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Review

Recent Advances in the Application of 3D-Printing Bioinks Based on Decellularized Extracellular Matrix in Tissue Engineering

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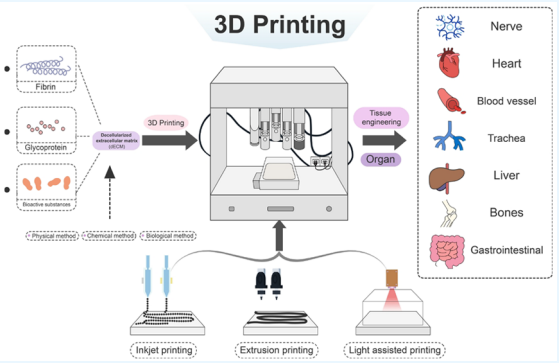
ABSTRACT: In recent years, 3D bioprinting with various types of bioinks has been widely used in tissue engineering to fabricate human tissues and organs with appropriate biological functions. Decellularized extracellular matrix (dECM) is an excellent bioink candidate because it is enriched with a variety of bioactive proteins and bioactive factors and can provide a suitable environment for tissue repair or tissue regeneration while reducing the likelihood of severe immune rejection. In this Review, we systematically review recent advances in 3D bioprinting and decellularization technologies and comprehensively detail the latest research and applications of dECM as a bioink for tissue engineering in various systems, with the aim of providing a reference for researchers in tissue engineering to better understand the properties of dECM bioinks.



1. INTRODUCTION

Tissue engineering is the use of cells, biomaterials, and biomolecules to construct biologically active tissues or substitutes.[1] In the early days of tissue engineering, cultured cells were first deposited on biological scaffolds to form cell−material complexes, after which the cell-containing scaffolds were implanted into the body, where the *in vivo* environment induced the formation of the corresponding tissues or organs.[2] However, conventional tissue engineering faces problems such as inaccurate spatial localization of cells, uncontrolled cell density deposition, and scaffold−tissue mismatch.[3] To address these difficulties, researchers are applying emerging 3D bioprinting technologies to tissue engineering to construct bionic tissues with precise control of the composition, spatial distribution, and structure.[4] 3D bioprinting is a technology that utilizes 3D-printing technology combined with "bioinks" to selectively deposit biomaterials, biologically active molecules, and living cells to create highly bionic, biologically functional artificial tissues and organ structures layer by layer.[5] Since 2003, when researchers at Clemson University first reported the printing of living cells,[6] 3D bioprinting with bioinks for tissue engineering has received increasing amounts of attention from researchers. dECM is an excellent candidate bioink because it is rich in a variety of bioactive proteins and bioactive factors,[7] which allows it to reduce severe immune rejection and provide a specific environment for tissue repair or tissue regeneration.[8] In this Review, we systematically review the latest applications of bioinks based on decellularized extracellular matrix in 3D-printed tissue engineering (Figure 1) for the treatment of cardiovascular, respiratory, digestive, and osteoarthritic diseases over the past five years, with the aim of providing new ideas for the selection and design of bioinks for 3D-printed tissue engineering (the main discussion points of this Review are summarized in Table 1).

2. ARTICLE RETRIEVAL AND BIBLIOMETRIC ANALYSIS

To grasp recent advancements in decellularized extracellular matrix-based bioinks in 3D printing for tissue engineering, we conducted a metrological analysis of the articles in this field, Utilizing the "Web of Science Core Collection" database, using the terms "((3D bioprinting) OR (3D printing)) AND ((decellularized extracellular matrix) OR (dECM)) AND (tissue engineering)", and we identified 309 publications spanning 2015−2024 and generated statistical charts and knowledge maps using Excel 2021 and VOSviewer.[9] Our analysis revealed a trajectory of initial rapid growth followed by stabilization in global publications, indicative of evolving trends and increased international collaboration. Notable contributors to this field include leading nations such as China, the United States, and South Korea, with significant contributions from esteemed institutions like Pohang University of Science and Technology, Yonsei University, Zhejiang University, and

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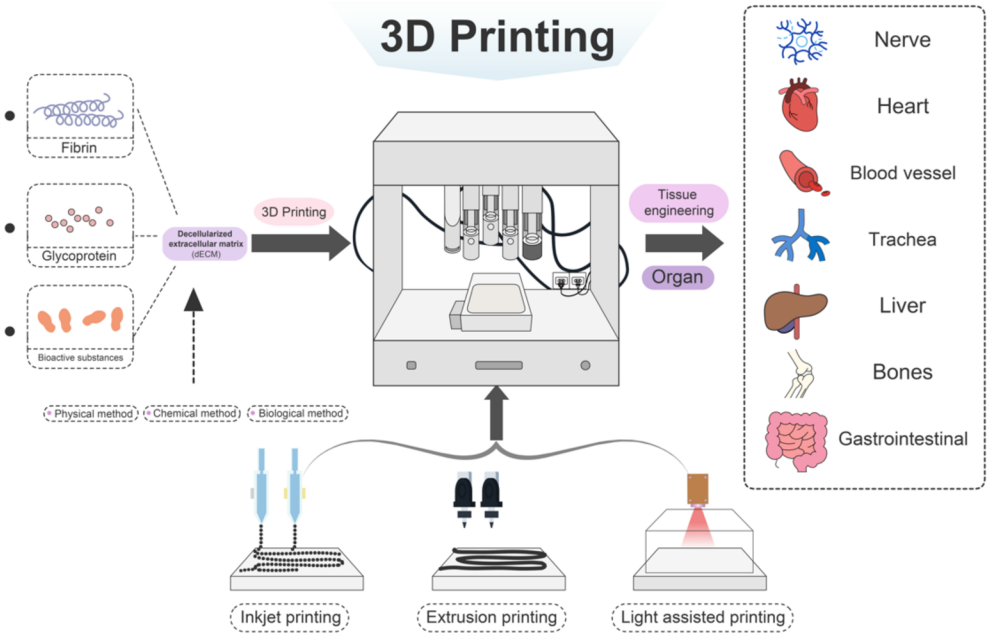

Figure 1. 3D-printing bioinks based on decellularized extracellular matrix in tissue engineering.

Table 1. Main Discussion Points of This Review

The main discussion points of this Review	Bibliometric analysis	Analysis of intercountry cooperation
		Analysis of interauthor collaboration
		Analysis of Institutional Published Literature
		Keyword co-occurrence analysis
	3D bioprinting	Inkjet printing
		Extrusion printing
		Light-assisted printing
	Bioinks based on dECM	Components of the dECM
		Functions of dECM
		Preparation of dECM
	Application of dECM as a bioink in 3D-printed tissue engineering	Utilization in the cardiovascular system
		Utilization in the respiratory system
		Utilization in the digestive system
		Utilization in Bones and muscles
		Utilization in the nervous system
		Utilization in skin and soft tissue
		Utilization in other systems and organizations

Shanghai Jiao Tong University. Particularly influential research teams led by Cho DW and Jang J have demonstrated substantial publication output and extensive collaborative efforts. Furthermore, our investigation delineates key research areas within the application of dECM-based bioinks in tissue engineering, emphasizing topics such as "3D cell printing", "scaffolds", and "mechanical properties". Statistical analyses, presented in tables and figures, offer valuable insights for further exploration and understanding.

Figure 2 displays the yearly publication statistics, indicating a rapid growth in publications on dECM-based 3D printing bioinks since 2015. This expansion peaked in 2022 and has since stabilized. The total number of articles published in 2024 remains undisclosed.

The total number of publications from the top 10 countries/regions is depicted in Figure 3. According to the publication distribution across countries, China (91), South Korea (87), the United States (87), India (19), and Germany (15) rank 1–5 in this field. Additionally, Figure 3 illustrates that China, South Korea, and the United States have the most extensive international collaboration, indicating a clear trifocus. This collaborative effort highlights the leading research position of these countries on a global scale.

In Figure 4, Jang J's team ranked first with the largest total strength of links (TLS = 152), followed by Cho DW's team in second place (TLS = 149). Jang J's team also published the highest number of papers (39), while Cho DW's team received the most citations, indicating their significant influence in the field. Consequently, they are prominently featured in the author collaboration graph.

Figure 5 displays the number of publications by each institution, revealing significant contributions from Pohang University of Science and Technology, Yonsei University, Zhejiang University, and Shanghai Jiao Tong University to the field.

Figure 6 examines the commonly utilized keywords in the relevant research articles. "3D bioprinting", "tissue engineering", "extracellular matrix", "scaffolds", and "bioink" emerge as popular keywords with high frequency. This suggests that researchers in the field of dECM-based 3D printing bioinks have a strong focus on various aspects, including the preparation of dECM 3D printing scaffolds and the application of hydrogel.

3. 3D BIOPRINTING

3D bioprinting is an emerging technology for the fabrication of artificial 3D tissue structures containing cells and hydrogels,

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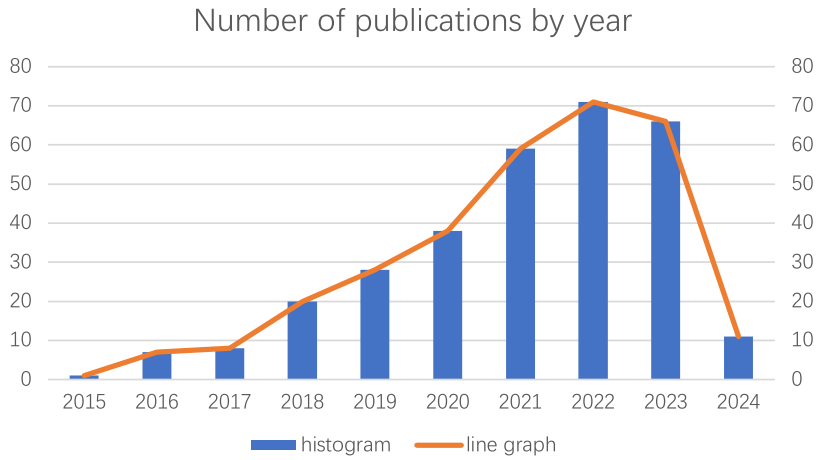

Figure 2. Number of publications from 2015−2024.

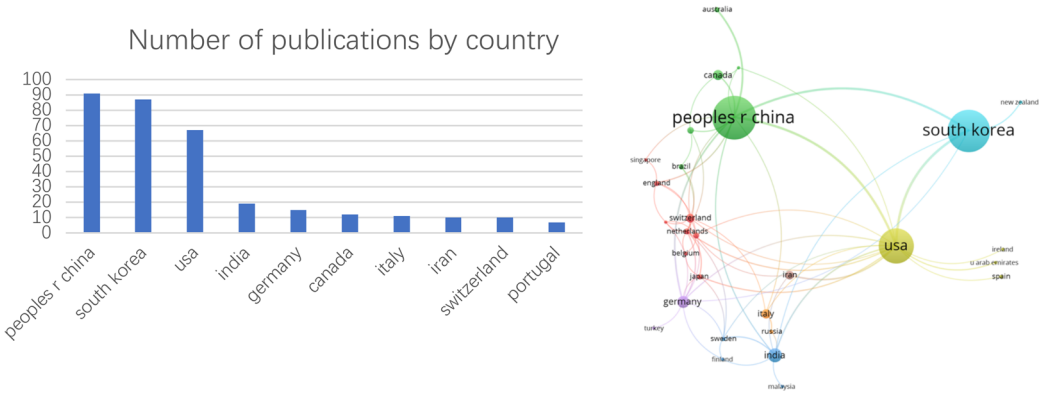

Figure 3. Number of publications by country and country cooperation network diagram

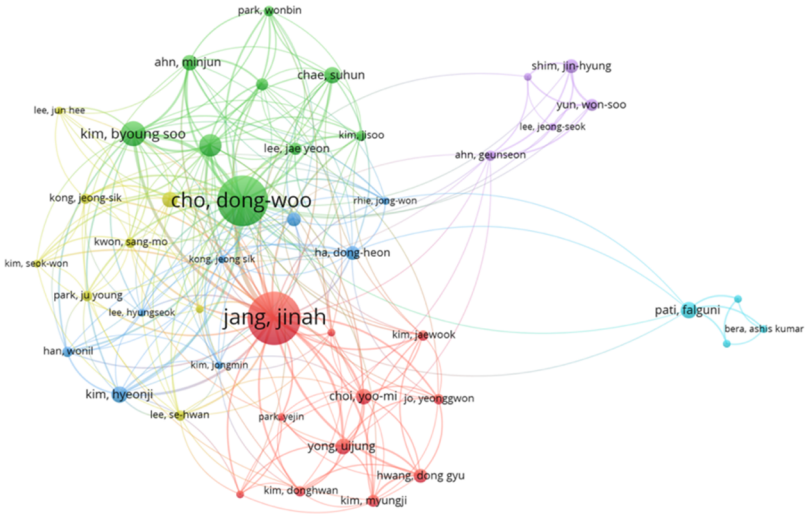

Figure 4. Author collaboration diagram.

which has applications in tissue engineering and regenerative medicine.[10] The development of 3D bioprinting has gone through five phases: materials without biocompatibility requirements (e.g., medical models/medical devices), biocompatible but nondegradable materials (e.g., permanent implants), biocompatible and resorbable-degradable materials (e.g., tissue-engineered scaffolds), live-cell bioink materials (e.g., in vitro biomimetic models), and miniature organoids.[11] At present, research on the first and second phases is very mature, while the construction of micro-organoids in the fifth phase is still in its infancy. Thus, the key directions of current research are the third and fourth phases, with a focus on biocompatible resorbable-degradable materials, such as tissue engineering scaffolds, and on *in vitro* biological modeling and other uses of live-cell bioink materials.[12] Accordingly, this paper explores the application of dECM-based bioinks in 3D-printed tissue.

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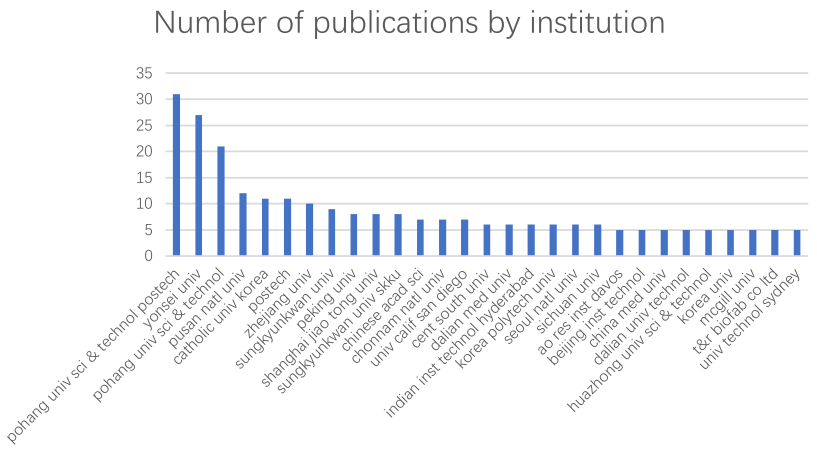

Figure 5. Statistics of published articles in the institutions.

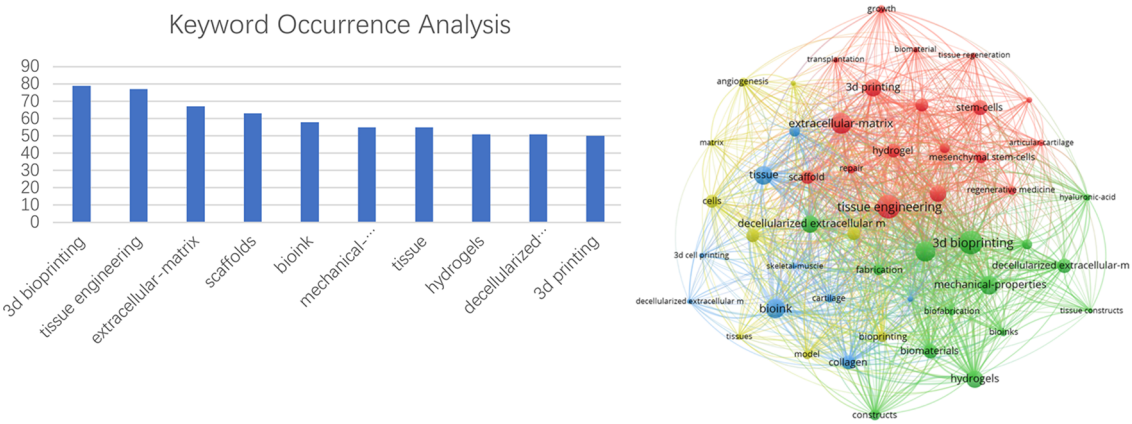

Figure 6. Keyword occurrence analysis.

To successfully perform 3D cell printing with dECM bioinks, the formulated bioinks need to be accurately and precisely deposited with the required spatial and temporal control. This process is primarily controlled by the print module, and the specific requirements vary depending on the operating principle of the print module used.[13] Currently, the commonly used modes of 3D bioprinting are inkjet printing, extrusion printing, and light-assisted printing. Inkjet-based printing methods were among the first used and depend largely on the physical properties (density, surface tension, and viscosity) of the bioink for the successful deposition of droplets onto the substrate. In inkjet-based bioprinting systems, the droplet size and deposition rate are largely dependent on the viscosity and surface tension of the biomaterial.[14] Therefore, this printing method is a test of the physical properties of the bioink itself.[15] Extrusion 3D bioprinting is currently a widely used 3D bioprinting mode.[16] Extruded 3D cellular bioprinting systems can be categorized as mechanical or pneumatic systems that continuously extrude cell-containing bioink through nozzle holes. The mechanical systems include piston-driven and screw-deposition systems. Piston-driven systems allow more direct manipulation of the flow of bioink, while deposition via a screw provides greater spatial control, allowing the printing of bioink at higher temperatures.[17] Due to the fragile nature of cell membranes, the intense forces generated by mechanical systems can lead to death of the encapsulated cells, limiting their application. Pneumatic systems can be controlled by air pressure to deposit a larger range of bioinks with different viscosities, thus promoting cell survival.[18]

Light-assisted bioprinting is a newer bio-3D printing paradigm, widely used in bio-3D printing research, and is based on tuning a light source to polymerize light-curable bioinks.[19] Light-assisted printing can be divided into three main modules: laser-induced forward transfer (LIFT), stereolithography appearance (SLA), and digital mirror device (DMD) modules. SLA is the most commonly used of the three techniques and is based on the principle of using a light source to selectively polymerize photo-cross-linking resins.[20] Most SLA printers are configured so that the light source polymerizes a bath filled with liquid resin onto a moving table. Once a layer is polymerized, the moving table is lowered to a predetermined distance (based on the vertical resolution of the SLA printer) to polymerize another layer. Although SLA has higher print speeds than other methods, it has lower resolution and lower biocompatibility. Continuous digital light printing (DLP) or DMD can improve light-assisted bioprinting by significantly shortening the curing process using computer-controlled arrays of micromirrors to generate dynamic masks while polymerizing a unit volume of resin.[21]

4. BIOINKS BASED ON DECM

The extracellular matrix (ECM) is a natural structure with biochemical and biomechanical functions that facilitates cell adhesion, proliferation, migration and differentiation.[22] The ECM consists of a variety of proteins, proteoglycans, growth

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factors and cytokines, and a wide range of other biologically active molecules that provide an important microenvironment for tissue survival, maintenance of function, and repair of damage.[7] However, since the native ECM has a complex structure and is prone to cause immune reactions with allogeneic tissues, researchers have prepared dECM by decellularization treatment, removing components of the native ECM that might induce immune reactions and retaining those that are conducive to cellular growth and adhesion for improved application in organ transplantation and tissue engineering.[23]

4.1. Components of the dECM. The composition of the dECM varies slightly from tissue to tissue, but it consists of two major classes of macromolecules, fibronectin (including collagen types I, II, III, IV, VI, X, and elastin) and glycoproteins (including proteoglycans, fibronectin, and laminin), as well as a number of other biologically active molecules.[24] These cell-associated proteins provide strength and perform space-filling functions in tissues and are involved in the regulation of protein complexes, cell signaling, and the binding of growth factors to promote cell adhesion.

Fibrin consists of collagen, elastin, and other proteins. Its high mechanical strength provides tensile and compressive resistance to tissues;[25,26] therefore, researchers have utilized the structural and mechanical properties of collagen and elastin in dECM in the design biomaterials. Lu X et al.[27] demonstrated that the elastin and collagen in the extracellular matrix of the pulmonary visceral pleura (PVP) are useful biomaterials with suitable mechanical compliance and the ability to promote the repair and regeneration of various tissues. Glycoproteins are glycosylated and form a complex structure in the dECM that contributes to a variety of biological processes, such as cell adhesion, migration, and signaling, and plays important roles in cell recognition, cell growth, and cell differentiation.[28]

In addition, dECM contains a variety of adhesion proteins, matrix receptors, and bioactive molecules, which, together with fibronectin and glycoproteins, form a complex organic network to support cell survival and activity. Therefore, dECM has attracted much attention from researchers as an important scaffold material in tissue engineering.

4.2. Functions of dECM. The main function of dECM is to provide structural support for tissues and organs to maintain their morphology. In addition, dECM can influence cell growth, adhesion, proliferation, differentiation, and other behaviors by regulating bioactive molecules, receptor levels, and microenvironmental pH.[29]

dECM provides spatial structural support to the cellular space, serves as a matrix for cell adhesion and migration, and participates in the transmission of biomechanical forces.[30] The physical properties of dECM, such as hardness, porosity, insolubility, and morphology, greatly influence the mechanical behavior of each tissue and its cellular activity. Kajtez J et al.[31] utilized the spatial structure-supporting function of dECM to construct a 3D-printed microgel-extracellular matrix composite for guiding the adhesion, growth, and differentiation of human neural stem cells. In addition, dECM can localize and present soluble growth factors. It regulates the secretion and degradation activities of cells, affecting the process of tissue repair and remodeling. In damaged tissues, the extracellular matrix promotes the cellular secretion of new matrix molecules and assists in the repair of damaged tissues.[32] Vincent TL[33] has demonstrated that the extracellular matrix of articular cartilage can control the bioavailability of pericellular matrix-bound growth factors to drive tissue homeostasis and repair *in vivo*.

4.3. Preparation of dECM. Decellularization of the ECM to remove cells and most of the major histocompatibility complexes can eliminate inflammatory reactions, foreign body reactions, and the risk of immune rejection, thereby facilitating the repopulation of new cells; thus, the dECM preparation process is crucial.[34] The dECM preparation process mainly includes pretreatment for decellularization, decellularization, and sterilization of the dECM. Each step can include multiple treatments.

Excess unwanted tissue and fat should be removed prior to decellularization by selecting an appropriate pretreatment protocol based on the tissue type to simplify the cell removal process and thereby reduce protein denaturation.[35] For example, thin tissue layers are often pretreated for decellularization by physical methods, which involve exposing the tissue to be decellularized to reagents for a relatively short period of time followed by rinsing. Thicker tissue layers, on the other hand, require extensive biochemical exposure and longer rinsing times, and adipose tissues and organs (e.g., brain adipose tissue) often require dehydration for pretreatment.[36,37]

There are various methods of decellularization, which can be divided into three main categories, physical, chemical, and biological, as well as decellularization by a combination of two or more methods.[38] Physical methods of tissue decellularization include freezing and thawing, mechanical massage or direct pressure, osmosis, and agitation, among others.[39] Physical treatments allow the disruption of cell membranes, and the release of cellular components, and they facilitate subsequent flushing to remove cellular components from the ECM. Reagents for chemical decellularization include acids and bases, decontaminants, and hypotonic or hypertonic solutions.[38] Different decellularization methods have different advantages and disadvantages, and we have organized the results in Table 2 for reference. Biological methods for decellularization include the use of enzymes and the induction of apoptosis, with enzymes being the most widely used.[40] The enzymes used for decellularization typically include trypsin, collagenase, lipase, and nucleases, both ribonuclease (RNase) and deoxyribonuclease (DNase). However, complete decellularization is difficult to achieve by a single passaging enzyme, and the presence of residual enzyme residues may hinder the decellularization of the ECM and even trigger an adverse immune response.[41] Apoptosis is a novel method of decellularization that utilizes exogenous or endogenous pathways to induce apoptosis, after which the cell membrane undergoes structural changes, resulting in the loss of contact between the cell and the ECM[42] and thereby achieving decellularization. In addition, since the cellular contents are strictly preserved within apoptotic vesicles and the cell membrane, immunogenic substances do not leak into the surrounding ECM.[43] Therefore, decellularization methods that control the activation of apoptotic pathways by delivering appropriate signals have been created. For example, the exogenous apoptosis induction pathway can be activated by specific ligands of the tumor necrosis factor superfamily death receptors. Intrinsic pathways can be activated by the application of environmental stresses or gene editing to place cells in an apoptotic state.[42]

The prepared dECM must be further sterilized to reduce the immune response as well as the possibility of infection.[44] Currently, commonly used sterilization methods for dECM

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Table 2. Fabrication of dECM

dECM fabrication step	methodologies	specific method	advantages and disadvantages
Preprocessing of dECM	Physical method	Squeezing, crushing, mixing	
	Chemical-biological method	Prolonged biochemical exposure and prolonged flushing	
Decellularization of dECM	Physical method	Freezing and thawing	Advantages: ease of operation Disadvantages: complete decellularization is difficult; further decellularization is often necessary
		Pressurization	Advantages: destroys cell membranes in tissues and organs while inactivating bacteria Disadvantages: decellularization may not be complete
		Mechanical crushing	Advantages: ease of operation and low cost Disadvantages: incomplete decellularization
	Chemical method	Acid-alkali method	Advantages: less expensive and less time-consuming Disadvantages: ECM architecture is damaged, affecting the content of the ECM[46]
		Detergent method	Advantages: can effectively remove cytoplasmic and nuclear material from tissues by dissolving cell membranes and dissociating DNA from proteins Disadvantages: tends to destroy growth factors, collagen, etc.[37,47]
		Hypotonic or hypertonic solution methods	Advantages: decellularization by osmotic effect, which can disrupt DNA−protein interconnections for cytolysis, particularly useful for removing cellular debris from tissues such as the cornea[48,49]
			Disadvantages: tends to lead to incomplete decellularization
	Biological method	Enzymatic decellularization	Advantages: highly specific decellularization, removes cellular and unwanted ECM components by disrupting cell-matrix junctions Disadvantages: complete decellularization is difficult to achieve with a single enzyme, and the presence of residual enzymes may prevent cellular decellularization and trigger adverse immune responses
		Apoptosis induction method	Advantages: immunogenic substances do not leak into the surrounding ECM Disadvantages: mechanisms of apoptosis are complex and not widely used

include autoclaving, dry heat sterilization, chemical reagent sterilization, electron beam irradiation, gamma irradiation, ethylene oxide sterilization, and supercritical carbon dioxide.[45] Each sterilization method has different advantages and disadvantages and is applicable to different tissues; therefore, the method should be selected according to the specific decellularized tissue.

5. APPLICATION OF DECM AS A BIOINK IN 3D-PRINTED TISSUE ENGINEERING

In tissue engineering applications, dECM-based bioinks are usually composed of dECM and hydrogels, which are used to enhance the mechanical properties and printability of the dECM. In addition, dECM can be combined with synthetic and other natural materials to form bioinks with different mechanical, printable, and electrical properties to meet different requirements for use in 3D printing for tissue engineering. The following is a detailed overview of the applications of dECM bioinks for 3D printing in tissue engineering over the past five years, categorized by system and organ.

5.1. Cardiovascular System. *5.1.1. Heart.* Various bioinks are currently used for cardiac tissue engineering via 3D cell printing technology; however, the lack of a suitable culture microenvironment results in poor functionality of the cultured cardiomyocytes. To solve this problem, researchers have focused on the ECM. The ECM plays a key role in regulating cardiomyocyte differentiation and maturation; therefore, dECM-based bioinks are considered promising candidates for improving the functionality of cultured cardiomyocytes in cardiac tissue engineering.[50]

Tissue-engineered hearts with excellent mechanical properties for cell and tissue regeneration have been prepared by using bioinks consisting of dECM combined with biopolymers (e.g., sodium alginate) or synthetic organic polymers (e.g., PCL, GelMA).[51] Basara et al.[52] developed a bioink consisting of decellularized human heart dhECM, and a gelatin methacryloyl (GelMA) or GelMA-methacrylated hyaluronic acid (MeHA) hydrogel double cross-linked with ultraviolet light and microbial transglutaminase (mTGase), which exhibited excellent mechanical properties and was compatible with human induced pluripotent stem cell-derived cardiomyocytes (iCMs) and human cardiac fibroblasts (hCFs). A breakthrough in improving the electrical properties of tissue-engineered hearts was made by Tsui JH et al.,[53] who created a hybrid hydrogel from porcine myocardial dECM and reduced graphene oxide (rGO) to provide a more suitable microenvironment for normal cell and tissue development. An experimental study revealed that tissue-specific protein profiles were retained after decellularization and that the mechanical and electrical properties of the hydrogels could be tuned by adjusting the rGO content and the degree of reduction. Engineered heart tissue (EHT) generated using dECM-rGO hydrogel scaffolds and human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes exhibited significantly increased contractility and upregulated expression of genes that promote contractile function. In addition, the hybrid biomaterial improved various aspects of electrophysiological function. Shin et al.[54] on the other hand reported a hydrogel bioink consisting of partially digested decellularized extracellular matrix (cdECM) from porcine heart tissue, Laponite-XLG nanoclay, and poly(ethylene glycol)-diacrylate (PEG-DA). Laponite facilitates extrusion-based 3D printing, whereas

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PEG-DA enables postpress photopolymerization, which allows a more flexible choice of 3D printing method to obtain tissue-engineered hearts from dECM bioinks. In addition, Kupfer M,[55] Noor N,[56] Silberman E,[57] and others have formulated dECM-based bioinks for cardiac tissue engineering using different materials and successfully obtained different properties (the specific studies are presented in Table 3).

5.1.2. Vasculature. Synthetic blood vessels made of polytetrafluoroethylene (ePTFE), which are currently commonly used in clinical practice, are prone to thrombosis and endothelial hyperplasia and have poor long-term patency rates, and their ability to meet clinical needs is severely limited. Therefore, tissue-engineered blood vessels, which are more closely related to natural tissues and have a high degree of biocompatibility, are needed to solve these problems.[58] 3D printing provides an excellent opportunity to fabricate biostructures with specific geometries, clinically relevant dimensions, and suitable functionalities for biomedical applications, and bioinks based on dECM can provide an environment that is conducive to cell survival, both of which provide new opportunities for tissue-engineered vasculature.[59] Isik M et al.[59] reported a 3D-printed hydrogel with high elasticity, self-recovery properties, excellent fluid dynamics, and improved biological activity. This hydrogel combines the fast gel dynamics of sodium alginate (Alg), the in situ cross-linking of hyaluronate-tyramine (HAT), and the temperature-dependent self-assembly and biofunctionality of decellularized aortic veins (dAECMs) to enable the fabrication of tissue-engineered vascular constructs using extrusion-printing-based methods, with potential applications in vascular tissue engineering and regenerative medicine. Kamaraj M et al.,[60] on the other hand, innovatively recovered discarded varicose veins for the isolation of endothelial cells and decellularized them to formulate dECM bioinks. The results showed that 3D-bioprinted blood vessels generated via this method can work well without secondary support and have potential as a tool for tissue-specific vascular regeneration. Cohen R,[61] Du J,[62] and others have also applied dECM for vascular tissue engineering, and we have described these studies in Table 3.

5.2. Respiratory System. *5.2.1. Trachea.* The trachea not only serves as a passageway for gas exchange but also performs functions such as regulating air temperature and humidity and removing foreign bodies. Structural problems of the trachea caused by congenital anomalies, developmental insufficiencies, trauma, chronic inflammation, or cancer are rare but life-threatening diseases.[63−65] Currently, the "gold standard" for the treatment of this type of disease is tracheotomy with end-to-end anastomosis.[66] However, when the lesion is more than half the length of the trachea in adults or one-third of the length of the trachea in children, there is not enough native trachea for anastomosis, and suitable tracheal substitutes are needed to reconstruct long tracheal defects.[67] In recent years, tissue-engineered trachea grafts developed on the basis of 3D-printing technology have been used to solve this problem. dECM has been used in bioink formulations due to its high biocompatibility and its ability to provide a favorable microenvironment for cell growth and reproduction.[68] De Santis MM et al.[69] designed a specific hybrid bioink consisting of the natural polymer alginate and dECM. They used this ink for the preparation of tissue-engineered trachea by 3D bioprinting and showed that the lumen of this trachea remained patent with living cells for 1 month in vitro, and there was evidence that the epithelial progenitor cells therein

Table 3. dECM-Based Bioinks in the Cardiovascular System

	researchers	date of publication	bioink composition	results and implications
Heart	Silberman E[57]	2023−08	Induced pluripotent stem cells (iPSC) and extracellular matrix (ECM)	The bioink has good mechanical properties and good shrinkage.
	Kupfer ME[55]	2020−07	ECM proteins, GelMA, LAP, etc.	Macroscale beating function in a geometrically complex, perfusable chamber structure was achieved for the first time. The bioink allowed extensive proliferation of stem cells prior to differentiation, resulting in a continuous muscle wall up to 500 μm thick.
	Noor N[56]	2019−06	Cardiomyocytes and endothelial cells and their ECM	Cardiomyocytes elongated and streaked with large amounts of actin after cardiac patch transplantation
Vasculature	Cohen R[61]	2023−10	Combination of natural ECM and alginate bound to the laminin adhesion molecule motif (YIGSR)	Blood vessels fabricated with YIGSR-ECM bioink exhibited satisfactory structural fidelity and supported the formation of monolayers of iPSCs-derived ECs on the inner surface of their channels.
	Du J[62]	2022−01	Decellularized porcine coronary artery (DPCA), hydrogels mixed with gelatin and sodium alginate in seven different ratios	With the increase of hydrogel content, the cells on the 3D scaffold formed cell colonies more quickly. The results show that the scaffold has high biocompatibility and can meet the needs of artificial blood vessel construction.

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progressively differentiated into mature epithelial cells, like those found in the natural human trachea. Weber JF[70] used a 3D-printed porcine-derived small intestinal submucosa (SIS) ECM patch in tissue-engineered trachea grafts. The experimental results showed that the SIS-ECM can promote tissue regeneration; however, further investigations are needed to determine how to match the microenvironment of the tissue-engineered trachea. Currently, the use of dECM-derived bioinks in tissue-engineered trachea tissue is an emerging technique, and in the future, the advantages of each method need to be integrated to construct biocompatible tissue-engineered trachea grafts with good pro-vascularity and chondrogenesis.

5.2.2. Lung Tissue. The increasing incidence of chronic lung disease has led to an increasing demand for lung transplantation, but because the number of natural donors is limited, the demand has promoted the development of lung tissue engineering.[71] Pouliot RA et al.[72] conducted a preliminary characterization of extracellular matrix hydrogels of porcine lung origin to explore the potential of decellularized matrices for use as bioinks. The results showed that in the process of lung dECM hydrogel preparation, the length of enzyme digestion time had a significant effect on the proteolysis, soluble protein distribution, gelation, mechanical and biological properties, and 12 h of pepsin digestion of porcine lung dECM was able to achieve an optimal equilibrium between the desired physical properties of ECM hydrogels and the effect on the behavior of lung cells. Noori A et al.[73] further found that lung dECM scaffolds promote the differentiation of human embryonic stem cells into alveolar progenitor cells, which provides guidance on the use of dECM for lung tissue engineering. In research on 3D printing practices, Falcones B and colleagues[74] developed a bioink based on decellularized porcine lung ECM hydrogel. This bioink was suitable for the 3D culture of lung MSCs without additional chemical or physical cross-linking. MSCs also exhibited good viability in the bioprinted scaffolds, and shrinkage assays revealed cell−matrix interactions. In the future, additional dECM-based bioinks will be applied in lung tissue engineering to provide safer and more functional graft donors for lung transplantation.

5.3. Digestive System. *5.3.1. Digestive Tract.* The application of dECM-based 3D printing bioinks for tissue engineering in the digestive tract has focused on tubular organs such as the esophagus and colon, but the overall number of such studies remains limited. Ha DH et al.[75] developed an esophagus-derived dECM (EdECM)-based hydrogel and fabricated an EdECM hydrogel-loaded scaffold using a combinatorial 3D printing system, which was implanted into a rat model of radiation esophagitis. The results of the study showed that delivery of the EdECM hydrogel through the scaffold platform resulted in rapid resolution of the inflammatory response, promoting a pro-regenerative microenvironment. This finding suggested that this approach is a promising therapeutic avenue for the treatment of radiation esophagitis. Researchers have also explored the use of dECM bioinks in gastric cancer models. Kim J et al.[76] developed a gastric tissue-derived decellularized extracellular matrix (g-dECM) bioink for the creation of 3D gastric tissue-specific microenvironments and found that the incorporation of cellulose nanoparticles (CNs) in the matrix made its mechanical properties more conducive to the progression of gastric cancer and that g-dECM bioinks with CNs could be used to print a variety of individual 3D shapes, including gastric wrinkles. This finding suggested that the proposed model can be used to develop physiologically relevant gastric cancer systems for use in future preclinical trials. For use in the colon, researchers including Han H[77] developed colon-derived decellularized extracellular matrix (colon dECM) bioink. This tissue-specific biomaterial exhibited the potential to guide the maturation of human intestinal cells, and human intestinal epithelial cells in 3D bioprinted intestinal tissue models spontaneously underwent 3D morphogenesis without any external stimuli. Thus, the system can be used as a platform for evaluating the effects of potential drug toxicity on human gut tissue and for creating coculture models of symbiotic microorganisms and immune cells for future therapies. Currently, few dECM-based 3D-printed digestive tracts have been used in animal experiments, possibly because of the complex composition of the natural digestive tract, the difficulty in establishing its microenvironment and the high requirements for mechanical properties. In the future, these problems are expected to be solved through continued exploration and attempts.

5.3.2. Liver. Previous studies have shown that most 3D-printed biomaterials partially or completely lack liver-specific ECM components, which may limit their bionic mechanical properties and biological functions.[78] Therefore, dECM-based bioinks are valued for their ability to provide a suitable microenvironment for cell growth and reproduction, as well as for their role in promoting tissue regeneration. Lee H et al.[79] developed 3D liver microarrays with multiple cell types for the coculture of hepatocytes and created vascular/biliary fluid channels for the vascular and biliary systems by utilizing the ability of liver dECM bioink to provide a suitable microenvironment for hepatocytes. The experimental results showed that compared with the controls, chips with biliary fluidic channels induced better biliary system creation and increased liver-specific gene expression and function. Khati V et al.[78] reported the 3D bioprinting of a PVA framework with decellularized liver extracellular matrix (dLM) hydrogel in its trilobal liver structure and the use of polyethylene glycol-based cross-linking agents and tyrosinase to fabricate robust multiscale 3D liver structures. This technique provides patency for medium perfusion and can be used to fabricate complex liver models. The approach can also be applied to other tissue types by using different biomaterials and multiple cells to support the creation of large, functionally complex tissues. Artificial tissues such as liver that are 3D printed with dECM-based bioinks are often hampered by their inherently poor mechanical properties. Although 3D bioprinting with dECM-based bioinks using additional scaffold modifications has advanced the development of load-bearing structures in recent years, these attempts to use dECMs have mostly been limited to low-temperature bioprinting, which is not conducive to the use of cells for longer print durations. To address this problem, Khati V and research team[80] developed a multimaterial decellularized liver matrix (dLM) bioink reinforced with gelatin and polyethylene glycol to improve the rheology, extrudability, and mechanical stability of the bioink. This shear-thinning bioink facilitates extrusion bioprinting with HepG2 cells at 37 °C to form 3D lattice structures and further increases cross-linking with mushroom tyrosinase for long-term applications. Compared to non-cross-linked dLM, the recross-linked structure presented a 16-fold increase in viscosity (2.73 Pas-1) and a 32-fold increase in the storage modulus while maintaining high cell viability (85%−93%) and liver-specific function.

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5.3.3. Pancreas and Islets. Due to the high prevalence of diabetes mellitus worldwide, pancreatic tissue engineering is currently focused mostly on creating islets for transplantation through tissue engineering, thus providing new opportunities for the treatment of diabetes mellitus. Pancreatic tissue-derived extracellular matrix is a potential candidate for mimicking the microenvironment in natural pancreatic tissue. Insulin secretion is strongly upregulated when pancreatic islet cells are cultured in pancreatic tissue-derived decellularized extracellular matrix (pdECM), and the possibility of fabricating therapeutically applicable graft-sized 3D constructs was validated by using this culture medium as a bioink for 3D cell printing technology.[81] Kim J et al.[82] detailed the fabrication of pdECM and its bioink, explored ways to increase the bioactivity as well as to provide a beneficial microenvironment for pancreatic islet cells, and investigated the whole process of generating 3D pancreatic tissue constructs using extrusion-based bioprinting. Wang D et al.[83] combined pancreatic extracellular matrix (pECM) and hyaluronan acid methacrylate (HAMA) to develop a new tissue-specific bioink for the construction of pancreatic islet-like organs by 3D printing and verified that the HAMA/pECM hydrogel could maintain islet cell adhesion and morphology in vitro through the Rac1/ROCK/MLCK signaling pathway, which contributed to the improvement of pancreatic islet function and activity and facilitated the formation of neovascular networks. Therefore, this study provides hope for the long-term efficacy of islet transplantation.

For more information about the application of dECM as a bioink in the respiratory and digestive systems, we have organized this material in Table 4 for the reader's reference.

5.4. Bones and Muscles. *5.4.1. Bones.* 3D-printing bioinks based on dECM have a wide range of applications in bone tissue engineering. In spinal fusion, Driscoll JA et al.[89] developed a 3D-printed ceramic demineralized bone matrix superelastic bone composite scaffold, which consisted of a composite scaffold containing different volume ratios of hydroxyapatite (HA) and human demineralized bone matrix (DBM) in a polypropylene glycolide-ethylene glycolide copolymer (poly(lactide-co glycolide)), for the treatment of spinal fusion. The experimental results showed that the HA:DBM composite at a ratio of 3:1 achieved the highest average fusion score and fusion rate (92%), which were significantly greater than those of the 3D-printed DBM-only scaffold (42%). More critically, the combination of HA and DBM resulted in the growth of bone-like bone needles within the DBM particles in the scaffold. In another study on bone regeneration scaffolds with HA and DBM,[90] the researchers also designed a 3D-printed bone regeneration scaffold composed of stoichiometric HA ceramic particles and DBM particles (HA-DBM). The first preclinical comparative assessment of HA-DBM versus the industry standard was conducted using a rat posterior lateral spinal fusion (PLF) model, and recombinant human bone morphogenetic protein-2 (rhBMP-2) was used as a positive control. Female Sprague−Dawley rats were subjected to bilateral L4-L5 PLF and implanted with either HA-DBM scaffolds or rhBMP-2. The experimental results showed that the HA-DBM and rhBMP-2 groups had similar outcomes in terms of unilateral fusion rates and significantly elevated expression of relevant osteogenic genes; however, the rhBMP-2 treatment showed greater stability than the HA-DBM scaffold. Thus, both studies suggest that HA- and DBM-based composite 3D-printed materials hold promise

Table 4. Utilization of dECM-Based Bioinks in the Respiratory and Digestive Systems

	researchers	date of publication	bioink composition	results and implications
Respiratory system				
Trachea	Nyirjesy SC[84]	2023−10	Decellularized tracheal grafts (PDTGs) and composite tracheal grafts (CTGs; PDTGs supported by 3D-printed external splints)	PDTGs and CTGs support tracheal endothelial cell regeneration and neovascularization
	Liu L[85]	2022−01	Partial decellularized tracheal graft (PDTG), composite tracheal graft (CTG) composed of 3D printed airway splints	CTG was easily implanted and did not result in vascular erosion, tracheal injury, or inflammation. Graft epithelialization and endothelialization were comparable to those in the CTG control group. No tracheal collapse was seen with the CTG. The composite tracheal stent combines a biocompatible synthetic scaffold with PDTG to support host epithelial cell regeneration while maintaining graft architecture.
Lung	Dabaghi M[86]	2021−06	Various dECM concentrations were tested to form physically stable and biologically responsive human lung dECM hydrogels	dECM-based hydrogels support the growth and proliferation of primary human lung fibroblasts in 3D cultures. dECM is also suitable for polyester membrane coating in inserts to improve cell adhesion, proliferation and barrier function of 2D primary human bronchial epithelial cells.
Digestive system	Sharma A[87]	2021−08	Soluble natural decellularized liver (DCL) matrix, filipin protein (SF), a mixture of SF with gelatin (8% w/v) were optimized using different percentages of DCL to obtain silk gelatin-DCL bioinks (SG-DCL)	SG-DCL 3D-printed scaffolds may provide a favorable microenvironment for increasing hepatocyte differentiation and function through the activation of the Wnt/β-catenin signaling pathway.
	Nam H[88]	2020−04	Esophageal dECM	Experiments confirmed the feasibility of the tissue-engineered esophagus with porous multilayer structure fabricated in this study to promote cell proliferation

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as recombinant growth factor-free bone graft alternatives for spinal fusion, but further optimization is needed. dECM-based bioinks have also been developed for use in maxillofacial bone tissue repair, and Dubey N et al.[91] used ECM and amorphous magnesium phosphate (AMP) to establish a novel bioink formulation (ECM/AMP) that combines a hydrogel containing 2% octapeptide FEFEFKFK and 98% ECM in water with AMP particles to achieve bioprintability with desirable cellular functionality. Experiments showed that this ECM/AMP bioink significantly increased bone formation and is expected to be useful for dental-specific bone tissue regeneration. In addition, dECM-based 3D printing bioinks for other bone tissues are emerging as potential substitutes for conventional artificial bone materials.

5.4.2. Cartilage. dECM-based 3D-printing bioinks are widely used in cartilage tissue engineering, such as the treatment of joints and auricles. Meniscus injuries are common in patients with orthopedic diseases. The anisotropy and structural inhomogeneity of the meniscus are the main challenges in its reconstruction. Accordingly, meniscal tissue engineering has emerged as a potential treatment for various meniscal diseases and injuries. Chae S et al.[92] developed a 3D cell-printed meniscus construct using a mixture of polyurethane and polycaprolactone polymers as well as cell-loaded decellularized meniscus extracellular matrix (me-dECM) as a bioink. The bioink was shown to provide a favorable biochemical environment for 3D cell-printed meniscus constructs, supporting cell growth and promoting the differentiation of encapsulated stem cells into fibrocartilage. In addition, researchers have explored the *in vivo* performance of 3D cell-printed meniscus constructs, which exhibit biocompatibility, excellent mechanical properties, and improved biological functionality. Similarly, Wang B et al.[93] investigated the application of dECM-derived 3D-printing bioinks in meniscus tissues by applying different lysis and decellularization methods to natural porcine meniscal tissue to produce highly concentrated dECM bioinks with different biochemical contents and printabilities. All the resulting dECM inks exhibited shear-thinning and thixotropic properties, and increased viscosity and improved printability were observed at higher pH values, especially after thermogelation at pH 11. The dECM bioinks allowed the fabrication of highly elastic meniscus tissues with compressive-mechanical properties similar to those of natural tissues. In addition to treatment for meniscal injuries, there are many other applications of dECM-based bioinks for 3D printing in cartilage tissue engineering. For example, in a recent study, Shanto PC et al.[94] used TEMPO oxidized cellulose nanofibers (TOCN), decellularized extracellular matrix (dECM), and sodium alginate (SA) to prepare 3D-printed scaffolds for the regeneration of cartilage tissues and explored the effect of varying the ratio of different components on the bioink properties. The results showed that increasing the ratio of TOCN to dECM significantly improved the viscoelasticity, stability, mechanical properties, and printability of the scaffolds. Zhang X et al.[95] developed a 3D-printed scaffold fabricated from bioink containing different concentrations of sericin proteins and decellularized extracellular matrix (SF-dECM) blended with bone marrow mesenchymal stem cells (BMSCs). The results showed that the SF-dECM bioink had suitable mechanical strength and a suitable degradation rate, and the expression of cartilage formation-specific genes was greater than that in the SF control group.

5.4.3. Muscles and Tendons. Compared to bone and cartilage, muscle and tendon are more difficult to 3D print for tissue engineering, but some studies have explored the possibility. Choi YJ et al.[96] used dECM 3D-printing bioinks to explore a novel approach for treating volumetric muscle loss (VML). 3D-printed muscle structures containing cells showed high cell viability in a rat model of VML, did not produce hypoxia, and enhanced the formation of new volumetric muscle. To improve functionality, researchers fabricated prevascularized muscle structures that mimic the layered structure of vascularized muscle by printing muscle and vascular dECM bioinks through coaxial nozzles. Experiments have shown that spatially printed tissue-specific dECM bioinks provide organized microenvironmental cues for cell differentiation and improve vascularization, innervation, and functional recovery. Thus, this study demonstrated that dECM-based 3D cell-printing bioinks can be effective for generating bionically engineered muscles for the treatment of VML injuries. On the other hand, Chae S et al.[97] developed tendon tissue-derived dECM bioink (TdECM) to 3D print tendon-bone interface patches for the treatment of chronic rotator cuff repairs. 3D-printed rotator cuff tendon-bone interface (TBI) patches were created by spatially aligning cell-containing tendon- and bone-specific bioinks in a hierarchical manner. This TBI patch provided a cell-friendly microenvironment characterized by high cell viability, high proliferative capacity, and the region-specific differentiation of encapsulated stem cells; thus, this study showed that repair via 3D-printed TBI patches with dECM bioink significantly accelerated and facilitated TBI healing in a rat model of chronic tear.

We have summarized additional studies on dECM as a bioink in bone and muscle in Table 5 for reference.

5.5. Nervous System. Neural tissue regeneration is currently a popular topic in medical research. dECM promotes tissue regeneration and is thus a key candidate ingredient of bioinks for 3D printing in neural tissue engineering. Bae M et al.[105] investigated the use of brain-derived tissue-specific bioinks for neural stem cell delivery and thus for the treatment of traumatic brain injury. For this purpose, they developed a bioink based on brain-derived decellularized extracellular matrix (BdECM). This BdECM bioink has shear-thinning properties for 3D cell printing, as well as physical properties and a fiber structure comparable to that of the native brain, which is important for tissue integration after implantation. Human neural stem cells (NSCs) (F3 cells) loaded with BdECM bioink were shown to differentiate fully into mature differentiated neurons with higher levels of markers than those observed with collagen bioprotein in vitro. In peripheral nerves, Liu S et al.[106] explored the regulatory effects of decellularized peripheral nerve matrix hydrogels and systematically investigated the gelation conditions (including digestion time and gel concentration) and the mechanical properties and stability (sol−gel transition temperature, gelation time, nanotopography, and energy storage modulus) of decellularized peripheral nerve matrix hydrogels (DNM-G). The results showed that the fully digested decellularized neural matrix solution exhibited improved mechanical properties, a shorter gelation time and a lower gelation temperature, and DNM-G significantly increased the length and penetration depth of dorsal root ganglion (DRG) synapses. Wang T[107] reported a hybrid hydrogel system consisting of dECM-Gs and photo-cross-linkable gelatin methacrylate (GelMA) to address the disadvantages of low mechanical stability and rapid

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Table 5. Utilization of dECM-Based Bioinks in Bones and Muscles

	researchers	date of publication	bioink composition	results and implications
Bone	Hua Y[98]	2022−12	Photocross-linked cartilage/bone-derived dECMs	The scaffold is capable of reconstructing a biphasic cartilage-osteomimetic microenvironment. The biphasic cartilage-osteomimetic scaffold provides a 3D microenvironment for osteochondral regeneration.
	Hwangbo H[99]	2022−10	Poly(L-lactic acid) (PLLA), HA, dECM	The flexural modulus of HA/PLLA/dECM composites increased by a factor of 2.1 compared with that of ordinary HA/PLLA composites. HA/PLLA/dECM biocomposite scaffolds are promising scaffolds for application to bone tissue regeneration.
	Lee J[100]	2020−12	Alginate, methacrylated-decellularized extracellular matrix (Ma-dECM) from bone tissue	Ma-dECM promotes printability and increases the viability of loaded cells in cellular 3D-printing bioinks.
Cartilage	Sang S[101]	2023−02	Gelatin methacrylate, hyaluronic acid methacrylate, chondroitin sulfate methacrylate, chondrocyte ECM	Photocross-linked ECM hydrogels with suitable degradation rates and excellent mechanical properties. 3D bioprinted ECM scaffolds exhibited good shape fidelity and improved basic synovial membrane properties, biological properties and cartilage formation.
	Yang Z[102]	2022−09	Cartilage-specific extracellular matrix (ECM), gelatin methacrylate (GelMA), transforming growth factor-β3 (TGF-β3)-embedded poly(lactic acid)-glycolic acid copolymer (PLGA) microspheres	The scaffold successfully promoted tissue repair in a sheep animal model, explicitly guiding organized neotissue formation to recreate the anisotropic structure of natural articular cartilage.
	Rathan S[103]	2019−04	Cartilage extracellular matrix (cECM), alginate bio	The bioink was demonstrated to be 3D printable and to support mesenchymal stem cell (MSC) loading and cartilage formation in vitro.
Muscles and Tendons	Monteiro RF[104]	2023−05	Tendon decellularized extracellular matrix (dECM)	The biophysical and biochemical properties of dECM hydrogels were shown to effectively induce the differentiation of human adipose-derived stem cells (hASCs) into the tendon cell lineage.
		2023−01	Tendon-derived dECM (TdECM)	TBI-specific environment coexisting with dECM bioink can modulate stem cell behavior to induce fibrocartilage formation that mimics TBI.

degradation. The experimental results showed that this system significantly increased printability and structural fidelity, and these premixed hydrogels retained high bioactivity and tissue specificity due to the presence of the dECM-Gs. Because hydrogels containing dECM-G derived from porcine peripheral nerves (GelMA/pDNM-G) effectively promoted axon growth and Schwann cell migration, neural cells can also be encapsulated in GelMA/pDNM-G hydrogels for 3D culture or cell-loaded bioprinting while maintaining high cellular viability, which endows these hydrogels with great potential for use in regenerative medicine. With the continuous exploration of dECM-based bioinks, brain and neural tissue engineering will more closely replicate the mechanical and biological functions of natural organs for eventual application in organ transplantation.

5.6. Skin and Soft Tissue. *5.6.1. Skin.* The number of patients with nonhealing skin wounds due to burns, trauma, or surgery is enormous, placing an enormous social and economic burden on patients and the health care system. Severe skin injuries represent a significant clinical challenge. Currently, researchers are working to create tissue-engineered skin that can be used for transplantation but are often limited by the inability to reproduce key biostructural features of the skin.[108] Since dECM is derived from natural tissues, it can provide a suitable microenvironment for the regeneration of skin tissues, and using it as a bioink in 3D-printing technology is expected to solve the problems of fabricating tissue-engineered skin. Sarmin AM et al.[109] performed an in-depth analysis of skin-derived dECM biomaterials. Researchers have extracted dECM materials from pig skin and quantified the major molecules in the ECM, including collagen types I, III, and VI, protofibrillar proteins, and basement membrane glycans (lumican), via mass spectrometry. Rheological analysis demonstrated the sol−gel and shear-thinning properties of the dECM materials, which indicated their physical suitability as tissue scaffolds. dECM materials are also compatible with existing advanced biomanufacturing techniques, including 3D printing in gelatin particle-supported baths, printing with sacrificial materials, or blending with other ECM molecules to obtain more complex compositions and structures. This study also demonstrated how dECM materials can be used to establish 3D skin wound healing models via 3D printing, paving the way for further research. Bashiri Z and team[110] explored skin tissue engineering with placental tissue-derived dECM. The researchers used sodium alginate, gelatin, and dECM derived from placental tissue at different concentrations (1.5%, 3%, and 5% w/v) to prepare a printable bioink for bionic natural skin and investigated the morphology, physical structure, mechanical behavior, biocompatibility, and angiogenic properties of the printed hydrogels. The researchers also applied 3D-printed scaffolds with an optimized ECM content (5% w/v) to whole-layer wounds in a mouse model, and the experimental observations showed that the dECM-based scaffolds provided a noncytotoxic microenvironment for cell adhesion, infiltration, angiogenesis, and proliferation with no signs of an immune response in the host. Compared to wounds implanted with printed scaffolds without dECM and with untreated wounds, deep wounds implanted with dECM 3D-printed scaffolds exhibited increased granulation tissue formation, angiogenesis, and re-epithelialization.

5.6.2. Soft Tissue. The regeneration of soft tissues, such as fat, is widely used in plastic medicine as well as in large soft tissue defects. Jiang X et al.[111] explored the mechanism by

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which decellularized adipose tissue (DAT) promotes fat regeneration and the mechanism of transplanted DAT survival *in vivo*. The researchers cocultured BM-MSCs, ADSCs and UCMSCs with DAT for 14 days and then stained them with oil red O. The lipogenic genes of the three types of MSCs were detected via RT−PCR, and DAT and adipose tissues were subcutaneously transplanted into the backs of nude mice to observe mid- and long-term morphological changes, vascularization and lipid formation efficiency. Mass spectrometry (MS)-based proteomics was used to analyze the content of lipogenic proteins in DAT and adipose tissue. The results showed that DAT did not contain any cellular components but was rich in collagen. MSC seeding experiments showed that DAT provided microenvironment that was conducive to adipogenesis and interacted with different types of MSCs, ultimately leading to adipose regeneration. The presence of multiple adipogenic proteins in DAT allows it to play an important role in adipose regeneration. Kim SH et al.[112] combined 3D-printing technology with decellularization to prepare dome-shaped elastic poly-PLCL scaffolds with channel and pore structures for patient-specific regeneration via a combination of 3D-printing technology and the gel pressing method. Researchers have combined PLCL scaffolds with adipose decellularized extracellular matrix (adECM), cardiac decellularized extracellular matrix (hdECM) hydrogels and human adipose-derived stem cells (hADSCs) to promote adipogenesis and angiogenesis. In vitro real-time PCR results showed that the dECM hydrogel mixture induced adipogenesis. In addition, *in vivo* studies at 12 weeks demonstrated that tissue-engineered PLCL scaffolds containing a hydrogel mixture (hdECM/adECM (80:20)) and hADSCs promoted angiogenesis and adipose tissue formation and inhibited apoptosis. Thus, the elastic scaffold and dECM hydrogel constructed in this study can be used clinically as materials for patient-specific large-scale adipose tissue regeneration.

5.7. Other Systems and Organizations. *5.7.1. Kidney.* The kidneys are responsible for maintaining the water balance of the body and for the excretion of waste products, and they contain glomeruli for filtration and tubules for reabsorption.[113] Kidney transplantation is considered to be the most effective treatment for patients with end-stage renal disease,[114] and the use of dECM as a 3D-printing bioink in renal tissue engineering is expected to enable the fabrication of suitable renal replacements. Soberiro-Almeida R et al.[115] decellularized, lyophilized, and digested porcine kidneys to produce a viscous solution. Enzymatic cross-linking of dKECM was then carried out using an agarose particulate-supported bath containing transglutaminase to optimize the bioprinting process and to allow the fabrication of constructs with good print resolution and high structural integrity. In addition, the encapsulation of primary renal progenitor cells resulted in high cell viability, enabling the generation of complex 3D structures over time to obtain a tissue-specific matrix that can influence cell growth and differentiation over time.

5.7.2. Testicles. Compared to other organs in the genitourinary system, artificial testes have been explored with dECM-based bioinks. Bashir Z et al.[116] decellularized fragments of testicular tissue from rams (RAM) using NaCl buffer, NaCl buffer-Triton, SDS and SDS-Triton. The effectiveness of decellularization was confirmed by DAPI and HE staining and assessment of DNA content, and T-ECM was confirmed to be well preserved by trichrome staining with Alcian blue, Orcein and Masson. Then, hydrogel scaffolds were printed using extracted T-ECM combined with alginate-gelatin. The printability and the morphological, mechanical and biological properties of the 3D-printed hydrogels were analyzed. The experimental results showed that 3D-printed scaffolds containing 5% T-ECM displayed uniform surface morphology in vitro and *in vivo* with high cell attachment and cell biocompatibility. From this, it can be concluded that T-ECM can be used as a biomimetic material to create artificial testes and potentially produce sperm in vitro. Surprisingly, this possibility of sperm production was confirmed by the team a year later.[117] In further studies, after evaluating the spermatogenesis process in testicular tissue, the authors still chose to decellularize Ram testicular tissues using a hypertonic solution containing Triton and used the extracted T-ECM as a bioink to print artificial testes. After cell adhesion and viability were assessed, pre- and postmeiotic cells in the study group were confirmed via PCR, flow cytometry, and immunocytochemistry, and the morphology of differentiated cells was assessed via transmission electron microscopy (TEM), toluidine blue staining, and hematoxylin and eosin (HE) staining. in the proliferation of testicular cells cultured on T-ECM-enriched scaffolds was confirmed by high cell viability and increased expression of colonization and premeiotic markers. In addition, researchers observed spermatogenesis by neonatal mouse testicular cells inoculated onto T-ECM-enriched scaffolds, and morphological assessment revealed that the structure of these cells closely resembled that of mature spermatozoa with a specialized tail structure. Hormonal analyses confirmed the production and secretion of testosterone and inhibin B by testicular supporting cells and mesenchymal stromal cells. This study confirmed previous speculation that T-ECM can be used as a biomimetic material to create tissue-engineered testes with the potential ability to produce spermatozoa in vitro, advancing the development of tissue-engineered testes and suggesting future approaches for treating human infertility.

5.7.3. Vagina. Vaginal detachment causes immense psychological and physical suffering to patients; therefore, vaginal reconstruction is necessary. Morphological repair and true functional reconstruction of the vagina are expected to be achieved with the help of 3D bioprinting.[118] Hou C et al.[119] prepared a bioink using decellularized vaginal matrix (AVM) to obtain bionic 3D vaginal tissues via 3D-printing technology. The bioink consisted of 15% gelatin and 3% sodium alginate mixed with AVM solution, and rheological analysis, scanning electron microscopy, and HE staining were performed to assess the viscosity, morphology, and biocompatibility of the bioink after configuration. The researchers also wrapped bone marrow mesenchymal stem cells (BMSCs) around 3D-printed scaffolds after 3D printing. Hematoxylin and eosin (HE), immunohistochemistry, and immunofluorescence staining showed that the 3D scaffolds wrapped with BMSCs had a significant positive effect on the vascularization and epithelialization of the printed vaginal tissues and that the BMSCs acquired the phenotype of vaginal epithelial and endothelial-like cells. Thus, this study suggested that the 3D printing of bionic vaginal tissues with AVM-based bioinks plus encapsulated BMSCs is a promising approach for vaginal reconstruction.

5.7.4. Cornea. Research on dECM-based tissue-engineered corneal bioinks is currently in full swing and is expected to bring hope to corneal transplant patients. Zhang M et al.[120] developed a novel corneal decellularized extracellular matrix/gelatin methacryloyl (CECM-GelMA) bioink by digital light

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processing (DLP) 3D bioprinting technology. It was shown to produce complex microenvironments with highly tunable mechanical properties while maintaining high light transmission. In addition, a composite hydrogel was loaded with human corneal fibroblasts (hCFs), and in vitro experiments showed that the hydrogel maintained high cell viability and expressed core proteins. *In vivo* experiments showed that the hydrogel could promote epithelial regeneration, maintain stromal alignment and restore transparency. Thus, modified bioinks for rapidly customizable artificial corneas have great potential in the development of in vitro corneal stromal analogs.

5.7.5. Retina. In addition to the cornea, researchers have made advances in the study of dECM-based bioinks for tissue engineering in the retina. Kim J and his colleagues[121] developed a retinal decellularized extracellular matrix (RdECM) from porcine retina, and the preservation of the ECM component of native retina without cellular components was evaluated. The researchers subsequently mixed RdECM with collagen to form a bioink and confirmed its suitability for 3D cell printing before further investigating the effect of the RdECM bioink on the differentiation of Müller cells and confirming the retinoprotective effects of the RdECM bioink in an animal model of retinal degeneration. Thus, this study demonstrated that RdECM bioinks are promising candidates for retinal tissue engineering. The retina is a complex structure, and the natural tissue origin of dECM provides advantages in 3D-printed tissue engineering.

6. RESULTS AND DISCUSSION

6.1. Challenges for dECM Bioinks. The current application of dECM as bioink in 3D printing tissue engineering faces some challenges. First, incomplete decellularization can easily lead to immune rejection, and different decellularization methods have different effects on different tissues.[122] Second, improper decellularization methods are prone to cause changes in the original dECM microenvironment, thus losing its role in promoting tissue repair and supporting cell growth.[123] Third, the mechanical properties of dECM as a bioink have been a long-standing issue, and the decellularization process and the effort to enhance the printability of bioinks will reduce the mechanical properties of dECM bioinks to varying degrees.[124] Finally, the evaluation of the biosafety of dECM as a bioink and its specific clinical applications are still faced with obstacles. In the future, researchers will focus on these issues and continue to optimize the preparation process of bioink based on decellularized substrates in order to improve their mechanical properties and printability based on complete decellularization.

6.2. Future Directions. *6.2.1. Optimizing the Preparation of Decellularized Matrix Bioinks.* To address the challenges of incomplete decellularization and destruction of the original extracellular matrix microenvironment, future research should explore additional decellularization methods, integrate different techniques, and combine multiple approaches for decellularizing various tissues to develop a combined decellularization method tailored to specific tissues, capable of preserving the original dECM microenvironment while ensuring complete decellularization.

6.2.2. Enhancing the Mechanical Properties of Decellularized Matrix Bioinks. Currently, researchers usually use the addition of auxiliary materials to improve the mechanical properties of dECM bioinks[125] such as the addition of nanoparticles such as graphene oxide, hydroxyapatite, magnesium oxide, and zinc oxide as the support materials.[126] However, the addition of auxiliary materials usually requires multiple attempts, and the types and amounts of materials to be added vary for different tissues. Therefore, in the future, it is necessary to further summarize the changes in the mechanical properties of dECM bioinks with the addition of different auxiliary materials, aiming to establish a unified and systematic standard and reduce the workload of subsequent studies.

6.2.3. Improving the Biosafety Assessment of dECM as a Bioink to Promote Its Specific Clinical Application. In recent years, research on 3D printed bioinks based on dECM has been emerging, mostly in the stage of animal experiments rather than clinical trials. The primary barrier to clinical translation is the lack of standardized biosafety assessment protocols. To advance in this field, future developments should focus on standardizing the selection of dECM sources, establishing uniform biosafety standards, and encouraging researchers to transition their findings into clinical trials, so as to obtain the tissue-engineered organs that can be used on a large scale in the clinic through the long-term follow-up on the regularity of the experimenters.

6.2.4. From 3D to 4D Bioprinting. Many biomedical applications require dynamic shape changes, which cannot be achieved with conventional 3D printing technologies.[127] To address this problem, the concept of 4D bioprinting has emerged, which extends 3D space to a fourth dimension that encompasses time enabling printed biomimetic tissues to change shape or function over time in response to stimuli,[128] so that printed biomimetic tissues can be preprogrammed and adapted to more closely resemble the original.[129,130] Smart design and smart materials are integral components of 4D bioprinting. Smart design involves predesigning the bioprinting process using computer-aided design to predict the deformation of a 3D object over time.[131] Smart materials, on the other hand, are materials that respond to external stimuli such as temperature, humidity, pH, light, pressure, or magnetic fields. They can change their geometrical shapes or properties accordingly. Examples of smart materials include shape-memory metal alloys, shape-memory polymers, stimulus-responsive hydrogels, dielectric elastomers, and smart nanocomposites.[132]

In the future, there's potential to combine dECM with smart materials to advance 3D printing capabilities further. This could lead to the development of 4D bioprinted tissue-engineered grafts with enhanced biological functions closer to native tissues.

7. CONCLUSIONS

dECM, with its origins in natural tissues, low immunogenicity, and capacity to provide a specific microenvironment for cell growth, has emerged as a promising candidate for 3D printing bioinks. At present, dECM applied to tissue engineering is still facing problems such as incomplete decellularization, insufficient mechanical strength, and difficulty in maintaining 3-dimensional structure, etc. In the future, with the iterative development of technological updates and the research and development of new auxiliary materials, these problems are anticipated to be solved one by one. We believe that with the rich interdisciplinary research in the fields of bioengineering, biomaterials, cytology, and clinical medicine, dECM will become an excellent candidate for 3D printing bioinks.

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Notes

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