gelatin methacryloyl; HA/HAMA, hyaluronic acid/hyaluronic acid methacrylate; HAp/nHAp, hydroxyapatite/nanohydroxyapatite; HITS-Bio, High-throughput Integrated Tissue Fabrication System for Bioprinting; hUCMSC, human umbilical cord mesenchymal stromal cell; HUVEC, human umbilical vein endothelial cell; LAB, laser-assisted bioprinting; MSC, mesenchymal stromal cell; PCL, polycaprolactone; PEG/PEGDA/PEGDMA, poly(ethylene glycol)/its diacrylate/its dimethacrylate; PEI, polyethylenimine; PLGA, poly(lactic-co-glycolic acid); RGD, arginine-glycine-aspartic acid; rhBMP-2, recombinant human bone morphogenetic protein 2; VEGF, vascular endothelial growth factor; VML, volumetric muscle loss; β -GP, β -glycerophosphate. The prefix p denotes a plasmid encoding the indicated growth factor.

2.5. Coupling technology selection with the material window

The printing technology defines an initial range of usable material properties, while the formulation determines the operating parameters required within that range. Extrusion requires a combination of nozzle geometry, pressure, and speed that delivers the intended volume without excessive shear or interruption; increasing cell density or mineral loading can narrow that window. Droplet delivery additionally depends on jetting stability and particle size. Photofabrication requires a formulation whose optical attenuation and curing kinetics are compatible with the available illumination. Consequently, a formulation optimized on one device cannot be transferred by preserving polymer concentration alone. Delivery should remain reproducible after the longest anticipated operative pause.^{22,24}

In surgery, this coupling must be tested as a sequence: delivery, temporary retention, definitive stabilization and contact with the tissue boundary. For example, the alginate/GelMA/PEGDA dual network separates rapid ionic stabilization from subsequent photocuring, whereas direct HAMA printing depends on accessible illumination.^{31,68} Increasing viscosity to reduce washout may demand a larger nozzle or slower deposition; increasing cure dose to improve fidelity may impair cells or adjacent tissue. The practical choice is therefore a device and formulation compatible with the surgical procedure, with defined intervals for delivery and fixation. Section 3 considers material families within that joint window.

3. Bioinks for in situ bioprinting

Bioink selection for surgery involves three distinct requirements: delivery through the device, rapid stabilization within the defect, and attachment to the surrounding tissue. Cell-adhesion motifs and bulk gel stiffness do not, by themselves, demonstrate wet-tissue adhesion. The gelation window must allow cartridge loading and delivery without premature blockage, yet provide retention before irrigation or tissue motion displaces the deposited material. The material families below are therefore assessed by their contribution to these requirements, together with biological function and remodeling. Testing should reproduce the intended tissue substrate, temperature, blood or fluid exposure, and fixation procedure.^{14,79,80}

3.1. Collagen, gelatin, and GelMA

Collagen, gelatin, and gelatin methacryloyl (GelMA) are among the matrix-forming materials most frequently used in in situ bioprinting. Type I collagen provides integrin-binding sites and an enzymatically degradable fibrillar network and

can carry cells, mineral particles, and bioactive molecules. However, fibrillogenesis may be too slow for immediate intraoperative fixation, and low-concentration formulations have limited yield stress and wet mechanical strength, making them prone to spreading, contraction, and detachment on curved or bleeding bone surfaces. Formulation strategies for *in situ* use therefore include increasing polymer concentration, adding minerals or thermoresponsive components, introducing a second network, or applying concurrent crosslinking to broaden the processing window.^{56,63,65,81}

Direct *in vivo* evidence for collagen derives from LAB in murine calvarial defects. Low-concentration type I collagen/nHAp and cell suspensions were deposited in a predefined sequence, and distinct cell arrangements produced different bone-formation patterns. Subsequent endothelial-cell patterning further illustrated the use of collagen as a carrier for spatially patterned cells and signals.^{56,57} The model, however, involves a small, open, low-load surface and cannot be extrapolated to deep, bleeding, or cyclically loaded defects. In addition to controlling spatial cell organization, collagen can be combined with chitosan, β -glycerophosphate (β -GP), and nHAp to form gene-activated biomaterial inks. Free plasmid DNA encoding platelet-derived growth factor B (pPDGF-B) and chitosan nanoparticle-encapsulated plasmid DNA encoding bone morphogenetic protein 2 (pBMP-2) were designed for early and sustained release, respectively.⁶³ Another study found that a cell-free route involving direct delivery of polyethylenimine (PEI)-pBMP-2 simplified the intraoperative workflow relative to a cellular route using pretransfected bone marrow mesenchymal stromal cells (BMSCs) and produced greater bone formation.⁶⁴ Because the direct gene-delivery formulation contains no cells, it is strictly a biomaterial ink. The findings support localized, sequential delivery but do not establish a benefit of a cell-laden bioink; PEI cytotoxicity, nonspecific uptake, ectopic bone formation, and the long-term safety of transgene expression also require evaluation.

Collagen concentration is a key variable in scaling the printing process. A composite containing collagen at 66 mg/mL together with chitosan, β -GP, and nHAp remained printable after the addition of up to 1×10^7 human adipose-derived stromal cells (hADSCs)/mL and maintained cell viability above 90% at 14 days. The cell-laden formulation was printed directly into rat calvarial defects. When scaled to an ovine craniomaxillofacial defect approximately 31 times larger than the rat defect, a six-axis robot completed deposition in approximately 5 min; a cell-free formulation containing

recombinant human BMP-2 (rhBMP-2) achieved approximately 80% defect coverage at 12 weeks and increased repair-site stiffness and peak load.⁶⁵ This scale-up comparison links a cell-laden bioink in rodents with an acellular biomaterial ink in sheep, but it did not test the cellular formulation in sheep, and the effect of rhBMP-2 cannot be attributed to collagen or printing alone. Because adding cells can alter modulus, degradation, and extrusion behavior, the formulation window must be redefined during scale-up.

In skeletal muscle repair, collagen functions primarily as an anisotropic template. The Pri-Actor system uses nozzle-induced shear to align type I collagen, human adipose-derived stem cells (hASCs), and human umbilical vein endothelial cells (HUVECs), with simultaneous genipin crosslinking. Direct printing into a rat temporalis muscle defect was associated with greater tissue regeneration.⁶⁹ These findings suggest that printing direction may provide a structural cue, but the temporalis model does not establish restoration of muscle force, reinnervation, or long-range force transmission in typical limb volumetric muscle loss (VML). Whereas collagen directly provides a fibrillar template, gelatin retains some collagen-derived adhesion and enzyme-sensitive sequences. Methacryloyl functionalization yields GelMA, which can be rapidly immobilized by photopolymerization and whose network properties can be tuned through polymer concentration and degree of substitution.⁸¹ Adding nanosilicates such as Laponite can improve the rheology and printability of GelMA⁸² (Figure 3A). Increasing polymer concentration or crosslinking dose can improve filament fidelity and washout resistance while reducing network mesh size and restricting cell spreading and matrix deposition. GelMA is therefore most useful as a cell-adhesive, tunable phase within a composite, with separate structural support where orthopedic loading requires it.

In porcine segmental tibial defects, an alginate/GelMA/poly(ethylene glycol) diacrylate (PEGDA) formulation was rapidly stabilized by a Ca^{2+} -mediated network and then photocured to create a stable covalent network, with direct *in vivo* deposition performed robotically.³¹ The animal formulation was acellular and therefore provides feasibility evidence of printing a biomaterial ink into a large irregular defect, but it does not establish load-bearing function. Beyond *in vivo* bone defects, GelMA has been used in the *ex vivo* fabrication of prevascularized bone and cartilage substitutes, either as the principal matrix in a BMSC-laden bone construct⁷⁵ or in combination with methacryloyl-modified acellular Wharton's jelly (AWJMA) to integrate ECM cues with

blue-light crosslinking.⁸³ Both constructs were printed *in vitro* and subsequently implanted and therefore do not address optical access in the operative field, wet fixation, or immediate loading. Together, these studies support the use of collagen and GelMA as cell-adhesive, biologically instructive, and remodelable components. Further clinical development would require control of raw-material sources, collagen concentration, GelMA degree of substitution, endotoxin, photoinitiator, and unreacted functional groups, together with release criteria linked to wet adhesion and degradation. For collagen/GelMA formulations, operative handling should specify cartridge temperature, warming at the tissue surface, the maximum delay before curing, and accessible illumination angles. Bulk shape retention should be evaluated separately from interfacial fixation; a cured GelMA plug can retain its geometry while still detaching from the defect wall. These tests would establish whether the chosen physical and covalent stabilization steps remain effective outside a controlled culture environment.

3.2. Alginate

Alginate is among the most widely used natural polysaccharides in bioink formulations. Its guluronic acid residues rapidly form ionic networks with Ca^{2+} , enabling mild cell encapsulation, filling of irregular defects, and early shape stabilization.⁸⁴ Unmodified alginate, however, lacks mammalian cell-adhesion ligands, and its network relaxes through ion exchange. Its main functions are therefore rheological support and rapid shape formation, whereas tissue induction, long-term stability, and cell adhesion require supplementation with a mineral phase, ECM protein, chemical modification, or a second network. Consistent with this role, direct *in vivo* evidence for cell-laden alginate is available from rabbit craniofacial bone defects. Alginate/HAp loaded with autologous adipose-derived stromal cells (ADSCs) was printed directly into the defects, producing more new bone at 6 weeks than either the acellular or empty-defect groups⁶⁶ (Figure 3B). This comparison supports an additional effect of cells within the same carrier; however, inflammation, encapsulation, and the absence of mechanical testing of regenerated bone indicate that short-term increases in new bone are insufficient to establish safety or functional recovery.

Combining alginate with a covalent photopolymerized network can reduce dimensional drift caused by relaxation of the ionic gel. In a 3D scanning-guided *ex vivo* study, partially Ca^{2+} -crosslinked alginate/PEGDA was used for conformal filling of bone defects; a subsequent porcine *in vivo* tibial study used alginate/GelMA/PEGDA to generate an ionically and covalently crosslinked network.^{31,70} These studies provide

separate evidence for geometric conformity and intraoperative deposition but do not demonstrate that the same processing window persists after the addition of cells or minerals. In cartilage, nanofibrillated cellulose (NFC)/alginate containing human chondrocytes was printed directly into osteoarthritic lesions in *ex vivo* tibial plateaus, preserving short-term cell viability and generating cartilage-like matrix.⁸⁵ NFC improved shear-thinning behavior and filament fidelity, whereas alginate provided crosslinking and encapsulation. The *ex vivo* model, however, lacks synovial-fluid washout, inflammation, motion, and cyclic loading and therefore cannot predict *in vivo* fixation or durability.

Other alginate systems primarily provide mechanistic evidence at the formulation level. Tyramine-modified alginate/gelatin and tyramine-modified carboxymethyl cellulose (CMC)/gelatin were crosslinked with a ruthenium/sodium persulfate (Ru/SPS) visible-light system to form an osteochondral gradient, with regional differentiation of human BMSCs observed over 3 weeks; however, the constructs were evaluated only *in vitro*.⁷¹ These data can guide formulation optimization but cannot replace *in vivo* printing evidence. Reporting only the mass concentration of alginate is insufficient for formulation reproducibility. The guluronic/mannuronic acid ratio, molecular weight, endotoxin level, oxidation or arginine-glycine-aspartic acid (RGD) modification, Ca^{2+} source, and crosslinking time all influence gelation and degradation. For articular and long-bone applications, reports should additionally include wet adhesion, volumetric swelling, particle sedimentation, interfacial failure under cyclic loading, and residual crosslinker. Alginate primarily provides rapid shape stabilization; cell–matrix interactions and durable mechanical function generally require additional components.⁸⁴ In practice, the Ca^{2+} source and its delivery sequence should be chosen to avoid gelation within the nozzle while achieving uniform stabilization throughout the deposit. Crosslinker concentration and exposure time should be assessed under fluid dilution, with residual crosslinker and washout measured alongside cellular tolerance. A second network may preserve bulk shape, but its contribution to tissue attachment must be tested independently.

3.3. Hyaluronic acid and HAMA

Hyaluronic acid (HA) is a major glycosaminoglycan in cartilage ECM and synovial fluid. It maintains hydration and viscoelasticity and regulates cell behavior through receptors such as CD44.^{86,87} Native HA turns over rapidly and lacks sufficient structural stability to retain a prescribed shape on a wet articular surface without

modification or support. Methacryloyl modification produces hyaluronic acid methacrylate (HAMA) and enables rapid stabilization by photopolymerization, but it may also alter degradation and cell-recognition properties. Molecular weight, degree of substitution, photoinitiator, and cumulative optical dose should therefore be treated as important formulation variables. A representative *in vivo* study used an acellular biomaterial ink composed of HAMA and four-arm acrylate-terminated polyethylene glycol; a six-degree-of-freedom robot filled rabbit knee cartilage defects in approximately 60 s. At 12 weeks, outcomes were comparable to those of a preformed hydrogel, suggesting that the observed contribution was primarily rapid geometric conformity rather than a demonstrated therapeutic advantage⁶⁸ (Figure 3C).

HAMA can provide a highly hydrated, cartilage-like environment, but hydration alone does not establish wet fixation, low friction, wear resistance, or integration with subchondral bone. Increasing the degree of substitution or optical dose may strengthen early stability while limiting cell infiltration. Future formulations should establish the minimum effective crosslinking dose for the selected initiator and wavelength, and combine tissue fixation with controlled enzymatic degradation and cartilage-bone zoning. Visible-light exposure should not be presumed safer without a formulation-specific assessment. Evaluation should include friction, wear, tidemark formation, subchondral-bone remodeling, and long-term gait alongside functional recovery. Acellular filling, cell delivery, and factor-delivery reservoirs should also be considered as distinct products with separate manufacturing and regulatory pathways.^{68,86}

3.4. Decellularized extracellular matrix

Decellularized extracellular matrix (dECM) retains tissue-derived collagen, glycosaminoglycans, matrix-binding proteins, and a proportion of growth factors, providing combinations of cues that are difficult to reproduce with a single polymer; it can also be processed into cell-laden inks.^{88,89} Decellularization, milling, enzymatic digestion, and sterilization, however, alter its structure and bioactivity. Digested dECM is generally low in viscosity, gels slowly at physiological temperature, and exhibits marked batch variability; on its own, it has limited resistance to washout in the operative field. It is therefore generally used to supply biological cues within a formulation that provides additional structural support.⁹⁰ This division of material functions is illustrated by an AWJMA/GelMA cartilage bioink: AWJMA provides ECM-derived cues from Wharton's jelly, whereas GelMA provides blue-light-mediated

fixation. BMSC-laden constructs implanted into rabbit cartilage defects were associated with improved repair⁸³ (Figure 3D). Porcine tendon dECM can likewise promote tenogenic differentiation of hASCs,⁶⁹ although neither approach has been validated by direct deposition into a living defect.

A key translational question for dECM is whether its biological complexity provides a reproducible functional benefit at a defined concentration. Clinical formulations would require control of tissue source, donor eligibility, residual DNA, α -Gal, endotoxin, viral safety, compositional fingerprints of key ECM components, and post-sterilization potency. High proportions of GelMA or synthetic networks introduced to improve printability may dilute tissue-specific signals. One strategy is to use the minimum necessary quantity of a defined network to correct rheological deficiencies and to demonstrate the contribution of dECM through lineage-specific potency assays rather than total protein content. For operative handling, a defined delivery and gelation window is also needed after reconstitution and during warming. If slow dECM gelation is supplemented by a rapidly crosslinking carrier, testing should determine whether the formulation resists dilution and washout while retaining its tissue-specific potency. The carrier should not be assumed to provide adhesion merely because it improves print fidelity.

3.5. Fibrinogen and fibrin

Thrombin-mediated conversion of fibrinogen produces fibrin, a cell-adhesive, hemostatic network susceptible to fibrinolytic degradation that forms rapidly under physiological conditions and provides a degree of wet adhesion. These properties may be useful at tendon ends, bone-soft tissue interfaces, and bleeding wounds. The network, however, contracts and degrades rapidly, has low mechanical strength, and may undergo further deformation under cell-mediated traction. Fibrin can provide adhesion and remodeling capacity when combined with a more stable polysaccharide network or surgical fixation.^{77,91} Consistent with this role, a rotator cuff enthesis study used fibrinogen/RGD-alginate to carry human umbilical cord mesenchymal stromal cells (hUCMSCs), with poly(lactic-co-glycolic acid) (PLGA) microspheres providing layer-specific delivery of basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF- β), and BMP-2. After *ex vivo* printing and implantation, the trilayer construct was associated with more extensive formation of a fibrocartilage gradient and improved biomechanical outcomes in a rabbit rotator cuff model.⁷⁷ Fibrin mediated adhesion and remodeling, alginate maintained geometry, and the microspheres

provided spatiotemporal signal delivery; the construct was not, however, printed directly at the footprint.

Translation of this strategy to arthroscopic or open in situ printing would require consideration of interactions between thrombin and coagulation status, fibrinolysis rate, dilution by blood, and load sharing between the printed layers and suture anchors. Evaluation should include cyclic fatigue, stiffness, interfacial sliding, and rerupture rather than only a histological gradient or single tensile test. The hemostatic and adhesive properties of fibrin may improve early retention but cannot replace mechanical fixation. Separate delivery of fibrinogen and thrombin can postpone gelation until deposition, but the mixing position and ratio must avoid premature nozzle occlusion and incomplete curing. The useful endpoint is retention of the printed layer during the intended irrigation and traction sequence, with an appropriate surgical fixation control.

3.6. Nanocellulose, carboxymethyl cellulose, and self-assembling peptides

NFC functions principally as a rheological modifier. Its high-aspect-ratio fibers undergo physical entanglement, increasing yield stress, shear-thinning behavior, and post-extrusion structural recovery and thereby improving filament fidelity without an excessive increase in crosslinkable polymer concentration.⁹² NFC/alginate formulations containing human chondrocytes have demonstrated printability and chondrogenic capacity⁹³ and have been used to fill *ex vivo* osteoarthritic lesions.⁸⁵ Existing evidence is confined to *in vitro* or *ex vivo* studies, and the degradation and clearance of NFC in mammals, foreign-body responses, and the risk posed by wear particles within a joint remain uncertain.^{85,93} CMC, another cellulose-derived material, is likewise used to increase viscosity and network stability. Tyramine-modified CMC/gelatin and tyramine-modified alginate/gelatin were crosslinked with visible light using Ru/SPS to form a continuous osteochondral interface. The stiffer CMC-rich region was assigned to bone, whereas the more highly swollen alginate-rich region was assigned to cartilage⁷¹ (Figure 3E). This study shows that a modified rheological component may also function as a spatially defined crosslinkable network; however, residual Ru/SPS, crosslinking uniformity, and gradient stability during physiological loading and degradation have not been validated in an *in vivo* in situ-printing model.

Self-assembling peptides are used less frequently but offer a distinct mechanism and well-defined chemistry. Chemically defined ultrashort peptides have demonstrated printability and stiffness-dependent chondrogenic differentiation.⁹⁴ FEFKFKFK

self-assembles into nanofibers and, when combined with amorphous magnesium phosphate (AMP), forms an ECM-mimetic bone ink that supports dental pulp stem-cell survival and osteogenic differentiation without exogenous osteogenic factors. *In vivo* testing used acellular prefabricated constructs and showed an AMP-associated increase in bone formation.⁹⁵ Shape retention *in vivo*, scale-up cost, consistency of assembly after sterilization, and resistance to washout remain to be established for peptide gels with low solids content. For both cellulose-modified and peptide-based inks, recovery of viscosity after extrusion should be distinguished from tissue adhesion. Assembly or gelation should be tested after contact with physiological salts and blood, because a formulation that retains shape *in vitro* may still detach or disperse in the operative field.

3.7. Inorganic mineral phases, synthetic networks, and delivery excipients

Hydroxyapatite (HAp/nHAp), β -tricalcium phosphate (β -TCP), and AMP primarily serve as dispersed phases in bone formulations, promoting protein adsorption and providing osteoconductive cues while altering viscosity and yield stress.⁹⁶ nHAp has been used as a mineral phase in collagen-based LAB⁵⁶ and in collagen/chitosan/ β -GP gene-activated matrices,⁶³ whereas HAp can increase the mineral content of alginate. In one formulation series, increasing HAp from 8% to 16% or 24% increased nozzle clogging and reduced mechanical performance.⁶⁶ Printability considerations may therefore impose an upper limit on mineral loading, and particle size, surface chemistry, sedimentation, nozzle abrasion, and intraoperative suspension stability should all be incorporated into quality control. The nonmineral components considered below serve three distinct functions: a covalent hydrogel network stabilizes the deposited biological phase, a thermoplastic framework can carry early structural loads, and a delivery carrier controls local signal exposure. In the first role, synthetic photocrosslinkable polymers such as PEGDA form a tunable second network that compensates for relaxation of the alginate ionic network and improves geometric stability.^{31,70} Although PEGDA offers favorable batch consistency and mechanical tunability, it lacks native adhesion sites, and high crosslink density restricts cell migration while increasing exposure to unreacted components and photochemical products. The PEGDA fraction should therefore be optimized for stability without unnecessarily restricting cell function or tissue remodeling. Mineral loading also changes light scattering in photocurable composites, so cure depth should be validated after adding particles rather than transferred from the particle-free formulation.

Sedimentation during operative pauses may change both mineral dose and crosslinking uniformity across the defect.

As structural components, thermoplastics such as polycaprolactone (PCL) form shells and porous frameworks that provide early mechanical support. They are structural inks, whose load-bearing and thermal requirements differ from those of the cell-containing hydrogel phase. Point-of-care cranial reconstruction used PCL to mimic the outer table and an autologous bone-particle/BMSC hydrogel to mimic cancellous bone⁷⁴ (Figure 3F), whereas a patient-specific PCL/ β -TCP scaffold used a dense exterior to limit fibrous-tissue invasion and a porous core to support bone and vascular ingrowth.⁹⁷ Both studies illustrate an approach to assigning load-bearing and biological functions in large-scale bone repair, but neither establishes the safety of molten deposition directly within a living defect. The third role is signal delivery: in the formulations discussed here, chitosan, PEI and PLGA carry genes or growth factors and control their local availability. Chitosan can also contribute to gelation, so these functional roles are not mutually exclusive. Chitosan contributes to thermosensitive gelation and forms pBMP-2 nanoparticles for sustained release.⁶³ PEI condenses pBMP-2 into polyplexes for intraoperative transfection, requiring the potential gain in expression to be balanced against cytotoxicity.⁶⁴ PLGA microspheres distribute bFGF, TGF- β , and BMP-2 across different enthesis layers.⁷⁷ Adding these delivery components can introduce further product-classification and quality-control requirements, depending on the cells, genes, or drugs included. Successful printing does not replace evaluation of carrier degradation, effective dose, ectopic ossification, inflammation, and systemic exposure.

Each additional component in a composite formulation increases raw-material variability and intermolecular interactions and broadens release-testing requirements; each component should therefore have a demonstrable contribution, assessed using appropriate component-omission controls. Material selection should be driven by the dominant failure mode of the target defect and should distinguish a cell-containing bioink from an acellular biomaterial ink used for geometric filling or factor delivery.⁷⁹ Evaluation should address sterility and lot control before printing, printability and wet fixation during printing,⁸⁰ and cell function, degradation, interfacial integration, and stability under cyclic loading after printing.^{98,99} Craniofacial bone repair prioritizes geometric conformity and osteogenic-vascular signaling; load-bearing bone requires a mechanical framework; cartilage requires low friction and osteochondral integration;

and tendon and skeletal muscle require anisotropy and force transmission. These differences inform the material considerations in the injury-specific appraisal in the next section. A practical selection sequence is to identify the immediate failure risk, select a compatible stabilization mechanism, and then establish retention and cell function under the complete surgical handling sequence. Deep defects with restricted illumination may require a non-optical initial fixation step, whereas exposed articular lesions permit photocuring but demand resistance to fluid washout and interfacial shear. These are indication-specific design choices; no single formulation in the cited evidence satisfies all surgical and tissue requirements.

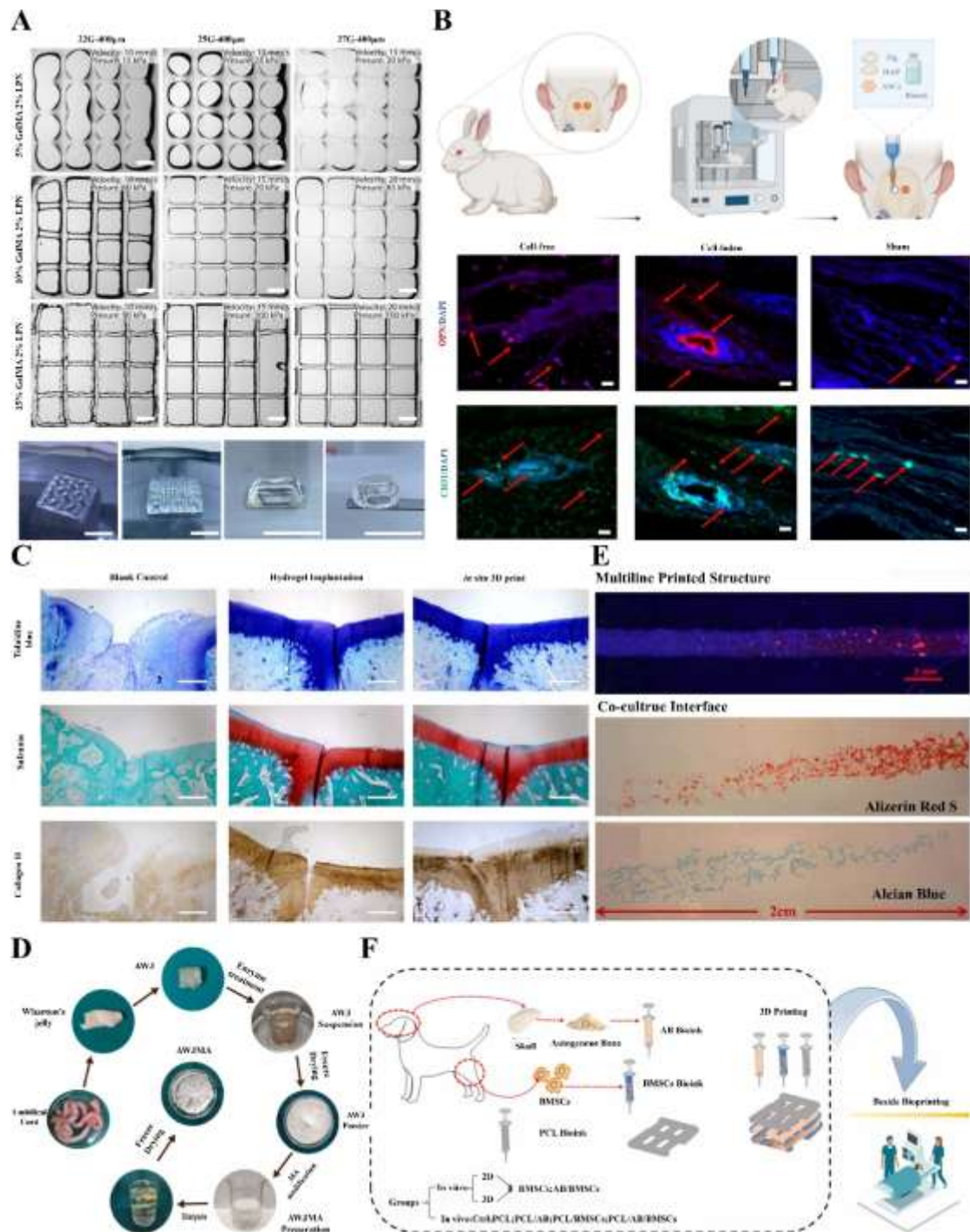


Figure 3. Representative bioink formulations and their roles in printability, spatial organization, and tissue-specific regeneration. (A) Laponite-containing GelMA bioinks illustrate improved printability and retention of three-dimensional geometry across formulations and printing conditions.⁸² (B) Direct printing of alginate/Hap bioink, with or without autologous adipose-derived stromal cells, into living rabbit calvarial defects is assessed by osteopontin and CD31 immunofluorescence.⁶⁶ (C) Histological and immunohistochemical staining at 12 weeks compares untreated defects with hydrogel implantation and in situ 3D-printed repair, showing greater defect filling and cartilage-specific matrix staining in the treated groups. Reprinted with permission from Ma *et al.*⁶⁸ Copyright © 2020 The Authors. (D) AWJMA synthesis comprises Wharton's jelly decellularization, enzymatic digestion, methacryloyl functionalization, dialysis, and lyophilization. Reprinted with permission from Hu *et al.*⁸³ Copyright © 2023 Elsevier Ltd. (E) Tyramine-modified CMC and alginate bioinks form a continuous osteochondral interface; region-specific osteogenic and chondrogenic differentiation was reported.⁷¹ (F) Point-of-care bioprinting combines a PCL shell with autologous bone and BMSC-laden components to represent the lamellar and cancellous compartments of the calvarium.⁷⁴ Evidence settings differ across panels: A and E

concern material or in vitro fabrication studies; B and C concern direct in vivo printing; D shows preparation of a bioink used for ex vivo printing followed by implantation; and F concerns point-of-care fabrication followed by implantation.

Abbreviations: AWJMA, methacryloyl-modified acellular Wharton's jelly; BMSC, bone marrow mesenchymal stromal cell; CD31, cluster of differentiation 31; CMC, carboxymethyl cellulose; DAPI, 4',6-diamidino-2-phenylindole; GelMA, gelatin methacryloyl; HAp, hydroxyapatite; OPN, osteopontin; PCL, polycaprolactone; AB, autologous bone; Alg, alginate; AWJ, acellular Wharton's jelly; LPN, Laponite; MA, methacrylic anhydride.

4. In situ bioprinting for musculoskeletal injury repair

4.1. Bone defects

Bone defects typically have irregular boundaries and substantial patient-to-patient geometric variation and impose concurrent mechanical and biological constraints. Repair generally requires reconstruction of the defect contour, maintenance of early stability, and creation of a local environment conducive to osteogenesis and vascular ingrowth. In situ bioprinting allows printing paths to be derived from medical imaging or intraoperative scanning and biomaterials to be deposited layer by layer according to the defect contour. Multimaterial and zonal printing may additionally deliver cells, mineral phases, and nucleic-acid or protein signals in predefined spatial relationships. Its potential contribution may extend beyond geometric conformity to irregular defects by providing a manufacturing route for coordinating structural filling, osteoinduction, and vascularization. Because craniofacial bone and load-bearing long bone impose different repair requirements, the following sections examine key technical implementations, principal outcomes, and limits of the evidence according to defect type; cross-cutting vascularization requirements are discussed in Section 5.3.

4.1.1. Craniofacial bone

Calvarial defects generally present an open, well-defined surface, are readily accessible for imaging and registration, and experience less cyclic bending and torsion than long-bone defects. They are therefore useful models for assessing the feasibility of *in vivo* printing and the biological effects of spatial control. In this setting, in situ printing has been used to examine whether spatial cell organization can alter bone regeneration. Laser-assisted bioprinting directly patterned MSCs and collagen/nanohydroxyapatite into mouse calvarial defects; despite similar biological components, different cellular arrangements produced distinct bone-regeneration outcomes⁵⁶ (Figure 4A). Subsequent work examined endothelial-cell patterning and angiogenic-osteogenic coupling. Endothelial cells were printed in disk or crossed-circle

patterns within a collagen matrix containing MSCs and vascular endothelial growth factor (VEGF) in murine critical-sized calvarial defects. Relative to random seeding, the disk pattern increased vascularization and bone regeneration by 203.6% and 294.1%, respectively, whereas the crossed-circle pattern increased the respective measures by 355% and 602.1%.⁵⁷ These findings suggest that the spatial organization of deposited cells may influence regenerative outcomes in addition to the effects of local cell delivery. Increased vascularization in a mouse calvarium, however, does not establish the formation of mature, patent, and durable vessels or demonstrate that the same effect can be reproduced at a clinically relevant scale.

Complementing spatial control, sequential delivery aims to coordinate angiogenic and osteogenic signals with tissue repair. One gene-activated matrix combined free pPDGF-B with pBMP-2 encapsulated in chitosan nanoparticles. The former was released over approximately 10 days *in vitro* and the latter over approximately 5 weeks, corresponding to early cell recruitment and angiogenesis followed by osteogenesis. At 6 weeks, sequential co-delivery produced approximately 40% new bone and 90% defect coverage, compared with approximately 10% and 25%, respectively, in empty defects.⁶³ A subsequent study compared two delivery routes for pBMP-2. Both groups underwent intraoperative printing: the *in situ* gene-delivery group received PEI-pBMP-2 directly within the ink, whereas the *ex situ* gene-delivery group received rat BMSCs transfected *in vitro* before incorporation into the bioink. Direct *in situ* delivery produced greater bone formation.⁶⁴ These findings are consistent with a role for localized intraoperative delivery of plasmids encoding growth factors and staged expression across distinct regenerative windows. Translation also requires assessment of carrier-related cytotoxicity and inflammation, the magnitude and duration of transgene expression, ectopic bone formation, and long-term gene-delivery safety.

Mineral-containing and growth-factor-delivery formulations offer complementary ways to retain material within wet defects while providing osteogenic cues. An ECM-mimetic hydrogel comprising the self-assembling octapeptide FEFEFKFK and AMP maintained approximately 90% cell viability and promoted mineralization and osteogenic-marker expression in dental pulp stem cells without exogenous growth factors. After implantation of acellular prefabricated constructs into rat calvarial defects, bone volume fraction (BV/TV) in the ECM/1.0AMP group at 4 and 8 weeks was approximately 1.7 and 1.4 times that of the AMP-free ECM group, respectively.⁹⁵ An alginate/collagen/CaSO₄ hydrogel used shear-thinning behavior, post-injection stability,

and further crosslinking at 37 °C to adapt to a wet defect and released BMP-2 over approximately 14 days. Following injection, more than 90% of hMSCs remained viable over 7 days, and greater repair of critical-sized rat calvarial defects was reported at 8 weeks.¹⁰⁰ These studies provide evidence of mineral induction without exogenous growth factors and stable delivery in a wet environment, respectively. The former used implanted acellular prefabricated constructs, and the latter was delivered by injection; neither can be directly extrapolated to multilayer *in situ* bioprinting. Future studies should jointly evaluate cellular function, shape fidelity, and tissue repair during prolonged extrusion and deposition on irregular bone surfaces.

Clinical translation also requires patient-specific defect conformity and durable host integration. Evidence toward this objective spans patient-specific prefabrication, point-of-care manufacturing, and direct printing within a living defect. An imaging-guided PCL/ β -TCP scaffold used a dense outer layer to limit competing fibrous-tissue invasion and a porous interior to support bone and vascular ingrowth; whereas control scaffolds formed new bone mainly at the margins, the biomimetic design achieved more complete defect filling.⁹⁷ Point-of-care printing subsequently combined hierarchical reconstruction of the calvarium with autologous bone particles and BMSC delivery: a PCL shell reproduced the outer table, and an autologous bone-particle/BMSC hydrogel reproduced cancellous bone. In beagle calvarial defects followed for 9 months, implanted BMSCs contributed to endothelial-, cartilage-, and bone-associated tissues, while the constructs also recruited host BMSCs⁷⁴ (Figure 4C). The former study suggests that a geometric barrier may influence competition between bone and fibrous tissue, and the latter provides longer-term large-animal observations of host integration. The first used a prefabricated implant and the second a point-of-care construct implanted after printing; neither involved real-time fabrication within a living defect. A subsequent *in vivo* rabbit study adapted a standard benchtop 3D bioprinter for use in critical-sized parietal defects and directly deposited bioink containing autologous adipose-derived stromal cells. The cell-laden *in situ* group showed greater bone regeneration than the sham-surgery and acellular-bioink groups on micro-computed tomography and histology⁶⁶ (Figure 4B). This study extends direct *in vivo* printing evidence from mouse and rat models to rabbits and provides an example of adapting conventional equipment for an *in vivo* procedure.

To translate patient-specific design into intraoperative deposition, the engineering system would need to accurately link the image, patient, and material-deposition

coordinate systems. A study using a patient-specific cranial phantom reconstructed from the computed tomography (CT) scan of a real trauma case reported a workflow for preoperative planning, fiducial registration, curved and overhanging path generation, and robotic deposition. For a defect of approximately 40 cm², in situ printing required approximately 1 h 40 min; the reported accuracies for 3D model acquisition, path planning, registration, and printing were 0.4 mm, <0.1 mm, approximately 1.5 mm, and approximately 1.5 mm, respectively⁷⁸ (Figure 4D). A subsequent study of the five-degree-of-freedom IMAGObot integrated extrusion, inkjet, valve-jet deposition, and photocrosslinking modules, together with radiofrequency identification (RFID) and automatic tool exchange, to perform continuous multimaterial, multiscale manufacturing in a cranial phantom in approximately 45 min⁶² (Figure 4E). Figure 4D addresses where the robot prints through registration and nonplanar path planning; Figure 4E addresses how it changes tasks while retaining a common coordinate frame. Automated exchange could allow a single procedure to alternate bulk filling, localized cell or factor delivery and curing without repeated manual mounting. RFID may help associate a tool with its intended material and parameters, but it does not verify sterility or the identity of the cartridge contents. For clinical use, each exchange would require secure docking, tool-tip calibration, priming and checks for residual material, leakage and collision. The relevant comparison is total procedure time and error frequency against manual exchange, including failed exchanges and rescue; the phantom demonstration does not establish a reduction in infection or operative time. These studies demonstrate complementary capabilities for registered, continuous manufacturing in phantoms. Because both studies were acellular phantom validations, however, they did not address blood obscuration, tissue deformation, dynamic registration, sterile tool exchange, optical dose, or the effects of material curing on living tissue.

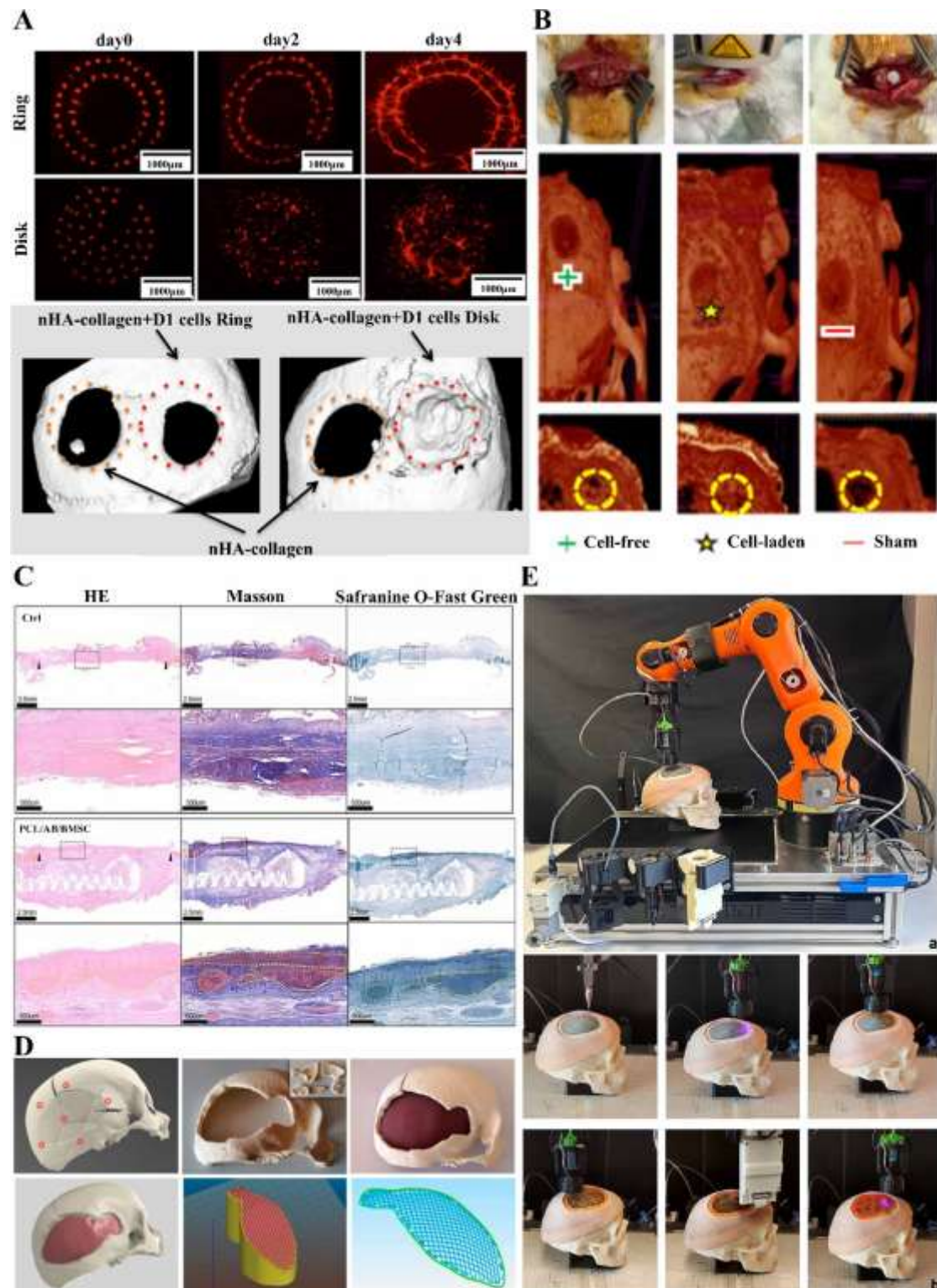


Figure 4. Representative strategies for craniofacial bone reconstruction using bioprinting. (A) D1 mesenchymal stromal cells printed in ring or disk geometries are shown during in vitro culture (upper panels); corresponding in vivo printing experiments show geometry-dependent bone formation in mouse calvarial defects (lower panels).⁵⁶ (B) Operative images show direct alginate/HAp deposition into living rabbit calvarial defects; postoperative micro-CT compares cell-free, autologous stem-cell-laden, and sham groups.⁶⁶ (C) Point-of-care-bioprinted PCL/autologous-bone/BMSC constructs are evaluated for long-term cranial-defect regeneration and host-tissue integration in a beagle model.⁷⁴ (D) CT-based modeling, fiducial registration, and nonplanar path planning are used for patient-specific reconstruction of a cranial phantom defect.⁷⁸ (E) A multitool robotic platform integrates extrusion, jetting, valve

deposition, and photocrosslinking for multimaterial, multiscale fabrication in a cranial phantom defect.⁶² Panels A and B provide direct *in vivo* printing evidence, whereas C involves fabrication followed by implantation and D and E are phantom validations.

Abbreviations: BMSC, bone marrow mesenchymal stromal cell; CT, computed tomography; HAp, hydroxyapatite; nHA/nHAp, nanohydroxyapatite; PCL, polycaprolactone; AB, autologous bone; Ctrl, control; HE, hematoxylin and eosin.

4.1.2. Large and load-bearing bone defects

Evaluation of segmental long-bone repair should move beyond technical feasibility to determine whether load-bearing and locomotor function are restored. A construct must bridge the defect and, together with internal fixation, withstand axial compression, bending, torsion, and repeated rehabilitation loads while developing a sustained blood supply. Robotic *in vivo* printing has been performed in long-segment defects of the porcine tibia, with deposition completed in approximately 12 min and improved tissue-repair measures reported at 3 months.³¹ This study provides feasibility evidence for direct deposition into a load-bearing long-bone defect but does not systematically report bending or torsional strength, fatigue resistance, or gait. The unresolved issue is whether procedural feasibility translates into durable mechanical and locomotor recovery.

Meeting early load-bearing requirements would likely require structural materials that reconcile printability, degradability, and mechanical stability. A poly(L-lactide-ran-p-dioxanone-ran-glycolide)/poly(D-lactide) (PLPG/PDLA) composite scaffold forms stereocomplex polylactide during processing, improving crystallinity and mechanical performance while retaining printability, degradability, and cytocompatibility.¹⁰¹ This material may serve as a candidate load-bearing framework for *in situ* hybrid manufacturing, but current evidence is limited to *in vitro* material and cell testing and does not constitute cell-laden bioprinting.

Alongside the selection of load-bearing materials, geometric reconstruction determines how closely a construct conforms to the actual defect boundaries. High-resolution 3D scanning of the defect and contralateral healthy anatomy, followed by Boolean operations, was used to generate the target volume of a segmental long-bone defect and to fill it conformally with a photocrosslinked hydrogel. The same workflow was applied to a free-form fracture defect of the femoral condyle and an International Cartilage Repair Society grade IV cartilage defect.⁷⁰ This work indicates that surface scanning may capture the actual defect geometry and guide conformal deposition; *ex*

vivo feasibility, however, does not establish fixation on a bleeding bone surface, cell viability or function, or stability under loading.

Because available evidence has separately addressed load-bearing materials and geometric reconstruction, one near-term integration target may be multimaterial hybrid manufacturing rather than full-scale direct printing with a single soft hydrogel. A biodegradable load-bearing framework could be combined with internal fixation, followed by deposition of cell- or factor-laden hydrogels and predefined vascular units into its pores, with an intraoperative vision system continuously correcting the path. Although developed for wounds rather than bone defects, the INSIGHT platform provides feasibility evidence for real-time recognition, continuous printing at multiple targets, and dynamic path adjustment through an eye-in-hand depth camera, a six-degree-of-freedom robot, and dual-mode extrusion and spraying.⁶¹ These capabilities may be transferable to orthopedic robots, but blood obscuration, specular reflection from bone, obstruction by instruments, and subtle limb motion are likely to be more difficult to manage in a deep operative field than in an open wound. This hybrid proposal remains an inference drawn from independent lines of evidence. Assessment of functional benefit would require load-bearing large-animal models, such as sheep or pigs, with concurrent reporting of radiographic bridging, vascular perfusion, bending and torsional strength, fatigue, gait, infection, and material degradation. Vascularization strategies and their implications for host integration are discussed in Section 5.3.

4.2. Osteochondral injuries

Osteochondral tissues comprise the superficial, middle, and deep cartilage zones, calcified cartilage, and subchondral bone. These layers differ in cell phenotype, ECM composition, water content, modulus, and vascular requirements. The cartilage compartment requires low friction, compressive resistance, and an avascular environment, whereas the bone compartment requires mineralization and perfusion; their interface must transmit shear and compressive loads. Osteochondral in situ printing must therefore reconstruct the articular surface and a stable compositional and mechanical gradient while restricting vascular invasion into cartilage and supporting perfusion in bone. Previous reviews have identified imaging, computer-aided design and manufacturing (CAD/CAM), vascularization, and clinical-scale manufacturing as areas requiring further attention before bone and cartilage bioprinting can be considered for in situ surgery.²¹

Accurate identification of defect geometry is an early requirement for reconstructing a patient-specific articular surface. Three-dimensional scanning, a healthy contralateral reference, and Boolean operations enabled conformal *ex vivo* filling of a free-form fracture of the femoral condyle and a grade IV cartilage defect.⁷⁰ A subsequent study of human osteoarthritic tibial plateaus compared CT, magnetic resonance imaging, and 3D surface scanning. Under the acquisition conditions tested, 3D surface scanning provided 2.77 times the point density of CT and was the only method to detect the osteoarthritic defect. After generation of computer-aided design files and G-code from the scan, human chondrocytes were printed directly into the lesion. Mean chondrocyte viability within the printed construct was 81% on day 5 of chondrogenic differentiation; cartilage tissue fragments had formed by 2 weeks and showed strong expression of the mature splice variant of type II collagen (COL2A1 type B) and aggrecan (ACAN)⁸⁵ (Figure 5A). This study provides an *ex vivo* demonstration linking diseased human tissue, patient-specific scanning, and direct deposition within the lesion. The resected tissue, however, does not reproduce synovial-fluid washout, inflammatory mediators, joint motion, or cyclic loading and thus cannot establish *in vivo* construct fixation or maturation.

Accurate filling must be complemented by a stable microenvironment that supports chondrogenesis through ECM-derived signals. Combining AWJMA with GelMA yields a bioink that couples print stability with human-derived ECM cues. In rabbit full-thickness cartilage defects, the implanted constructs supported BMSC survival, spreading, and chondrogenic differentiation and were associated with improved repair⁸³ (Figure 5B and 5C). These findings support the inclusion of Wharton's jelly ECM cues in a photocrosslinkable cartilage formulation. The constructs were nevertheless printed *in vitro* before implantation, evaluation focused primarily on the cartilage layer, and the calcified-cartilage-subchondral-bone unit was not fully reconstructed.

Direct *in vivo* studies have also tested the procedural feasibility of intra-articular filling. Using a six-degree-of-freedom robot, one study printed HAMA hydrogel directly into living rabbit knee-cartilage defects and completed filling in approximately 60 s. At 12 weeks, the *in situ*-printed group and the prefabricated-hydrogel group had comparable biochemical and biomechanical outcomes⁶⁸ (Figure 5D). The study provides evidence of the procedural feasibility of direct *in vivo* intra-articular printing, but the comparable outcomes also indicate that an additional therapeutic benefit over

prefabricated implantation has not been established. The complete cartilage-calcified cartilage-subchondral bone gradient was not reconstructed, and long-term wear, gait, and joint function were not assessed.

Reconstructing the osteochondral unit also requires a stable cartilage–bone gradient. An aspiration-extrusion microcapillary method aligned tyramine-modified alginate/gelatin and tyramine-modified CMC/gelatin within a single microcapillary and crosslinked them *in situ* under visible light using Ru/SPS, thereby producing a continuous osteochondral gradient. After 3 weeks of culture, hBMSCs exhibited chondrogenic and osteogenic phenotypes in the respective regions, indicating that material stiffness, chemical induction, and spatial composition may jointly influence region-specific chondrogenic and osteogenic differentiation.⁷¹ This study provides mechanistic evidence for formation of a continuous gradient interface but remains a small-scale *in vitro* investigation and does not establish whether the gradient can persist in a load-bearing joint.

Osteochondral studies thus provide separate evidence for defect identification,^{70,85} cartilage formation and chondrogenic phenotype,^{83,85} feasibility of direct *in vivo* filling,⁶⁸ and continuous regional differentiation,⁷¹ but these elements have not yet been integrated within a single *in vivo* platform. Future studies should directly compare *in situ* printing with clinical options such as microfracture and osteochondral transplantation in load-bearing large-animal joints. Evaluation should concurrently address early construct fixation; surface friction and wear; zonal cartilage-matrix organization; tidemark formation; subchondral-bone reconstruction; aberrant vascular invasion; magnetic resonance imaging and histological assessments; and gait or joint function.

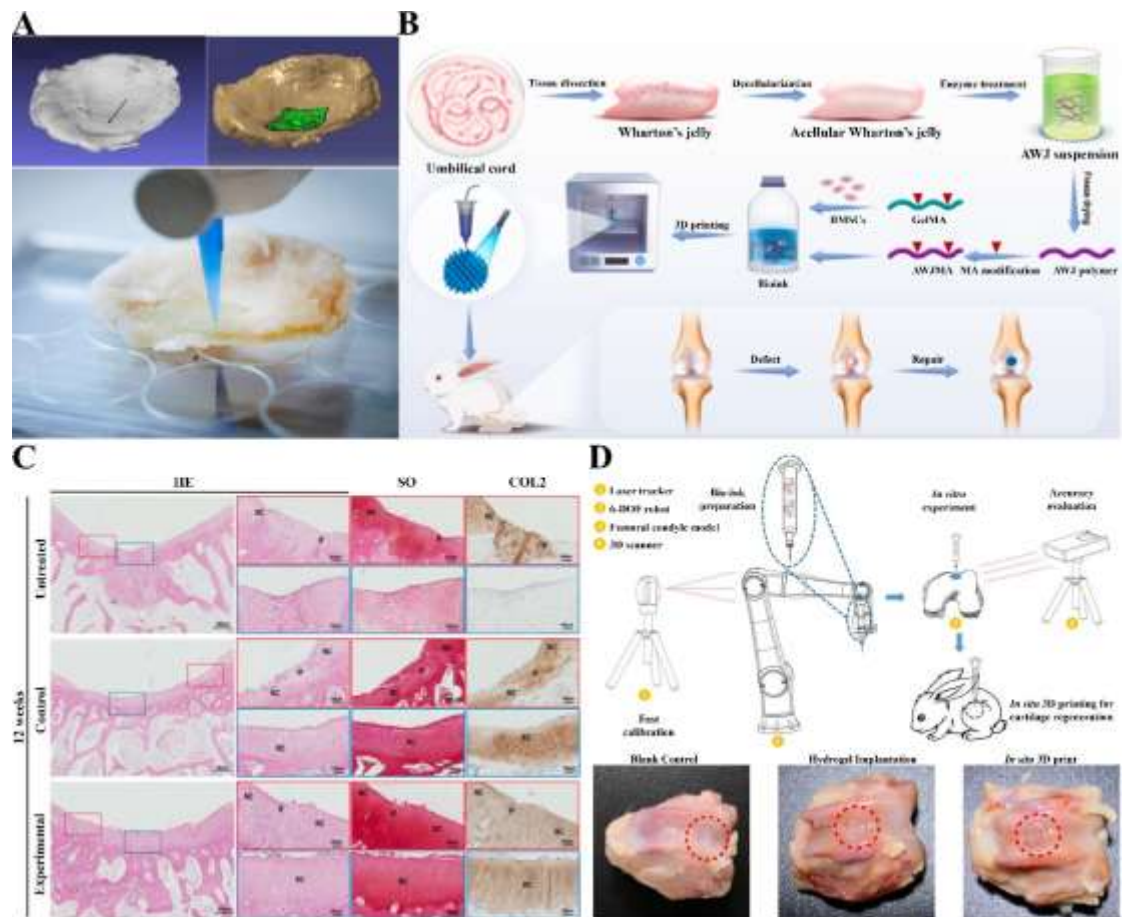


Figure 5. Representative bioprinting strategies for articular-cartilage defect repair. (A) Three-dimensional surface scanning and digital reconstruction identify a patient-specific osteoarthritic chondral lesion and guide direct *ex vivo* bioprinting into the human tibial plateau.⁸⁵ (B) The workflow depicts AWJMA/GelMA bioink fabrication, BMSC loading, 3D printing, and implantation for repair of full-thickness rabbit articular cartilage defects. Reprinted with permission from Hu *et al.*⁸³ Copyright © 2023 Elsevier Ltd. (C) At 12 weeks, hematoxylin and eosin, Safranin O, and type II collagen staining shows defect filling and cartilage-matrix formation in the AWJMA/GelMA construct group. Reprinted with permission from Hu *et al.*⁸³ Copyright © 2023 Elsevier Ltd. (D) Six-degree-of-freedom robotic *in vivo* bioprinting is used for direct filling of rabbit knee-cartilage defects with HAMA hydrogel; representative gross specimens are shown at 12 weeks. Reprinted with permission from Ma *et al.*⁶⁸ Copyright © 2020 The Authors. Panel A is an *ex vivo* human-tissue study, panels B and C concern printing followed by implantation, and D provides direct *in vivo* printing evidence. Cartilage repair in these studies does not establish reconstruction of the entire osteochondral unit.

Abbreviations: AWJMA, methacryloyl-modified acellular Wharton's jelly; BMSC, bone marrow mesenchymal stromal cell; GelMA, gelatin methacryloyl; H&E, hematoxylin and eosin; HAMA, hyaluronic acid methacrylate; COL2, type II collagen; DOF, degrees of freedom; IF, interface; NC, native cartilage; RC, regenerated cartilage; SO, Safranin O.

4.3. Tendon and tendon-bone enthesis injuries

For the tendon-bone enthesis, the primary repair objective is to reconstruct a continuous tissue gradient along the loading direction. The tendon region requires restoration of aligned type I collagen bundles, whereas entheses such as the rotator cuff require a continuous tendon-unmineralized fibrocartilage-mineralized fibrocartilage-bone transition. One study used a custom microfluidic printhead to

fabricate trilayer living-tissue constructs. PLGA microspheres carrying bFGF, TGF- β , or BMP-2 were embedded within the corresponding layers of an RGD-alginate/fibrinogen system, providing layer-specific sustained release for approximately 42 days and inducing region-specific tenogenic, chondrogenic, and osteogenic differentiation of hUCMSCs. At 12 weeks in a rabbit rotator cuff tear model, the construct produced a fibrocartilage gradient more closely resembling the native enthesis, with improvements in collagen deposition and alignment, bone integration, and biomechanical repair. The ultimate failure load of the trilayer growth-factor construct reached 154.3 ± 9.5 N, approaching that of the native enthesis⁷⁷ (Figure 6A and 6B). This study links layered factor delivery and regional differentiation with gradient-tissue reconstruction and mechanical readouts, providing functional animal evidence relevant to enthesis repair. The construct, however, was printed *ex vivo* and then implanted; the term *in situ* referred to regional stem-cell differentiation after implantation, not direct printing at the rotator cuff footprint.

Repair of the tendon proper requires aligned collagen bundles and a microenvironment that supports tenogenesis. Studies using the Pri-Actor platform reported a tenogenic phenotype in hASCs cultured with porcine tendon dECM bioink without additional differentiation medium. In the same platform, shear generated by microscale nozzle extrusion and concurrent crosslinking fixed the orientation of cells and collagen⁶⁹ (Figure 6C). The findings suggest that biochemical cues from tissue-specific dECM and structural cues from printing-induced cell and collagen alignment may jointly contribute to a local niche favorable to tenogenic differentiation. This study did not establish therapeutic efficacy or mechanical restoration in an animal model of tendon injury. Current evidence therefore supports the narrower conclusion that bioprinting has been used to fabricate implantable constructs with enthesis gradients and tenogenic niches, but the cited studies do not demonstrate tendon or enthesis repair by direct intraoperative printing.

Future *in situ* strategies for tendon and tendon-bone enthesis repair should address three clinical requirements: alignment of printed fibers with the patient's loading axis; construct stability during suturing, early traction, and rehabilitative loading; and coordination of factor release with inflammation, cell recruitment, fibrocartilage formation, and mineralization. Outcome assessment should not be limited to single-pull tensile strength but should include cyclic fatigue, stiffness, rerupture, fatty infiltration of the associated muscle, and long-term limb function.

4.4. Skeletal muscle injuries

For VML, the objective of in situ printing should be to reconstruct tissue units that contract in a defined direction, are perfusable, and transmit force to tendon, rather than simply increasing myogenic-marker expression. The Pri-Actor system couples microscale nozzle extrusion with simultaneous genipin crosslinking. Nozzle-induced shear aligns type I collagen and cells along the printing direction, and concurrent crosslinking fixes this topology. Collagen constructs containing hASCs and HUVECs showed expression of myogenic and angiogenic markers and were associated with greater muscle regeneration after direct in situ printing in a rat temporalis muscle-defect model⁶⁹ (Figure 6D). Processing can influence cell and collagen orientation as well as matrix stiffness. These structural cues may support differentiation, although their ability to shorten *in vitro* predifferentiation remains unproven. The *in vivo* experiment provides direct-printing feasibility evidence in an accessible temporalis muscle defect; it does not establish functional recovery in a representative limb VML model. Future studies should evaluate muscle force, fatigue, reinnervation, perfusion, and gait and should not infer efficacy solely from histological volume or increased marker expression.

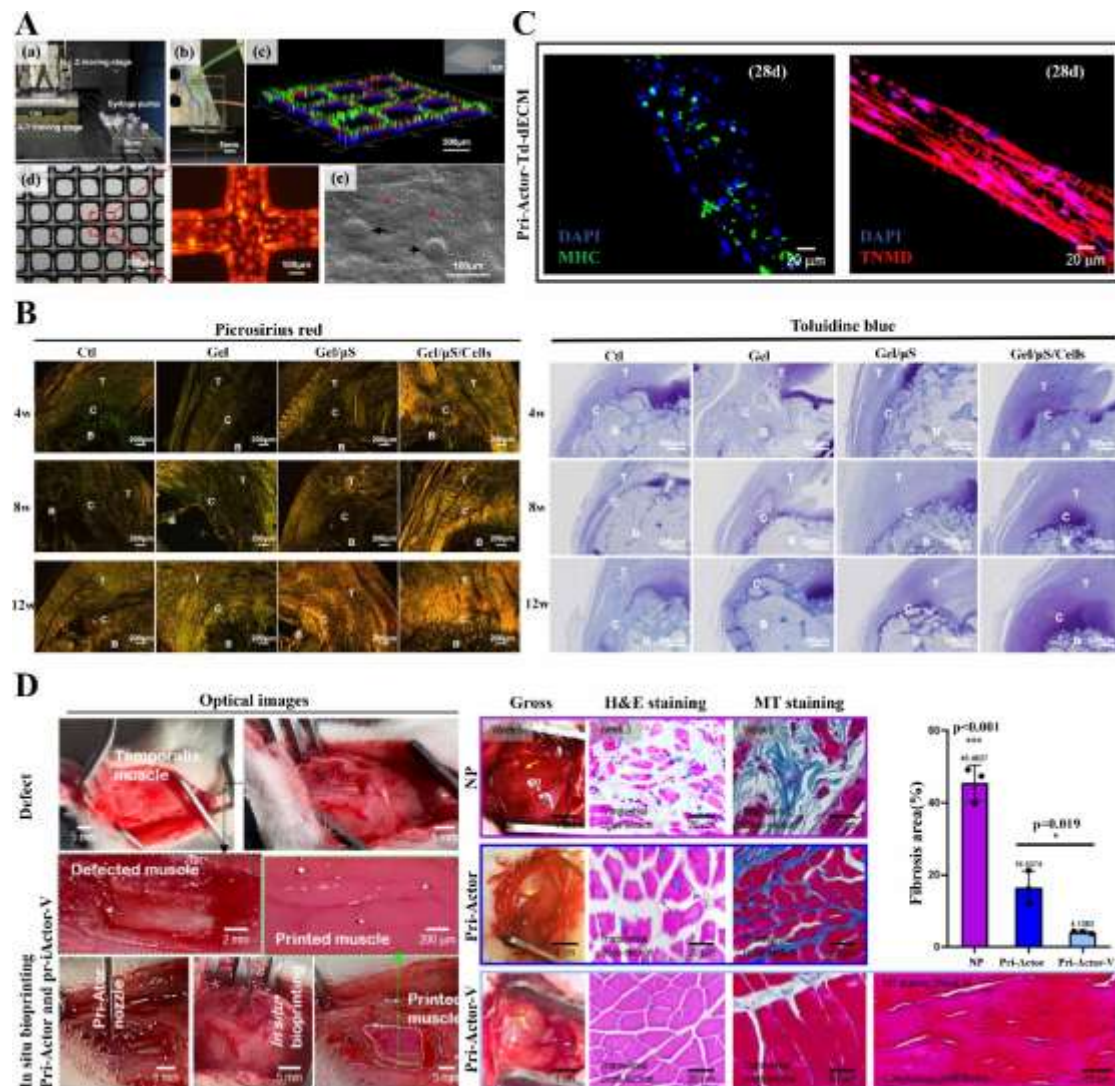


Figure 6. Bioprinting strategies for tendon-bone enthesis, tendon, and skeletal muscle regeneration. (A) Microfluidic multilayer bioprinting produces layer-specific living constructs containing hUCMSCs and growth-factor-loaded PLGA microspheres for enthesis repair. Reprinted with permission from Bai *et al.*⁷⁷ Copyright © 2022 Acta Materialia Inc. (B) Histological evaluation over 4-12 weeks shows greater collagen organization and fibrocartilage reconstruction across the tendon-bone interface after layer-specific factor delivery. Reprinted with permission from Bai *et al.*⁷⁷ Copyright © 2022 Acta Materialia Inc. (C) Pri-Actor-printed tendon-derived dECM bioink is associated with tenogenic differentiation of hASCs in a tissue-specific microenvironment. Reprinted with permission from Kim *et al.*⁶⁹ Copyright © 2021 Elsevier B.V. (D) Direct in situ printing of collagen containing hASCs alone (Pri-Actor) or hASCs plus HUVECs (Pri-Actor-V) into rat temporalis muscle defects was associated with greater muscle regeneration and less fibrosis, with Pri-Actor-V showing the smallest fibrotic area. Reprinted with permission from Kim *et al.*⁶⁹ Copyright © 2021 Elsevier B.V. Panels A and B concern prefabricated constructs implanted for enthesis repair, C provides in vitro evidence of a tenogenic niche, and D shows direct printing in a living muscle defect. The muscle result does not establish direct-printing efficacy for tendon or enthesis repair.

Abbreviations: dECM, decellularized extracellular matrix; hASC, human adipose-derived stem cell; hUCMSC, human umbilical cord mesenchymal stromal cell; HUVEC, human umbilical vein endothelial cell; PLGA, poly(lactic-co-glycolic acid); DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; MHC, myosin heavy chain; MT, Masson's trichrome; TNMD, tenomodulin; μ S, microspheres.

5. Challenges and future perspectives

The translational evidence remains uneven across musculoskeletal indications. Direct *in vivo* printing has been demonstrated most often in accessible bone and cartilage defects, whereas the cited tendon and enthesis studies provide *in vitro* or prefabricated-implant evidence. Direct printing has also been explored in a rat temporalis muscle defect, but its relevance to functional limb VML repair remains unproven. Even within bone and cartilage, technical feasibility, tissue formation, and clinical benefit are not equivalent endpoints. Future development should therefore distinguish direct printing within a living defect from *ex vivo* printing, point-of-care fabrication followed by implantation, and phantom or systems validation, and should match each claim to the evidence supporting it.

A central translational risk is failure of the complete procedure despite satisfactory performance of individual components under laboratory conditions. A geometrically accurate print can still be displaced before fixation, remain unperfused, lose strength during rehabilitation, or impose an impractical sterile workflow. The following sections link these failure mechanisms to measurable acceptance criteria, then consider adverse-event reporting, operating-room resources and indication-specific development priorities.

5.1. Intraoperative sensing and process control

A printing path derived from a preoperative image may no longer fit after debridement, tissue retraction, or movement. Intraoperative scanning should therefore establish the final target and registration should be checked during deposition, particularly after instrument contact or a change in limb position. Phantom systems demonstrate coordinate registration and tool scheduling, but they do not reproduce blood obscuration or deforming tissue.^{62,78} A closed-loop platform should detect loss of registration or blocked delivery, pause safely, and allow the surgeon to resume printing or convert to conventional reconstruction. Validation should report errors at the tissue interface, the frequency and duration of interruptions, and total workflow time, including scanning, material exchange and curing, rather than deposition time alone.

Artificial intelligence could support three linked tasks: identifying the defect boundary, updating a feasible deposition path and detecting deviations during delivery. Conventional computer-vision guidance should be distinguished from machine-learning-based segmentation. INSIGHT extracts color and contour information, reconstructs the target surface and updates printing coordinates; its wound

experiments do not demonstrate an AI model validated for musculoskeletal defects.⁶¹ A separate BioLite U-Net preprint reports segmentation of nozzle, extruded bioink and background in 787 printing images, with inference taking 335 ms per frame on a Raspberry Pi 4B.¹⁰² This is an enabling monitoring study, not an intraoperative defect-segmentation trial or evidence of autonomous repair in living tissue.

For a musculoskeletal application, a machine-learning model would need to identify the post-debridement boundary despite blood, glare, retraction and partial instrument occlusion. Its output should be checked in three dimensions against a surgeon-approved target before a collision-aware planner generates a path. Path updates would then be constrained by nozzle clearance, deposition rate, curing access and the permitted change in delivered dose. Training and test sets should be separated at the animal or patient level and, where applicable, by procedure; adjacent frames from the same sequence should not be split across these sets. External testing should assess generalization across instruments and imaging conditions. Boundary error at the tissue interface, update latency, missed obstacles and the frequency of unsafe or unnecessary pauses are more clinically informative than segmentation overlap alone. Low-confidence or out-of-distribution inputs should trigger a pause and review, not an unconstrained correction.

5.2. Wet fixation and safe stabilization

Rapid crosslinking must prevent washout without shortening the delivery window to the point of nozzle blockage or increasing photochemical, thermal, or chemical injury. Neither a high bulk modulus nor integrin-binding sequences establish adhesion to a wet defect. Candidate formulations should undergo retention and interfacial testing on the relevant tissue with controlled blood contamination, irrigation, and early cyclic loading; failure within the gel should be distinguished from detachment at the tissue boundary. Layer thickness, time to fixation, crosslinker residues, swelling, and cell function should be measured together. The Biopen and dual-network studies demonstrate feasible stabilization strategies,^{18,19,31} but their success does not establish a universal dose or gelation time for deeper defects, different ink compositions, or arthroscopic irrigation.

Stabilization introduces a trade-off between immediate retention and subsequent integration. Strong crosslinking can prevent immediate washout yet limit interfacial cell migration or produce a stiff plug with a mechanically weak tissue boundary. A useful test would reproduce the complete interruption sequence, including a clogged

nozzle, a curing delay and irrigation before the next layer, rather than assess only uninterrupted deposition. Acceptance limits should specify the maximum displaced volume, interfacial failure load and allowable temperature or light exposure for the target tissue. A rescue procedure should establish whether an incomplete print can be removed without extending the defect and whether conventional fixation or reconstruction remains possible.

5.3. Vascularization and host integration

Vascularization constrains the survival and remodeling of large bone and muscle constructs and should be considered across defect types. The requirement is tissue dependent: bone needs perfusion, whereas the cartilage compartment of an osteochondral repair should resist inappropriate vascular invasion. Printed channels and endothelial markers are intermediate endpoints; functional integration requires connection to the host circulation within a timescale compatible with survival of the implanted cells.

Current efforts to construct perfusable vascular networks capable of connecting to the host circulation can be grouped into three strategies: direct patterning of endothelial cells within a defect,⁵⁷ spatially controlled endothelialization of internal channels in printed bone scaffolds,⁷⁵ and preassembly of highly cellular bone organoids capable of rapid vascular integration.^{76,103} These approaches emphasize vascular geometry, luminal interfaces, and developmentally inspired tissue units, respectively, and their outcomes do not provide equivalent evidence of vascular function. With otherwise similar biological components, an in situ printing experiment that altered only the spatial organization of endothelial cells provides supportive evidence that predefined microvascular geometry may influence angiogenic-osteogenic coupling.⁵⁷ In another study, BMSC-laden GelMA and an endothelial-cell-laden thermoresponsive templating ink were deposited in alternating lines and successive layers. After photocrosslinking, removal of the templating hydrogel at 37 °C allowed endothelial cells to attach to the channel walls; subsequent culture generated vascular-like networks with improved endothelial-cell distribution and seeding efficiency. RNA sequencing indicated coordinated upregulation of osteogenic and angiogenic processes, and the implanted constructs promoted new bone formation in rat calvarial defects⁷⁵ (Figure 7C). This strategy extends conventional surface seeding to spatially controlled endothelialization of internal lumina, but predefined channels are not equivalent to mature vessels that can immediately anastomose, remain perfused, and resist thrombosis.

A third strategy uses bone organoids to provide both highly cellular tissue units and a prevascular network. Prevascularized bone organoids assembled from MSCs, HUVECs, and osteogenic microparticles may serve as cell-dense building blocks. In this system, graphene oxide was reported to promote osteogenesis primarily through focal adhesion and PI3K/Akt signaling. Organoid-laden hydrogels were printed into complex constructs and were associated with accelerated vascularized bone formation in rat calvarial defects⁷⁶ (Figure 7A). A separate Neo-Organoid Visualization and Assembly (NOVA) strategy combined bioprinting with hydrogel bioadhesion to form tissue-scale tubular bone-organoid grafts with unidirectionally guided vascularization. The approach was designed to mitigate central hypoxia in tissue-scale organoid constructs and to promote vascularization and ectopic bone formation after subcutaneous implantation in mice; orthotopic bone-defect repair was not evaluated¹⁰³ (Figure 7B and 7D). The first approach emphasizes prevascularization and osteogenic induction, whereas the second uses modularity and directional architecture to support tissue-scale assembly and directional vascular organization. Both, however, are more accurately described as printing or assembly followed by implantation and *in vivo* maturation than as direct fabrication within a living defect. They also introduce concerns relating to nanomaterial dose, organoid batch consistency, immune compatibility, and manufacturing quality control for these complex products.

Across these three strategies, vascularization should be evaluated in terms of function rather than vessel abundance alone. CD31-positive area and vessel-like structures alone do not establish functional vascularization. Evaluation of a vascular network's translational relevance should include early perfusion, host anastomosis, maintenance of barrier integrity over time, and predefined directionality where required. Additional endpoints should include the time to host anastomosis, central oxygen tension, risk of thrombosis and leakage, and the quantitative relationship between improved perfusion and mechanical maturation of new bone.

The translational question is whether these strategies can provide sufficient early perfusion without a prolonged *ex vivo* maturation phase. Studies should relate perfused vessel distribution to viable tissue depth, host integration, and later mechanical function, rather than infer adequate supply from vessel density alone. For muscle repair, vascular integration must also be coordinated with reinnervation and force transmission. Organoid and prevascularization methods remain candidate components of an

intraoperative system until their delivery, retention, and survival have been demonstrated in that setting.

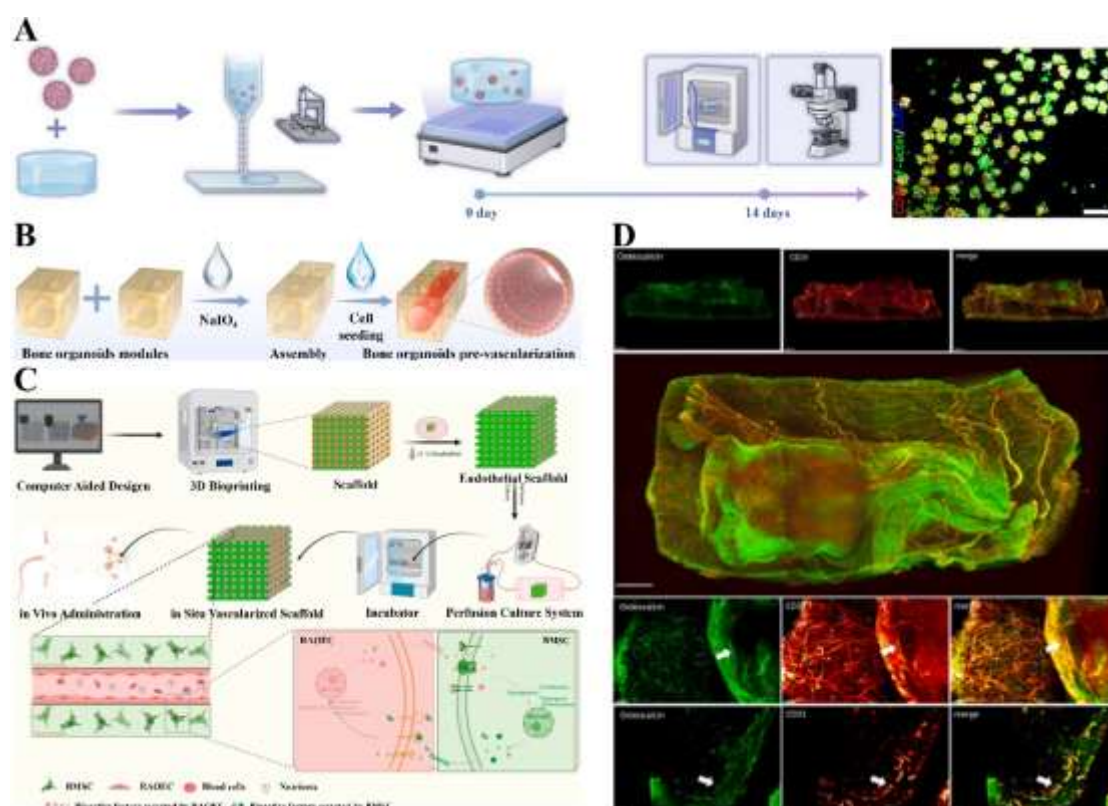


Figure 7. Vascularization strategies relevant to musculoskeletal repair. (A) Prevascularized bone organoids form printable, cell-dense constructs. Reprinted with permission from Duan et al.⁷⁶ Copyright © 2025 Wiley-VCH GmbH. (B) NOVA uses bioadhesive modular assembly and endothelial-cell seeding to create prevascularized tubular bone-organoid constructs. Reprinted with permission from Ju et al.¹⁰³ Copyright © 2026 The Authors. (C) Co-printing of BMSC-laden GelMA and an endothelial-cell-laden sacrificial ink enables channel endothelialization after template removal, followed by culture and implantation. Reprinted with permission from Shen et al.⁷⁵ Copyright © 2022 The Authors. (D) Whole-mount immunofluorescence shows osteocalcin-positive bone and CD31-positive vasculature after subcutaneous implantation in mice. Reprinted with permission from Ju et al.¹⁰³ Copyright © 2026 The Authors. All panels concern constructs fabricated before implantation; they do not demonstrate intraoperative printing.

Abbreviations: BMSC, bone marrow mesenchymal stromal cell; CD31, cluster of differentiation 31; NOVA, Neo-Organoid Visualization and Assembly; RAOEC, rat aortic endothelial cell; OCN, osteocalcin; NaIO₄, sodium periodate.

5.4. Mechanical support and tissue-specific function

A printed construct must remain stable while its material phase degrades and newly formed tissue acquires strength. This requires matching degradation and matrix maturation to the loads imposed by fixation and rehabilitation. For segmental bone, internal fixation or a structural framework should carry early loads while the biological phase supports repair; direct deposition of a hydrogel does not establish independent

load-bearing capacity.³¹ Cartilage studies should assess friction, wear and interfacial shear in addition to compression. Enthesis and tendon studies require cyclic loading and rerupture assessment, while muscle studies require active force, fatigue resistance and reinnervation. Evidence from implanted enthesis constructs and direct printing in a temporalis muscle defect can guide these endpoints, but neither establishes the performance of intraoperative repair across tendon, enthesis and limb muscle injuries.^{69,77}

The vulnerable period may occur after an apparently successful early repair, when scaffold degradation precedes maturation of the new matrix. Mechanical testing at prespecified time points should therefore be aligned with material loss and rehabilitation, with predefined failure criteria such as delamination, collapse, rerupture or loss of active force. For a composite construct, failure location matters: a strong polymer frame can conceal poor integration of the biological phase, while abundant new bone does not establish torsional load transfer across a segmental defect. Repeated loading and movement should be evaluated in addition to endpoint histology, using a comparator that controls for fixation and growth-factor exposure.

5.5. Aseptic manufacturing and quality control

The operative field combines a device, disposable fluid paths, biomaterials and, in some products, living cells, genes or drugs. Aseptic cartridge loading, validated sterilization of compatible components, defined material hold times, and traceable tool exchange must therefore be designed as part of the procedure. Cell-containing products cannot be assumed to tolerate terminal sterilization. Pre-use controls should address identity, sterility assurance, endotoxin, composition and relevant potency; intraoperative records should capture delivered volume, pressure, temperature, registration status and curing exposure. Material substitutions or changes in cell density may require revalidation because they alter delivery and crosslinking. Regulatory classification and release requirements will depend on composition, intended action and jurisdiction, so a single pathway should not be presumed for acellular fillers and cell- or gene-containing constructs.^{79,99}

Aseptic manufacture at the bedside moves some release decisions into the operation, where final culture-based sterility results for material prepared during surgery will generally be unavailable before use. Assurance must therefore rest on a validated process, qualified materials, closed or protected fluid paths and traceability of each cartridge and tool. The maximum validated material hold time should account for

cell settling, temperature changes, and the longest expected pause. The team also needs a predefined response to a breached drape, unexpected tool contact or a failed cartridge check. Automated exchange can reduce handling steps in principle, but an inadequately protected docking interface or a line opened during priming can introduce contamination; these risks need simulation and microbiological validation rather than an assumption that automation is inherently aseptic.

5.6. Failure conditions and adverse-event reporting

Reported fabrication limits already identify plausible failure conditions. In the rabbit alginate/HAp study, higher mineral loading caused nozzle clogging and inconsistent extrusion during formulation testing. At 6 weeks, cell-laden material was associated with capsule formation and substantial inflammatory-cell infiltration, despite increased bone formation.⁶⁶ These observations show why a favorable regenerative endpoint cannot be treated as comprehensive biocompatibility evidence. The inflammation was not, by itself, evidence of infection. Reports should distinguish transient postoperative inflammation from persistent foreign-body reaction and microbiologically supported infection, and should relate each finding to composition, delivered dose and residual material.

Printing failure should be reported for all attempted procedures, including abandoned deposition, blocked delivery, registration loss, incomplete curing, washout and conversion to conventional repair. The denominator should include failures before implantation and animals excluded after complications, with reasons and timing. Mechanical or optical injury, wound dehiscence, infection, delayed inflammation, and revision procedures should be recorded separately from regenerative outcomes. The evidence summarized here does not provide a harmonized denominator for estimating these complication rates; an unreported event cannot be counted as zero. Routine perioperative antibiotics and short follow-up further limit what an apparently uncomplicated animal experiment can establish.

Heterotopic ossification requires particular attention when BMP-2 protein or osteogenic gene delivery is used.⁶³⁻⁶⁵ Exposure of adjacent tissues to osteoinductive signals is a potential mechanism of ectopic bone formation. This risk warrants assessment, but the cited studies do not establish its incidence after in situ printing. For bone repair, surveillance should include adjacent soft tissues as well as the defect; for osteochondral or enthesis repair, it should assess unintended mineralization within cartilage or tendon. Dose-matched, nonprinted controls would help distinguish effects

of the biological payload from those of deposition. Imaging and histology should cover the release and degradation period, while gene-delivery studies should also examine persistence and distribution of expression.

5.7. Operating-room integration and economic value

Operating-room integration depends on the complete sequence of care. Patient positioning, debridement, scanning, draping, cartridge loading, calibration, printing, curing and wound closure compete for access to the same operative field. The printer must leave room for anesthesia equipment, imaging, instrument exchange and rapid surgical intervention. Workflow studies should record the personnel required, which preparatory steps can occur in parallel, and how long the wound remains exposed. A faster nozzle trajectory is of little practical value if setup, tool changes or repeated registration extend anesthesia. The cranial phantom systems provide engineering starting points for this analysis, but their reported deposition times are not clinical operating-time estimates.^{62,78}

Economic evaluation should compare the full treatment pathway with an indication-specific standard, such as graft reconstruction or a composition-matched prefabricated implant. Relevant costs include equipment depreciation and utilization, maintenance, sterile disposables, aseptic processing and release testing, cell or factor preparation, staff training, additional operating-room time and failed prints. Potential cost offsets, including reduced graft-harvesting requirements, shorter implantation time, or fewer revisions, should remain model assumptions until comparative evidence supports them. Early studies can use microcosting and cost-consequence analysis; later cost-utility analyses require credible functional and quality-of-life outcomes. Transparent reporting of perspective, comparator, time horizon and uncertainty should follow CHEERS 2022.¹⁰⁴

The most informative early economic question is often the threshold for value: how much additional procedure time or consumable cost could be justified by a specified reduction in complications or improvement in function? Sensitivity analyses should vary case volume, print failure, cartridge wastage, staffing and revision risk. Low utilization can make an expensive robotic platform difficult to justify even when an individual print is efficient; a handheld system may reduce equipment needs but require more operator training and process control. These trade-offs require comparative measurement before either approach can be considered cost-effective. The studies summarized in Table 1 do not establish comparative clinical cost-effectiveness.

5.8. Readiness assessment and validation priorities

A–D evidence categories should be complemented by readiness assessment, but they should not be converted directly into TRL numbers. The general TRL framework distinguishes experimental proof of concept, integration of components and testing in increasingly realistic environments.¹⁰⁵ For bioprinting, the assessment unit should be a specified device, formulation, tissue indication and surgical workflow; a grade for extrusion or robotics alone is too broad. We propose three development milestones informed by this framework: reproducible delivery and cell compatibility in bench testing; validated integration of sensing, deposition and stabilization; and direct testing under the relevant biological and mechanical conditions. These milestones are an engineering interpretation for this review, not a formal or independently validated TRL assessment.

The current evidence supports different next steps for specific combinations. Craniofacial extrusion and laser printing have direct animal feasibility and tissue-formation evidence; further progress requires reproducible, sterile performance at the intended scale with functional comparators.^{56,63,65,66} Robotic hydrogel deposition has been demonstrated in living porcine tibial defects, but cyclic loading and locomotor recovery remain decisive gaps.³¹ Biopen and robotic HAMA delivery for cartilage require evidence of durability under joint motion and benefit over established repair or matched implants.^{19,68} For tendon and enthesis, direct intraoperative delivery and fixation remain unproven by the cited in vitro or implant studies.^{69,77} Temporalis muscle printing requires testing of reinnervation and force recovery in a relevant limb VML model.⁶⁹ Cranial phantom platforms require validation in living tissue before their geometric accuracy can support therapeutic claims.^{62,78}

A numerical TRL would become defensible only after the relevant environment, critical components and pass criteria are specified and the supporting evidence is assessed against each requirement. A mature robot arm does not establish the readiness of a cell-containing surgical process, and a small-animal biological result does not demonstrate the safety of automatic operation. Readiness should therefore be judged against the least validated critical component. Reporting completed milestones and unmet requirements provides a defensible basis for development until formal, indication-specific TRL assessment is possible.

Development should progress from realistic wet-tissue testing to direct printing in an indication-matched animal defect and then to longer-term functional comparisons.

Large-animal testing is particularly relevant where joint size, access or loading cannot be represented adequately in a small model. To isolate the contribution of printing, studies should compare direct deposition with a composition-matched prefabricated implant when feasible, alongside the appropriate standard treatment. Randomization, blinded outcome assessment, sample-size justification, predefined exclusions and reporting of failed procedures would make these comparisons more interpretable. Follow-up should cover material degradation and rehabilitation rather than stop at early histological repair.

The minimum evidence package should combine procedural feasibility and safety with tissue-specific mechanical and functional outcomes. Operative time, additional equipment and personnel, material wastage, ease of rescue after a failed print, and reproducibility across operators should also be assessed. The immediate priority is an integrated workflow for a defined, accessible indication with a credible fixation strategy, a defined rescue procedure and quantified operating-room resource requirements. Expansion to deep, highly loaded or multi-tissue defects should depend on demonstrated benefit at the preceding stage, not simply on higher printing resolution or more complex bioink composition.

6. Conclusion

In situ bioprinting offers a distinct route to musculoskeletal repair by linking final defect geometry with spatially controlled delivery of structural and biological components at the point of care. The strongest direct evidence concerns procedural feasibility in accessible bone and cartilage defects. Evidence for load-bearing bone and skeletal muscle is more limited, while the cited tendon and enthesis studies involve in vitro testing or prefabricated implants. Translation will therefore depend on more than printability or short-term tissue formation. Clinically relevant platforms must integrate reliable imaging and registration, wet-tissue fixation, vascularization, sterile and traceable manufacturing, appropriate mechanical support, and closed-loop process control within acceptable operative times. Their value should ultimately be demonstrated against standard treatments in load-bearing large-animal models using long-term structural, mechanical, and functional endpoints. Accordingly, in situ bioprinting is best viewed as a complementary surgical manufacturing platform that combines digital reconstruction, regenerative materials, and precision delivery rather than as a stand-alone replacement for established reconstruction.

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Conflict of interest

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