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Advancements Of 3D Bioprinting In Regenerative Medicine

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A B S T R A C T

3D bioprinting has unlocked new possibilities for generating complex and functional tissues and organs. However, one of the greatest challenges lies in selecting the appropriate seed cells for constructing fully functional 3D artificial organs. Currently, there are no cell sources available that can fulfill all requirements of 3D bioprinting technologies, and each cell source possesses unique characteristics suitable for specific applications. In this review, we explore the impact of different 3D bioprinting technologies and bioink materials on seed cells, providing a comprehensive overview of the current landscape of cell sources that have been used or hold potential in 3D bioprinting. We also summarized key points to guide the selection of seed cells for 3D bioprinting. Moreover, we offer insights into the prospects of seed cell sources in 3D bioprinted organs, highlighting their potential to revolutionize the fields of tissue engineering and regenerative medicine.

Keywords:
3D bioprinting,
Seed cells,
Bioink materials,
Cell lines,
Stem cells,
Primary cells,

1. Introduction

3D bioprinting is an emerging technology that enables the precise layer-by-layer deposition of bioink, a specialized material consisting of living cells, biomaterials, and bioactive molecules [1–6]. This advanced technique allows for the fabrication of complex and functional tissues or organs. The implications of 3D bioprinting are vast, with the potential to generate tailored and functional tissues for diverse applications, including regenerative medicine [7–9], drug discovery [10–13], and toxicology testing [14,15]. However, there are still several challenges that need to be overcome to unlock the full potential of 3D bioprinted tissues and organs.

Regardless of the rapid advancements in additive manufacturing technologies, obtaining suitable seed cells persists in the pursuit of creating fully functional organs through 3D bioprinting [16–18]. Seed cells play an essential role in 3D bioprinting technology since cells are the fundamental units of life. High quality cells are indispensable for generating applicable 3D bioprinted tissues and organs. An ideal cell source possesses several key characteristics.

1. Printability: The cells should demonstrate the ability to withstand the rigorous printing process, including shear stress, pressure, and temperature variations, without compromising their viability or functionality. Although suitable bioink materials could help to increase the printability of cells, it is still important for cells utilized for the generation of organs and tissues.

2. Proliferation: For organ and tissue fabrication, cells need to possess a proliferation capacity in order to expand to the required cell number and adequately populate the 3D bioprinted tissue. However, it is also important to have control over the proliferation of the seed cells. Excessive proliferation could result in hyperplasia which can disrupt the structure and function of 3D bioprinted tissues.
3. Functionality: The cells should either possess functional attributes or have the capability to differentiate into mature functional cells, thereby establishing the desired functionality of the 3D bioprinted organs.
4. Safety: When constructing transplantable normal tissues for therapeutic purposes, it is crucial to use cells with a normal karyotype, non-tumorigenic properties, and devoid of phycological toxicity. Immunological rejection induced by allogeneic or heterogeneous cells is also a serious problem, which should be carefully considered.
5. Economy: The construction of large-scale organs necessitates a substantial number of seed cells. Therefore, the cost-effective large- scale expansion of seed cells is crucial for the applications of 3D bioprinted organs.
6. Self-assembly ability: The microstructure of 3D bioprinted tissues plays a crucial role in achieving full functionalization. The microstructure is primarily formed through the natural self-assembly and organization of cells, which cannot be precisely designed using the current resolution capabilities of 3D bioprinting techniques. The vessel networks of 3D bioprinting vascular tissues mainly relied on the cells [19,20].

An appropriate cell source is key for the successful development of fully functional 3D bioprinted organs. By meticulously selecting seed cells that possess essential characteristics, including printability, proliferation, functionality, safety, economic feasibility, and self- assembly ability, researchers can overcome a significant hurdle in achieving the desired microstructure and multi-functionality of 3D bioprinted tissues and organs [21,22]. In this review, we explored the impact of various 3D bioprinting technologies and bioink materials on seed cells and provided a comprehensive overview of the currently available options for cell sources in 3D bioprinting. Furthermore, we offer insights into the prospects of seed cells for 3D bioprinted organs, highlighting their potential to revolutionize tissue engineering and regenerative medicine.

2. Cell requirements in different 3D bioprinting techniques

In recent years, significant advancements have been made in 3D-bioprinting techniques, revolutionizing the field of bioartificial organ construction. 3D bioprinting involves the precise deposition of bioinks composed of living cells and biomaterials, enabling the fabrication of complex tissue structures [23–25]. However, it is important to note that different bioprinting technologies possess unique characteristics, resulting in distinct requirements for seed cells (Table 1).

Extrusion-based bioprinting has gained widespread popularity as a versatile and straightforward 3D-bioprinting technique, capable of creating stable structures [26–28]. This strategy involves the controlled ejection of bioinks from nozzles, which are subsequently cured and stacked layer by layer on the printing plane to build a predefined 3D construct (Fig. 1a) [29,30]. Notably, extrusion-based bioprinting offers a broader selection of bioinks compared to other techniques, facilitating the achievement of high-cell-density printing [31]. However, the pressure and shear stress during the extrusion process could damage the cells. Additionally, the temperature fluctuations or UV stimulation often used for the solidification of the bioink can impose further stress on the cells [32–34]. Therefore, by cooperating with bioinks, cells used in this approach

must possess suitable size, shape, and viscosity to be ejected as droplets without compromising their viability or functionality. They should also be capable of withstanding the polymerization process of bioink materials [31,35].

Inkjet bioprinting offers a notable advantage in the precise delivery of biological inks to specific locations according to a determined scheme, allowing for the creation of multifunctional bionic structures while avoiding disruption to existing constructs (Fig. 1b) [36]. This technique enables control of ink droplet sizes as small as picoliters, making it feasible to fabricate artificial tissues and organs at the microscopic scale [37,38]. While inkjet bioprinting is capable of achieving microstructures with small ejected liquid droplets, it can also be utilized for printing large-sized tissues. However, the successful printing of large tissues requires a substantial number of seed cells and bioink [39]. Moreover, for inkjet bioprinting, cells must meet stringent criteria, including compatibility with the bioink materials and the ability to withstand the jetting process [39,40].

Laser-assisted bioprinting (LAB) is capable of generating high-resolution 3D bioprinted tissues, allowing for precise patterning of individual cells and smaller structures [41]. One distinguishing feature of LAB is the elimination of nozzles for bioink deposition, which reduces the risk of contamination and lowers fabrication costs (Fig. 1c) [42]. Additionally, LAB's high printing frequency makes it suitable for constructing high-resolution structures, such as capillary systems, and its in situ capability minimizes the potential for secondary damage to transplant recipients [43,44]. However, it is crucial that the cells used in LAB possess the ability to withstand the

Table 1
Requirements of cell sources of different 3D bioprinting techniques.

Technique	Requirements	Ref.
Extrusion-based bioprinting	Suitable size shape, viscosity Stable viability and functionality	[32–34]
Inkjet bioprinting	Compatibility with bioink materials Jetting process resilience	[39,40]
Laser-assisted bioprinting	Stable viability and functionality Laser resilience	[45,46]
Stereolithography bioprinting	Compatibility with light-sensitive materials Light exposure resilience	[50]

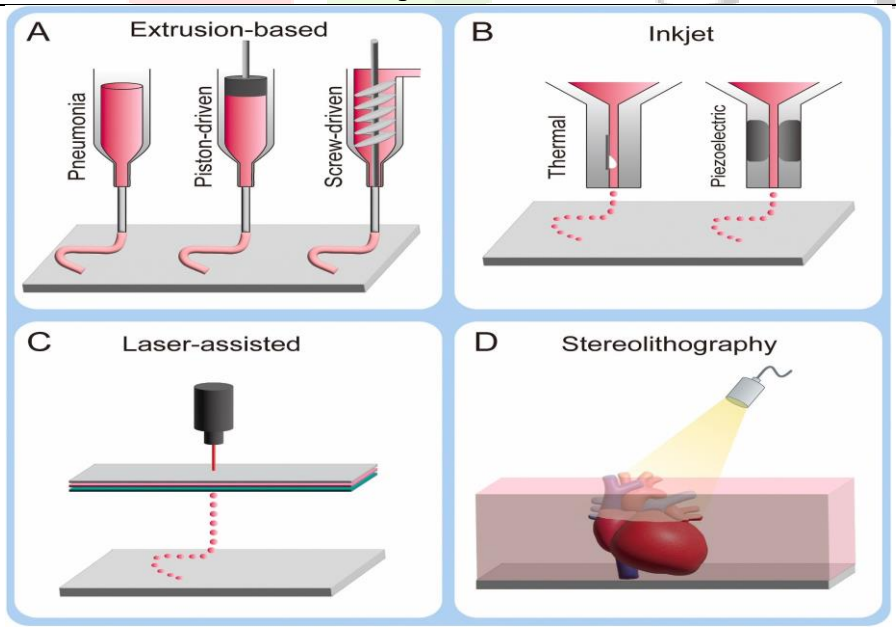

Fig. 1. Representative 3D bioprinting techniques. (A) Extrusion-based bioprinting; (B) Inkjet bioprinting; (C) Laser-assisted bioprinting; (D) Stereolithography bioprinting.

laser energy applied during the printing process. It is also important to note that the laser-induced transfer process can potentially disrupt cell structure [45,46]. The influence of laser and UV on the DNA of cells should also be considered when applying the laser-assisted bioprinting technique [47].

Stereolithography bioprinting utilizes digital micromirror arrays to control the light intensity in each pixel, enabling the polymerization of light-sensitive polymer materials (Fig. 1d) [48,49]. This technique provides several advantages such as high printing efficiency, exceptional resolution, and a stable model framework, making it well-suited for fabricating large tissues and organs. For this strategy, cells must be compatible with the light-sensitive polymer materials, capable of enduring the polymerization conditions induced by light exposure, and able to maintain their viability, functionality, and structural integrity throughout the printing and post-printing stages [50].

While 3D bioprinting technology holds promise in alleviating the scarcity of organs for transplantation, challenges persist in the preoperative fabrication, *in vitro* cultivation, and transplantation processes, posing considerable risks and limitations [51,52]. The emerging in situ 3D bioprinting technology, which allows direct organ printing at the transplant site, shows the potential to mitigate these issues to some extent. Currently, various studies have explored the application of in situ 3D bioprinting in skin repair and bone reconstruction. The use of in situ 3D bioprinted skin [53] and bones [54] has demonstrated its ability to expedite wound healing and reduce immune rejection reactions. Beyond skin and bones, in situ, 3D bioprinting technology proves highly effective in specific applications within oral medicine and certain ophthalmic implants [55,56]. With the continual enhancement of 3D printing precision, it is anticipated that in situ 3D bioprinting technology will find an even broader range of applications.

Novel bioprinting techniques, such as acoustic bioprinting [57–59], magnetic bioprinting [60–62], electrohydrodynamic bioprinting [63,64], and other innovative approaches, are emerging to address the constraints of conventional methods and open up new possibilities in the creation of complex tissue. A significant focus of these advancements is the integration of precise control mechanisms to improve cell viability and functionality when fabricating intricate tissue architectures.

3. Influences of bioink materials on cells

Bioink, a combination of seed cells and bioprinting materials, plays a core role in achieving high-density cell distribution within bioprinted structures while maintaining their biological function throughout the 3D-bioprinting process [65,66]. Serving as a delivery and support system, bioink materials ensure the transportation and sustenance of seed cells during bioprinting. Moreover, bioink materials regulate biocompatibility, mechanical properties, and rheological characteristics of the bioprinting structure [67–69]. Additionally, bioink materials create a supportive microenvironment for the cells, which is essential for achieving functional and physiologically relevant 3D-bioprinting tissues and organs. An ideal bioink material should be able to mimic the intricate extracellular matrix (ECM) of the target tissue or organ while exhibiting appropriate rheological properties that enable extrusion or deposition through the bioprinting system [70–72].

The utilization of natural materials in 3D bioprinting has garnered significant attention due to their exceptional biocompatibility, biodegradability, and potential to enhance cell survival, function, adhesion, and self-organization [73–75]. Extensive research and optimization have been conducted on several natural materials, including collagen, gelatin, alginate, hyaluronic acid, and chitosan, to develop them as bioink materials for 3D bioprinting [76–84]. Acellular tissue matrix, which contains mixed tissue-specific ECM components, is often incorporated into the bioink materials to provide a tissue-specific environment with signaling and mechanical properties [85,86]. Natural materials generally exhibit low immunogenicity, and reduce the risk of causing adverse reactions [68,87]. Furthermore, their ability to undergo biological degradation over time is crucial for establishing cell-cell interactions and ECM remodeling during the functionalization of 3D bioprinted organs [88,89]. Natural materials also offer the advantage of incorporating bioactive molecules, growth factors, and cytokines into their structures, further enhancing the microenvironment for seed cells (Fig. 2). However, the relatively limited options of natural materials cannot meet the increasing demand for diverse mechanical properties and precise manufacturing controls [90–92].

Synthetic materials, typically derived from non-biological sources through chemical synthesis or manufacturing processes, offer flexibility in tailoring their mechanical properties to adapt different 3D bioprinting approaches [93]. These materials can be engineered to possess specific characteristics such as

stiffness, toughness, elasticity, and crosslinking modes [87]. Due to their superior mechanical properties and consistent quality, synthetic materials find wide application in the 3D bioprinting of large-scale tissues and organs [94,95] (Fig. 2). Among the synthetic polymers used in 3D bioprinting, polycaprolactone (PCL) stands out for its excellent biocompatibility and ease of shaping. However, it exhibits limited capability in effectively encapsulating cells. With a low melting

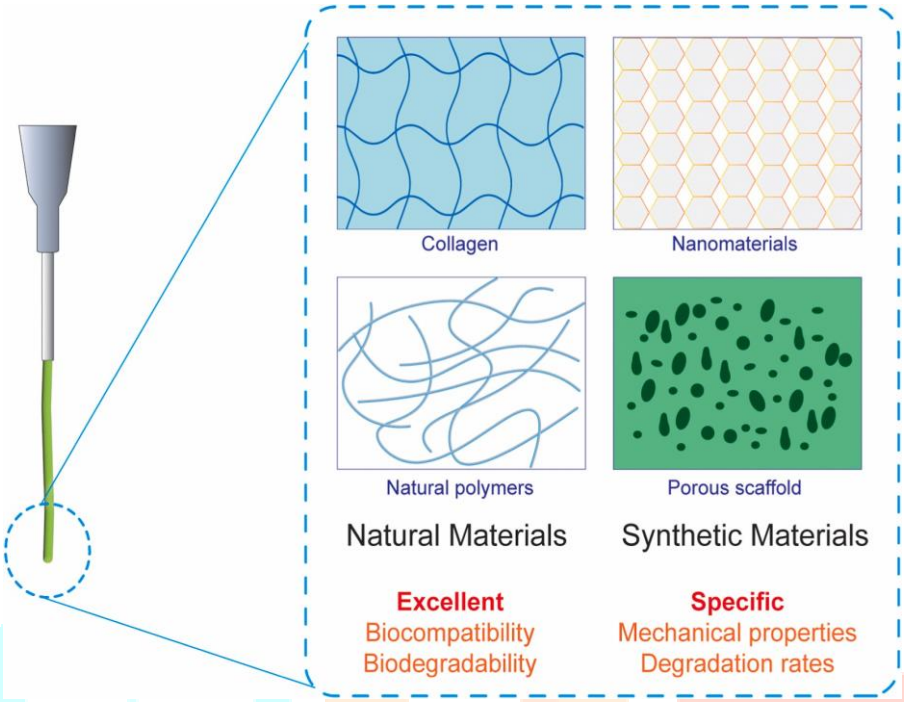

Fig. 2. Advantages of different bioink materials for 3D bioprinting.

point, PCL is suitable for extrusion-based 3D bioprinting and finds applications in printing scaffolds for bone, cartilage, and vascular tissue engineering [96–98]. Polyethylene glycol (PEG), known for its hydrophilicity and cell-friendly nature, allows for cell attachment and proliferation. PEG-based bioink materials can be customized to exhibit specific mechanical properties and degradation rates, making them suitable for vascular, cartilage, and heart bioprinting, as well as for use in bioprinted organ scaffolds [99–101]. Poly (lactic-*co*-glycolic acid) (PLGA), on the other hand, has been utilized in scaffold development for nerve tissue regeneration [102–105]. Synthetic materials used in 3D bioprinting must demonstrate high biocompatibility, low immunogenicity, and good tolerance by cells and tissues [106–108].

Achieving the multifunctionality of complex tissues and organs using a single type of bioink poses several challenges [109–111]. Simply increasing the concentration of bioinks to enhance the mechanical properties of bioprinting structures lacks the ability to selectively couple small molecules or dynamically alter the printed structures [112–114]. To address these limitations and enhance the specificity and controllability of bioinks, the utilization of chemically modified and environmentally responsive materials holds promise. By incorporating such materials, it becomes possible to improve the performance of bioinks, allowing for precise modulation and responsiveness to specific cellular environments and stimuli [115–119].

4. Cell sources for 3D bioprinting

Cells are the fundamental building blocks for 3D bioprinted tissues and organs [120,121]. Despite the explosive development of various approaches to generate stem cells or functional cells for generative medicine purposes, many cell types are currently not suitable for 3D bioprinting due to their fragility during the printing process, limited proliferation ability, inability to adapt to the bioink materials, insufficient functionality, tumorigenic risk, costly culture system, and other limitations [28,122]. In this context, we provide a summary of commonly used cell types as well as potential cell types that show promise for use in 3D bioprinting applications.

4.1. Cell lines

Cell lines are populations of cells that maintain stable phenotypes and functions over an extended period of time. They are frequently employed as models in the development of 3D bioprinting technologies, especially because primary cell types are usually difficult to maintain and expand *in vitro*. There are many advantages of cell lines, such as easy handling and cost-effectiveness [123, 124]. Their culture does not require complex conditions and is robust against environmental changes and mechanical stresses. Various cell lines have made significant contributions to the establishment and optimization of 3D bioprinting technologies (Table 2) . For instance, beta-TC60, an immortalized mouse cell line capable of insulin secretion was utilized in the initial inkjet bioprinting experiments . Human umbilical vein endothelial cell lines, smooth muscle cell lines, and skin fibroblast cell lines have been widely employed in the development of bioprinted vascular systems . HepaRG cells, an immortalized hepatic cell line that maintains many hepatic functions, were utilized in the construction of a transplantable 3D bioprinted liver. When transplanted into a liver injury mouse model, this 3D bioprinted bioartificial liver persisted for over 8 weeks *in vivo* and demonstrated therapeutic effect [136].

Numerous cell lines are derived directly from tumors, while others are generated from primary cells of normal tissues by disabling cell cycle arrest or senescence genes such as p53, Rb, and p16 . This can be achieved through spontaneous mutations, shRNA, gene editing, or the overexpression of Simian Vacuolating Virus 40 large T antigen . Therefore, cell lines are capable of

Table 2
Cell lines used in 3D bioprinting.

Cell lines	Bioprinting techniques	Bioink materials	Tissues or Organs	
MCF-7, MDA-MB-468, MCF-12A	Extrusion-based	ECM	Mammary organoids and tumoroids	**Advantages:** Diverse sourcing options
Glioblastoma stem cells	Digital light processing (DLP)-based	Photocrosslinkable native ECM derivatives	Biomimetic 3D cancer microenvironment	Cost-effectiveness Easy cultivation
Mouse myoblast cells (C2C12s)	Extrusion-based	Oxidized alginate-gelatin (ADA-GEL) hydrogel	Muscle tissue	Stable proliferation Excellent homogeneity
Huh7 & HepaRG	Extrusion-based	Fibrinogen, gelatin & alginate	Skeletal muscle-like bundles Bone-like tissue with vascular lumen	**Disadvantages:** Carcinogenic risk Lack certain organ-specific functions
HUVECs	Extrusion-based	GelMA	Aortic valves tissue	
Valvular interstitial cells (VICs)	Extrusion-based	Alginate & gelatin		
Caco-2 cells	Extrusion-based	GelMA	Intestinal villi and hair follicles	
MCF-7 & BT-474	Extrusion-based	Mammocult & Matrigel Alginate hydrogel	Breast adenocarcinoma tissue	
Beta-TC60	Inkjet		Single-cell based microparticles	
HepaRG	Extrusion-based	Gelatin & sodium alginate	Liver	
HEK 293FT	Extrusion-based	Gelatin & sodium alginate	Kidney tissue models	

Huh7 & HepaRG	Extrusion-based	Gelatin methacryloyl (GelMA)	Liver model
HepG2	Drop-on-Demand (DoD)	Agarose, gelatin & collagen	Functional liver carcinoma model

continuous proliferation, making it easy and cost-effective to produce a large quantity of cells. However, the high proliferative capacity of cell lines may not always be advantageous for 3D bioprinting. While many cell lines exhibit contact inhibition and cease proliferation after population of the 3D bioprinted constructs, others, particularly those derived from tumors such as HepG2 cells, can continue proliferating and give rise to neoplasms. Therefore, it is crucial for cells to proliferate and populate the 3D bioprinted construct appropriately, with proliferation ceasing after the formation of functional structures.

Another notable advantage of cell lines is their homogeneity . Cell lines have the ability to undergo clonal expansion, generating populations of cells with identical genetic profiles . This uniformity allows for the fabrication of homogeneous tissues and organs, ensuring consistent and reliable outcomes in the printed constructs. Moreover, the utilization of homogenous cells offers stability and high reproducibility when developing new 3D bioprinting technologies.

However, when it comes to constructing transplantable 3D bioprinted tissues and organs for therapeutic purposes, there are certain challenges to consider. Many cell lines exhibit abnormal karyotypes or carry mutations in oncogenes and tumor suppressor genes, increasing the risk of tumor formation . For example, the human HEK 293 cell line, used in the construction of 3D kidney models, is a hypotriploid cell line with 64 chromosomes . A karyology analysis of the human umbilical vein endothelial cells (HUVEC) cell line (CRL-1730) provided by the American Type Culture Collection (ATCC) showed that 87.8 % of the cells are hypodiploid or polyploid .

Another disadvantage of cell lines is their inability to fully replicate the mature and specialized functions of their *in vivo* counterparts. Although many cell lines retain some marker genes associated with their origin cell types, they often lack or have significantly reduced functional characteristics. For instance, HepG2 cells, a commonly used hepatoblastoma cell line that has been used for constructing a 3D liver-like model, express the *ALBUMIN* gene, a common hepatic marker, but they exhibit defects in drug metabolism, urea metabolism, coagulation system, and more . Thus, it is nearly impossible to reconstruct a fully functional artificial liver based on HegG2 cells. Consequently, it is crucial to explore safer cell sources that are capable of generating mature functional cells with controlled proliferation.

4.2. Stem cells

Stem cells, possessing the remarkable ability to self-renew and differentiate into various cell types, offer tremendous potential as cell sources for 3D bioprinted tissues and organs (Table 3) .

One of the most discussed advantages of stem cells is their theoretically unlimited proliferation capability, which holds the potential for generating an unlimited number of functional cells. This feature becomes particularly valuable when it is challenging to culture their counterpart cells *in vitro*. In recent years, numerous technologies have been developed to induce the differentiation of ESCs and iPSCs into various functional cell types, such as neurons, cardiomyocytes, hepatocytes, β cells, muscle cells, and endothelial cell.

Table 3
Stem Cells used in 3D bioprinting.

Cell types	Bioprinting techniques	Bioink materials	Tissues or Organs	
hiPSC-derived neural progenitor cells	Microfluidics - based	Fibrin-based bioink	Neural tissue	**Advantages:** Easy accessibility
Human Adipogenic Mesenchymal Stem Cell (hADMSCs)	Extrusion-based Drop-on-demand	Pluronic acid as the sacrificial material and type I collagen	Cardiac Purkinje System	Pluripotency Strong proliferation Stable genetic
iPSs	Extrusion-based		iPS tissue	Mature functionality post-differentiation
iPSC-derived cardiomyocytes	Extrusion-based Extrusion-based Microextrusion-based	Alginate hydrogel Hydroxypropyl chitin (HPCH)	iPS tissue Neural tissue	**Disadvantages:** Carcinogenic risk High cultivation costs
iPSC-derived neural cells		Gelatin Collagen I and Matrigel	Cardiac Neural tissue	
iPSCs-derived cardoimyocytes (CMs) and ECs	Extrusion-based	Gelatin Patient-derived dECM & gelatin	Cardiac tissue	
iPSC-derived mesenchymal stem cell	Extrusion-based	Alginate & gelatin	Endometrium tissue	
iPSC-derived neural aggregates	Extrusion-based	Alginate, fibrin & genipin	Neural tissue	
hiPSCs	Sequential printing in a reversible ink template (SPIRIT)	GelMA, Alginate & Gelatin	Vascular tissue	
iPS Cells-derived cardiomyocytes and hepatocytes	DLP-based	Decellularized extracellular matrices (dECM)	Myocardium tissue, liver	
Mouse embryonic stem cells	Extrusion-based	Gelatin & alginate	N/A	

Recently, iPSCs have been effectively differentiated into endothelial cells and retinal pigment epithelial cells, which, when combined with pericytes and fibroblasts, can generate a 3D bioprinting tissue that mimics the outer-blood-retina-barrier in the back of the eye .

However, the proliferation capability of stem cells decreases as they undergo differentiation. Once fully differentiated, these cells typically lose their ability to divide and become unable to populate the 3D constructs. To address this challenge, a compromised strategy is to guide the stem cells to differentiate into committed stage or progenitor cells that retain the ability to proliferate. After the initial population of the 3D bioprinting structure, further differentiation and maturation can be induced, allowing for the development of functional and mature tissues and organs.

However, due to the complex and time-consuming nature of the iPSC generation process, the current focus does not prioritize the production of patient-specific 3D bioprinted organs based on iPSCs.

Before the clinical application of ESC or iPSC-based 3D bioprinting technologies, several safety concerns need to be addressed. These include the risk of genetic and epigenetic abnormalities and the potential tumorigenicity of undifferentiated pluripotent stem cells. In recent years, significant efforts have been dedicated to enhancing the safety. Researchers are working on developing ESC and iPSC strains with normal karyotypes, avoiding tumorigenic mutations, and implementing good manufacturing practices (GMP) during the production process to meet the requirements for clinical applications.

In addition to pluripotent stem cells, there are many other stem cells available for use as cell sources in 3D bioprinting, including adult stem cells such as mesenchymal stem cells (MSCs). Among them, MSCs have the potential to differentiate into multiple cell lineages and promote tissue regeneration, which made them widely used in 3D bioprinting technology development and tissue repair research .

4.3. Cells derived by transdifferentiation

Mammalian cells have demonstrated the ability to undergo transdifferentiation, which offers a new avenue for cell sources in regenerative medicine . By overexpressing lineage-specific transcription factors, somatic cells have successfully been converted into neurons, hepatocytes, cardiomyocytes, endothelial cells, and myofibroblasts . Transdifferentiation technology provides a simpler and less time-consuming approach to generating patient-specific functional cells, bypassing the pluripotent stage and avoiding associated risks. In recent clinical experiments, hepatocytes generated through transdifferentiation have shown remarkable therapeutic effects in bioartificial liver support systems, offering promising prospects for advanced interventions .

However, one limitation of transdifferentiated neurons, cardiomyocytes, and hepatocytes, is their limited potential for *in vitro* proliferation. To address this issue approaches such as silencing p19Arf or overexpressing SV40 large T antigen have been used to expand transdifferentiated hepatocytes, although this raises additional safety concerns .

4.4. Primary cells

Primary cells derived from adult normal tissues are highly regarded as valuable cell sources due to their excellent safety profile and functional performance . However, their practical application has been hindered by the limited availability of suitable donor sources and the challenges associated with *in vitro* culture and expansion. Freshly isolated primary cells are often delicate and vulnerable to the stresses imposed by temperature, pressure, and shear forces during 3D bioprinting, leading to high mortality rates .

Nonetheless, significant progress has been made in recent years with continuous *in vitro* expansion and culture techniques for various primary cell types. Hepatocytes, muscle satellite cells, small intestine stem cells, and lung stem cells have seen breakthroughs in large-scale expansion *in vitro* . These *in vitro* expanded primary cells demonstrate improved adaptability to the 3D bioprinting process and exhibit relatively higher cell survival rates when subjected to *in vitro* conditioning . Furthermore, these cells maintain a normal karyocyte post-expansion and exhibit functional maturity or the ability to differentiate into mature functional cells . Notably, they possess enhanced self-assembly capabilities, enabling the formation of intricate microstructures.

The remarkable progress has led to the inclusion of these cells in clinical trials for *in vivo* cell transplantation therapies, underscoring their favorable safety profiles and the potential for smooth translation into clinical applications in the future. This promising development paves the way for their utilization of cutting-edge medical treatments, providing hope for improved patient outcomes and advancements in regenerative medicine. We summarized the key benefits of utilizing primary cells in 3D bioprinting as follows.

(1) Better physiological relevance: Primary cells retain the *in vivo* phenotype and function of native tissues, ensuring a closer representation of physiological conditions.
(2) Tissue-specific functions: Primary cells exhibit tissue-specific functions, making them well-suited for fabricating organ-specific structures and functions, offering a more accurate and specialized tissue model.

(3) Patient-specific: Primary cells can be sourced directly from a patient's tissue, allowing for the generation of personalized organ constructs that closely mimic the individual's unique biological characteristics. This personalized approach holds immense promise for regenerative medicine applications.

(4) Better integration: Primary cells possess remarkable integration capabilities within host tissues, minimizing the risk of immune rejection and enhancing the overall functionality of the 3D-bioprinting tissue or organ. This improved integration promotes long-term tissue function and enhances the potential for successful transplantation.

By harnessing the unique characteristics of primary cells, several kinds of primary cells had been utilized in the 3D bioprinting of tissues and organs (Table 4). Primary human hepatocytes and hepatocellular carcinoma cells were applied in constructing the liver and liver cancer models . Primary fibroblasts and intestinal cells have also been employed in the generation of skin tissues and intestinal models .

Despite these unique strengths of primary cells, several limitations need to be addressed to fully harness their potential . Primary cells are difficult to obtain, especially from human tissues, and their isolation and expansion are often time-consuming and labor-intensive. Besides, the heterogeneity of primary cells makes their behavior vary depending on the donor, tissue source, and culture conditions.

Primary cells have a limited lifespan, and their replicative potential decreases with each passage. The differentiation capacity of primary cells can be affected by the culture conditions, resulting in variability in the quality of 3D-bioprinting tissue or organ constructs. The biggest limitation of the usage of primary cells is the ethical concerns, especially for the cells sourced from human tissues, as the collection of tissues may require informed consent and approval from ethical review boards.

The use of primary cells in 3D bioprinting has the potential to revolutionize the field of regenerative medicine. However, to fully harness their potential, limitations such as their limited availability, heterogeneity, and differentiation capacity need to be addressed through the development of standardized isolation and culture protocols .

4.5. Trends and prospects of cell sources

Finding a suitable cell source for 3D bioprinting is a critical decision that greatly influences the success and functionality of generated tissue or organ. Different cell types bring their own set of advantages and limitations to the table (Fig. 3). Understanding these factors and selecting the most suitable cell type for a particular application is paramount in achieving the desired outcome of functional and viable tissue models .

Table 4:
Primary Cells used in 3D bioprinting.

Cell types	Bioprinting techniques	Bioink materials	Tissues or Organs	
Human mesenchymal stem cells (hMSCs)	Extrusion-based	Alginate- and silk-based	Smart dual scaffold	**Advantages:** High safety
hMSCs	Femtosecond-laser- assisted	Tunicate cellulose nanofibrils &	Cell-laden corneal tissue	Stable functionality
Human primary dermal fibroblasts	Inkjet	Alginate-based ECM-like	Soft tissue	Patient-specific
Primary normal human fibroblasts (NHF)	Magnetic-based	BioInk	models Skin	Excellent *in vivo* integration capability
Primary human hepatocytes	Extrusion-based	Collagen	tissue	**Disadvantages:** Limited cell sources
	Extrusion-based	GelMa	Liver	High cost
		GelMA	Skeletal muscle	

Primary Skeletal Muscle Progenitor Cells			Weak proliferation ability High heterogeneity
Primary human osteoblasts	Extrusion-based	GelMA	Osteoblasts scaffold
Primary human keratocytes	Extrusion-based	Collagen-based	Corneal stromal tissue
hMSCs	Extrusion-based	GelMA	Compact macrotissue
HUVECs	Extrusion-based	GelMA-Fibrin	Vascular tissue
Human umbilical vein & artery cells	Microfluidic bioprinting	Alg/Gel/GelMA	Vascular tissue
Human nasoseptal chondrocytes	Extrusion-based	Nanocellulose-alginate	Rounded chondrogenesis
Hepatocellular carcinoma (HCC) cells	Extrusion-based	Gelatin & sodium alginate	HCC Model
Primary intestinal cells	Extrusion-based	Novogel	Intestinal models

It is crucial to carefully consider the advantages and limitations of each cell type to the specific requirements of the tissue or organ being generated. Factors such as printability, proliferation capacity, functionality, safety, and economic considerations should be taken into account during the selection process.

By making informed decisions regarding cell types, researchers can optimize the outcomes of 3D bioprinting, leading to the fabrication of functional and viable tissue models that hold immense potential for various applications, including drug screening, disease modeling, personalized medicine, and regenerative therapies.

5. Discussion and future perspectives

The 3D bioprinting technique is experiencing rapid and remarkable advancements, holding the potential for swift application in drug screening and evaluation processes. Researchers have successfully bioprinted vascularized tissues and organs, improving cell survival and functionality . Multicellular bioinks that mimic native tissue microenvironments have been developed, enabling the creation of bioprinted constructs resembling native organs . These advancements bring us closer to viable organ transplantation alternatives and personalized medicine. While 3D-bioprinting organs for *in vivo* transplantation is still a work in progress, future research will concentrate on overcoming key challenges.

One critical area is vascularization, where the establishment of a functional vascular network capable of connecting with the body's native blood vessels becomes essential. A multi-level vascular network is needed to ensure the long-term survival and growth of the 3D- bioprinting organ, incorporating smooth muscle and composition of vascular endothelial cells within the blood vessels.

Another crucial step is massification, requiring a substantial quantity of cells. This places greater demands on the *in vitro* expansion culture of cells. Finding ways to reduce the production cost of a significant cell quantity becomes an important consideration.

Ensuring safety is another essential aspect. The production process must avoid immune problems stemming from animal-derived components to safeguard cell safety effectively.

Additionally, achieving functionalization poses a challenge in current 3D bioprinting. The current technology lacks the ability to establish functional connections between cells. Consequently, cells need to

self-organize and proliferate to form mature functional microstructures, often necessitating additional *in vitro* culturing of 3D bioprinting organs and further functionalization post-

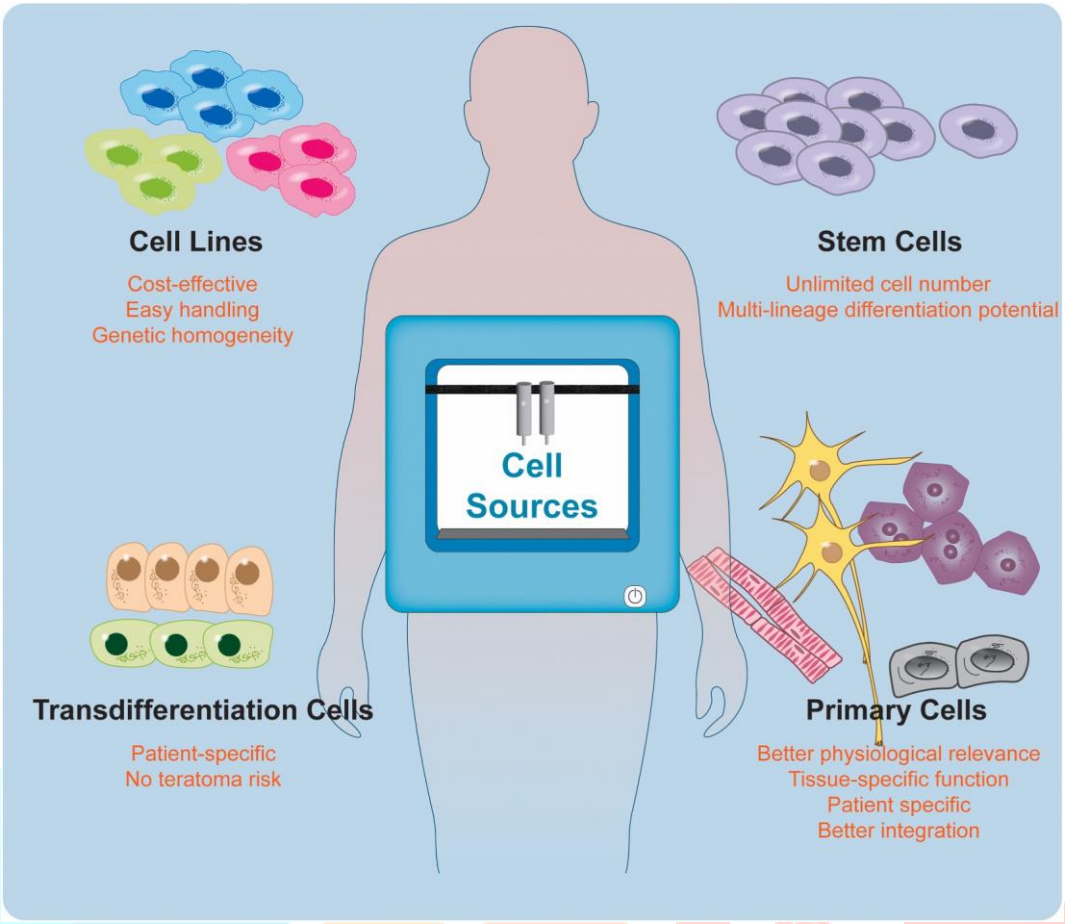

Fig. 3. Advantages of different cell sources available for 3D bioprinting.

transplantation. Furthermore, cell sources for 3D bioprinting require a high level of functionality, prompting exploration of methods to enhance the functional capabilities of cells.

Addressing these challenges will be crucial to advancing the field of 3D bioprinting and realizing the development of functional and transplantable artificial organs. Through ongoing research and innovation, the goal of creating viable, safe, and fully functional 3D- bioprinting organs will come within closer reach.

6. Conclusion

3D bioprinting technology holds immense potential for organ fabrication. A crucial challenge in successful 3D bioprinting is the availability of a substantial quantity of cells. Different cell types offer unique advantages and face their limitations. With ongoing advancements in cell isolation and culture technologies, the challenges of cell sources in 3D bioprinting will be overcome, significantly impacting the field of regenerative medicine.

Ethics approval and consent to participate

An ethics statement is not applicable.

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Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Yue Ma: Writing – review & editing, Writing – original draft. **Bo Deng:** Writing – review & editing, Writing – original draft. **Runbang He:** Writing – review & editing, Writing – original draft. **Pengyu Huang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition.Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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