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REVIEW ARTICLE

Advances in decellularized extracellular matrix bioinks for regenerative medicine applications

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Abstract

Organ transplantation serves as a critical life-saving intervention. However, the persistent global shortage of donor organs continues to result in high mortality rates. This pressing clinical challenge has fueled the search for alternative therapeutic strategies. Among these strategies, three-dimensional (3D) bioprinting has emerged as a transformative technology capable of fabricating complex tissue constructs using bioinks composed of living cells and supportive biomaterials. Notably, recent advancements have highlighted the incorporation of decellularized extracellular matrix (dECM) as a bioactive component, significantly enhancing biocompatibility, structural integrity, cellular support, and the formation and maturation of vascular networks. In this review, we detail the pivotal role of the ECM as a dynamic reservoir of biochemical signals and mechanical cues that regulate cellular behavior through mechanotransduction. These processes guide essential functions including gene expression, tissue development, and remodeling, thereby ensuring tissue-specific mechanical properties such as elasticity and tensile strength. We highlight how dECM-based bioinks can retain the native structural and molecular features of the ECM, making them ideal for replicating physiologically relevant microenvironments. Representative studies demonstrate the successful application of dECM bioinks in engineering complex *in vitro* 3D tissue models. Furthermore, we address current challenges in tissue engineering, including the standardization of bioink formulations, the refinement of decellularization techniques, and the enhancement of the mechanical and architectural properties of scaffolds. Finally, we explore emerging solutions—such as artificial intelligence-guided optimization, *in situ* bioprinting, and

the development of patient-specific bioinks—as promising avenues to overcome current limitations and drive the clinical translation of 3D-bioprinted tissues.

Keywords: Decellularized extracellular matrix; Decellularized extracellular matrix-based bioinks; Regenerative medicine; Three-dimensional bioprinting; Tissue regeneration; Vascularization

1. Introduction

Organ transplantation has become a definitive treatment for end-stage organ or tissue failure,[1] significantly improving patients' quality of life and preventing death.[2] However, access to transplantation remains a major challenge.[3] In 2022, approximately 3777 individuals in Canada were on the transplant waiting list, while in the United States, this number exceeded 103,000 by 2024.[4,5] Each year, hundreds of patients die while waiting for an organ to become available.[6] Consequently, there is an urgent need to explore new avenues to address this critical supply issue.

Three-dimensional (3D) bioprinting is an emergent technology capable of replacing or restoring dysfunctional tissues and organs, and represents a key advancement in the field of regenerative medicine.[7,8] In cases of organ failure, regenerative medicine aims to restore healthy bodily function by implanting tissues that have been engineered through 3D bioprinting in the laboratory.[9] This technique can reduce the risk of organ rejection[10] by using patient-derived induced pluripotent stem cells (iPSCs) for tissue engineering.[11,12] Since its development, regenerative medicine has emerged as a promising field for treating organ failure[9] and addressing other medical challenges, including the development of disease models for drug testing[13,14] and the treatment for autoimmune disorders,[15] such as type 1 diabetes,[16] Crohn's disease,[17] and rheumatoid arthritis.[18,19]

The technique of 3D bioprinting fabricates tissue constructs through the layer-by-layer deposition of cells within printable biomaterials.[20] These printable biological materials, termed "bioinks," consist of cell formulations compatible with automated biofabrication systems. Bioinks may incorporate biologically active components and supportive biomaterials, providing essential properties including structural support, controlled biodegradability, biocompatibility, and minimal immunogenicity.[21,22]

Researchers are currently developing novel bioinks by incorporating biological components such as decellularized extracellular matrices (dECM) to better recapitulate native tissue characteristics. The decellularization process removes cellular components from native tissues while preserving the ECM-rich non-immunogenic scaffold architecture. Depending on the specific decellularization method used, these scaffolds can retain natural vascular templates along with biological cues that support cell differentiation. Additionally, these mixtures could maintain native tissue architecture, including critical mechanical properties such as strength and stiffness, while simultaneously promoting vasculogenesis during the reseeding process.[23] The biological compatibility between seeded cells and the ECM scaffold remains paramount, as it directly enables the cellular adhesion, proliferation, and migration necessary for forming a functional endothelial lining along vascular structures.[24]

Thus, dECM-derived materials represent a promising strategy for optimizing bioink formulations and producing more physiologically relevant scaffolds.[25] These advanced scaffolds simultaneously enhance key biophysical properties, including stiffness and viscoelasticity, to better match native tissue mechanics, while preserving crucial biochemical components that support tissue growth, functionality, and regeneration. Importantly, most dECM preparations naturally contain angiogenic factors that actively promote vascularization following transplantation.[26] This review explores the use of dECM materials to enhance bioink formulations in combination with various 3D bioprinting techniques. Additionally, it examines how dECM scaffolds can create an optimal microenvironment to promote vascularization and enable functional tissue regeneration.

2. Three-dimensional bioprinting techniques for vascularized scaffolds

The application of cutting-edge material design and precise manufacturing processes can create vascularized structures for tissue engineering. The process of vascularization is vital for the survival of tissues, as it enables the transportation of oxygen, nutrients, and waste products. However, replicating the intricate shape and layered structure of natural vascular systems remains challenging. To address this issue, several 3D printing methods have been used to make structures with chosen structure details, clarity, and material mix (Figure 1). These techniques include extrusion-based

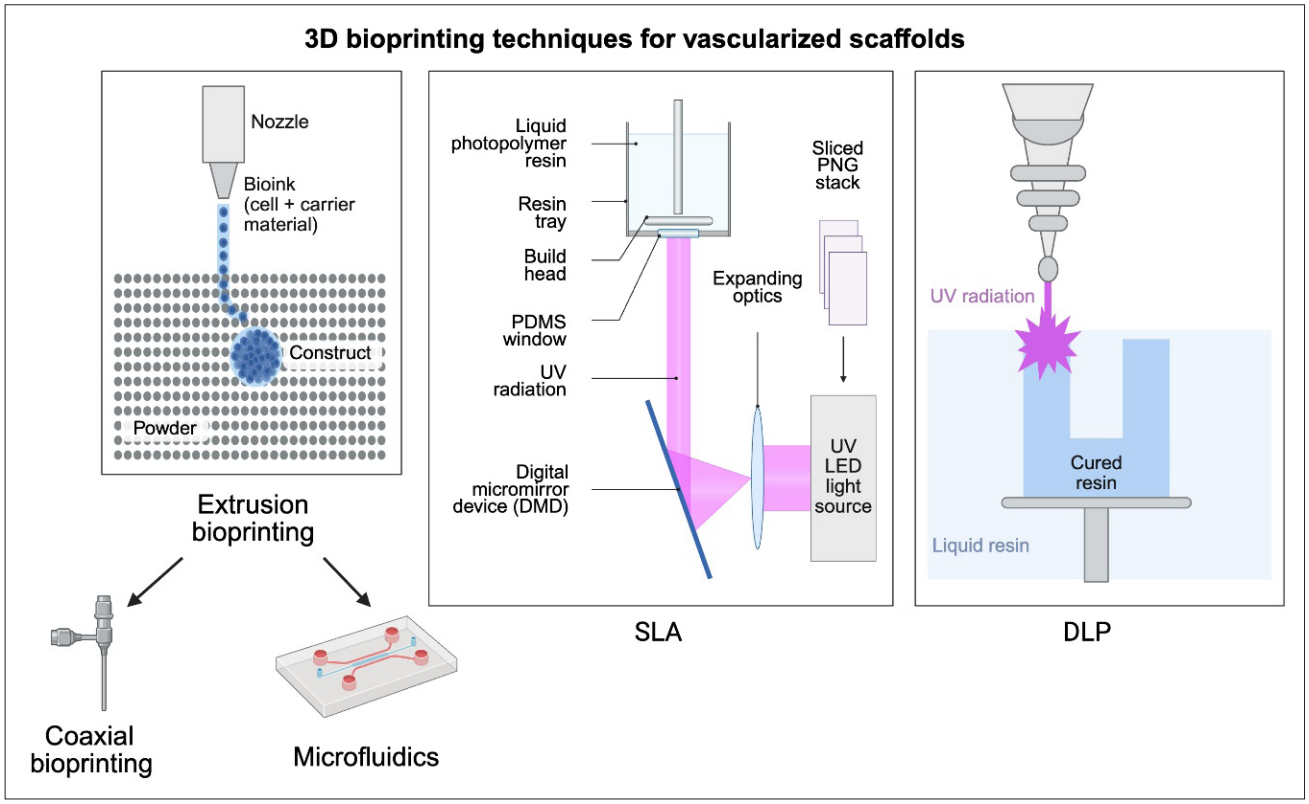

Figure 1. Overview of 3D bioprinting techniques for fabricating vascularized scaffolds. This schematic illustrates key bioprinting modalities. Extrusion-based bioprinting enables layer-by-layer deposition of cell-laden bioinks into sacrificial or coaxial geometries for vascular channel formation. DLP and SLA employ photopolymerization of light-sensitive resins using projected or scanned UV light for high-resolution fabrication of complex vascular structures. Created in BioRender. Willerth, S. (2025). https://BioRender.com/27bauwd. Abbreviations: 3D, three-dimensional; DLP, digital light processing; LED, light-emitting diode; PDMS, polydimethylsiloxane; SLA, stereolithography; UV, ultraviolet.

printing, stereolithography (SLA), digital light processing (DLP), coaxial printing, sacrificial printing, microfluidic printing, and multi-material ways (Table 1). By integrating dECM bioinks into each of these techniques, their biological significance can be improved. dECM provides native biochemical and mechanical signals that stimulate the development of new blood vessels and promote tissue regeneration. By combining these bioprinting techniques with bioactive bioinks, it becomes feasible to create vascularized constructs that closely resemble the intricate structure and functionality of natural tissues.

Sections 2.1–2.4 describe each 3D bioprinting technique in more detail, along with their advantages and limitations. Comparisons between techniques are summarized in Table 1, including defining quantitative parameters such as resolution, cell viability, tolerance, and throughput. **Section 2.5** provides a brief overview of new and emerging 3D bioprinting techniques.

2.1. Extrusion-based bioprinting

Extrusion-based bioprinting, a frequently employed technique for tissue engineering, is able to handle diverse biomaterials, including high-viscosity hydrogels, bioinks with cells, and composite materials.[29] This technique delivers bioink through a nozzle, driven by pneumatic, mechanical, or piston-powered pressure. It enables an accurate and step-by-step creation of complex 3D structures. Its compatibility with dECM bioinks—which are derived from tissues like adipose, cartilage, and cardiac tissues—makes it particularly appealing for creating vascularized tissue constructs. dECM bioinks provide specific biochemical signals and support cell differentiation, while their adaptable rheological properties allow precise printing of cells with compatibility and accuracy.[39,40] Notably, extrusion-based bioprinting has been employed to successfully create vascularized adipose and cardiac patches, aiding in the organization of endothelial cells and the formation of tubules.[40]

While extrusion-based bioprinting provides scalability and versatility, it faces challenges in terms of resolution, affecting the precise alignment of fibers and the ability to control microarchitecture for the creation of vascular networks.[41] Furthermore, shear stress during extrusion can

Table 1. A comprehensive overview of three-dimensional bioprinting techniques using decellularized extracellular matrix bioinks in regenerative medicine

Bioprinting technique	Advantages	Limitations	Typical applications	Quantitative parameters	References
Extrusion-based bioprinting	Widely used due to its simplicity, cost-effectiveness, and ability to print high-viscosity bioinks, including decellularized extracellular matrix. It supports the creation of large, cell-laden constructs with good mechanical properties	Lower resolution; shear stress during the printing process negatively affects cells	Commonly used for fabricating bone, cartilage, and skin tissues. It is particularly effective in creating vascularized structures when combined with coaxial bioprinting	Resolution: ~50–200 μm; cell viability: 40–80%; shear tolerance: low; throughput: medium (slow filament deposition)	[27–30]
Inkjet bioprinting	Offers high resolution and speed, suitable for low-viscosity bioinks. It allows precise placement of cells and bioactive molecules	Limited to low-viscosity bioinks; has the potential for nozzle clogging	Ideal for creating skin and cartilage tissues, and for applications requiring high cell viability and precise patterning, such as drug testing models	Resolution: ~20–100 μm; cell viability: ~90%; shear tolerance: low/moderate; throughput: high (up to ~10,000 droplets/s)	[28,31–33]
Laser-assisted bioprinting	Provides high resolution and cell viability, as it minimizes mechanical stress on cells. It is suitable for printing complex structures with high precision	Expensive equipment; potential thermal damage to cells	Used in skin tissue engineering and for creating intricate vascular networks, as well as in applications requiring high precision, such as neural tissue engineering	Resolution: ~20 μm or better; cell viability: >95%; shear tolerance: high; throughput: Medium (droplet-by-droplet)	[28,31–33]
Digital light processing and stereolithography	·Both techniques use light to cure photosensitive bioinks, offering high resolution and smooth surface finishes. They are suitable for creating complex geometries	Potential ultraviolet damage to cells; limited to photopolymerizable bioinks; potential cytotoxicity from photoinitiators	Effective in fabricating bone and dental tissues, where precision and surface quality are critical	Resolution: ~1–200 μm (often tens of μm); cell viability: >90%; shear tolerance: high; throughput: high (fast layer-by-layer)	[28,34,35]
Coaxial bioprinting	A subtype of extrusion-based bioprinting, it allows the creation of hollow structures and vascular networks by printing concentric layers of bioinks	Complex setup; limited to specific bioink combinations	Particularly useful for vascular tissue engineering and creating constructs with integrated vascular channels	Resolution: ~200 μm (core–shell fiber diameter); cell viability: ~80–90%; shear tolerance: moderate; throughput: medium	[36,37]
Sacrificial bioprinting	Involves printing a sacrificial material that is later removed to create hollow structures or channels. It is useful for creating complex internal geometries	Additional steps for material removal; potential structural instability post-removal	Used in creating vascularized tissues and complex organ models, such as liver and kidney constructs	Resolution: ~400–550 μm (hollow-channel diameter); cell viability: ~80–90%; shear tolerance: moderate; throughput: medium	[34,35,38]
Microfluidic bioprinting	·Allows precise control over the microenvironment and cell distribution, enabling the creation of highly detailed tissue constructs	Complex device fabrication; limited scalability	Suitable for creating organ-on-a-chip models and for applications requiring precise control over cell placement and microenvironment, such as cancer research	Resolution: ~10–250 μm; cell viability: ~85–95%; shear tolerance: low; throughput: medium	[34–36]

negatively impact cell viability, and the resulting constructs may have inadequate porosity and mechanical strength.[41] Innovative approaches like coaxial extrusion—which allows the creation of perfusable vascular channels by extruding bioinks and crosslinking agents simultaneously—have been developed to tackle these problems.[42] Additionally, sacrificial bioinks such as Pluronic F127 can be used to create temporary structures that can be removed post-printing, leaving behind perfusable channels.[41] These methods also improve the alignment of collagen fibers, enhancing cell proliferation and tissue integration.[41] Additionally, various crosslinking methods, such as ultraviolet-assisted or chemical crosslinking, are commonly used to improve the mechanical strength and stability of the printed material. Selecting an appropriate bioprinting strategy is critical for optimizing fiber orientation, porosity, stiffness, and vascular architecture to meet specific tissue engineering goals.[29,43,44]

2.1.1. Coaxial bioprinting

Coaxial bioprinting has attracted considerable attention due to its ability to produce hollow, tubular structures that closely mimic the natural structure of blood vessels.[45] It provides the ability to manipulate vessel geometry, lumen size, and wall thickness with precision. This technique utilizes concentric nozzles to simultaneously inject core and sheath bioinks, enabling the creation of perfusable vascular conduits (Figure 2). The bioink usually contains endothelial cells along with proangiogenic factors, while the secondary component provides structural support or acts as a sacrificial material. Alginate and calcium chloride have been extensively employed in coaxial bioprinting due to their quick ionic crosslinking and compatibility with biological systems.[41,46] dECM-derived bioinks have been combined with coaxial printing to promote endothelial cell adhesion, migration, and vessel formation, with studies demonstrating enhanced tubulogenesis and vascular endothelial growth factor receptor 2 (VEGFR2) expression.[47] Nevertheless, recreating the intricate branching structures of natural blood vessels and replicating their long-term mechanical stability pose significant challenges.[48] Sacrificial bioprinting is frequently employed to generate vascular channels by depositing temporary materials that are subsequently removed to form hollow conduits. Materials like DNA hydrogels, gelatin microparticles, and carbohydrate glass have been utilized to construct perfusable networks.[49,50] dECM bioinks are commonly used to enclose these sacrificial structures, providing biochemical signals that promote endothelial cell adhesion and the formation of a vascular network.[51]

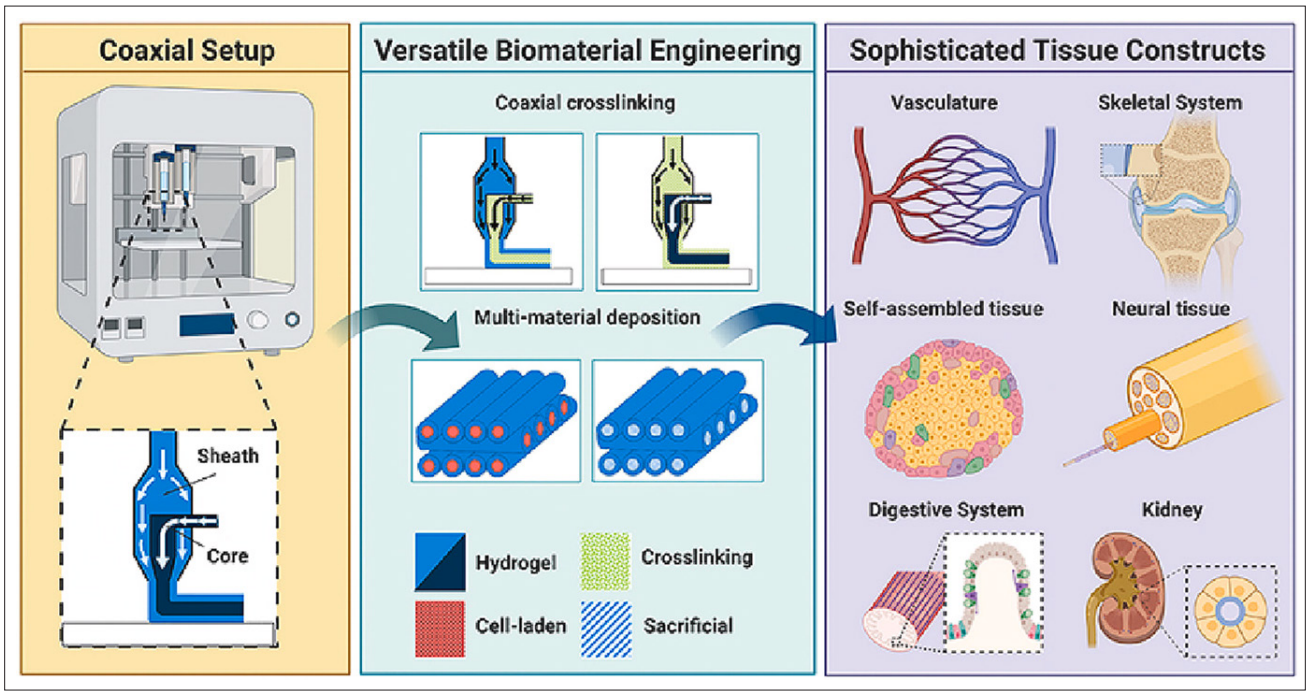

Figure 2. Schematic representation of coaxial bioprinting for tissue engineering applications. The diagram illustrates a coaxial bioprinting setup enabling core–sheath extrusion, followed by versatile biomaterial engineering strategies such as coaxial crosslinking and multi-material deposition. This approach facilitates the fabrication of sophisticated tissue constructs, including vasculature, skeletal, neural, digestive, and renal tissues. Reprinted with permission from Kjar *et al.*[52] Copyright © Elsevier 2020.

2.1.2. Microfluidic bioprinting

Decellularized ECM bioprinting, when combined with microfluidic and organ-on-a-chip technologies, offers a powerful platform for advanced drug testing and disease modeling. Microfluidic bioprinting utilizes microfluidic platforms to fabricate structures with intricate capillary-like features, achieving high precision in their design. These devices enable the accurate handling of bioinks and growth factors, facilitating the creation of complex vascular networks.[36] Incorporation of dECM bioinks into microfluidic systems has been shown to stimulate angiogenesis and promote the growth of endothelial cells,[53] enhancing the biological relevance of *in vitro* tissue models. Furthermore, dECM-based constructs integrated with organ-on-a-chip platforms can replicate both the mechanical and biochemical cues of native tissues, making them highly suitable for simulating physiological and pathological conditions in a controlled setting. This synergy enhances the fidelity of models used for studying disease progression and screening drug responses. However, microfluidic bioprinting still faces key obstacles related to scalability, efficiency, and consistency,[54] which must be addressed for broader adoption in clinical and pharmaceutical research.

2.2. Stereolithography and digital light processing

SLA and DLP are light-based bioprinting techniques that employ photopolymerization to create intricate 3D structures with high resolution. These techniques have become valuable tools for generating microvascular networks due to their high precision and ability to create complex shapes. SLA can achieve resolutions as fine as 5–50 μm,[55] while advanced DLP systems can reach 0.6–30 μm.[56] In SLA, a focused laser beam selectively polymerizes photosensitive bioinks layer by layer, while DLP employs a digital light projector to cure entire layers simultaneously.[57,58] Both techniques have been employed to generate vascularized scaffolds by incorporating endothelial cells into photopolymerizable hydrogels. dECM-based bioinks, such as cardiac-derived dECM hydrogels, have been incorporated into SLA systems to support endothelial cell adhesion, proliferation, and tubulogenesis.[39,40] The main benefit of SLA and DLP is their capability to create detailed and precise 3D models with complex vascular systems. Nevertheless, the restricted range of photopolymerizable bioinks and the potential cytotoxicity of photoinitiators present substantial obstacles.[58,59] Ongoing efforts are centered around creating biocompatible photoinitiators and hybrid bioinks that combine dECM with synthetic polymers to improve printability and functionality.

2.3. Sacrificial bioprinting

Sacrificial bioprinting is commonly used to produce vascular channels by depositing temporary materials that are later eliminated to create hollow conduits. Materials like DNA hydrogels, gelatin microparticles, and carbohydrate glass have been utilized to construct perfusable networks.[49,50] dECM bioinks are frequently employed to enclose these sacrificial structures, offering biochemical signals that facilitate endothelial cell adhesion and the development of a vascular network.[51] Sacrificial bioprinting allows the creation of complex vascular networks, but challenges persist in maintaining channel patency, mechanical stability, and immune compatibility.[50,60]

2.4. Multi-material bioprinting

Multi-material bioprinting merges different bioinks to create tissues with complex structures, consisting of various materials.[61] This method combines bioinks with synthetic and nanostructured materials to create tissues with unique biochemical and mechanical characteristics.[62,63] Advanced bioinks that react to external factors have been created to improve blood vessel growth and facilitate dynamic tissue changes.[31] Although multi-material bioprinting holds great promise, there are still obstacles to overcome, such as ensuring compatibility between different bioinks, optimizing mechanical properties, and establishing standardized processes.[64,65] The combination of 3D bioprinting technologies and dECM bioinks signifies a groundbreaking approach to engineering vascularized tissues. Each bioprinting technique presents unique benefits and obstacles, emphasizing the necessity for hybrid systems that integrate the strengths of multiple approaches. Future studies should concentrate on refining bioink compositions, creating biocompatible photoinitiators, and improving vascularization techniques to enhance the performance and practical application of engineered tissues.

2.5. Emerging innovations in three-dimensional bioprinting

Recent advances in 3D bioprinting include a wave of transformative technologies that address limitations in resolution, cell viability, and structural complexity. One exciting development is the Freeform Reversible Embedding of Suspended Hydrogels method, which allows printing of soft, liquid-like bioinks within a yield-stress bath, preserving construct fidelity and resolution while supporting anatomically accurate structures.[45,66] Similarly, liquid-in-liquid bioprinting also utilizes a bath to ensure electrostatic stabilization. The deposition of high-cell-density bioinks in the granular bath has resulted in cell proportions exceeding 90% v/v.[67]

There are also newly emerging techniques that are specifically tailored to create vascularized tissue models. For example, suspension-based bioprinting techniques, such as suspension bath-based 3D bioprinting, utilize starch hydrogel baths to create vascularized, multicellular constructs with smooth, removal-free support structures.[68] Similarly, infiltration-induced suspension bioprinting employs hyaluronic acid's shear-thinning and self-healing properties to fine-tune scaffold microarchitecture and mechanical strength while maintaining high-resolution endothelial cell patterning.[69] In addition, sacrificial bioprinting enables the fabrication of perfusable, tubular structures by serving as transient supports in soft matrix printing.[70] Sequential printing in reversible ink template is another new technique that uses biphasic microgel-based ink as both medium and bioink to build complex tissues with perfusable vasculature, including cardiac organoids.[71]

In addition, certain techniques that enable the construction of multi-layered tissue models, which are especially important when trying to replicate the complexity of human organs, are currently being developed. For example, multi-material bioprinting enables co-deposition of diverse bioinks containing different cell types to recreate native tissue heterogeneity with spatial precision.[72] In addition, volumetric bioprinting surpasses traditional layer-by-layer constraints by rapidly forming centimeter-scale structures via photopolymerization.[73] Microfluidic-assisted bioprinting provides control over microarchitecture through hierarchical bioink patterning.[74] Lastly, programmable granular hydrogel inks, with tunable porosity and stimuli-responsiveness, are paving the way for advanced four-dimensional bioprinting that enables the alteration of the bioink's mechanical properties during tissue maturation *in vitro* or following implantation *in vivo*.[75]

These emerging technologies have enabled the creation of skin models, vascular tissues, and organoids with increased fidelity and functionality, significantly advancing applications in regenerative medicine and personalized therapy.[33,76] Future efforts will aim to address scalability, material limitations, and regulatory challenges, while integration with artificial intelligence and bioinformatics promises to optimize design, printing parameters, and translational potential.[77,78]

3. The role of decellularized extracellular bioinks in vascularization

3.1. Structural and functional properties of native extracellular matrix and its role in vascularization

The ECM plays a central role in regulating cellular behavior and tissue development, extending far beyond its structural function. Composed of proteins, glycoproteins, and proteoglycans, the ECM modulates angiogenic signaling by sequestering growth factors and guiding cell communication within the native microenvironment.[79–81] This intricate regulatory capacity is largely enabled by the ECM's fundamental yet adaptable architecture, which typically comprises two interconnected compartments: the interstitial matrix and the basement membrane. While these compartments serve as common organizational principles, their specific molecular composition and structural arrangement are tailored to meet the unique functional demands of each tissue.[82] For instance, the interstitial matrix forms the primary structural scaffold of tissues, composed of collagen, elastin, and adhesive glycoproteins like fibronectin that enable cell adhesion, migration, and mechanical integrity. On the other hand, the vascular basement membrane—a dense sheet underlying endothelial cells—primarily contains laminin and collagen IV, both of which regulate cell anchorage and vascular barrier function.[83,84] Crucially, both compartments contain glycosaminoglycans and heparan sulfate proteoglycans, which stabilize growth factors and ensure precise spatiotemporal control over angiogenic signaling and endothelial morphogenesis.[83]

The ECM composition is continuously remodeled through a feedback loop between mechanical and biochemical signals. Cells sense and respond to matrix stiffness and external stresses via surface receptors such as integrins, ion channels, and primary cilia, activating mechanotransduction pathways.[85] These cues activate intracellular signaling cascades that influence gene expression and drive ECM remodeling.[86] This dynamic reciprocity maintains tissue-specific mechanical properties, such as elasticity and tensile strength, which are essential for function.[87]

The interplay between cells and their matrix environment gives rise to ECM specializations tailored to distinct tissue needs. For instance, in cartilage, the mechanical environment dictates chondrocyte behavior and ECM synthesis, with collagen types, elastin, and small leucine-rich proteoglycans shaping tissue stiffness and flexibility, distinguishing hyaline, elastic, and fibrocartilage, and influencing their respective load-bearing capacities.[88,89] Similarly, the skin's ECM exhibits a multi-layered complexity, where laminin-332, collagen IV, and collagen VII anchor the epidermis to the dermis via the basement membrane.[90] Fibroblasts further contribute to fibrillin-rich microfibrils and elastic fibers, while crosslinking enzymes like lysyl oxidases and small leucine-rich proteoglycans reinforce collagen organization—properties vital for skin elasticity and resilience.[91]

The ECM's tissue-specific adaptations extend to various other tissues. Bone, for example, is a mineralized connective tissue composed primarily of collagen I, hydroxyapatite, and proteoglycans such as decorin and biglycan, which support mineralization and biomechanical strength.[89] Moreover, the diverse functional roles shaped by ECM organization and composition are exemplified by the cornea, whose transparency relies on the precise alignment of collagen fibrils and the presence of anti-angiogenic molecules, such as thrombospondins and matrix metalloproteinases, which are essential for preventing vision-impairing vascularization.[92]

This structural diversity is particularly evident in vascular tissues, where ECM cues directly orchestrate angiogenesis. For example, the interaction between integrins (e.g., αvβ3 and αvβ5) and growth factor receptors modulates receptor tyrosine kinase activity, such as that of VEGFR.[93] Upon ligand binding, VEGFR2 triggers downstream signaling pathways that drive angiogenesis and endothelial cell organization into vascular structures.[94] These finely tuned interactions demonstrate how ECM composition and structure regulate vascularization at the molecular level.

Preserving the ECM's biochemical and mechanical cues is vital for directing cellular responses involved in tissue development and remodeling. As a central reservoir of bioactive molecules, the ECM orchestrates mechanotransduction and signaling pathways that regulate gene expression and morphogenesis.[85] dECM, which can retain native structural and molecular features, has therefore emerged as a promising bioink component for recapitulating vascularization in engineered tissues.[23]

3.2. Decellularized extracellular matrix as a bioactive bioink biomaterial to promote vascularization

Decellularized ECM has emerged as a promising biomaterial for regenerative therapies, with several formulations already approved by the Food and Drug Administration.[95] Despite differences in decellularization methods, their shared goal is to eliminate cellular and immunogenic components while preserving the native ECM's structural and biochemical properties. The resulting matrices retain essential components—such as collagen, fibronectin, laminin, glycosaminoglycans, and growth factors—that support tissue-specific cellular functions.[24] These retained cues are particularly critical for promoting angiogenesis, positioning dECM as a promising element in the development of biomaterials aimed at supporting vascular network formation.

A critical challenge in engineering functional tissues is the limited diffusion of oxygen and nutrients within the created 3D structures. In living organisms, cells must reside within a short distance (typically 100–200 μm) of capillaries to ensure adequate supply; beyond this range, they suffer from hypoxia and necrosis.[96,97] Therefore, a crucial goal in tissue engineering is promoting the formation of robust, functional vascular networks throughout engineered scaffolds. One approach involves the growth and formation of new blood vessels from existing ones, known as angiogenesis.[98] Achieving this vascularization requires a two-pronged approach: first, the strategic delivery of angiogenic factors to stimulate blood vessel growth, and second, the effective recruitment and support of endothelial cells, such as human umbilical vein endothelial cells, which exhibit a strong capacity to form vascular structures within environments that mimic natural ECM environments.[99] Furthermore, for these engineered tissues to successfully integrate and function long-term within a host, the biomaterials used must possess a balanced set of properties, including biocompatibility, low immunogenicity, and controlled biodegradation, allowing effective tissue remodeling and seamless integration.[100]

In addition to enhancing regenerative outcomes, dECM biomaterials must recapitulate the biochemical and biophysical features of native ECM. Mechanical properties such as stiffness and viscoelasticity, along with microstructural features like topography, influence how cells sense and respond to their environment. These physical cues regulate critical cellular processes—including migration, proliferation, and morphogenesis—by modulating intracellular signaling through integrin and other mechanotransduction pathways.[101] Incorporating these properties into dECM-based bioinks ensures that the scaffold not only supports vascular network formation but also provides a dynamic microenvironment conducive to tissue regeneration.

3.3. Decellularized extracellular matrix-based bioink formulation

Both the source tissue and the decellularization protocol must be carefully selected for preparing dECM, as they significantly influence the biochemical composition, structural integrity, and immunogenic profile of the final material.[102] dECM can be derived from three main sources: cell-derived matrices, animal tissues, and human tissues. Cell-derived matrix is produced *in vitro* from cultured cells and offers a controllable and reproducible matrix rich in signaling molecules; however, it often lacks the mechanical robustness of native tissue and has limited scalability.[103,104] Animal-derived dECMs, such as porcine bladder and heart, bovine tendons, goat lung and

kidney, or rat liver and lungs, are widely used due to their availability and structural similarity to human tissues. Nonetheless, they present potential challenges, including immunogenicity, cross-species disease transmission, and batch variability.[103,104] Human-derived dECMs, obtained from cadaveric tissues, diseased or discarded organs, or from tissue banks, offer a superior physiological relevance and lower immunological risk, making them particularly valuable in applications related to organ repair and transplantation. However, their availability is limited and subject to strict ethical and regulatory oversight.[103] The selection of the source should therefore align with the intended clinical application, especially in contexts like organ transplantation, where immunocompatibility and structural fidelity are paramount.

Decellularization is equally important as the source of the material, removing all cellular and immunogenic material while preserving the native ECM's 3D architecture, biochemical complexity, and mechanical strength. Decellularization strategies encompass physical methods (e.g., freeze–thaw cycles, agitation, hydrostatic pressure), chemical agents (e.g., detergents, acids, bases), and enzymatic treatments (e.g., nucleases and proteases), often applied in combination to maximize efficacy while minimizing damage to bioactive components.[102,105,106] In contrast to the often more intensive protocols required for decellularizing animal and human tissues with their cellularly dense structure, cell-derived matrices—owing to their relatively less compact and *in vitro*-assembled structure—typically necessitate gentler decellularization procedures. These milder approaches often exclude harsh mechanical disruption, such as crushing, potentially leading to better preservation of the ECM's delicate ultrastructure and molecular composition.[107] Effectiveness is typically assessed through metrics such as residual DNA content, ultrastructural analysis, mechanical property retention, and preservation of key ECM constituents like collagen, fibronectin, laminin, and glycosaminoglycans.[40] Inadequate decellularization can result in immune rejection, while overexposure to harsh treatments can compromise the matrix's biological function.[108]

Once decellularized, dECM-based biomaterials can be processed into either powdered or hydrogel form, each suitable for different biofabrication applications. In their research, Mesquita *et al.*[109] followed a protocol in which decellularized animal tissue was lyophilized, cryomilled, and sieved, yielding powdered particles with sizes ranging from 45–1000 μm. Alternatively, dECM can be solubilized through pepsin digestion, neutralized to promote self-assembly, and then induced to form a hydrogel through incubation at 37°C.[110] These processing endpoints are particularly valuable for bioink development as they preserve the native ECM architecture, a crucial factor for successful tissue regeneration. Figure 3 highlights the different processes used to formulate dECM-based bioink.

Preserving the native ECM architecture is crucial for using dECM in biofabrication, particularly in 3D bioprinting. When properly processed, dECM retains essential biochemical and mechanical signals that promote cell adhesion, proliferation, differentiation, and angiogenesis, which are key for regenerating functional tissues. However, native dECM often lacks the mechanical robustness and printability required for clinical-scale constructs.[40] To address this, researchers have developed composite bioinks that integrate dECM with reinforcing materials to improve rheological properties, extrusion stability, and cellular mechanosensing. These enhancements are vital not only for structural fidelity (mechanical strength) but also for creating a microenvironment conducive to vascular development.[111] Shin *et al.*[112] demonstrated this approach using a bioink comprising porcine cardiac dECM, Laponite-XLG nanoclay, and poly(ethylene glycol)-diacrylate. This formulation achieved enhanced printability and mechanical properties matching both healthy (~5–15 kPa) and fibrotic cardiac tissue (~30–100 kPa), while maintaining cell viability and functionality.[112] Similarly, Alibeigian *et al.*[114] addressed dECM limitations by combining calcium phosphate cement with bone-derived dECM hydrogel. Subcutaneous implantation in rats showed no toxicity or inflammatory responses, with radiographic and computed tomography analyses demonstrating superior bone formation in dECM/calcium phosphate cement-hydrogel and mesenchymal stem cells-incorporated groups compared to controls.[113,114] Figure 4 highlights some of the diverse tissue origins of dECMs used in various tissue engineering applications.

3.4. Decellularized extracellular matrix-based bioinks: advancing vascularization and tissue regeneration in 3D bioprinting

Decellularized ECM can enhance vascularization and tissue regeneration when incorporated into bioinks. For example, You *et al.*[115] developed a liver dECM-gelatin-alginate scaffold that promoted osteogenic differentiation of rat bone mesenchymal stem cells. *In vivo* studies using a calvarial defect model revealed enhanced vascularization and bone regeneration.[115] Martinez *et al.*[116] similarly showcased dECM's potential through reproductive tissue-mimicking hydrogels combining ovarian, oviductal, and endometrial dECM with 1% alginate. These scaffolds maintained viability of multiple cell types and supported microvascularization without supplemental growth factors, highlighting dECM's inherent bioactivity.[116] Additionally,

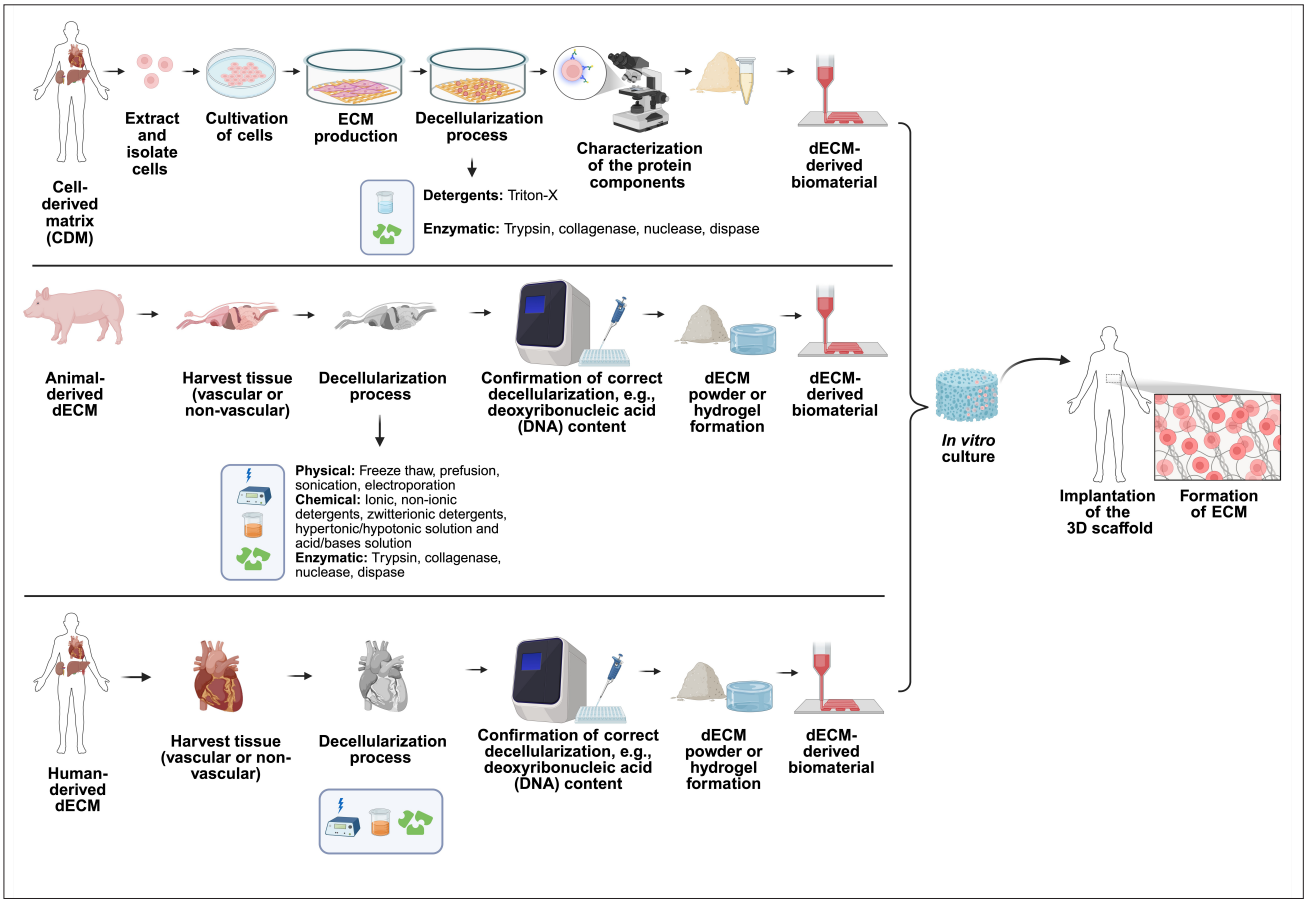

Figure 3. Decellularized extracellular matrix (dECM)-based bioink formulation. The schematic figure shows the key steps in preparing dECM-based bioink from different tissue sources (cell-derived, animal-derived, or human-derived). The process involves decellularization using physical, chemical, or enzymatic methods to remove cellular material while preserving ECM composition and structure, followed by processing into either powdered form (via lyophilization, cryomilling, and sieving) or hydrogel form (via pepsin digestion, neutralization, and thermal gelation). The resulting bioink retains native ECM components (e.g., collagen, fibronectin, glycosaminoglycans) and can be used for tissue engineering applications. Created in BioRender. Willerth, S. (2025). https://BioRender.com/hs597ud.

Kim *et al.*[61] developed a bioink from pancreatic dECM to replicate *in vivo* pancreatic tissue for islet transplantation as a treatment for type 1 diabetes mellitus. The inclusion of human umbilical vein endothelial cells in the bioink promoted vascularization and reduced necrosis in the 3D cultured islets. These results demonstrate the potential of dECM to provide a supportive microenvironment for pancreatic cells, offering promise for pancreatic regeneration and improved islet transplantation outcomes. These studies collectively demonstrate the potential of dECM-based bioinks in supporting tissue-specific cell functions, promoting vascularization, and ultimately improving tissue regeneration across a range of tissue engineering-based applications.

The development of dECM-based bioinks marks an advancement in 3D bioprinting, as they uniquely integrate native biochemical signaling with tunable mechanical properties. However, achieving functional vascularization requires more than an optimized bioink formulation. It also requires precise control over 3D bioprinting parameters to engineer scaffolds with biologically relevant structural features. Key printing considerations, including resolution, pore architecture, and fibrin alignment, must be systematically optimized to facilitate essential physiological processes such as nutrient diffusion, cell migration, and angiogenic sprouting.[103] For example, the combination of dECM with human smooth muscle cells and endothelial cells could be used to create capillary-like networks within the scaffold, which would be critical for ensuring the long-term viability of implanted tissues. This approach builds on previous studies that have shown that dECM's properties support cell attachment, migration, and proliferation—all of which are crucial for successful vascularization.[116] Moreover, using dECM from different tissues, such as cardiac or liver tissue, can be tailored to support specific

tissue types, enhancing the scaffold's ability to replicate the physiological microenvironment of the engineered tissue (Figure 4). Adding this level of vascular support could significantly improve the long-term functionality and tissue regeneration capabilities of 3D-bioprinted constructs, especially when designing scaffolds for complex tissue engineering applications such as wound healing or organ regeneration. By integrating cell assembly strategies with dECM scaffolds, these engineered tissues can achieve advanced levels of vasculature, mimicking the hierarchical architecture of native blood vessels.

4. Challenges in three-dimensional bioprinting vascularized scaffolds with decellularized extracellular matrix bioinks

The advancement of 3D bioprinting technologies and dECM-based bioinks has significantly enhanced the development of vascularized tissue constructs. Despite their biomimetic advantages, dECM-based bioinks face several challenges that hinder their widespread use in tissue engineering and regenerative medicine. Key limitations include poor mechanical properties, difficulties in vascular network maturation, immunogenicity concerns, and scalability issues—all of which pose barriers to clinical translation. Additionally, optimizing bioink rheology, crosslinking kinetics, and printing temperature is critical to ensure scaffolds can accurately replicate native vascular ECM organization. These interdependent factors highlight the need for a synergistic approach, combining advanced material design with precision fabrication techniques to achieve successful vascularization strategies.

4.1. Printability and mechanical properties

The printability of dECM bioinks is influenced by rheological properties, crosslinking mechanisms,

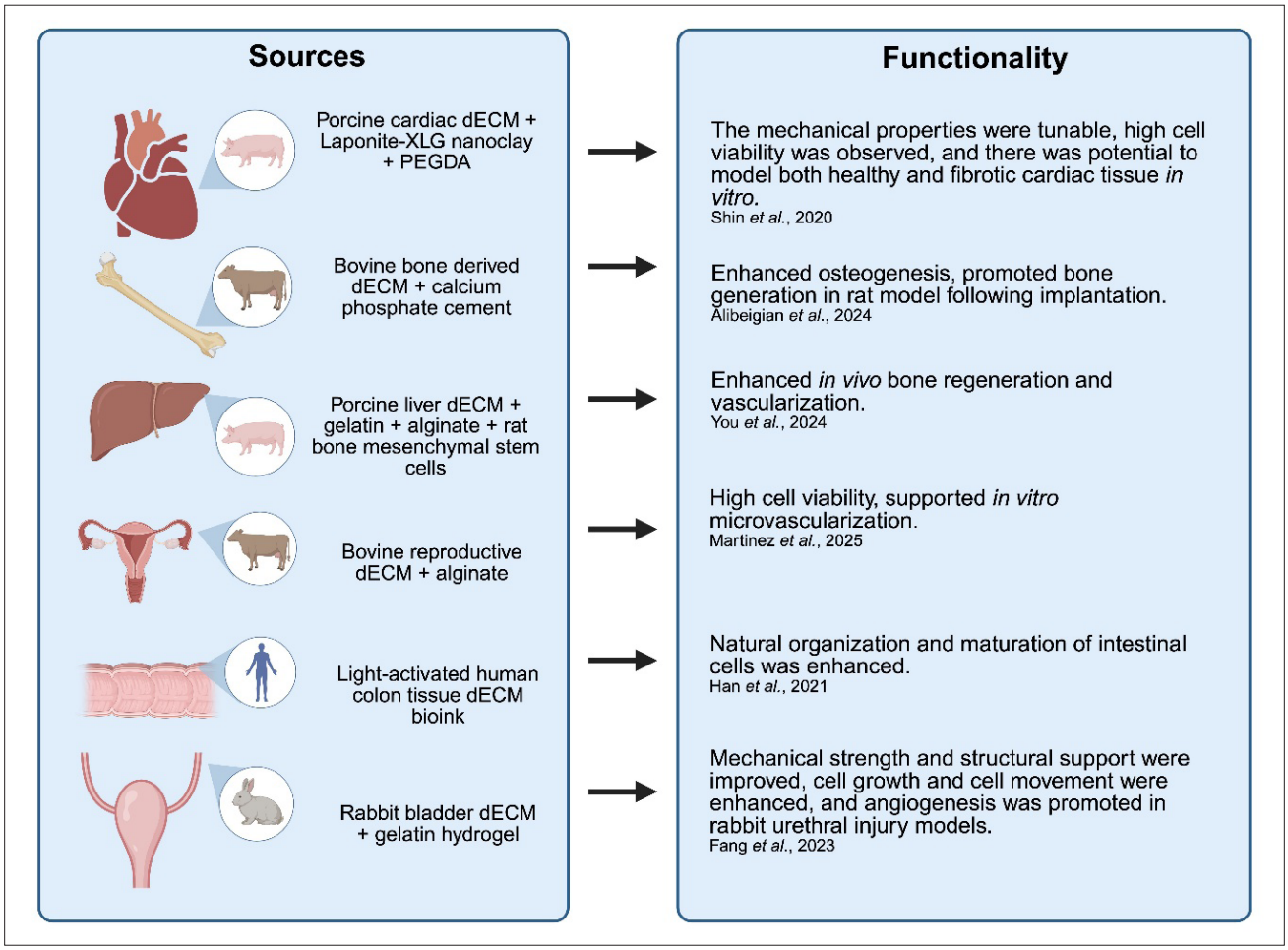

Figure 4. Overview of decellularized extracellular matrix (dECM)-based bioinks and their applications in tissue engineering. Applications for dECM bioinks may span cardiovascular, skeletal, hepatic, reproductive, intestinal, and urinary systems, highlighting its diverse benefits, including tunable mechanical properties, enhanced vascularization, cell viability, and structural support. The studies included in the figure validate these functionalities in both *in vivo* and *in vitro* models. Created in BioRender. Willerth, S. (2025). https://BioRender.com/mmkf47d

and extrusion parameters. All of these factors govern the structural integrity and resolution of bioprinted constructs.[117] However, dECM bioinks lack intrinsic mechanical strength, making it difficult to fabricate scaffolds with high shape fidelity and long-term stability.[117,118] The loss of structural proteins during decellularization results in weak gelation kinetics, leading to deformation or collapse of printed structures, particularly in complex vascular architectures.[118]

To improve printability, researchers have explored dual crosslinking mechanisms that incorporate photo-crosslinking and thermal gelation, thereby enhancing mechanical properties while preserving bioactivity.[118] Additionally, rheological tuning through the incorporation of reinforcing agents and viscosity modifiers has been investigated to optimize the flow behavior and shear-thinning properties of dECM bioinks.[119] However, balancing mechanical reinforcement with biological functionality remains a critical challenge, as excessive crosslinking or material modifications can compromise cell viability and ECM bioactivity.

4.2. Vascular network maturation

Vascularization is essential for the functionality and longevity of bioprinted tissues, yet generating hierarchically organized, perfusable vascular networks remains a major hurdle. dECM bioinks, while rich in angiogenic factors and ECM-based biochemical cues, lack the mechanical stability necessary for sustained vascular lumen formation and perfusability.[117,120] As a result, bioprinted vasculature often exhibits premature collapse, limited branching complexity, or poor endothelialization, limiting its functionality *in vivo*.[47,121] Efforts to enhance vascular maturation have focused on coaxial bioprinting and sacrificial templating approaches, which enable the fabrication of hollow, perfusable vascular conduits with tunable lumen sizes and wall thicknesses.[121,122] Furthermore, the incorporation of endothelial cells, pericytes, and angiogenic growth factors within dECM bioinks has been explored to promote endothelial sprouting and vessel stabilization.[118] Notably, Shiwarski *et al.*[45] demonstrated that combining photocurable, cell-laden hydrogels with high-resolution DLP bioprinting enables the fabrication of perfusable vascular networks with improved mechanical fidelity and biological relevance. However, even with these advancements, functional integration with host vasculature remains a significant challenge, requiring precise biochemical and mechanical signaling to achieve successful anastomosis and long-term viability.

4.3. Immunogenicity and standardization

Although dECM bioinks are derived from native ECMs, they are not entirely free from immunogenic concerns. Residual cellular debris, nucleic acids, and immunogenic proteins from the decellularization process can elicit inflammatory responses and graft rejection when introduced into a biological system.[51,119] Immunogenicity is further exacerbated by batch-to-batch variability, which results from differences in tissue sources, decellularization protocols, and processing conditions, ultimately impacting the reproducibility and safety of dECM bioinks.[123]

The source of the antigen, whether it comes from an animal or a human, has a significant impact on its ability to trigger an immune response. Animal-derived dECM, such as porcine or bovine, often contains species-specific antigens, such as α-gal epitopes, which can cause strong immune reactions in humans, resulting in inflammation and potential graft rejection. In contrast, human-sourced dECM lacks these foreign antigens, which reduces the risk of immune rejection and improves biocompatibility. For instance, research has demonstrated that ECM from humans has a substantial impact on reducing the risk of immune rejection when it comes to xenogenic ECM, such as the response to α-gal epitopes. Consequently, while animal-derived dECM has benefits in terms of availability and scalability, human-derived dECM is commonly chosen for clinical applications where immunogenicity is a significant concern.[124]

The lack of standardized procedures for the generation of dECM represents another major challenge.[95,125] Standardizing dECM production is impeded by wide variation in tissue properties and decellularization outcomes. For instance, a protocol optimized for porcine hearts required extended sodium dodecyl sulfate treatment and additional steps to process human hearts, yet still resulted in markedly different levels of cellular residues among human donors.[95] Likewise, applying a single detergent regimen to five human livers yielded five distinct scaffold outcomes (with only one liver completely devoid of cells).[126] Moreover, if dECM is improperly decellularized, residual immunogenic material may further complicate standardization. Donor-specific antigens, including DNA fragments and human leukocyte antigen/major histocompatibility complex molecules, can provoke host immune responses if not eliminated, but methods harsh enough to remove them often concurrently strip away essential bioactive ECM components.[95]

Standardization of bioink processing and bioprinting protocols is critical for addressing current inconsistencies and ensuring the clinical feasibility of dECM-based constructs. Recent research has focused heavily on optimizing decellularization techniques to minimize immunogenic components while preserving bioactive ECM components.[117] Furthermore, the implementation

of biochemical assays, biocompatibility testing, and quality control measures are also essential in establishing standardized manufacturing and regulatory guidelines.[119,123]

4.4. Scalability and clinical translation

Despite promising advancements in 3D bioprinting and dECM bioink development, scaling up the fabrication process for clinically relevant tissue constructs remains a significant challenge. Current bioprinting platforms are primarily optimized for small-scale constructs, and achieving large-scale, vascularized tissues suitable for implantation requires improvements in bioink stability, tissue perfusion, and automated fabrication techniques.[119,123] The inherent biological variability of dECM bioinks further complicates scalability, as maintaining consistent composition and mechanical properties across batches is difficult. From a regulatory standpoint, clinical translation of bioprinted tissues requires extensive preclinical validation, safety assessments, and compliance with Good Manufacturing Practices. Unlike synthetic biomaterials, dECM-based scaffolds must demonstrate long-term biocompatibility, functional integration, and reproducibility across multiple production cycles to receive regulatory approval.[123]

Real-world commercial products such as CorMatrix® ECM (used in cardiovascular and soft tissue repair) and MatriStem® UBM (a urinary bladder matrix used in wound healing and hernia repair) exemplify how dECM-based materials have successfully navigated some of these translational hurdles. These products, derived from porcine tissues and processed under rigorously standardized conditions, highlight the clinical viability of dECM scaffolds and reinforce the importance of quality control, reproducibility, and long-term safety. Several ongoing clinical trials (e.g., NCT04023254 investigating dECM hydrogel for myocardial infarction repair) further illustrate the potential of these materials in regenerative therapies.

Current efforts are directed toward developing standardized decellularization methods, optimizing biofabrication workflows, and incorporating real-time quality control measures to ensure the scalability and regulatory compliance of dECM-based bioprinted tissues. Ensuring the long-term stability of dECM-based scaffolds—defined by the retention of native matrix architecture, mechanical integrity, and bioactivity during extended implantation—is essential for successful clinical translation. Strategies such as incorporating polymer-dECM composite bioinks and applying post-fabrication crosslinking have been developed to enhance scaffold durability. For example, fibrinogen-dECM hybrid hydrogels have demonstrated improved mechanical modulus and extended structural stability compared to pure dECM gels.[24] Moreover, careful optimization of decellularization protocols, such as minimizing enzymatic degradation from agents like trypsin, helps preserve critical ECM components and mechanical strength.[103] To ensure quality and monitor scaffold behavior over time, advanced imaging modalities are being integrated as non-destructive, real-time analytical tools. High-resolution microscopy techniques such as scanning and transmission electron microscopy are used to confirm the preservation of scaffold microarchitecture post-implantation.[127] In parallel, non-invasive, *in vivo* imaging platforms—including optical coherence tomography, multiphoton microscopy, and ultrasound—are being employed to longitudinally track scaffold degradation, tissue maturation, and host integration. Functional imaging methods such as magnetic resonance imaging and photoacoustic imaging further enable dynamic assessment of vascular perfusion, metabolic activity, and cellular functionality within the developing constructs.[24,127]

4.5. Regulatory and ethical considerations

Decellularized ECM can pose significant risks and ethical concerns when introduced back into the human body due to its bioactive nature. In 2001, a clinical trial using Synergraft® decellularized porcine heart valves passed regulations and gained approval in Europe. However, it was later understood that the valves were insufficiently decellularized, and upon implantation, they resulted in the death of three out of four patients due to immunotoxicity.[128] This case study highlights the importance of thorough regulation and testing prior to the implantation of dECM products.

The introduction of implantable 3D-bioprinted dECM products to the market is currently regulated by both national and international organizations. The International Organization for Standardization (ISO) introduced the ISO 10993 standard for the biological evaluation of medical devices.[121] The ISO 10993 comprises a series of frameworks and tests to assess the safety of medical devices that come in contact with the human body, including tests for immunotoxicity, sensitivity, degradability, and implantation studies. The framework has been incorporated into pre-existing policies by the European Union's European Medical Devices Regulation (MDR), Health Canada's MDR, and the United States' Food and Drug Administration.[129] These regulatory bodies ensure that new medical devices demonstrate safety across applicable ISO 10993 standards by requiring that manufacturers submit extensive biocompatibility data and undergo chemical characterization testing. Independent of the ISO 10993, the Food and Drug Administration and European and Canadian MDR also possess their own safety regulations and requirements, including a

risk-based classification system for new products and post-market tracking.[130–132] Many other countries also use the ISO 10993 as a policy guideline for medical devices, including China's National Medical Products Association, India's Central Drugs Standard Control Organization, and Japan's Pharmaceuticals and Medical Devices Agency. In summary, fulfilling the rigorous evaluation requirements of ISO 10993 is a time-intensive and expensive process, but it is a necessary safeguard to ensure human safety.

In addition to regulatory considerations, there are also multiple ethical considerations that must be taken into account when considering the development of 3D-bioprinted dECM tissues. Firstly, the tissue source for dECM poses significant ethical challenges. dECM has three main sources; however, human-derived dECM is the most beneficial for clinical applications. The most common human dECM sources include cadaveric tissues, diseased, and discarded organs, and must therefore adhere to guidelines surrounding informed consent, confidentiality, and bias minimization.[133] Some researchers are also advocating for the ability of donors to choose how their tissues are used over the long term[134] to better address ethical concerns surrounding human tissue usage. Secondly, the source of the cells needed for recellularization of the dECM construct also poses ethical challenges. iPSCs are a promising avenue to reseed dECM, as they can be derived from pre-established human cell lines and have a reduced risk of immunotoxicity.[127] However, the most significant drawback of iPSCs' use in 3D-bioprinted constructs is the risk of tumor formation.[135] Significant advancements have been made in recent years, with studies successfully reseeding iPSCs into dECM scaffolds while avoiding tumor formation.[136–138] Despite this, tumorigenesis still remains a pressing ethical challenge in iPSC research.[139] While recellularization of dECM is generally considered to be a beneficial process,[127] another growing area of research is the use of dECM scaffolds that are not reseeded with cells.[125] This process involves implanting a cell-free scaffold into the body and recruiting cells from the surrounding tissue. This technique has demonstrated wide success in the cases of skin (AlloDerm® [BioHorizons], Oasis® [Smith & Nephew]), tendon and ligament (GraftJacket® [Wright Medical], Allopatch HD™ [MTF Sports Medicine]), and repair and regeneration.[103,125,127] However, recellularized dECM constructs are preferred when manufacturing multilayered, thick, or complex organ models, where recruiting a sufficient number of endogenous cells would be a challenge.[125]

Lastly, the general risks associated with 3D-printed dECM implantable constructs, such as immune responses,[140] dislodgement, and migration of the implants,[141] must also be considered from an ethical standpoint. Ethical guidelines for 3D-printed tissues are primarily provided by national organizations and at institutional levels; however, certain prominent international organizations, such as the United Nations Educational, Scientific, and Cultural Organization's International Bioethics Committee and the European Molecular Biology Laboratory Ethics Board, also play important roles. At a national level, the risks associated with dECM 3D-printed implantable constructs are evaluated by the Institutional Review Boards in the United States, the Research Ethics Board in Canada, and in Europe, by the Independent Ethics Committee, and through general guidelines created by the European Commission. These ethics boards carefully review each proposed technology and weigh the benefit versus the potential harm caused, monitor ongoing projects, and ensure informed consent and the protection of vulnerable populations in research.[142–144] However, rapid advancements in the fields of tissue engineering and regenerative medicine have led some researchers to propose a specialized ethics framework to ensure that all technological advancements in the field are guided through careful consideration of their societal implications.[141] For example, one specific area of ethical concern is ensuring that tissue engineering technology is made accessible to all members of the population, regardless of differing socioeconomic status.[141,145]

In summary, the incorporation of dECM into regenerative medicine technologies introduces unique ethical challenges, including the dECM tissue source, the source of cells used during recellularization, and the risks associated with implantable constructs. Well-established regulatory and ethical organizations at both international and national levels currently dictate the development of 3D-bioprinted dECM products. However, ethical challenges persist and will continue to grow as the field rapidly develops.

4.6. Summary of challenges

Although 3D bioprinting with dECM bioinks shows great potential for vascularized tissue engineering, several obstacles must be overcome to enable its widespread use in clinical settings and large-scale applications. Future research should focus on improving the printability and mechanical stability of 3D-printed objects by refining crosslinking strategies and rheological properties. This will improve shape accuracy and structural strength. In addition to promoting the growth of vascular networks, it is crucial to focus on improving their stability and functionality. This can be achieved by developing advanced techniques that allow the creation of interconnected and functional vascular systems that can seamlessly integrate with the existing vasculature. Furthermore, minimizing immunogenicity and variability is crucial, and this can

be accomplished by refining decellularization protocols and implementing standardized quality control measures to guarantee consistent results across different batches. Ultimately, overcoming scalability and regulatory hurdles will necessitate the creation of efficient, automated bioprinting platforms and strict adherence to clinical safety protocols. By successfully addressing these obstacles, bioinks and 3D bioprinting technologies can play a crucial role in creating functional, vascularized tissues for regenerative medicine and organ transplantation.

5. Future perspectives

The rapid advancements in bioink design and bioprinting are revolutionizing tissue engineering and regenerative medicine. Future research is expected to focus on developing hybrid bioinks that enhance both biological functionality and mechanical properties, smart bioinks capable of controlled biomolecule release, *in situ* bioprinting for direct tissue repair, artificial intelligence-driven biofabrication optimization, and patient-specific bioinks for personalized regenerative therapies. These innovations aim to overcome current limitations in bioprintability, vascularization, and clinical translation, paving the way for more effective and scalable tissue engineering solutions.

5.1. Advanced bioink design

The development of hybrid bioinks that integrate dECM with synthetic polymers is a promising strategy for enhancing both bioactivity and structural integrity. dECM provides essential biochemical cues that support cell adhesion, proliferation, and differentiation, mimicking native ECM. However, its inherent weak mechanical properties and limited printability necessitate the incorporation of synthetic polymers such as poly(ethylene glycol) diacrylate, gelatin methacryloyl, and nanocellulose, which enhance viscosity, shear-thinning behavior, and crosslinking efficiency.[117,123] Recent advances in modular bioinks, where dECM microgels are incorporated within composite hydrogels, have demonstrated improved post-printing cell viability and tissue-specific functionality.[20] Additionally, the integration of nanomaterials, such as carbon nanotubes and graphene oxide, has been explored to enhance mechanical strength and electrical conductivity, particularly for applications in neural tissue engineering and biosensing.[146,147] Tissue-specific dECM bioinks further refine this approach by providing biologically relevant microenvironments tailored to specific organ systems, such as liver, cartilage, and cardiac tissues, improving their regenerative potential.[119]

5.2. Smart bioinks

The emergence of smart bioinks introduces new opportunities for controlled biomolecule delivery and enhanced tissue functionality. Stimuli-responsive bioinks, designed to release growth factors or other bioactive molecules in response to environmental cues, offer precise spatiotemporal control over tissue development.[148] An important application of smart bioinks is the controlled release of angiogenic factors, such as VEGF, which plays a crucial role in promoting vascularization within bioprinted constructs.[149] Aptamer-based bioinks have demonstrated the ability to sequester and release VEGF in response to specific triggers, guiding endothelial cells in forming hierarchical vascular networks. Furthermore, bioinks incorporating pH-sensitive polymers enable VEGF release in response to the slightly acidic microenvironment of regenerating tissues, optimizing localized angiogenesis.[150,151] Other innovations include bioinks embedded with extracellular vesicles or liposomes that deliver microRNAs involved in angiogenesis regulation, allowing sustained modulation of gene expression in encapsulated cells.[151] These smart bioinks hold significant potential for improving vascular integration, reducing ischemic tissue loss, and enhancing overall tissue maturation in complex bioprinted constructs.

5.3. *In situ* bioprinting

In situ bioprinting represents a transformative approach to tissue engineering by enabling direct deposition of bioinks at the site of injury, eliminating the need for pre-fabricated scaffolds and reducing the time required for tissue maturation. This technology is particularly beneficial for treating large-scale tissue defects, burn wounds, and cartilage injuries, where immediate bioprinting at the injury site enhances integration with host tissues.[119,152] The development of portable and robotic bioprinting systems has significantly improved the precision and feasibility of *in situ* bioprinting, allowing real-time adjustments based on anatomical variations and patient-specific needs. These systems often incorporate advanced imaging techniques, such as optical coherence tomography and real-time ultrasound, to ensure accurate deposition of bioinks within complex tissue geometries.[152] Additionally, patient-specific dECM bioinks used in conjunction with *in situ* bioprinting further enhance tissue integration by providing a biomaterial that closely matches the patient's native ECM composition.[123] As *in situ* bioprinting technology continues to advance, its applications are expected to expand into regenerative therapies for musculoskeletal, cardiovascular, and dermal tissue repair.

5.4. Integration with artificial intelligence and machine learning

Artificial intelligence and machine learning are increasingly transforming bioprinting by optimizing bioink formulations and printing parameters with greater precision and efficiency.[153] Artificial intelligence-

driven models utilize large datasets to predict key bioink properties—such as rheology, printability, and mechanical stability—minimizing trial-and-error experimentation and enhancing reproducibility.[24,46] Notably, Bayesian optimization techniques have enabled the fine-tuning of bioink compositions, ensuring optimal viscosity and crosslinking behavior tailored to specific applications.[24,154] Neural network-based models have also emerged to evaluate cell viability under varying printing conditions, allowing real-time adjustments that support high post-printing cell survival.[155] Furthermore, artificial intelligence-integrated bioprinting platforms now offer real-time feedback and error correction, dynamically adjusting parameters to improve construct fidelity and mechanical integrity.[46,156] Alongside these technological innovations, materials science is also advancing rapidly, particularly in the development of personalized bioinks that enhance tissue compatibility.

5.5. Personalized medicine

The use of patient-specific dECM bioinks is revolutionizing personalized regenerative medicine by enabling the creation of biologically relevant, immune-compatible tissue constructs. Unlike traditional bioinks, which rely on allogeneic or xenogeneic ECM sources, patient-specific dECM bioinks are derived from the individual's own tissues, ensuring a tailored biochemical and biomechanical environment that promotes better tissue integration and function.[123,147] These bioinks offer significant advantages in reducing immune rejection, enhancing cell–matrix interactions, and improving overall regenerative outcomes. Additionally, the mechanical and structural properties of patient-specific dECM bioinks can be customized to match the requirements of specific tissue types, such as increased stiffness for bone regeneration or enhanced elasticity for soft tissue repair.[117,147] The combination of patient-specific dECM bioinks with *in situ* bioprinting has demonstrated great promise in clinical applications, particularly in reconstructive surgery, chronic wound healing, and cardiovascular tissue engineering.[119,152] As the field progresses, personalized biofabrication approaches are expected to become a cornerstone of precision medicine, offering tailored regenerative solutions for a wide range of patient-specific needs.

6. Conclusion

This review highlights the importance of ECM as a dynamic reservoir of biochemical and mechanical cues that regulate cellular behavior through mechanotransduction. These processes play a fundamental role in guiding tissue function by influencing gene expression, cell adhesion, proliferation, migration, and maturation. We also address the ongoing challenges in 3D tissue bioprinting, particularly the difficulty in generating vascularized networks that ensure the compatibility and functionality of bioprinted constructs for transplantation. dECM-based biomaterials have emerged as promising candidates due to their ability to preserve the native structural and molecular characteristics of ECM, thereby enhancing vascular network formation and tissue integration. However, several hurdles remain. These include the standardization of decellularization protocols and dECM sources to minimize immunogenic responses, as well as the development of optimized bioink formulations that improve the mechanical stability of 3D scaffolds while preserving the biological cues necessary for cellular support and tissue maturation. Emerging technologies and strategies such as artificial intelligence, machine learning, *in situ* bioprinting, and personalized medicine offer promising strategies to address these limitations by enabling more precise optimization of bioprinting parameters and reducing immune-related complications.

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Conflict of interest

Dr. Willerth is the C.E.O. and co-founder of Axolotl Biosciences, a biotechnology company that sells novel bioinks. The rest of the authors declare they have no competing interests.

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