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REVIEW ARTICLE

3D bioprinting-driven strategies for tissue regeneration and controlled immune modulation

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Abstract

Tissue loss, fibrosis-prone repair, and immune-mediated graft failure remain persistent obstacles in regenerative medicine. Within this context, 3D bioprinting is shifting from structure-centric fabrication to a platform for programmed immune modulation. This review synthesizes evidence across materials, architecture, and living components to delineate how bioprinted constructs can steer host responses toward resolution and durable function. We first examine events at the blood–biomaterial interface, including protein corona formation, complement–coagulation crosstalk, and leukocyte recruitment, and map them to tunable parameters, such as chemistry, stiffness, degradability, topography, and pore geometry that direct macrophage and dendritic-cell programs. We compare natural and synthetic bioinks, emphasizing printability windows, batch control, and impurity management as prerequisites for interpretable immunological readouts. We survey stimuli-responsive inks triggered by pH, reactive oxygen species, enzymes, light, or magnetic fields to deliver cytokines, chemokines, and metabolites with temporal precision, and highlight architected lattices and gradients that guide cell trafficking, vascular and lymphatic integration, and mechano-immune conditioning. Cell- and signal-centric strategies include immune–stromal coprinting, extracellular vesicle embedding, membrane cloaking for immune stealth or targeting, and synthetic circuits that sense inflammation and secrete immunoregulatory payloads. Finally, we identify translational bottlenecks and outline opportunities in 4D bioprinting, AI-assisted design, digital twins, and *in situ* printing. Treating immunity as a primary design variable is essential for predictable, durable, and clinically credible bioprinted therapies.

Keywords: 3D bioprinting; Bioinks; Foreign body response; Immunomodulation; Macrophage polarization; Regenerative medicine

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1. Introduction

Clinical translation of tissue engineering remains constrained by recurrent regenerative failure, chronic inflammation leading to fibrotic encapsulation (foreign body reaction, FBR), and graft rejection with immune-related complications that impair function and necessitate re-interventions.[1-3] Upon implantation or blood contact, adsorbed protein layers rapidly form on material surfaces and trigger complement–coagulation–contact pathway crosstalk, amplifying leukocyte recruitment, thrombosis, and fibrosis that undermine long-term performance.[4,5] These realities highlight that structural reconstruction alone does not guarantee durable functional recovery without parallel control of host immunity.[3]

Three-dimensional (3D) bioprinting—defined as digitally guided, layer-by-layer deposition of living cells, bioactive matrices ("bioinks"), and signaling cues to build tissue-like architectures—has matured from proof-of-concept to versatile biofabrication for preclinical modeling and regenerative therapy.[6,7] Contemporary reviews catalog technique families (extrusion, inkjet, light-based), material classes, and cross-scale design rules that enable spatial control of mechanics, porosity, and cell distribution.[8] Beyond geometry, emerging work positions bioprinting as a platform to encode immunological functions into constructs, aligning scaffold architecture with immune-instructive chemistry and controlled release to steer healing trajectories.[6,7]

Converging evidence shows that biomaterial physicochemical cues—stiffness, topography, wettability, and pore architecture—direct macrophage polarization and dendritic-cell priming, thereby dictating downstream angiogenesis, matrix remodeling, and scarring.[9,10] Immunomodulatory strategies now integrate cytokines (e.g., IL-4/IL-10), chemokine gradients, and pro-resolving mediators within bioinks to achieve spatiotemporal control of innate and adaptive responses.[11,12] In parallel, delivery materials are being engineered to recruit and reprogram immune and stromal cells (macrophages, T cells, MSCs), enabling "materials-as-medicine" concepts that couple regeneration with immune homeostasis.[13] Together, these advances motivate the design of immune-competent constructs—printed tissues capable not only of providing structure but also of sensing, modulating, and stabilizing host immune signaling for durable repair.[9,12]

This review therefore addresses the central question: how can 3D bioprinting move beyond structural replacement to enable immune-controlled regeneration? We analyze early events at the blood/material and tissue interfaces—including complement activation and protein corona formation—as programmable targets, and we map how construct architecture, surface chemistry, and payload release can de-escalate acute inflammation, prevent chronic FBR, and promote functional integration.[1,5,14,15]

Scope and search strategy. We surveyed peer-reviewed literature across PubMed, Scopus, and Web of Science from January 1, 2015 to November 5, 2025, using combinations of keywords ("3D bioprinting/bioprinted," "bioink," "immunomodulation/immunoengineering," "macrophage polarization," "complement activation," "foreign body reaction," "immune-competent constructs," "organoids/organ-on-chip"). Inclusion criteria were: (i) English-language original experimental or clinical studies, or reviews, that involved 3D bioprinting or bioprinted constructs and (ii) reported at least one immune-related outcome (e.g., immune-cell phenotype, cytokine/chemokine profiles, complement/coagulation activation, or histological inflammation or fibrosis). We excluded duplicate records, conference abstracts, nonpeer-reviewed theses or preprints, purely manufacturing or process-optimization papers lacking any biological or immunological validation, purely computational articles without experimental confirmation, and additive-manufacturing reports unrelated to immunity. Within this narrative synthesis, we applied an a priori hierarchy of evidence adapted from evidence-based medicine levels-of-evidence, classifying key studies as Level 1 (systematic reviews or randomized clinical trials of bioprinted constructs), Level 2 (prospective nonrandomized clinical studies or controlled *in vivo* animal studies with predefined endpoints), Level 3 (exploratory *in vivo* animal studies and *ex vivo* organoid/organ-on-chip models), Level 4 (mechanistic *in vitro* cell–material interface investigations), and Level 5 (methodological, conceptual, or commentary articles). Within each level, we qualitatively considered methodological signals of quality (e.g., explicit randomization or allocation concealment, blinding of outcome assessment, justification of sample size, and reproducible reporting of dosing/printing parameters) when deciding which studies to feature in detail, whereas small, single-arm or incompletely reported experiments were treated as hypothesis-generating. Reporting of the search and selection approach follows PRISMA 2020 guidance for transparency.[16,17] Evidence was graded using a simplified scheme adapted from the 2011 Oxford Center for Evidence-Based Medicine (OCEBM) levels of evidence.[16] Briefly, Level 1 denotes randomized or prospective clinical studies, Level 2 other human observational studies or systematic reviews, Level 3 controlled *in vivo* animal experiments, Level 4 *in vitro* or *ex vivo* mechanistic studies, and Level 5 methodological or conceptual papers. For each group of findings, we explicitly indicate in the text and tables whether the supporting data are clinical (Levels

1–2), preclinical *in vivo* (Level 3), or *in vitro*/mechanistic only (Levels 4–5). Reporting of the search approach follows PRISMA 2020 guidance for transparency.[17,18]

2. Conceptual framework of immuno-modulated regeneration

After tissue injury, the host response can be parsed into overlapping hemostasis, acute inflammation, proliferative tissue formation, and remodeling phases orchestrated by innate immune and stromal cells. Rather than detailing every cytokine and cell subset, for 3D bioprinting it is most important that (i) early danger sensing and neutrophil/monocyte recruitment clear contaminants but also condition the matrix, (ii) resolution programs reprogram macrophages and fibroblasts from proinflammatory to proregenerative phenotypes, and (iii) vascular and lymphatic ingrowth restore perfusion and immune surveillance. These process-level concepts, synthesized in canonical wound-healing reviews, provide the background for understanding how printed constructs can either accelerate resolution or lock tissues into chronic foreign-body fibrosis.[19-21]

In 3D-bioprinted constructs, the blood–material interface is the first decision point for immune compatibility. Protein adsorption, complement activation, and contact coagulation occur within seconds of implantation and are strongly influenced by surface chemistry, topography, and degradation products. Here, we therefore focus on two practical axes: control of the adsorbed protein corona, which shapes downstream leukocyte recruitment and macrophage polarization, and modulation of complement–coagulation crosstalk, which can be harnessed for hemostasis and vascularization but, if excessive, drives thromboinflammation and fibrotic encapsulation. Mechanistic work on biomaterial–complement interactions now provides concrete design rules—such as hydrophilic or zwitterionic coatings and immobilized complement regulators—that are directly transferable to bioink and scaffold optimization.[4,14,22]

A third pillar of our framework is innate–adaptive crosstalk and immune memory. Rather than exhaustively cataloging T-cell and antigen-presenting-cell subsets, we emphasize that macrophage–dendritic-cell–T-cell loops and stromal niches together determine whether implanted constructs induce acute rejection, immune tolerance, or "trained" hyper-responsiveness. Recent work on trained immunity highlights how prior exposures imprint long-lived metabolic and epigenetic programs in innate cells; in the context of bioprinting, this implies that the same scaffold can elicit very different responses in naïve versus pre-inflamed tissues, and that dynamic control of danger signals, antigen load, and metabolic cues is essential to maintain a regenerative rather than pathologic memory state.[23,24]

Finally, immune outcomes—tolerance versus trained immunity—link early programming to long-term graft performance. Inappropriately engaged trained innate responses can perpetuate chronic inflammation around implants, whereas antigen-specific tolerance mediated by Tregs and tolerogenic DCs supports durable integration; designing 3D-printed constructs that avoid maladaptive "training" while locally favoring context-appropriate tolerance is therefore central to achieving immune-competent regeneration.

To connect materials, printing parameters, and immune outcomes in a more systematic fashion, we treat "immune-competency" as a quantifiable design objective rather than a binary pass/fail property.[25-27] At the materials/printing level, each construct can be described by a shared set of physicochemical and architectural descriptors, including surface chemistry and charge, crosslink density and stiffness, degradation kinetics, pore size and interconnectivity, and the spatial–temporal presentation of ligands, cytokines, and other bioactive cues. At the biology level, we advocate a tiered immune readout panel that captures (i) early protein corona and complement–coagulation activation, (ii) innate-cell phenotypes and secretomes (e.g., macrophage and dendritic-cell states), (iii) adaptive immune activation versus tolerance (T-cell activation, exhaustion and regulatory markers, and antigen-specific responses), and (iv) tissue-level endpoints such as vascularization, fibrotic capsule thickness, and integration with host parenchyma across *in vitro*, *ex vivo*, and *in vivo* models. Aggregating these variables into indication-specific composite indices—provisionally conceptualized here as an "immune-competency score"—would allow comparison of bioprinted constructs across materials and printing modalities and provide a bridge between mechanistic immunology, pharmacology, and regulatory evaluation.

3. Bioinks and smart biomaterials

To keep the focus on immune design, we now summarize base bioink classes only briefly. Natural matrices such as collagen/gelatin, alginate, and tissue-specific decellularized extracellular matrix (dECM) provide native ligands and cytokine reservoirs but show batch-to-batch variability and potential residual immunostimulatory contaminants, whereas synthetic platforms including poly(ethylene glycol) (PEG) and degradable polyesters poly(lactic-co-glycolic acid) (PLGA) polycaprolactone (PCL) offer more predictable mechanics and degradation at the expense of

intrinsic bioactivity; detailed pros and cons are consolidated in Table 1 rather than described extensively in the main text. To avoid conflating mechanistic and translational findings, in this and subsequent technical sections we specify whether cited studies are based on *in vitro* experiments (Level 4–5), preclinical *in vivo* models (Level 3), or human data (Levels 1–2), according to the OCEBM-derived evidence scheme outlined in the Introduction.[16]

Printability and the "4S" properties define engineering constraints and clinical readiness. Strength and shape fidelity arise from rheology (shear-thinning/yield stress) and crosslinking kinetics that maintain filament integrity and pore geometry postdeposition.[28] Signaling capacity depends on retained ECM motifs, tethered cytokines, and degradation-controlled release.[29] Safety requires validated processes to remove cells/DNA from dECM (commonly <50 ng DNA/mg dry ECM; fragments <200 bp) and to eliminate endotoxin or residual photoinitiators that confound immune readouts.[30,31] Harmonized biocompatibility testing for cell-laden inks is essential to link material variables with host immune outcomes.[28]

Immunofunctionalization strategies fall on a spectrum from "immune-silent" to "pro-immunomodulatory." PEG or zwitterionic coatings can minimize protein opsonization and complement activation, reducing foreign-body responses.[32] Conversely, proactive designs harness topography, degradability, and ligands (e.g., CSF-1/IL-4-mimetic cues or arginyl-glycyl-aspartic acid motifs) to steer macrophage polarization, angiogenesis, and resolution of inflammation within printed constructs.[33] With natural polymers, purification and tailored chemistry (e.g., ultralow-endotoxin alginate; selective oxidation of ECM) help avoid unintended TLR-driven activation while preserving prohealing signaling.[34,35] Key base materials and their immune footprints are summarized in Table 1.

Stimuli-responsive ("smart") bioinks enable on-demand, spatiotemporal immune control.[39] A highly self-supporting, rapidly reversible chitosan-based hydrogel network—driven by weak electrostatic interactions and hydrogen bonding—enables high-fidelity prints (e.g., microtubes, multicomponent structures). Incorporation of AgNPs and a HIF-1α stabilizer (VH298) confers antimicrobial activity and prohealing efficacy *in vivo* (Figure 1). pH-responsive networks release anti-inflammatory agents preferentially in acidic, inflamed niches.[40] Reactive-oxygen-species (ROS)-responsive hydrogels scavenge oxidative stress and couple this with timed delivery of immunoreparative payloads in wounds and bone defects.[41,42] Enzyme-cleavable (e.g., MMP-responsive) linkers align drug release with matrix-remodeling phases to coordinate macrophage transition and vascularization.[43] Light-responsive, photocrosslinkable systems provide surgical-time activation with attention to initiator dose to protect resident immune cells.[44] Magnetic cues can remotely modulate macrophage infiltration and phenotype within printed gels, adding a noninvasive control layer for immune-competent constructs.[45] Representative smart bioinks and triggers are summarized in Table 2.

Table 1. Immunomodulatory bioinks and base materials: composition, immune footprint, printability, and evidence.

Bioink/material (type)	Immune-facing features	Printing suitability/notes	Key immune outcomes/readouts	Model/context	Refs.
Tissue-specific dECM (natural)	Native ligands/cytokine reservoirs; tissue-matched cues	Extrusion/DLP compatible; batch-to-batch bioactivity variance	Guides macrophage and T-cell behavior to pro-regenerative states	dECM bioinks overview & tissue-matched immunocues	29,35,36
PEG-based hydrogels (synthetic)	Inert backbone for precise ligand/peptide display; reduced opsonization	Broad modality compatibility; tunable crosslinking	Minimizes protein adsorption/complement activation; modular immunoregulation	Immuno-inert coatings & hydrogel platforms	32
PLGA / PCL (polyesters)	Structural robustness; degradable	Good filament fidelity; acidic PLGA by-products to buffer	Acidic degradation biases M1-like macrophage programs unless neutralized	Immunological effects of polyester degradation	37
Alginate (natural)	Widely used hydrogel; ionic crosslinking	Extrusion-friendly; viscosity adjustable	Impurities/endotoxin can activate innate immunity—need ultralow-endotoxin grades	Innate activation by unpurified alginate	34
Heparin-affinity hydrogels	Affinity capture & sustained release of cytokines (e.g., IL-4)	Photocrosslinkable or enzyme-set matrices	Sustained IL-4 polarizes macrophages and accelerates bone repair	Bone immuno-regeneration with IL-4 retention	38
Decellularization quality controls	DNA/endotoxin removal	GMP relevance; QC thresholds	Prevents confounded immune readouts	dECM safety and process validation	30,31

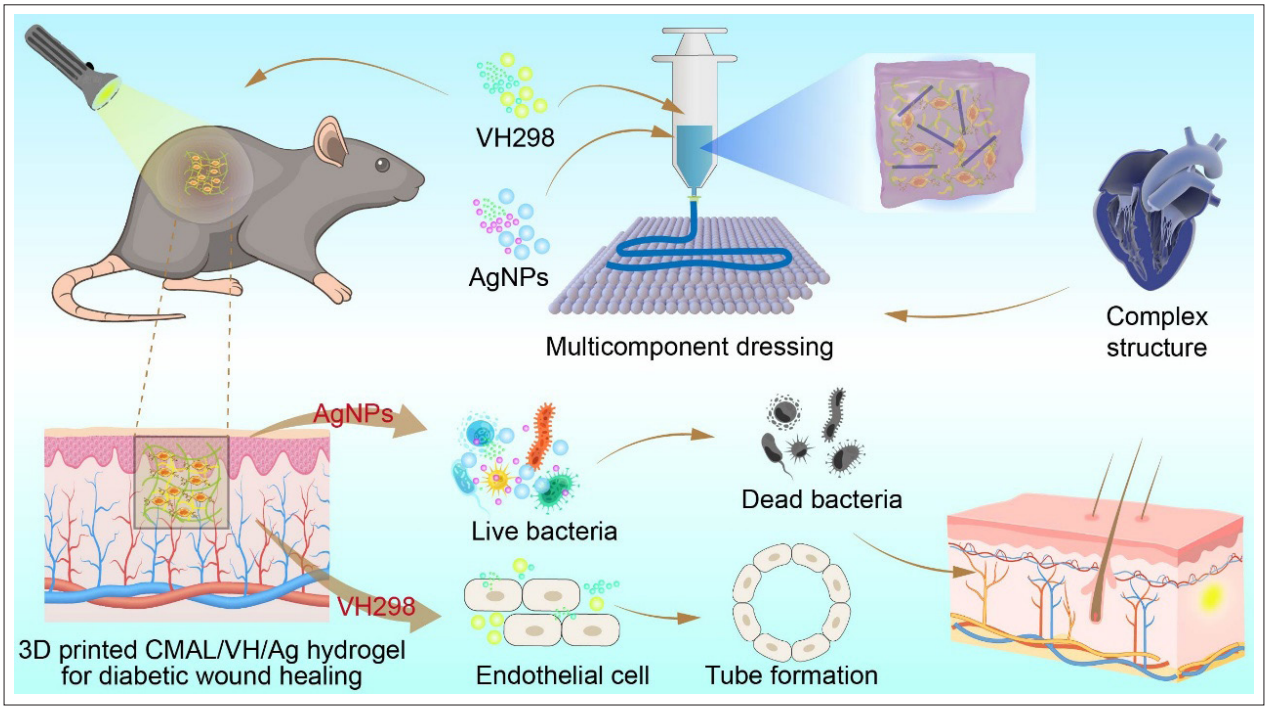

Figure 1. *In vivo* application of highly self-supporting chitosan-based hydrogel ink. The highly self-supporting chitosan-based hydrogel ink possesses a strong and rapidly reversible physical crosslinking network driven by dense weak electrostatic interactions and hydrogen bonds. This network facilitates a variety of high-fidelity direct 3D-printing processes, such as microtube fabrication and multicomponent or complex structure printing. Incorporating silver nanoparticles (AgNPs) and a hypoxia-inducible factor-1α (HIF-1α, VH298) stabilizer into the CMAL ink results in excellent therapeutic efficacy *in vivo*.

4. Printing modalities and architectures

Modality comparison. Extrusion bioprinting remains the workhorse for building centimeter-scale tissue constructs because it tolerates viscous, cell-dense bioinks, and supports multimaterial codeposition, though shear exposure during nozzle transit is a key limitation for delicate immune and stromal cells.[57] Inkjet (jetting) enables noncontact, high-throughput patterning with fine droplets but typically requires low-viscosity inks and offers limited cell loading.[58] Digital light processing (DLP/SLA) projects entire layers with rapid, high-resolution curing and is well-suited to architected microstructures, with attention to photoinitiator choice and dose to minimize phototoxicity.[59] Two-photon polymerization (TPP) pushes resolution into the submicrometer regime for microvasculature-like and lymphatic-scale features, at the cost of slower build rates.[60] Across platforms, coordinated multimaterial printing (e.g., structural ink + immune-modulatory ink) is increasingly used to spatially segregate mechanical support and immunological functions.[61] Immune-relevant trade-offs across printing modalities are compared in Table 3.

Architectural parameters and immune guidance. Porosity and pore size strongly condition innate immune set-points: precision-templated scaffolds with uniform ~40 μm pores reduce foreign-body reaction and increase vascular density relative to smaller or larger pores,[62] and large-pore additively manufactured PEEK implants used as nonbioprinted load-bearing scaffolds bias macrophages toward prohealing M2 polarization at the bone–implant interface, improving osteogenesis and angiogenesis.[63] In this context, PEEK is processed as a high-temperature, rigid implant material rather than as a cell-laden bioink, and the Yang *et al.* study is cited to illustrate how precisely controlled pore architecture in a structural frame can be leveraged in future hybrid systems where a soft, bioprinted hydrogel is integrated with a PEEK cage to decouple mechanical and immunological design spaces.[63-65] Anisotropy and surface alignment further steer leukocyte trafficking; aligned conductive polyurethane conduits enhance macrophage recruitment and M2 polarization, supporting peripheral nerve repair.[66] At the cell scale, shape/topography cues modulate macrophage phenotype independent of soluble cytokines, enabling "mechanotype-aware" immuno-instruction via ridge, pit, or fiber geometries.[67] Gradients of stiffness or ligand density (mechanical and biochemical) can serve as "immune taxis" to bias dendritic/macrophage migration and localization within printed tissues.[68] Architecture-level cues and immune effects are compiled in Table 4.

Table 2. Summary of reported bioinks.

Bioink (material)	Representative composition/variant	Crosslinking/curing	Key printability/biological notes & typical use	Refs.
Alginate	Alginate (various MW; M/G ratios)	Ionic (Ca^{2+}) ± secondary	Printability strongly depends on MW and M/G; tunable mechanics for extrusion	46
Gelatin methacryloyl (GelMA)	GelMA (various % solids)	Photopolymerization (UV/visible)	Cell-adhesive (RGD); widely used; supports neurogenesis/neuronal models	47
Hyaluronic acid (HAMA)	HAMA ± tissue-specific ECM blends	Photopolymerization	HA derivatives printable; HAMA+pECM supports islet applications	48
Collagen	Type I/II/III collagen inks; FRESH-compatible	pH/thermal; embedded (FRESH)	Native ECM protein; improved fidelity via embedded printing; broad TE use	49
Fibrin/Fibrinogen	Fibrinogen→Fibrin (± blends)	Enzymatic (thrombin); *in situ*	Biocompatible but soft; strategies include blends/support baths to boost fidelity	50
Decellularized ECM (dECM)	Organ-specific dECM inks	Thermal/enzymatic/photocuring (formulation-dependent)	High biomimicry; often low mechanics—blend/modify to improve printability	36
Chitosan (derivatives)	Methacrylated/phenol-modified chitosan	Visible–light radical (e.g., SPS/$Ru(bpy)_3$)	Works for extrusion & vat printing; antimicrobial/biodegradable hydrogels	51
Nanocellulose (NFC/CNF)	Nanocellulose–alginate blends	Ionic (Ca^{2+})	Good shape fidelity; supported chondrocytes and complex ear/meniscus prints	52
Gellan gum	Gellan gum ± laminin-peptide tethering	Ionic (Ca^{2+}) ± peptide tethering	Shear-thinning; peptide-tethered variants improve adhesion & mechanics	53
κ-Carrageenan	κ-Carrageenan (pristine or MA-modified)	Ionic/dual crosslinking or photo	Printable scaffolds with high water content; tunable 24–100 kPa modulus	54
Agarose (incl. carboxylated)	Carboxylated agarose (CA)	Thermal/photochemical (variant-dependent)	Free-standing, high-stiffness structures via microextrusion; good fidelity	55
PEG-based (synthetic)	PEGDA/PEGNB ± RGD/gelatin fragments	Photo-crosslinking (thiol-ene/MA)	Chemically defined; mechanics & bioactivity tunable; broad TE models	56

From an engineering standpoint, the spatial patterning of immune cues is ultimately constrained by the lateral and axial resolution of multimaterial bioprinting. Comparative analyses of bioprinting modalities indicate that continuous inkjet typically generates droplets on the order of ~100 μm, whereas drop-on-demand (DOD) inkjet can reproducibly form much smaller droplets (~20–50 μm), allowing different cell- or cytokine-laden inks to be placed side-by-side at tens-of-micrometer spacing.[69] Microfluidics-enabled maskless stereolithography (MMSLA) further improves discretization in light-based systems: by digitally switching between multiple photocurable bioinks, Miri *et al*. demonstrated voxel-wise patterning of several hydrogel compositions with inter-material interfaces registered within a single projected pixel (tens of micrometers in the x–y plane) while maintaining cell viability.[70] At the single-cell scale, Kim *et al*. used a 30-μm DOD inkjet nozzle to achieve "cell-level" deposition, with ~1–3 mammalian cells per droplet in 20 × 20 arrays, illustrating that immune cells, such as macrophages could in principle be patterned with ~30–60 μm center-to-center accuracy.[71] In contrast, extrusion-based multimaterial printheads remain limited by nozzle diameters and filament spreading, so that adjacent immune-modulatory strands typically have effective feature sizes in the 100–300 μm range for cell-laden hydrogels.[69] Collectively, these data suggest that current high-definition inkjet and light-assisted systems can already pattern distinct immune bioinks at length scales comparable to small clusters of immune cells (tens of micrometers), whereas replicating the full 3D hierarchy of larger immune niches (hundreds of micrometers) still requires combining printed architectures with subsequent cell self-organization and remodeling.

Vascular and lymphatic microstructures. Functional immunity in engineered tissues requires rapid perfusion and drainage. Extrusion with coaxial nozzles yields perfusable, branching channels that support endothelialization and flow,[72] while light-based μCOB/DLP prints prevascularized microarchitectures at high speed and fidelity.[73] Prevascularization strategies integrated into bioprinting improve inosculation and immunocompetence by shortening hypoxic/inflammatory windows postimplantation.[74] Beyond blood vessels, "lymph-mimetic" features are emerging: tumor-on-a-chip models with a bioprinted blood–lymphatic vessel pair recapitulate interstitial transport and drug

clearance, highlighting the role of blind-ended lymphatic channels in solute drainage.[75] New lymphatics-on-a-chip platforms demonstrate engineered lymphatic vessels capable of draining nanoparticles and modeling immune cell trafficking, offering templates for lymph-inclusive printed constructs.[76]

Inline quality control and print-path–immune coupling. Real-time, *in situ* monitoring is moving from aspiration to practice: embedded bioprinting systems with on-board imaging quantify strand fidelity and defects as they occur[77]; *in situ* photorheology reveals crosslinking kinetics and viscoelastic evolution during light-based printing, enabling dose-to-property control.[78] Frameworks that connect rheology/flow to viability now span experiments, analytics, and machine learning to predict printability and cell survival.[79,80] Crucially, nozzle shear and postdeposition flows are not only cytotoxic risks—they can shift macrophage programs toward inflammatory states, linking print path and extrusion conditions to downstream immune outcomes.[81,82] As such, interpretable models that map toolpath, speed, pressure, and exposure to cytokine readouts will be essential for designing "immune-competent" prints with reproducible host responses. To give these relationships quantitative context, microvalve-based extrusion studies that explicitly controlled nozzle wall shear report that maintaining average wall shear stresses below ~5 kPa preserves >90% stem cell viability, whereas stresses in the 5–10 kPa range cause modest losses and ≥10–20 kPa are associated with a steep decline in viability and proliferation.[83]

Table 3. Printing modalities: process constraints and immune considerations.

Modality	Typical ink properties	Cell viability (immune/stromal)	Immune-specific concerns	Typical feature resolution	Representative outcomes/notes	Refs.
Extrusion	Viscous, shear-thinning, cell-dense	Good for immune/stromal coprinting; shear stress is key	Nozzle shear & postflow can bias macrophages proinflammatory	≈ 50–500 μm (strand diameter / nozzle-limited)	Map toolpath/pressure to cytokine readouts	[57,81,82]
Inkjet (drop-on-demand)	Low-viscosity, low cell density	High patterning throughput; gentler shear	Limited cell loading; droplet impact on cells	≈ 20–100 μm (droplet-limited)	Useful for gradiented immunocues	[58]
DLP/SLA (light-based)	Photocurable, moderate viscosity	Layer-wide curing, high resolution	Photoinitiator dose–immune cell compatibility	≈ 10–50 μm (projected pixel-limited)	Architected microstructures for immune guidance	[59]
Two-photon polymerization (TPP)	Specialized resins; submicron	High precision; limited throughput	Long exposure; potential photostress	≈ 0.1–1 μm (hundreds of nm; sub-100 nm demonstrable)	Microvasculature/lymphatic-scale features	[60]
Multimaterial coordinated printing	Orthogonal inks in one build	Spatially segregate mechanical vs. immuno-functional inks	Cross-talk between inks; curing compatibility	Set by underlying modality (typically ≈ 10–500 μm; can reach sub-μm if TPP integrated)	Immune "hotspots" encoded in specific regions	[61]

Table 4. Architecture-guided immune modulation in printed constructs.

Architectural cue	Design parameter	Immune effect	Tissue/functional outcome	Notes	Refs.
Precision-templated porosity	Uniform ~40 μm pores	Reduced FBR; balanced myeloid influx	↑Vascular density; less fibrotic encapsulation	Size-dependent immune set-points	[62]
Large-pore PEEK scaffolds	Increased pore size/porosity	Bias toward M2-like macrophages	Improved osteogenesis & angiogenesis	Osteoimmunology link	[63]
Anisotropy/alignment	Aligned conduits/surfaces	Enhanced recruitment + M2 polarization	Better nerve repair outcomes	Conductive PU example	[66]
Micro/nano-topography	Ridges/pits/fibers	Mechanotype-dependent macrophage programming	Cytokine profile shift without soluble cues	Geometry as immune "language"	[67]
Stiffness/ligand gradients	Mechanical/biochemical gradients	"Immune taxis" of DCs/macrophages	Region-specific localization/function	Gradiented guidance in lattices	[68]

5. Engineering the immune microenvironment

Innate immune modulation. Sterile inflammation from DAMPs often converges on the NLRP3 inflammasome and complement—the two systems also intersect with coagulation at blood–material interfaces, amplifying thromboinflammation.[4,84,85] Surface strategies that recruit or present complement regulators (e.g., factor H–binding peptides) or use nanoscale topographies can curb alternative-pathway C3 activation and C5a generation, reducing leukocyte recruitment and clotting on implants.[4,85] Lactate signaling via the GPR81 axis can directly suppress TLR-primed NLRP3 activation and IL-1β release, suggesting a metabolite-based route to dampen early sterile inflammation in printed constructs.[84]

Adaptive immune modulation. Biomaterial delivery of tolerogenic cues can steer effector–regulatory balance. IL-10–functionalized PEG hydrogels support dendritic cells that drive $CD25^{+}FoxP3^{+}$ Treg induction, offering a materials handle to tilt responses away from Th1/Th17-dominant inflammation.[86] Localized release of TGF-β1 from microporous scaffolds reduces peri-implant cytokines and leukocyte infiltration and improves graft function, illustrating that protolerogenic cytokines can be embedded without blunting tissue integration.[87] Nanoparticles codelivering IL-2 and TGF-β to $CD4^{+}$ T cells expand functional Tregs *in vivo*, a concept readily translatable to bioink-compatible depot systems for printed grafts.[88]

Spatiotemporal "signal-release" modules. Sequential or gradiented cytokine delivery can choreograph the innate–adaptive handoff. IL-4–programmed release (alone or in IFN-γ→IL-4 sequences) polarizes macrophages toward pro-regenerative phenotypes that favor constructive remodeling around scaffolds.[89] Heparin-affinity hydrogels tuned for IL-4 sustain bioactivity and accelerate bone repair, underscoring the value of affinity-based temporal control.[38] Embedding TGF-β1 or IL-10 in defined depots within lattice architectures enables local tolerance while preserving peripheral immunity, a design principle for immune-competent bioprinted tissues.[86,87]

Immunometabolic targeting within constructs. Immune cell fate is metabolically gated; leveraging this in 3D bioprints adds a powerful, orthogonal control layer. Lactate accumulation can epigenetically reprogram macrophages via histone lysine lactylation to induce wound-healing gene programs, providing a rationale for lactate-releasing matrices in late repair phases.[90] Conversely, succinate stabilizes HIF-1α to boost IL-1β, so buffering or scavenging succinate around grafts may limit necroinflammatory loops.[91] Short-chain fatty acids (SCFAs), such as butyrate and propionate promote extrathymic Treg differentiation through histone deacetylase (HDAC) inhibition and G protein–coupled receptor (GPCR) signaling; incorporating SCFA reservoirs or SCFA-releasing promaterials into bioinks could couple metabolic and epigenetic tolerance at the host–graft interface.[92] Finally, material chemistry that modulates complement opsonization on particles/scaffolds can secondarily shape humoral immunity, offering routes to avoid unwanted B-cell activation and antibodies against the graft.[93]

Key takeaways for 3D bioprinting. Combining surface complement-control, Treg-skewing cytokine depots, and metabolite-guided reprogramming (lactate/SCFAs) yields immune-competent, yet quiet, niches that support integration and long-term function of printed tissues.[86,87,90,92]

6. Cells and immune-smart constructs

Immune-smart bioprinted constructs aim to actively shape host responses by coprinting or sequentially seeding immune cells and by embedding programmable cues that steer inflammation toward resolution and regeneration.[94] Coprinting macrophages, dendritic cells, NK, Treg, or B cells—together with stromal populations—enables spatiotemporal "training" of innate and adaptive immunity; in bone repair models, 3D-printed scaffolds that bias macrophages toward an M2-like phenotype enhance angiogenesis and tissue integration.[95,96]

Designing staged delivery (e.g., early CSF-1R inhibition to curb M1 activation, followed by proresolving cues) within printed architectures exemplifies how immune education can be scripted into the build plan.[95] Mesenchymal stromal cells (MSCs) are central "conductors" of this dialogue: they suppress antigen-presenting cell activity and induce IL-10–dominant programs via PGE2/IDO/TGF-β, thereby expanding Tregs and tempering Th1/Th17 responses.[97] Crucially, much of the MSC effect is paracrine; MSC-derived extracellular vesicles (MSC-EVs) reproduce immunomodulation with lower immunogenicity and are increasingly integrated into bioinks for cell-free yet immune-active constructs.[98] Recent analyses detail EV dosing, release kinetics, and cargo engineering (miRNAs, TGF-β/IL-10) compatible with extrusion and light-based printing workflows, supporting scalable incorporation into printed scaffolds.[98] Membrane cloaking offers another layer of immune stealth and targeting. Cell-membrane-coated nanoparticles and microgels—leveraging red blood cell, platelet, or leukocyte membranes—inherit "self" proteins (e.g., CD47) to reduce opsonization, complement activation, and phagocytic clearance, while enabling homotypic adhesion to inflamed or tumor endothelium.[99] Within regenerative settings, platelet-membrane coatings confer lesion homing via P-selectin/ICAM interactions and can be adapted to hemostatic or prorepair payloads,

a concept extensible to printable hydrogel carriers.[100] Broader overviews emphasize that membrane sources (RBC, platelet, neutrophil, macrophage) and hybrid coatings can be matched to indication-specific immune barriers, informing design libraries for "immunity-aware" bioinks.[101]

Finally, synthetic biology endows living components with on-demand immunoregulatory outputs. Engineered living materials equipped with CRISPRa/i or sensor-actuator circuits can sense inflammatory mediators or damage-associated signals and secrete immunomodulators (e.g., IL-10, TGF-β, CXCL12) accordingly, closing the loop between sensing and response *in situ*.[102] Programmable synthetic receptors—including synNotch and next-generation context-sensing designs—allow cells within printed constructs to decode microenvironmental cues and trigger tailored cytokine release or differentiation programs, improving safety and precision.[103] Cell- and signal-centric strategies for immune-competent constructs are outlined in Table 5. At the same time, these synthetic-biology–driven circuits generally depend on stable genome editing or vector integration in therapeutic cells, which introduces risks of off-target edits, large structural variants and insertional mutagenesis that require rigorous mapping and long-term follow-up before clinical use.[104] Recent work on engineered living materials further highlights the importance of controlling mutation, horizontal gene transfer, and ecological containment when living cells are embedded in macroscopic constructs.[105] Beyond technical issues, biomedical synthetic biology raises participant-safety, biosafety, and biosecurity concerns that demand dedicated governance frameworks extending beyond conventional biomaterials regulation.[106]

In sum, immune-smart constructs integrate (i) immune-cell coprinting and temporal "training," (ii) MSC/EV paracrine orchestration, (iii) membrane cloaking for immune evasion and targeting, and (iv) gene-circuit logic for closed-loop cytokine delivery—collectively advancing bioprinted tissues from passive grafts to active, immune-competent therapeutics.[94,97,99,102]

7. Applications and disease contexts

Skin and mucosa. Bioprinted, vascularized skin substitutes now integrate keratinocytes, fibroblasts and endothelium to accelerate re-epithelialization while supporting immune surveillance and angiogenesis.[107] Clinical priorities include infection control (e.g., antimicrobial bioinks) and scar immunobiology—shifting macrophage phenotypes and

Table 5. Cell- and signal-centric strategies to build immune-competent constructs.

Strategy	Implementation in bioprints	Primary immune target	Key result/readout	Application context	Refs.
Staged macrophage programming	Early CSF-1R inhibition → later IL-4 (or IFN-γ→IL-4 sequence) depots	M1→M2 transition; resolution	Reduced pro-inflammatory cytokines; pro-healing remodeling; early IFN-γ priming induces a transient M1 phase (debris clearance, angiogenic cue release), followed by IL-4-driven M2 transition that supports constructive tissue remodeling	Bone/soft-tissue repair	[89,95]
Treg-skewing cytokine depots	IL-10–functionalized PEG gels; localized TGF-β1 release	Tolerogenic DCs, $CD25^{+}FoxP3^{+}$ Tregs	↑Tregs, ↓Th1/Th17; improved graft function	Local immune tolerance near graft	[86,87]
MSC coprinting / paracrine orchestration	MSC-laden inks or postseeding	APC suppression; Treg expansion	IL-10/IDO/PGE2-mediated dampening	Broad regenerative contexts	[97]
MSC-EV–integrated bioinks	EVs embedded with controlled release	Low-immunogenic immunoregulation	Dose/release-controlled IL-10/TGF-β cargo effects	Cell-free immune-active constructs	[98]
Membrane cloaking (RBC/platelet/leukocyte)	Membrane-coated microgels/particles within constructs	Reduced opsonization/ complement; lesion homing	↓Complement activation; improved "self" signaling	Immune stealth & targeting	[99-101]
Synthetic biology circuits	Sensor–actuator cells (e.g., synNotch; engineered living materials) coprinted or embedded	On-demand cytokine secretion (IL-10/ TGF-β/CXCL12)	Closed-loop immunomodulation *in situ*	Adaptive, programmable grafts	[102,103]

TGF-β signaling to reduce hypertrophic scarring and fibrosis.[108]

Bone and cartilage. Inflammatory milieus (e.g., rheumatoid arthritis) uncouple osteoblast–osteoclast crosstalk, driving erosions, and systemic bone loss; osteoimmunology highlights TNF/IL-17–enhanced osteoclastogenesis as a dominant axis.[109] Bioprinted immunomodulatory scaffolds that cue macrophage polarization (spatiotemporal M1→M2 or pro-osteogenic subtypes) restore osteogenesis/chondrogenesis under inflammation and improve integration at the bone–implant interface.[110] Xingge Yu and colleagues developed a bioink based on a 10% gelatin methacryloyl (GelMA)/5% strontium-doped calcium silicate (Sr-CSH) nanocomposite hydrogel to encapsulate bone marrow mesenchymal stem cells (BMSCs), and used extrusion-based 3D bioprinting to fabricate biomimetic bone tissue for bone defect repair and regeneration[111] (Figure 2). In addition to sterile inflammatory conditions, infection-associated bone and periodontal defects require scaffolds that integrate antibacterial activity with immune modulation. For example, Guo *et al.* designed infection-sensitive 3D-printed PLGA scaffolds coated with superparamagnetic iron oxide nanoparticles (SPIONs), which resist bacterial adhesion, shift macrophages toward IL-10–producing M2 phenotypes, and enhance periodontal regeneration in a Porphyromonas gingivalis infection model.[112] Recent work by Qiao and colleagues reported composite poly(L-lactic acid) (PLLA)/Pearl scaffolds loaded with rifampicin- and moxifloxacin-PLGA microspheres; these 3D-printed constructs achieved local bacterial eradication and simultaneous bone defect repair in a rabbit infected bone defect model of the radius, illustrating how antibiotic delivery can be integrated into architected scaffolds for orthopedic infections.[113] Related studies on PLGA/Cu(I)@ZIF-8 nanocomposite scaffolds and broader reviews of antimicrobial, biodegradable 3D-printed scaffolds for orthopedic infections further highlight how scaffold chemistry, porosity, and degradation products can be tuned to couple anti-infective function with proregenerative immunological conditioning.[114,115]

Myocardium and vasculature. Postischemic repair demands time-staged control of immune clearance, neovascularization, and scar remodeling. Bioprinted

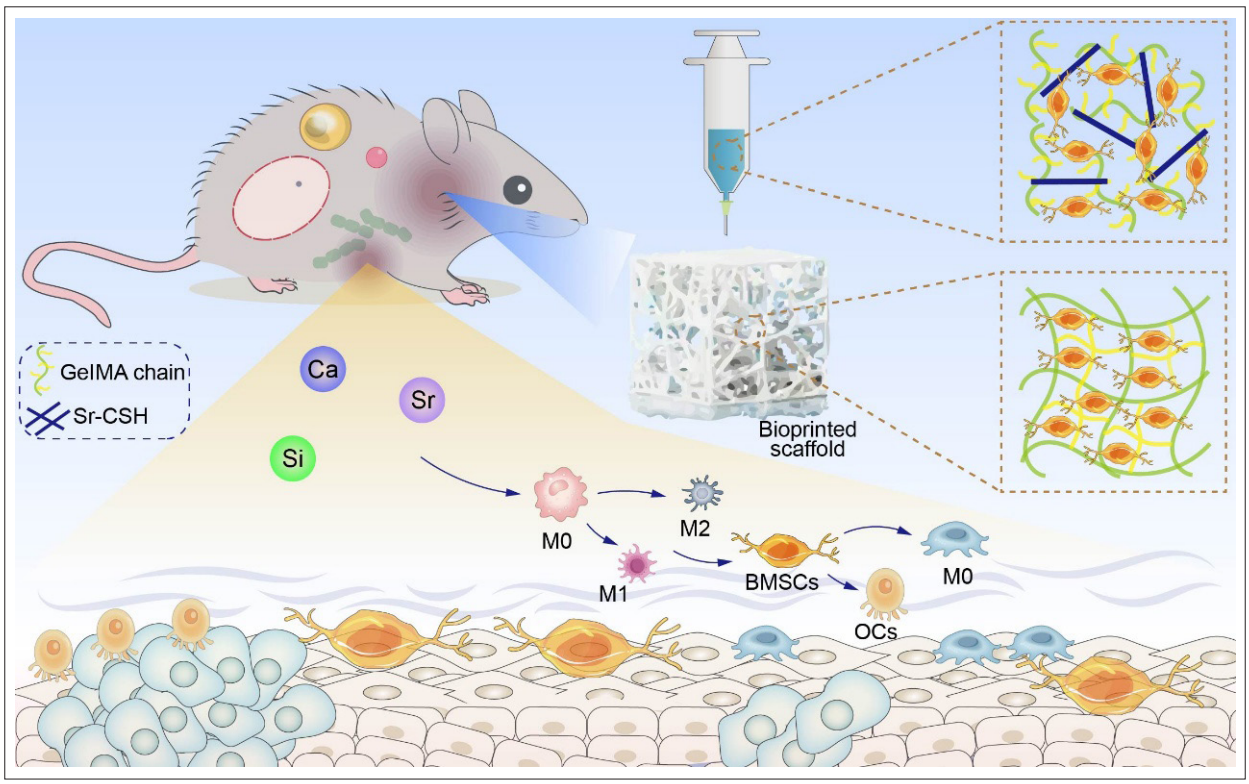

Figure 2. 3D bioprinting for constructing biomimetic bone tissue. A novel GelMA-Sr-CSH nanocomposite bioink improves printing precision and mechanical performance while exhibiting excellent biocompatibility. Sr-CSH induces macrophage M2 polarization: by releasing active ions, Sr-CSH modulates the inflammatory microenvironment and promotes the osteogenic differentiation of BMSCs. 3D-bioprinted scaffolds achieve complete bone repair: in a rat model, GelMA-Sr-CSH bioink scaffolds loaded with BMSCs demonstrated superior bone regeneration capacity.

cardiac patches combining aligned cardiomyocytes with conductive/vasculogenic architectures improve perfusion and electromechanical coupling.[116] Parallel work maps innate/adaptive responses to bioprinting biomaterials, guiding choices that limit FBR, fibrosis, and thrombosis after epicardial implantation.[117]

Liver/kidney/gut models for pharma–toxicology and immune safety. Humanized 3D liver models (e.g., hiHep-based or xeno-free hepatocyte cocultures) improve CYP activity, bile acid handling, and detection of drug-induced liver injury (DILI)phenotypes relevant to cholestasis and idiosyncrasy.[118,119] Bioprinted proximal tubule tissues with endothelial–interstitial compartments capture transporter-dependent nephrotoxicity and fibrotic responses for hazard identification.[120] Immunocompetent intestine-on-chip and bioprinted intestinal tissues enable mucosal barrier, microbiota interaction, and cytokine readouts for complement/inflammation risk assessment.[121,122]

Obstetrics and gynecology. For endometrial injury and Asherman's syndrome, bioactive, cell-laden scaffolds (e.g., hMSC-printed constructs) enhance vascularization and immunomodulation, supporting glandular regeneration, and antifibrotic remodeling.[123] Pelvic floor/perineal reconstruction leverages mechanically robust, biocompatible meshes, and emerging bioprinted muscle/tendon constructs, with attention to chronic inflammation and foreign-body responses.[124] Bioprinted placental organoids and maternal–fetal interface models incorporate trophoblasts, endothelial and immune cells to probe tolerance, complement activation, and drug safety in a physiologic context.[125]

Tumor-margin regeneration. After oncologic resection, multifunctional 3D scaffolds can couple tissue regeneration with local chemo-/immunotherapy to suppress recurrence while supporting prohealing immunity.[126] In parallel, bioprinted tumor microenvironments with stromal and immune compartments serve as prescreening platforms for surveillance, checkpoint response, and relapse biology at the resection edge.[127] Schematic of an implantable 3D-printed hydrogel that delivers local chemotherapy and self-amplifying immunomodulation at the resection bed, illustrating immune-cell recruitment, controlled drug release, and suppression of postsurgical tumor recurrence *in vivo* (Figure 3).

Together, these scenarios position 3D bioprinting not only as "structure reconstruction," but as a platform for **immune-competent** regeneration and drug testing across dermatologic, musculoskeletal, cardiovascular, hepatic–renal–intestinal, reproductive, and oncologic indications.

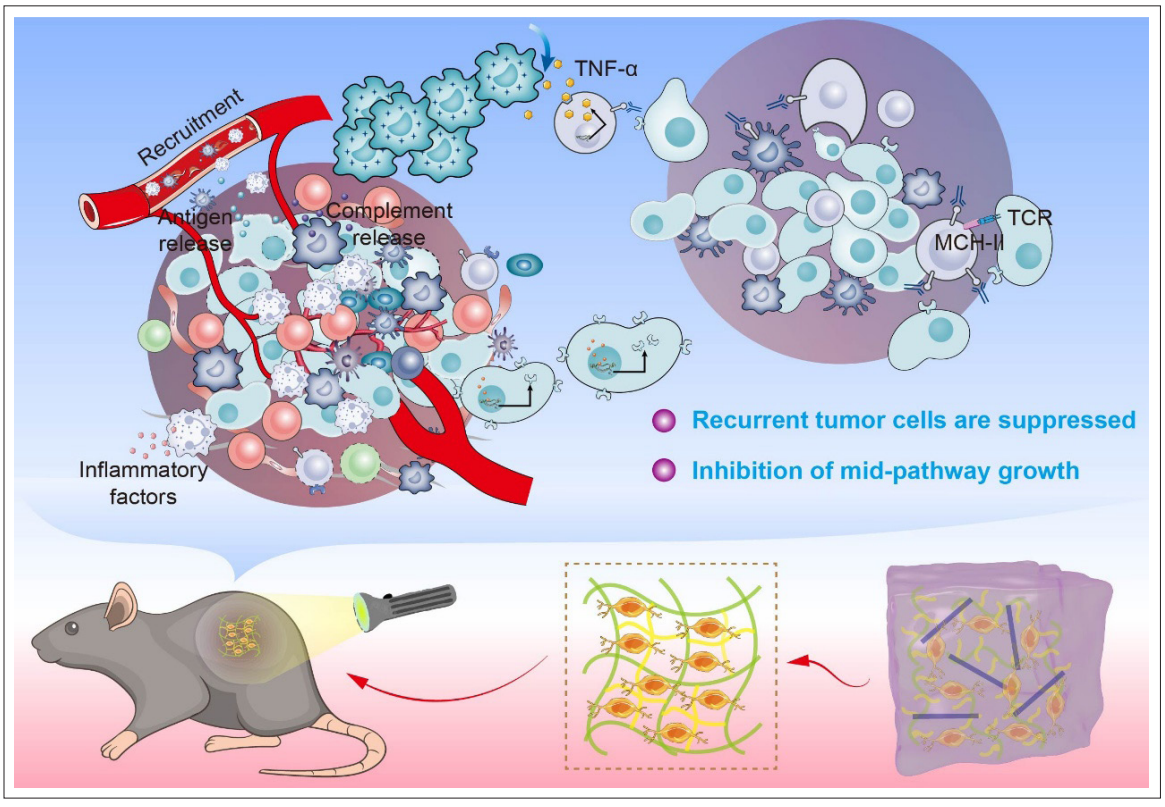

Figure 3. *In vivo* antitumor application of 3D-printed hydrogels.

8. Pharmacology, efficacy, and immunotoxicity

Immunocompetent organoids and microtissues now enable mechanism-aware pharmacology for immunotherapies by preserving native lymphoid components and physical barriers that dictate drug penetration and cell–cell interactions.[128] Patient-derived, T-cell–retaining tumor organoids capture antigen-specific cytotoxicity and allow assessment of small-molecule and biologic combinations in the context of endogenous T cells, offering a more faithful readout than cytotoxicity assays alone.[129] Notably, patient-derived glioblastoma organoids cotreated with autologous CAR-T cells mirrored clinical pharmacodynamics, including target loss, cytolysis, and cytokine signatures that correlated with patient cerebrospinal fluid profiles, supporting their utility for efficacy ranking and cytokine-release–related risk evaluation.[130] Across the evidence map synthesized in this review, only a minority of bioprinting-based immune-modulation strategies are currently supported by human clinical data (Levels 1–2), such as early trials of bioprinted skin substitutes and tumor resection guides. By contrast, most examples remain at the stage of controlled animal models (Level 3) or *in vitro*/*ex vivo* mechanistic work (Levels 4–5). We therefore report the highest OCEBM-derived evidence level supporting each key conclusion and treat lower-level data as mechanistic reinforcement rather than definitive proof.[16]

Conceptual view of 3D-printed organoids in which living cells and bioinks are assembled layer-by-layer to create perfusable, physiologically relevant mini-organs that retain immune components for efficacy and immunotoxicity testing (Figure 4). Multimodal analytics strengthen these platforms. High-content, live 3D imaging frameworks such as BEHAV3D classify engineered T-cell behaviors and link them to tumor organoid death dynamics, enabling quantitative mode-of-action mapping and early detection of inefficient infiltration or synapse formation.[131] A standardized protocol further operationalizes this approach for reproducible screening across donors and products.[132] Single-cell and spatial transcriptomics reveal how cellular composition and spatial organization steer immunotherapy response, supplying biomarkers for pharmacodynamic (PD) stratification and resistance mapping.[133] Complementarily, immunometabolomics exposes metabolic bottlenecks in CAR-T and tumor-infiltrating T cells—such as nutrient competition and inhibitory metabolites—that blunt efficacy and can be leveraged to guide combination strategies.[134,135] Together,

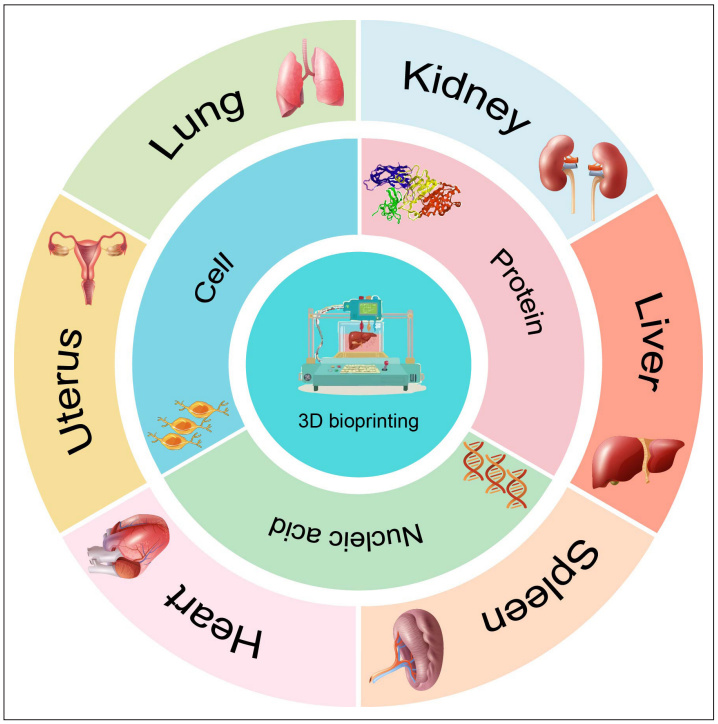

Figure 4. 3D-printed organoids. Biological 3D printing is a technology that uses 3D printing to construct biologically active tissues or organs. Through bio-3D printing, living cells (such as stem cells or primary cells) and biocompatible materials (bioinks) are assembled layer by layer to form miniature organ models with 3D structures and physiological functions. The core of this technology lies in seeding living cells into scaffolds, allowing them to grow *in vitro* and ultimately form functional tissues.

these readouts create an integrated pharmacology stack that couples behavior, transcriptional state, spatial context, and metabolism to actionable PD endpoints.

To reduce animal use while improving human predictivity, organ-on-chip (OoC) and organoid-on-chip (OrgOC) systems provide controlled perfusion, immune–stromal crosstalk, and multiorgan coupling for absorption, distribution, metabolism, and excretion (ADME)-tox, efficacy, and immunotoxicity testing.[136] OrgOC frameworks fuse organoids with microfluidics to maintain tissue fidelity under flow and support patient-specific testing; they are increasingly positioned for precision medicine applications and cross-donor "bioequivalence-like" comparisons.[137,138] Crucially, coupling OoC outputs to physiologically based pharmacokinetic (PBPK) models enables PK/PD translation, scenario testing (dose, schedule, route), and in silico sensitivity analyses that anticipate off-target exposure and cytokine-storm liabilities.[139] In practice, an integrated print–organoid–chip pipeline can expose 3D constructs (including immune-competent tissues) to candidate therapies under human-relevant flow and barrier conditions, while multiomics and imaging deliver PD fingerprints aligned to modeled exposure, supporting go/no-go and combination design with improved external validity.[136,137]

Organoids and organoids-on-chip are increasingly adopted in traditional Chinese medicine (TCM) research to dissect efficacy, mechanisms, and toxicity of multicomponent formulations while preserving human-relevant tissue architecture and immune context.[140] Hepatic organoid-based high-content imaging has differentiated stereoisomer-dependent hepatotoxicity of stilbene glucosides from Polygonum multiflorum (He Shou Wu), offering a predictive platform for herb-induced liver injury and early de-risking of candidate preparations.[141] Complementarily, recent studies show raw versus processed P. multiflorum exhibit distinct hepatotoxicity with a ferroptosis signature, underscoring how processing alters safety profiles that can be quantified on organoid/OoC pipelines.[142] On the immunopharmacology side, flavonoids from Scutellaria, such as baicalin and baicalein attenuate inflammasome-driven inflammation—baicalin inhibits NLRP3 activation in macrophages, and baicalein suppresses NLRP3/AIM2 assembly and pyroptosis—providing tractable readouts for cytokine kinetics and myeloid programming in immunocompetent organoids,[143,144] Astragaloside IV modulates tumor-associated macrophage polarization and suppresses protumor M2 programs, a mechanism that can be ranked in patient-derived tumor organoids retaining endogenous T cells and myeloid cells to couple efficacy with immunotoxicity screening.[145] Together, integrating TCM compound libraries with printed organoids/OoCs and single-cell/spatial analytics can rationalize multitarget actions while flagging herb-specific immunotoxic liabilities for translation.[140,141]

Building on these platforms, we propose a unified "immune-competency" readout panel for preclinical testing of 3D-bioprinted grafts.[25,26,146] As a minimal set, studies should report (i) acute innate activation, including complement split products and, where appropriate, neutrophil recruitment, and NETosis markers; (ii) quantitative spectra of macrophage and dendritic-cell phenotypes (e.g., M1/M2-like indices, antigen-presenting capacity, and secretion of IL-1β, TNF-α, IL-10, and TGF-β); (iii) adaptive immune activation versus tolerance, including effector/Treg ratios, exhaustion markers, and antigen-specific responses; and (iv) histological or imaging-based features of vascularization, matrix remodeling, and fibrotic encapsulation. Normalizing these measurements to application-appropriate controls and combining them into organ- or indication-specific immune-competency indices would facilitate cross-study comparison and benchmarking of printed constructs. Importantly, such scorecards can be harmonized with established osteoimmunomodulatory and foreign-body-response assessment frameworks, providing a practical path to standardizing how constructive versus pathological host responses to biomaterials are quantified.

9. Limitations and challenges

A central, practical barrier is unrecognized immunogenic impurities and batch variability in commonly used bioinks and scaffolds. Endotoxin contamination of polymers and metals is frequent and can confound *in vitro* readouts and *in vivo* host responses unless rigorously quantified and removed, yet many studies do not report limulus amebocyte lysate testing or surface-bound endotoxin controls.[31] For dECM inks, incomplete cell removal and altered matrix composition vary with tissue source and protocol, driving heterogeneous immune activation and remodeling outcomes across lots.[30] Batch-to-batch biochemical differences in dECM bioinks remain a nontrivial source of experimental variability and translational risk that is often underappreciated at the design stage.[147]

Processing steps that enable printing can themselves prime inflammation. Terminal sterilization alters mechanics, swelling, and even printability of hydrogels: ethylene oxide or autoclaving can reduce stiffness or cell viability in GelMA constructs, whereas γ-irradiation may increase stiffness but impair sol–gel transition and degrade print performance.[148-150] Photopolymerization adds another layer of risk: commonly used photoinitiators

and UV/visible light doses exhibit cell-type-dependent cytotoxicity and can generate residual radicals if conversion is incomplete, with Irgacure 2959 generally more toxic than LAP at equivalent concentrations [151,152]. In practical terms, shorter-wavelength UV systems (~365–405 nm) support high theoretical feature resolution but often yield relatively shallow penetration in cell-dense, scattering hydrogels, so that achieving full-depth cure may require higher doses that increase phototoxic and immunotoxic risk. By contrast, visible–light initiation (~405–450 nm) combined with appropriately tuned initiator chemistry can provide deeper, more homogeneous curing at lower photon energy, which is advantageous for preserving viability and function of professional immune cells in thick constructs, albeit sometimes at a modest cost in achievable voxel resolution. Without reporting of light spectrum, dose, initiator concentration, and postcure washing, reproducibility and immune safety are difficult to evaluate across studies.[151]

Beyond impurities and processing-induced epitopes, a fundamental constraint in immune-instructive 3D bioprinting is the "printability window" in which sufficient viscosity, yield stress and crosslinking are achieved for strand stability and shape fidelity, without imposing excessive shear stress or over-stiffening that compromise cell viability and ECM-encoded bioactivity. Increasing polymer content and crosslinking density improves filament stability but typically requires higher extrusion pressures and produces stiffer networks, which can damage cells, denature growth factors, and alter mechano-immunological signaling, whereas overly dilute inks preserve native ligands yet collapse under their own weight.[79,153,154] For ECM- and especially dECM-based bioinks, recent design strategies aim to decouple printability from biochemical content by blending dECM with rheology-modifying but relatively inert carriers (e.g., gelatin, alginate, gellan gum, silk), optimizing the degree of enzymatic digestion, and using interpenetrating or double-network architectures so that mechanical support is provided by a secondary network while dECM concentration remains high enough to retain glycosaminoglycans and matricellular proteins.[36,155,156] In parallel, embedded and granular printing approaches—including support-bath/FRESH methods and jammed microgel or microparticle bioinks—allow very soft, highly bioactive matrices to be printed within a mechanically supporting phase, thereby achieving high shape fidelity while keeping local stiffness and shear stresses low.[157,158] Finally, fragile immunomodulatory signals such as cytokines, chemokines, or exosomes can be protected during printing by affinity-based hydrogels (e.g., heparin-containing matrices) or nano/microparticle carriers, which shield bioactive factors from thermal, photochemical, and shear damage and enable controlled release that favors proregenerative macrophage and lymphocyte responses.[38,159] Together, these strategies illustrate how rheological tuning, printing mode, and factor-delivery design can be combined to balance printability with preservation of native ECM bioactivity and immunocompatibility in dECM-rich bioinks.

Printing physics also modulate immunity in ways that are still being systematized. Extrusion shear and nozzle geometry can push macrophages toward a proinflammatory phenotype, with downstream effects on endothelial barriers and fibrosis, implying that shear history is an immunomodulatory variable—not just a fabrication parameter.[160] Fluid-dynamics modeling and controlled bioprinting experiments indicate that, under typical nozzle diameters of 200–400 μm and shear-thinning hydrogel bioinks, calculated wall shear stresses commonly fall in the low-to-mid-kPa range, and conditions that exceed ~10 kPa coincide with marked drops in cell viability and function.[83] Beyond cell stress, architecture matters: precision-templated scaffolds with ~40-μm interconnected pores reduce chronic inflammation/foreign-body reaction compared with larger or smaller pores, highlighting a narrow structural "safe window" for host integration.[62] Relatedly, appropriately sized pores in 3D-printed PEEK implants fabricated by high-temperature additive manufacturing (nonbioprinted) promote macrophage M1→M2 transition and better osseointegration at load-bearing interfaces, but effects are material- and context-dependent, complicating generalization across platforms.[63] These rigid PEEK devices are widely used as structural spinal and orthopedic implants rather than as bioinks, yet they provide a useful paradigm for how pore metrics in a supporting frame could be codesigned with bioprinted, cell-laden hydrogels to program peri-implant immune responses.[63,64] These data argue for reporting shear rates, dwell times, and pore metrics alongside standard biological endpoints to interpret immune outcomes credibly.[62]

For constructs that contact blood, complement–coagulation crosstalk and the adsorbed protein corona remain under-tested liabilities. Surfaces and nanoparticles can activate complement via classical or alternative pathways (e.g., through C1q-mediated binding), recruiting platelets and leukocytes and amplifying thromboinflammation under flow.[4,161,162] Even when tested, complement analytics are prone to preanalytical and assay pitfalls that yield false activation signals or mask true ones, confounding risk assessment and comparability across labs[163] Routine hemocompatibility testing under physiologic shear, with pathway-specific complement readouts and anticoagulant controls, is still not standard in many bioprinting studies.[162]

Finally, preclinical translation is constrained by model choice and manufacturing control. Rodent inflammatory programs frequently diverge from humans, while humanized mice correct only part of the gap; contradictory meta-analyses mean that immune findings in small animals demand careful human validation before clinical claims.[164,165] On the manufacturing side, regulators continue to grapple with how to classify and control living, patient-specific printed products as advanced therapy medicinal products and to define robust release criteria and comparability requirements for multicomponent constructs.[166,167] Translating immunomodulatory bioprinted grafts from bench to bedside will therefore likely require adopting pharmaceutical Quality-by-Design (QbD) and process analytical technology (PAT) principles to explicitly link critical material attributes (e.g., bioink rheology, crosslink density, encapsulated-cell dose) and critical process parameters (e.g., extrusion pressure and temperature, light dose, culture duration) to immunological critical quality attributes, such as defined cytokine set-points, macrophage polarization profiles, or antigen-specific tolerance markers.[168,169] Recent work in regenerative medicine manufacturing illustrates how sensor technologies and robotics-driven production lines can be combined into closed, automated workflows with in-line imaging, electrical, or optical readouts to support nondestructive, real-time assessment of constructs and enable scalable, GMP-compatible bioprocessing for living implants.[170,171] In parallel, standardized biocompatibility and immunotoxicity test batteries inspired by ISO 10993 "Big Three" panels (cytotoxicity, sensitization, and irritation) are being refined for medical devices and provide a starting point for developing dedicated assays tailored to bioprinted, cell-laden constructs.[172] Although *in-situ* monitoring (e.g., ultrasound- or fluorescence-based process analytics) is emerging to detect defects and heterogeneity in real time,[138,139], such tools are not yet widely integrated into bioprinting platforms operating under full GMP control, and harmonized reporting of process and quality-control metrics will be essential for cross-study comparability and regulatory approval.[166,170] Collectively, these practical hurdles—contaminants, processing-induced bioeffects, architecture–immunity coupling, hemocompatibility pitfalls, model limitations, and immature process controls—define the near-term bottlenecks for immune-competent bioprinting and should be addressed explicitly in study design and reporting.[166]

Engineered cell reinfusion, immunogenicity, and genetic drift. For immune-programmed bioprinted constructs that incorporate gene-edited immune or stromal cells (e.g., CAR-T/Treg or sensor–effector mesenchymal stromal cells), reinfusion, or transplantation of long-lived engineered cells introduces specific safety and regulatory challenges beyond those of degradable scaffolds.[173] Genome editing and integrating viral vectors can generate off-target mutations, chromosomal rearrangements and high-copy insertions that favor clonal outgrowth, as illustrated by case reports and integration-site analyses in CAR-T trials.[104,174,175] Regulators therefore require extensive release testing (vector copy number, karyotyping, insertion-site mapping), GMP-compliant manufacturing and long-term pharmacovigilance registries for cell and gene therapies, and similar requirements are likely to apply to synthetic-biology-enhanced bioprinted products.[173] Immunogenicity is an additional bottleneck: patients may mount antibody and T-cell responses against nonhuman gene-editing components, CAR constructs or synthetic receptors, which can drive cytokine release, loss of persistence, or loss of efficacy upon repeat dosing.[104,176] Mitigation strategies under active investigation include humanized or fully human receptor designs, hypoimmunogenic Cas variants and reducing the exposure window by using mRNA or ribonucleoprotein delivery rather than integrating vectors.[104,176] Finally, engineered cells residing within 3D-printed tissues may undergo phenotypic drift and accumulate genetic or epigenetic changes under chronic inflammatory or regenerative selection, making serial sampling, single-cell multiomics and functional assays essential to monitor genetic drift and to define stopping rules for long-term follow-up.[173,177] Taken together, these considerations suggest that early synthetic-biology-enabled bioprinted constructs should follow stepwise implementation pathways aligned with existing advanced-therapy medicinal product and cell/gene-therapy guidance, rather than entirely novel regulatory routes.[173]

In addition to these practical constraints, the literature underpinning this review is inherently heterogeneous. The included studies span diverse bioprinting modalities, scaffold chemistries, cell sources and species, implantation sites, and immune readouts, with inconsistent reporting of effect sizes and variance. As a result, we did not attempt formal meta-analysis, statistical heterogeneity metrics, or funnel-plot-based publication-bias tests; instead, we synthesized evidence qualitatively, emphasizing convergent trends across independent platforms and explicitly noting where findings are supported by single studies or remain contradictory. Moreover, the preclinical field is likely affected by selective reporting and a bias toward positive immune outcomes, which should be taken into account when extrapolating to clinical translation. Future evidence-based biomaterials and bioprinting reviews with pre-registered protocols, standardized immune outcome sets, and sufficient homogeneity within narrowly defined

questions will be needed to support rigorous quantitative heterogeneity and publication-bias assessment.

10. Outlooks

Four-dimensional (4D) bioprinting adds the time dimension to 3D constructs by combining stimuli-responsive biomaterials, cell-generated forces, and programmed degradation so that shape, stiffness, and factor release evolve *in vivo*. Recent reviews highlight classes of 4D architectures highly relevant to immune modulation: shape-morphing scaffolds that can be delivered minimally invasively and then deploy to restore anatomical defects; hydrogels that stiffen or soften in response to enzymes, pH, or reactive oxygen species characteristic of inflamed tissue; and constructs whose porosity and ligand display change over time to gradually shift macrophage and T-cell phenotypes from inflammatory to regulatory states.[178-180] For programmed immune modulation, these systems enable temporal separation of functions—for example, early burst release of anti-inflammatory or tolerogenic cues to dampen acute damage, followed by slower presentation of proregenerative growth factors and, finally, late-stage signals that promote tissue-resident memory without chronic activation. Translationally, 4D bioprinting raises specific regulatory questions on how to characterize time-dependent deformation and release under clinically relevant stimuli, how to guarantee long-term predictability of actuation, and how to integrate mechanical and immune endpoints into good-laboratory-practice test batteries. Current regulatory science is beginning to address these issues, suggesting that the most realistic near-term path will be relatively simple, mechanically robust 4D functions with clearly demonstrable clinical benefits, such as reduced re-operation rates or improved graft integration.[178,179] In practice, 4D immune-instructive constructs are designed by first specifying the target initial and final configurations of the scaffold, then mapping the stimulus type and dose (e.g., temperature, pH, enzymatic activity, magnetic field) to spatiotemporal changes in modulus, crosslink density, and ligand exposure that can be predicted by finite-element and multiphysics models.[178,181] For immunomodulatory applications, design variables, such as stimulus threshold, actuation kinetics, and sequence of epitope/ligand unveiling can be explicitly linked to quantitative readouts including macrophage M1/M2 ratios, dendritic-cell maturation markers, and cytokine trajectories, allowing 4D printing to be framed as an optimization problem over dynamic immune responses rather than static morphology alone.[178,181]

AI-assisted design and digital twins provide a complementary layer for de-risking complex immunomodulatory constructs before they enter the clinic (Figure 5). At the preclinical stage, supervised and reinforcement-learning models can link bioink composition, rheology, and toolpath parameters to print fidelity, cell viability, and early immune readouts, thereby

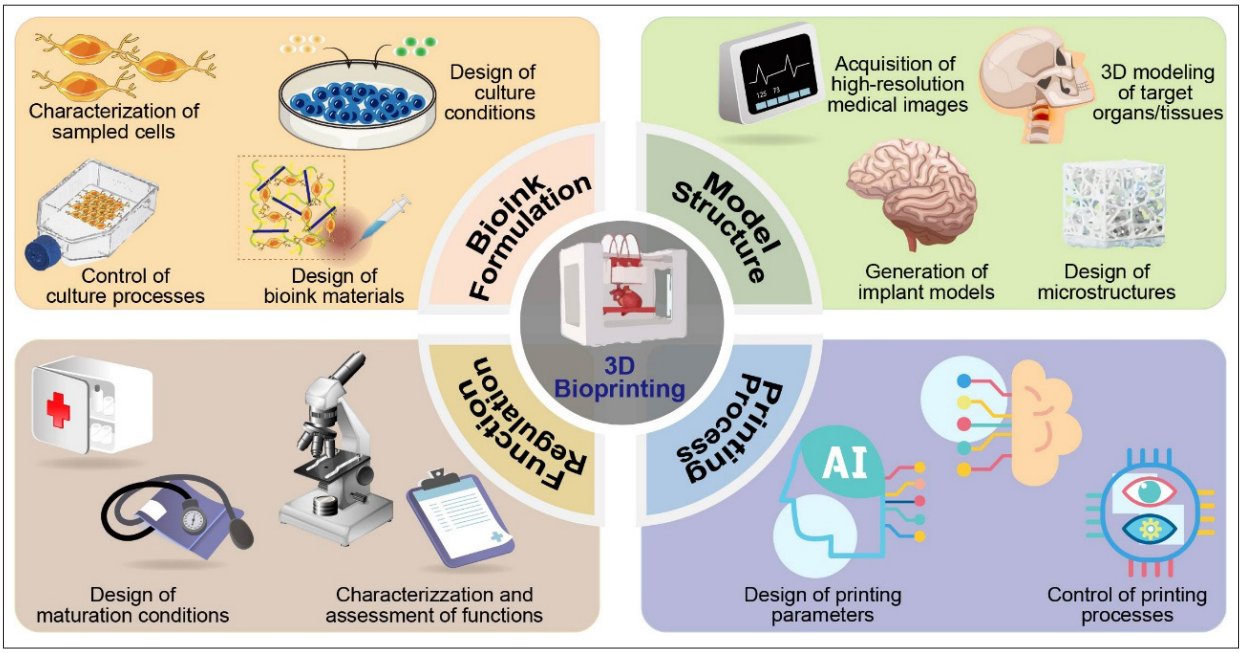

Figure 5. Schematic illustration of the integration between 3D bioprinting and artificial intelligence (AI) technologies.

shrinking the design space and enabling quality-by-design workflows for 3D and 4D bioprinting.[182,183] More recent frameworks couple patient-specific imaging, multiomics, and biomechanical simulations into "bioprinting digital twins" that simulate how a printed graft will deform, vascularize, and interact with the host immune system over months, allowing virtual testing of multiple designs and dosing schedules before first-in-human trials.[183,184] In our conceptual roadmap, such AI-guided and model-based tools are not merely add-ons but core components of the clinical translation pathway: they support automated in-line quality control during manufacturing, stratify patients according to predicted benefit–risk profiles, and generate the quantitative evidence needed for regulators to evaluate long-term safety and performance of immune-programmed bioprinted products.[182,184] Recent work has shown that machine-learning models can map topographical and physicochemical descriptors of biomaterial surfaces—such as multiscale roughness, skewness, contact angle, and surface energy—to quantitative macrophage polarization indices and cytokine ratios (e.g., IL-10/TNF-α), offering a concrete example of how high-dimensional design variables can be linked to immune readouts.[185] Analogously, AI-guided design of hydrogels and other soft biomaterials increasingly encodes polymer chemistry, crosslinker content, mesh size, and degradation constants as model inputs and predicts cell viability, inflammatory mediator release, and tissue-integration scores as outputs, providing a template for multiobjective optimization of "materials–structure–immune" performance in 3D/4D bioprinted constructs.[185]

In-body and on-body bioprinting will shorten the distance from CAD to care, with handheld or robotic systems depositing cell-laden bioinks directly onto wounds or organ surfaces to better match native curvature and mechanics.[186] After implantation, closed-loop constructs that sense local biomarkers (e.g., IL-6, TNF-α, pH) and trigger on-demand drug release can maintain immune homeostasis and prevent flares.[187] Experience from glucose-sensing, implantable biosensors underscores materials challenges (biofouling, drift, stability) and control algorithms needed for safe, autonomous immunotherapy—knowledge that can be ported to immune-responsive implants.[188]

Microbe–metabolite coengineering offers a powerful axis for immunometabolic reprogramming of printed grafts. Short-chain fatty acids (SCFAs) modulate barrier integrity, Treg induction, and inflammasome activity; embedding SCFA-releasing depots or prebiotic niches into bioinks could bias local immunity toward tolerance and repair.[189,190] Bile-acid signaling via FXR and TGR5 shapes innate and adaptive responses and is perturbed by dysbiosis; printing scaffolds that tune bile-acid gradients or present BAR-targeted ligands may rebalance Th17/Treg and macrophage states at tissue-device interfaces.[191] Together, metabolite-aware bioinks move beyond passive scaffolding toward active, immune-smart microenvironments.[191]

Finally, synthetic-immunology-enabled living constructs point to "programmable biologic drugs" embedded within printed tissues. Engineered immune cells with sensor–effector circuits (e.g., synNotch) can detect pathological cues and secrete IL-10, GM-CSF, or checkpoint modulators on demand, enabling local, titratable therapy with reduced systemic exposure.[192] In parallel, engineered-living-materials strategies use hydrogel containment to host therapeutic microbes with defined sensing-secretion programs, offering manufacturable, biocontained "living dressings" for chronic wounds or mucosal repair.[193] Integrating these cells with 4D, AI-optimized architectures could deliver self-tuning grafts that learn and adapt to patient-specific immune dynamics.[192,193]

Realizing these visions will require dedicated industry–academia–regulator consortia that treat immuno-active bioprinted products as a distinct class of advanced therapies rather than isolated case-by-case prototypes. Building on public–private partnership models already established for additive manufacturing and biomanufacturing (e.g., BioFabUSA/ARMI and NIIMBL), such alliances can align academic centers (mechanistic immunology, bioink discovery, *in vitro*/*in vivo* immune modeling), industrial partners (GMP bioink and printer platforms, scalable workflows, process analytical technologies), and regulatory agencies (definition of reference materials, immune potency assays, and critical quality attributes for batch release and change control) around shared translational roadmaps.[194] Within these consortia, regulatory-science innovations could include Quality-by-Design-based "platform" approvals for families of related scaffold architectures, regulatory sandboxes for AI-assisted design tools and immune digital twins, and the structured use of in silico virtual patient cohorts to complement preclinical and clinical data in benefit–risk assessment, thereby accelerating safe bench-to-bedside translation while maintaining rigorous oversight.[194-196]

11. Conclusion

3D bioprinting is reshaping regenerative medicine by coupling architectural precision with intentional immune modulation. The central lesson emerging across applications is that structure alone rarely ensures function; durable regeneration depends on orchestrating the early inflammatory cascade, guiding innate–adaptive crosstalk, and fostering tissue-specific tolerance. A "structure +

immunity" dual objective reframes design criteria: bioinks, architectures, and embedded payloads must be selected not only for mechanical fidelity and cell viability, but also for their capacity to dampen foreign body reactions, polarize macrophages toward proregenerative states, calibrate complement/coagulation interfaces, and ultimately support vascular and lymphatic integration.

This dual objective also changes how success is measured. Beyond histology and biomechanics, critical quality attributes should include immune kinetics (cytokine dynamics, complement activity), spatial phenotypes (myeloid and T cell distributions), and durability of immune homeostasis. Platforms that are "immune-competent by design"—whether through dECM-based inks, responsive release of immunoregulatory cues, or coprinted immune cells—offer realistic paths to reduce chronic fibrosis, improve graft take, and accelerate functional recovery.

Translationally, the roadmaps are clearer: quality-by-design frameworks, standardized immune assays, and modular release testing can align manufacturing with clinical endpoints. Digital design loops—AI-assisted material selection, multiphysics printing simulators, and data-driven immune prediction—will shorten iteration cycles and improve reproducibility. In parallel, 4D bioprinting, *in situ* printing, and closed-loop constructs that sense inflammatory mediators and respond with on-demand payloads can move the field from static implants to adaptive therapies. From a translational standpoint, embedding QbD- and PAT-informed control strategies together with robotics-assisted, closed bioprocessing platforms into bioprinting workflows will be as critical as innovations in bioink chemistry for delivering reproducible, regulator-ready immune-modulating constructs at clinical scale.[168,169,171]

Key challenges remain—long-term immune surveillance, patient-to-patient immune heterogeneity, scale-up under GMP, and cost/access—but they are tractable with coordinated standards, shared datasets, and early engagement with regulators. Ethically, longitudinal monitoring and transparent risk–benefit communication are essential as constructs become "living medicines."

In sum, the decisive gains in clinical translation will come from designs that treat immunity as a primary design variable rather than a downstream constraint. Achieving this requires tight collaboration among immunologists, bioengineers, clinicians, and regulators. When structure and immunity are co-optimized from the outset, 3D bioprinting can deliver not just repaired tissues, but resilient, self-maintaining organ function.

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The authors declare they have no competing interests.

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