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A focused review on three-dimensional bioprinting technology for artificial organ fabrication

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Three-dimensional (3D) bioprinting technology has attracted a great deal of interest because it can be easily adapted to many industries and research sectors, such as biomedical, manufacturing, education, and engineering. Specifically, 3D bioprinting has provided significant advances in the medical industry, since such technology has led to significant breakthroughs in the synthesis of biomaterials, cells, and accompanying elements to produce composite living tissues. 3D bioprinting technology could lead to the immense capability of replacing damaged or injured tissues or organs with newly dispensed cell biomaterials and functional tissues. Several types of bioprinting technology and different bio-inks can be used to replicate cells and generate supporting units as complex 3D living tissues. Bioprinting techniques have undergone great advancements in the field of regenerative medicine to provide 3D printed models for numerous artificial organs and transplantable tissues. This review paper aims to provide an overview of 3D-bioprinting technologies by elucidating the current advancements, recent progress, opportunities, and applications in this field. It highlights the most recent advancements in 3D-bioprinting technology, particularly in the area of artificial organ development and cancer research. Additionally, the paper speculates on the future progress in 3D-bioprinting as a versatile foundation for several biomedical applications.

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1. Introduction

3D printing, commonly known as additive manufacturing, is a rapidly growing field of research that is now playing a pivotal role in medicine, especially in organ printing.[1] A 3D-printed organ model can be designed by printing functional 3D structures layer by layer from materials using computer-aided design modeling. Bioprinting is a technique that allows one to create biological structures using bio-inks that have been infused with stem cells or other living cells.[2] The biological material is deposited in a stack of individual layers to form skin, tissue, or an organ. Human livers, kidneys, and hearts are now being bio-printed in laboratories and research centers worldwide.[3,4] The goal is to create long-term, sustainable solutions appropriate for transplantation and eliminate the need for real organs. This technology would be useful for dealing with the scarcity of organ donors and for better studying and understanding certain diseases.[5]

Due to global organ scarcity caused by the shortage of available organ donors, thousands of individuals who suffer from the consequences of catastrophic accidents, illnesses, or genetic abnormalities are left without healthy organs or tissues.[6] Unfortunately, many such patients die before organ transplantation can be performed and this has stimulated the quest for solutions to replace human organs with suitable alternatives. One is tissue engineering; this is a new discipline focused on developing engineered tissue and organ alternatives that can be used to repair or permanently replace damaged tissue.[7] Biomedical engineers are designing 3D temporary organ scaffolds to regenerate injured tissues, leading to the development of artificial organs.[8] It is also possible to utilize nerve healing in biomaterial-based structures in conjunction with other tissues for advanced tissue engineering. Furthermore, 3D printing is being progressively adopted in medical imaging. In clinical practice, 3D printed prototypes

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are effective and beneficial for medical treatment and medical study.[9] The use of 3D printing for developing instruments to facilitate and enhance clinical processes also has a long history. Accidents in daily life are causing severe damage to nerves, and dealing with nerve injury complications can be risky. So experiments on rats demonstrated that in the case of an injury damaging a nerve, the gap could be successfully repaired. Huang *et al.* implanted Spidrex conduits 10 mm in length to bridge an 8 mm gap in the rat sciatic nerve and proved that Spidrex conduits could promote axonal regeneration, which could lead to the loss of muscular function or sensation.[10] In this situation, a scaffold can connect the two phases of the damaged nerve, especially in the case of severe nerve injury. Mobaraki *et al.* used 3D bioprinting technology to produce a porous structure composed of the patient's neural cells and a biopolymer that served as a bridge over a wounded nerve.[11] The overall foundation of 3D-bioprinting relies on the controlled placement of biological elements, biochemicals, and living cells in layers. Autonomous self-assembly, biomimicry, and mini tissue building blocks are the primary methodologies used in 3D-bioprinting.[12]

In the modern era, much progress in the 3D printing of organ models has been made.[13] An artificial organ is a technologically advanced engineered device that is transplanted or assimilated into the human body interacting with biological tissues to interchange a natural organ and emulate a specific function or multiple functions *via* biomimetic concepts.[14] These functions help the patient to recover and continue with normal life quickly. Many clinicians or researchers prefer the personalization of organ models to ensure a better treatment methodology for patients. 3D printing approaches easily and quickly prepare different organ models or blood vessels without any excess use of biomaterials.[15,16] Owing to the superior properties of mimicking the real physical properties of the organs, the models can be employed for interoperative applications or give an overview of presurgical requirements.[17] Surgeons can easily analyze or simulate operations in 3D printed organ models leading to improved skills and avoiding any post-operational risk.[18] Beyond surgery, these organ models can be employed for teaching medical students, repeating biological experiments, testing drugs, *etc.* The biological 3D printing approach can promote collaboration between engineering and medicine. In the near future, 3D-printed organ models will improve human life and health.[19]

3D bioprinting has received a lot of coverage in the biomedical field in recent years; various review articles have concentrated on specific aspects such as fundamentals and procedures. However, recent advances in the 3D bioprinting of organ models and insights into its medical usage need to be discussed. In this context, we elucidate the feasibility of organ models in medical applications, transplantation, and cancer research by employing 3D bio-printing technology. Next, we categorically discuss the advantages and challenges of 3D-printed organ models. Finally, we present our perspective on future directions. The scope and main content are summarized in Fig. 1.

2. Fundamentals of 3D bioprinting

The 3D printing process relies on the accurate placing of biological components, biomolecules, and living cells layer by layer, which makes it superior to other technologies and it involves the placement of specific compositions onto the fabricated 3D structure. Some physical phenomena in 3D/4D bioprinting (*e.g.*, droplet/filament formation, droplet impact on the material, self-deformation induced by stimulation) are strongly related to dynamics and therefore will impact the printing resolution and adherence of printed


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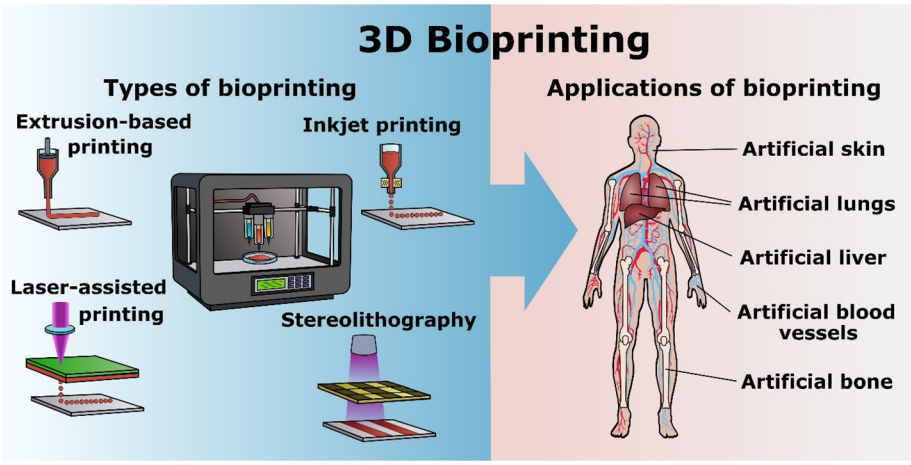

Fig. 1 An overview of the 3D-bioprinting techniques and their applications presented in this paper.

bioconstructs.[20–22] It uses three distinct approaches: biomimicry or biomimetics, active and self-assembly, and small building blocks.

2.1 Biomimetics

Biomimicry (*i.e.*, mimicking nature) is a technique that emulates aspects of the natural world to find the best solution to human issues. Many believe there are various blueprints from natural systems that will enable humans to design sustainable processes. The integration of biomimetic components into a bio-printed structure affects the ability of both native and foreign cells to attach, migrate, proliferate, and function.[23] The materials involved in cell attachment and determining cell size and shape also play a key role in the creation of a robust scaffold in that it permits the management of proliferation and differentiation.[24] Additionally, characteristics at the nanoscale, such as ridges, steps, and grooves, may affect cell adhesion, proliferation, and cytoskeletal assembly.[25] Secondly, the 3D environment can influence cellular morphology and development in a stem cell composition.[26] A biomimetic technique in 3D bioprinting requires an understanding of the fundamental collagen content of the organ of interest.[27] In short, it promotes the replication of identical cellular and extracellular components of tissues or organs based on a detailed examination of the corresponding natural objects. Successful biomimicry implies the accurate reproduction of tissue-specific functional components. Thus, the components utilized in this method influence cell attachment, cell size, and morphology, while the scaffold determines the control of cell proliferation and differentiation. A complete analysis of the cellular environ-


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ment, including cell type arrangement, extracellular matrix composition, a curve of soluble and insoluble factors, and the existence of biological forces.

Three-levels of biomimicry include (1) mimicking forms, materials, or functions of one specific organism, (2) copying the behavior of one organism or its surrounding environment, and (3) replicating various components in an ecosystem.[28] For the utilisation of 3D printing in artificial organs, the ideas of the last two levels should be the main approaches as organs are composed of various components and the organs should work well with the human body, which is a delicate and complex system. Biomimicry could be used as a transition or pathway toward success. Tissue-specific functional components of body tissue must be accurately reproduced to perform their functions successfully.[29]

2.2. Active and self-assembly

It is possible to reproduce an organ or tissue *in vitro* by employing a process known as autonomous self-assembly, which is analogous to how a developing embryo makes organs.[30] The cytoskeletal components and suitable cell signals necessary for the autonomous organization and segmentation of the desired tissue are produced by early cell organelles of a developing tissue.[31] The utilization of self-assembling cells can now be considered a practical means of both conducting histogenesis and manipulating the many features of the tissue, including composition, location, and structure. However, a deeper understanding of the mechanics of fetal organogenesis and the capacity to manipulate the environment and control those mechanisms is difficult to achieve. A crucial element of tissue engineering is the scaffold, which is essentially a three-dimensional, highly porous substrate.[32] After living cells are cultured, typically in a suspension phase, the cells are put on the scaffold. The formation of the new tissue is promoted by the scaffold as it enables the cells to attach, reproduce, and grow. The internal architecture of the scaffold material helps to manage and adjust the biological features of the cell.[33] Table 1 compares the various types of scaffolds as well as their merits and demerits in 3D-bioprinting.

In summary, the method of replicating biological tissue using embryonic tissue development and the growth of organs as a model is known as autonomous self-assembly. A cellular component of the developing tissue generates its extracellular matrix and cell signals, allowing the independent entity and sequencing to form the desired microarchitecture. A scaffold-free version is created during the process by using self-assembling cellular spheroids that differentiate and organize to form the desired tissue. It considers the cell as a primary driver for tissue creation, directing the localization, function, and structure of the resulting tissue. This method can shed light on understanding embryonic tissue development and organogenesis.[34]

2.3. Small building blocks

The small tissue building blocks approach combines both previously described strategies. Small building blocks, which are small functional units of tissues and organs, are produced using this bioprinting method. The basic structural and functional units of the organs, such as the kidney neuron, are represented by small tissues. This micro tissue can then be created using either self-assembly or biomimicry. The bioprinting process begins with the assembly of micro tissues into macro-tissues based on biologically inspired organization, fol-

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Table 1 Overview of some bio-printing scaffolds

Bioprinting scaffold	Description	Printing ability	Advantages	Disadvantages	Examples
Natural	Derived from natural or biological materials	Difficult	Biodegradable, good mechanical strength and stability, promote adhesion, cost-effective	Difficult to modify as per specific needs, high chances of clogging	Agarose, gelatine, collagen, hyaluronic acid, matrigel
Synthetic	Derived from synthetic materials	Easy	Customizable to meet certain criteria demands (functional groupings), at an increased price	Not biodegradable	PEG-based bio-inks (PEG diacrylate), piezoelectric polymers
Hydrogel	Derived from synthetic materials that are hydrophilic	Easy	Permit gas, vitamin, mineral, and immunoisolation exchange, and regulate consistency	Poor mechanical properties, poor cell seeding, costly	Keratin or collagen biocompatible polymers, which may be built on poly(acrylic acid) hydrochloride

lowed by the replication of tissue units that can self-assemble to form structural components.[35] It was demonstrated that lattice, honeycomb, and fibrous bundle patterns could be printed using a small-scale laboratory printer.[36] Then, it was possible to translate them to a larger scale with a high throughput-printing platform. It shows a digital image of uniform linear and circular templates using gelatin–alginate bioink, gross morphologies, and SEM images of the cross-linked and non-crosslinked structures. The structures, obtained through these two various approaches, were investigated using scanning electron microscopy (SEM). The difference in the average pore diameters of the printed structures was found to be statistically insignificant.

3. Types of bioprinting

3D bioprinting is the topmost fabrication method that achieves precise stacking of biomaterials to create tissue-mimetic structures. There are three primary types of bioprinting, with inkjet and laser-assisted techniques being the most common.[37] Table 2 presents an overview and compares different types of bioprinting technologies. Despite the availability of various bioprinters, their basic concept remains the same: depositing materials to create a layered 3D structure.

3.1. Inkjet printers

Inkjet printers utilize droplet-on-demand (DOD) technology that enables the precise placement of tiny droplets of ink on a page.[38] The inkjet method may generate droplets that range in size from picolitres (average 13 μm), dropping numerous times within a few seconds, and achieve non-contact printing. Inkjet printing has been used extensively in the printing of text and graphics ever since it became available.[39] Applications of the technique have increased as technological capacity has evolved from two-dimensional (2D) to three-dimensional (3D), which facilitated the creation of electrical device components.[40] Researchers in the field of biological sciences and tissue engineering understood the usefulness of this technology before the end of the 20th century, due to its potential to deposit biological components using its picolitre-level printing unit.[41] Modern medicine has started using inkjet technology to manufacture drugs, build scaffolds, and deliver cells.[42] In order of increasing complexity, inkjet bioprinting can be applied to create generic polymers, biomolecules, DNA, and cells. The obstacles this technology currently faces, and their potential solutions are explored. Gravity and the impact force between printed droplets and the substrate are the two most significant factors limiting resolution and fidelity. Yuan *et al.* reported upward bioprinting, in which the bioprinter's nozzle was turned upside down and the ejection direction was opposite to gravity. As a result, it enhanced the resolution and fidelity of droplet-based bioprinting.[43]

Inkjet-based bioprinting was investigated for application in a novel concept, biopixels, based on the inkjet printing of basic biological components.[44] Control of the inkjet process is divided into two parts: 1. the development of individual droplets targeted to a specific substratum area; and 2. the for-

Table 2 Comparison of different types of common bio-printing technologies

Type of bioprinting	Throughput	Droplet size	Cell viability	Cell density	Print/fabrication speed	Print cost
Inkjet bioprinting	High	50–300 μm	>85%	Low, $<10^5$ cells per mL	Medium	Low
Microextrusion printing	Medium	5 μm to millimeters wide	40–80%	High	High	Medium
Laser-assisted bioprinting	Low to medium	>20–80 μm	>95%	Medium, 10^8 cells per mL	Low	High
Stereolithographic method	Medium	~1.2 μm	>85%	Low–moderate, 10^6 cells per mL	High	Low–moderate

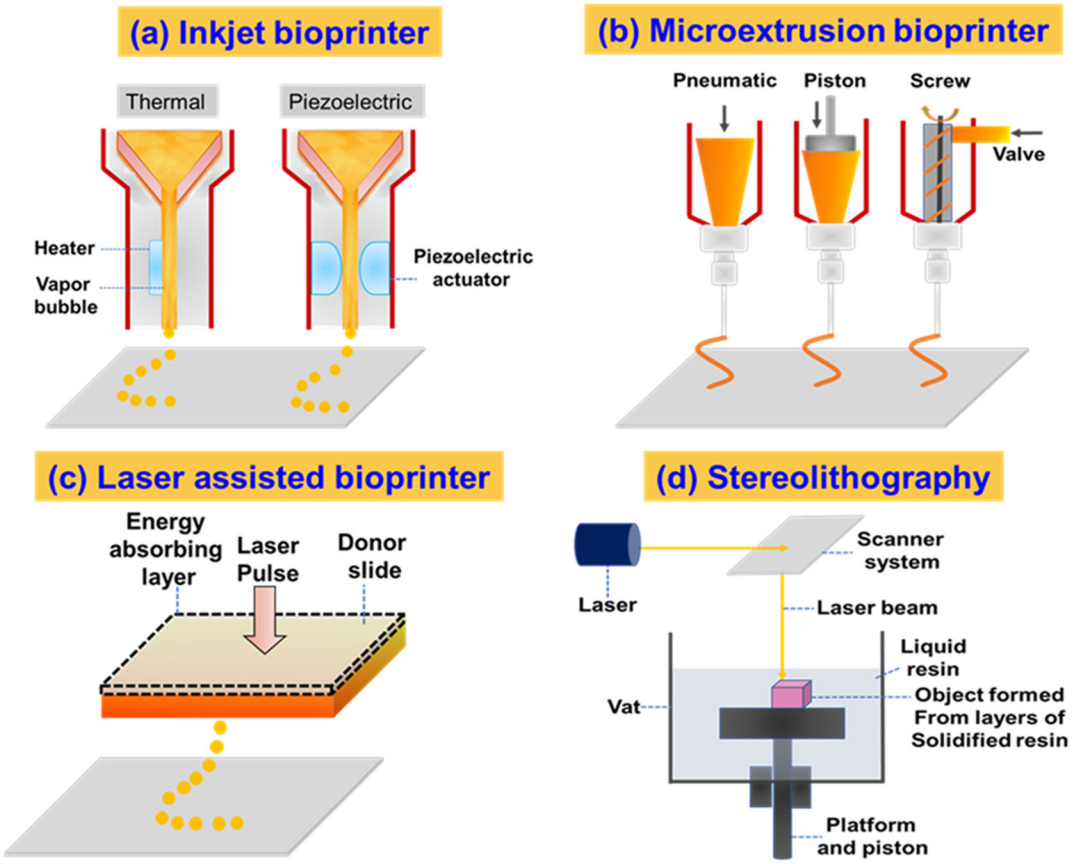

Fig. 2 Schematic representations of (a) inkjet bioprinting, (b) microextrusion bioprinting, (c) laser-assisted bioprinting, and (d) stereolithography.

mation of contact between droplets and substrates. There are two approaches for generating droplets. The continuous inkjet (CIJ) uses Rayleigh-plateau instability, a naturally occurring phenomenon that leads to the spontaneous change of a stream of liquid into a train of discrete drops.[45] CIJ printing is schematically presented in Fig. 2a. The drop-on-demand (DOD) inkjet, however, prints a droplet only when needed, and droplet deposition is carried out by moving the nozzle away from the target spot and then ejecting a droplet.[46]

DOD inkjet bioprinting could be additionally classified based on diverse droplet propulsion methods, such as thermal, piezoelectric, and electrostatic.[47] Thermal inkjet bioprinting technology is widely employed for proteins, cells, and various biologics.[48] Piezoelectric inkjet bioprinting uses a piezoelectric actuator to produce droplets.[49] When an impulsive voltage is applied to a piezoelectric crystal, a rapid and reversible deformation occurs, causing a sudden change in the volume of the chamber, which leads to the transmission of acoustic waves that provide the necessary pressure pulse to surpass the surface tension at the injector inlet.[50] Kim *et al.* demonstrated that polymer micro-patterning by inkjet printing controlled the cell adhesion geometry as shown in Fig. 3.[51] Electrostatic inkjet bioprinting is also capable of causing an instant increase in volume that aids in ejection; this is achieved by applying an impulse voltage to a baseplate and a motor, which causes a bending of the baseplate and the extrusion of bio-ink.[52]

3.2. Extrusion-based printers

Extrusion-based bioprinting (EBB) utilizes pneumatic or mechanical pressure for dispensing biomaterials with the help of a vessel.[53] Due to its potential to generate appropriate structures with a preferred internal structure, high accuracy in the microstructural establishment and cellular configuration, and flexibility in biodegradable polymers, viable cells, and preservative drug usage, EBB has grown into a leading technique in the field of biomedical engineering.[54] EBB has indeed been used in the regeneration of damaged tissues and organs, as well as the generation of *in vitro* tissue for drug delivery and clinical diagnostics. Extrusion-based methods are currently the most widespread and favored.[55] After cell suspensions are implanted in biomaterials, a combination of material characteristics and printer configurations, such as nozzle outlet diameter, material concentration, and working temperature, are employed to implant the cells and influence the cells' viability when extruded. Furthermore, these parameters affect the biomaterials' potential to form precise geometric shapes, known as the printing ability.[56] Until now, EBB parameter tuning was performed by systematic wet-lab research. Such a procedure may take a long time, and it can be challenging to apply the

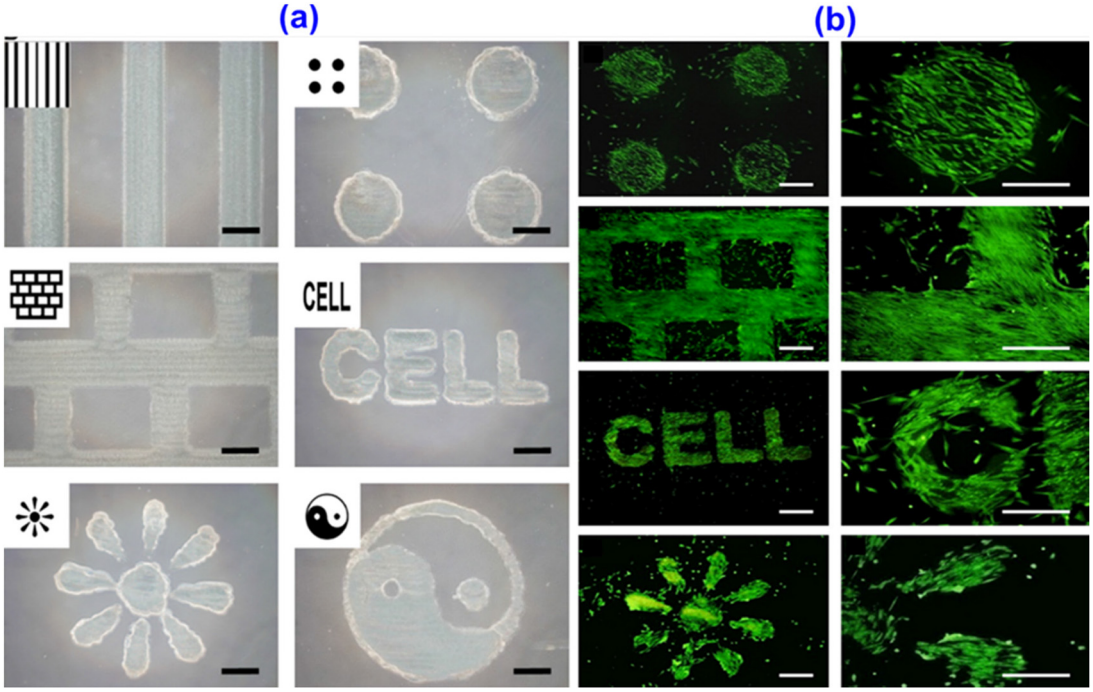

Fig. 3 (a) Optical micrographs of inkjet-printed PLGA patterns (scale bar is given by white horizontal bars showing 500 μm); (b) fluorescence microscope images of the human adipose-derived stem cells stably attached and proliferated within the different patterns of the PLGA on the PS substrate: dot pattern, brick pattern, "CELL" letter pattern, and flower pattern. White horizontal bars represent 500 μm. Reprinted from ref. 51 with permission from Elsevier. Copyright (2010) Elsevier.

results to diverse biomaterials and printers. EBB printing is schematically presented in Fig. 2b.

The extrusion of a solid filament material under pressure leaves a single strand behind. Through micro-extrusion printing, ink is used to create biomaterial structures with ink cartridges, and nozzles/needles linked to them.[57] Heterogeneous materials can be printed with multiple cartridges by loading them into the printer. To print cell-containing materials through bioprinting, the cells must be mixed in a bioplastic solution. To preserve the cells from the shocks they endure during printing, a substance called bio-ink is employed to enclose them and create a conducive extracellular matrix (ECM) environment.[58]

3.3. Laser-assisted printer

Laser-assisted bioprinting (LAB) is cutting-edge technology based on a laser-assisted hydrogel microdroplet transfer method. Laser-assisted bioprinting (LAB) makes use of a laser accurately placing biomaterials on a substrate. Pulsed laser sources, ribbons loaded with liquid biological components, and reception substrates are all often part of this process.[59] Specifically, the laser heats the ribbon, evaporating the liquids to make them available on the detecting substrate in the form of droplets. A biodegradable polymer or cell culture medium is present in the substrate to keep cells from sticking or growing when they are transferred first from the ribbon. For the printing of gels, cells, proteins, and ceramic materials, LAB utilizes ultrafast lasers with ultraviolet or similar wavelengths as energy sources.[60] LAB printing is schematically presented in Fig. 2c.

This technology is of special importance and has been popular in past years for advancing 3D biomimetic *in vitro* models. Such models play a significant role in a variety of biomedical engineering applications such as *in-vitro* diagnostics, high-throughput drug screening, cell-based therapies, and revealing key characteristics of various pathologies.[60] Bioengineering methodologies like the fabrication of topographically 3D engineered constructs form models with well-controlled features mimicking the same *in vivo* conditions. The re-emergence of recycled multicellular aggregates and more complex organoids can contribute to our understanding of 3D cell cultures.[61]

3.4. Stereolithography (STL)

Stereolithography (STL) is a stable and freeform (nozzle-free) method to create 3D structures from a wide range of biological and non-biological materials. This technology is suitable for producing complex parts with great accuracy and typically uses hydrogels sensitive to light, which are placed layer by layer to form a 3D structure. Using biological materials, is characterized by a rapid efficacy of approximately 40 000 mm s^{-1} and by over 90% cell viability.[62]

Stereolithographic 3D printing is a stable manufacturing technology that was pioneered in 1986 by the manufacturing company 3D systems.[63] Stereolithography, the most widely used form of solid fabrication technology, has undergone gen-

erations of refinement in precision and reliability, resulting in a performance similar to that of the conventional machine grinding process and making it the most commercially feasible fabrication technique currently accessible. The various developments in this discipline, as well as the benefits associated with this flexible production method, stimulate its extensive application and adaptability in a diverse range of industries, with biomedical and biochemical engineering being two of the most significant applications.[64]

Stereolithographic systems use photopolymerization, also known as light-initiated polymerization, which is classified into two categories: single-photon and multiphoton technologies. These two technologies differ by the method of light excitation and absorption that promotes polymerization.[65] Single-photon methods are further classified as follows: (1) visible radiation systems that utilize the visible light range; (2) conventional stereolithography systems, which utilize ultraviolet (UV) radiation; (3) infrared (IR) stereolithography systems, which use infrared (IR) radiation; and (4) stereothermal lithography systems, which employ a combination of UV and IR radiation to promote polymerization.[66] The production principle relies on polymerization (hardening) of a fluid photosensitive polymer with light-sensitive compounds when it is exposed to light (Fig. 2d). The intensity of light can be adjusted by utilizing a digital micromirror array. Such a process enables the creation of arbitrary-shaped 3D models and is further utilized to produce many animal tissues/organs.

3.4.1. Single photon stereolithography. In this process, excitation of the photoinitiator is influenced by the absorption of a single photon, thus justifying the name "single-photon stereolithographic fabrication processes". Such a type of photopolymerization includes UV light-based stereolithography as well as visible light-based stereolithography.[67] Laser-assisted writing and mask-based UV light-based stereolithography are two commonly known methods for single-photon photolithography in biomedical uses.[68]

3.4.2. Multiple photon stereolithography. Two-photon stereolithographic fabrication processes represent the most basic example of multiphoton absorption because they involve the sequential or concurrent absorption of two relatively low-intensity photons to excite a light-sensitive resin to a high-energy radical state. This method of excitation is quadratically dependent on incident light intensity (as opposed to a linear relationship for single-photon stereolithography), allowing for extremely fast 3D fabrication with submicron resolution.[69]

3.4.3. Interference lithography. Interference lithography is a novel type of photolithography that involves making a constructive interference among multiple cohesive visible light rays to generate a sequence of high and low-intensity light fringes.[70] Light-sensitive adhesives exposed to this interference-derived light pattern are thus polymerized in high-intensity fringe regions. This technique can be used to create patterns at nanoscale resolution and provides the added benefit of faster polymerization than conventional stereolithography. Tissue engineering of cancellous/trabecular bone, for example, necessitates the formation of an ossified "spongy" scaffold with a repetitive porous structure. Apparatus for interference lithography could be used to quickly and accurately fabricate this type of scaffold, as well as other scaffolds that require similar repetitive porous structures.[63]

4. Bio-inks in bioprinters

3D bioprinting uses various biological materials known as bio-inks to produce complex designs of tissues.[71] The term "bio-ink" is used to refer to both the cellular material employed in manufacturing and the other chemicals that aid in the development of the cells.

The bioprinting materials need (1) to be strong and durable to ensure high-quality shapes of produced parts and at the same time (2) must have properties similar to those of the living tissue, so the final tissue structures are accurately modeled. To satisfy the first requirement, bio-ink components must reveal tunable gelation and stability.[62] While for the second requirement, the bio-inks must be biocompatible and able to degrade in a natural microenvironment to mimic the natural healing process. Besides, chemical changes should permit bio-inks to produce specific tissues[35] and the rate of degradation should imitate the organic microenvironment to meet tissue-specific needs. Thus, the choice of an appropriate bio-ink is a critical phase and should be based on the physical, biochemical, biological, and viscoelastic qualities of the materials[72,73] (Fig. 4). These properties result in tissue constructs with appropriate mechanical strength and robustness while retaining tissue-matching mechanics, preferably in a tunable manner with adjustable gelation and stabilization to facilitate the bioprinting of structures with flexible biological properties. The rate of degradation of tissues imitating the organic microenvironment of the suitability of these tissues for chemical modifications in order to meet tissue-specific needs. Furthermore, standardised bio-ink formulations that can be used in a variety of bioprinting applications are urgently needed.[74] This requires accurate modelling of final tissue structures.

To enable suitable growth and development of cells, commercial materials used in bioprinting constitute a 3D molecular scaffold that is composed of biopolymer gels. Such biopolymers comprising a bio-ink are important because they help retain water inside a created tissue (depending on hydrophilicity), thereby ensuring its mechanical stability as well as maintaining embedded living cells. Das *et al.* developed a silk fibroin–gelatine-based bioink that differentiated encapsulated stem cells for targeted tissue formation as shown in Fig. 5.[75]

The costs of bio-inks are determined by the raw materials used. Cell-laden bio-inks, for instance, are relatively expensive because cell incorporation necessitates accurate control, advanced and powerful instrumentation, and skilled labour. Each batch's cell number restoration also requires precise control. In addition, the cost of a bio-ink is determined by cell type, doubling time, culture media, growing environment, and

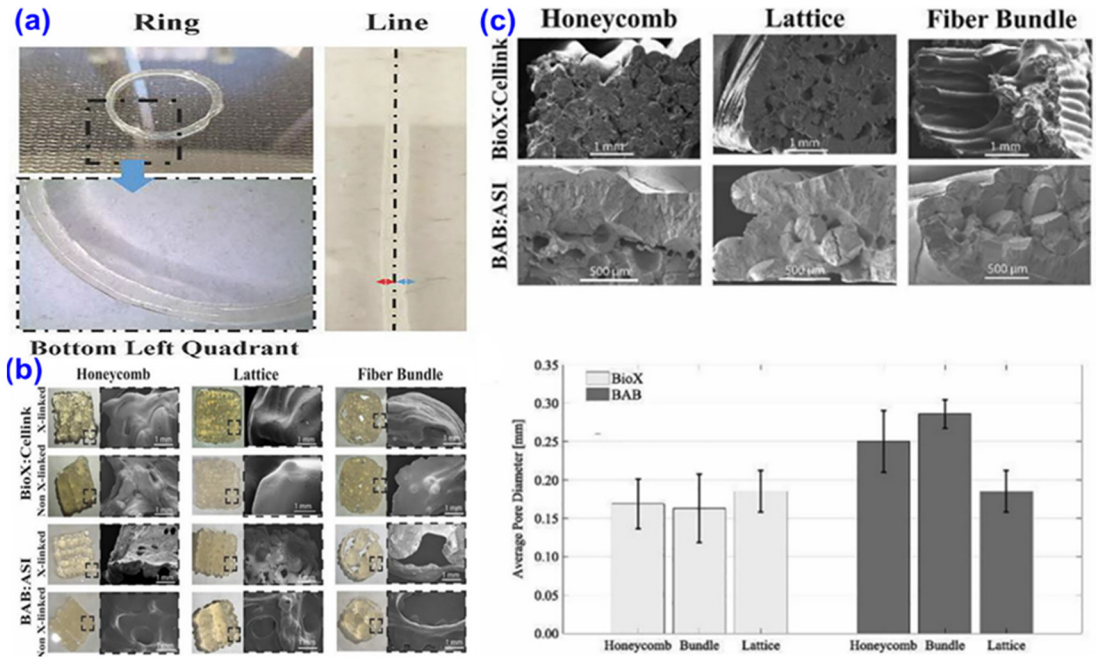

Fig. 4 (a) Digital image of uniform linear and circular templates using gelatin–alginate bioink, (b) gross morphologies and SEM images of the cross-linked and non-crosslinked structures, (c) cross-sectional SEM images and average pore diameters of the patterns were printed using two different bioprinting platforms: a small-scale laboratory bioprinter (BioX) and the high throughput printing platform BioAssembly Bot (BAB). Reprinted from ref. 72 with permission from Elsevier. Copyright (2020) Elsevier.

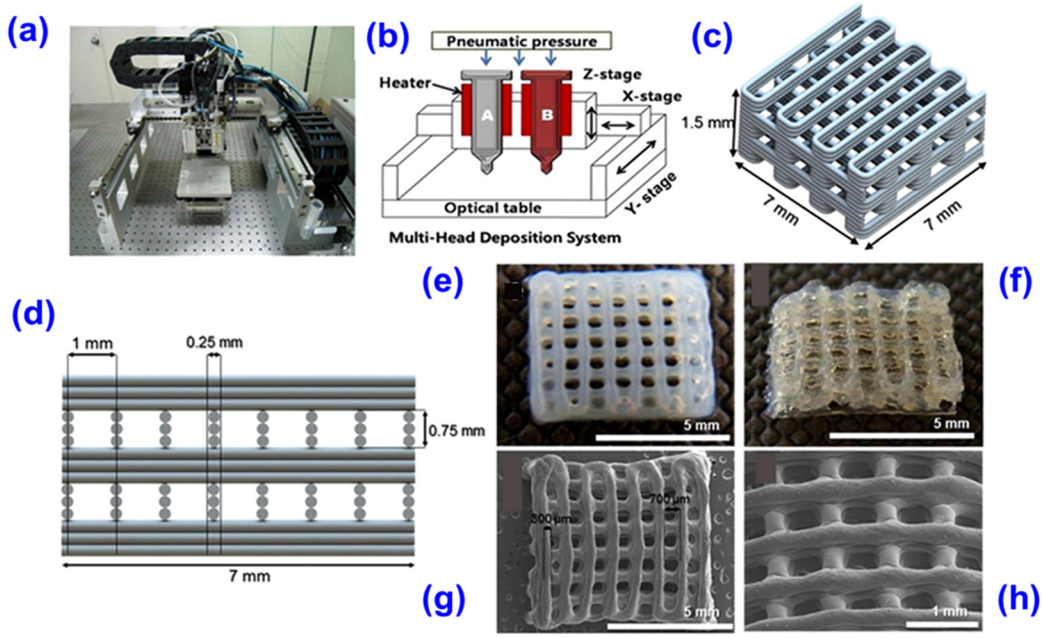

Fig. 5 A photograph (a) and scheme (b) of the multi-head deposition system used for 3D-bioprinting of the silk–gelatin constructs. Schematic diagrams (c and d) of the printed structures. Representative images of self-standing silk fibroin–gelatin (SF–G) constructs: (e) sonication-induced β-sheet crystallized SF–G and (f) tyrosinase crosslinked SF–G constructs. SEM images (g and h) of the tyrosinase crosslinked SF–G constructs. Reprinted from ref. 75 with permission from Elsevier. Copyright (2015) Elsevier.

speed of ECM deposition. Except for some natural hydrogels, such as collagen, laminin, and hyaluronic acid, which are prohibitively expensive due to their complex separation protocols, the majority of widely viable customizable hydrogels are inexpensive even without cell incorporation.[76] The availability of biopolymers eventually impacts on the cost of bio-printed

Table 3 Different types of bio-inks, bio-printers, cell viability percentages, and cell types

Type of bio-ink	Biomaterials	Bio-printing method	Targeted cell/tissue type	Cell viability	Ref.
Natural	Agarose/chitosan	Extrusion-based bioprinting	hMSCs	≥95%	182
	Alginate	Laser-assisted bioprinting	Human breast cancer cells	≥85%	183
	Gelatine	Extrusion-based printing	MSC cartilage	around 80%	184
	Collagen	Custom-made drop-on-demand bioprinting extrusion-based printing	MSC bone	>95%	185
	Fibrinogen/fibrin	Custom-made inkjet bioprinter	Cartilage	>80%	186
Synthetic	PEG	Extrusion-based printing	hBMSCs	>75%	187
	PVP	Inkjet bioprinting	HFF-1 (fibroblasts)	—	188
	Pluronic	Extrusion-based printing	Cartilage	62% for pure, 86% for modified	189
Commercial	Novogel	Extrusion-based printing	Aorta	>80%	190
	Dermatrix	Inkjet based bioprinting	Myogenic cells	—	191

structures. Synthetic polymers, for instance, are more widely available but can be toxic to the environment, whereas natural biomaterials are environmentally friendly, but they are often limited resources. Nonetheless, a few pure polymers generated from natural sources (*e.g.*, alginate and chitosan) are very affordable due to more standardized and convenient extraction techniques. They are derived from an abundance of resources. Table 3 shows the various bio-inks, types of bioprinters, cell viability percentages, and cell types used in 3D-bioprinting.

5. 3D bioprinting procedures

The entire process of producing bio-composites using the bioprinting technique comprises numerous phases that involve multiple technical fields. Pre-bioprinting, bioprinting, and post-bioprinting are the three phases of the process. Every step enhances the efficiency of the produced structures and can influence others. Tissue dissection and cell growth procedures, for example, are important in pre-bioprinting as they ensure that a large number of cells are available for mass organ creation. Medical imaging is also necessary since high-resolution images are required to bio-print them precisely. To obtain structures with excellent cell survival and adhesion, biomaterial compatibility must be coordinated with solidification kinetics during the bioprinting step. There is a need to develop bioprinters that are more mobile, can operate over many hours without malfunction, and are compact and affordable. Finally, the range of assessment even during the post-bioprinting phase regulates the maturation of bioprinter cells. Three steps are needed to complete the 3D-bioprinting process: pre-bioprinting, bioprinting, and post-bioprinting.

6. Advantages of 3D bioprinting

3D bioprinting is one of the most impressive and groundbreaking developments in tissue engineering.[77] Once it enables the reproduction of living organs, such as the heart and lungs, or replaces damaged skin, it could become life-changing technology that was previously imagined to be purely science fiction.[78] One of the most important keys to the progress made in bioprinting is the growth of technology capable of carefully and precisely constructing living tissue.[79] For this purpose, sub-micron cells must be placed properly and repeatedly. One of the ways to achieve it is to carefully dispense bio-ink, composed of living cells, into a bio-paper gel scaffold, which keeps the layers together. When cells are dispersed using non-contact jetting methods, the tissue quality also improves as well.[80] Recent, developments in 3D bio-printing technology have enabled this degree of precision, and a remarkable example is from Izumi International, Inc., which offers some of the most modern biomedical dispensing equipment. The following are a few instances where this amazing technology may have an impact on future progress.

6.1. Potential of bioprinting to replace organ donors

In 2009, 154 324 patients in the United States were registered on the human organ transplant waiting list. Fewer than 27 000 of them obtained the organs needed to live. Of the remaining patients expecting to be next on the waiting list, unfortunately, 8863 of them died.[81] If 3D-bioprinting were adopted, all of those patients might receive organs within days rather than years, as one of the aims of bioprinting is to fabricate living organs, including livers, kidneys, and lungs, of the human body.[82] This technology has the potential to decrease or even eliminate the shortage of organs for transplant, providing everyone with a fair chance at a new life. In addition, a separate line of research is underway to develop skin, the largest and most fragile human organ. Progress in this direction could help scientists and clinicians repair wounds faster and more effectively.[83]

6.2. Potential for bioprinting to prevent cell rejection

The creation of human tissue that functions normally is very challenging, and the chances of finding a donor with appropriate tissue cells are also limited. Incompatibility with foreign cells can cause the immune system to harm the body, which significantly complicates the process of organ transplant.[3] If the immune system attacks the new addition, it results in complications and health issues, and a patient will need to undergo a new transplant (*i.e.* time-consuming and painful approach) or continue to take immunosuppressants throughout their life. In contrast, 3D bio-printing technology enables cells to be cultured directly from the patient. This ensures that

the transplant will not be rejected by the body after the transplantation procedure.[84]

6.3. Bioprinting to eliminate animals and humans from testing laboratories

In the United States, laboratory testing causes suffering and death to 100 million animals.[85] A lot of scandals emerged from cosmetic research laboratories, following this, the L'Oréal Company became the first makeup company to test its products on bio-printed tissue. As tissue manufacturing continues to advance and become more commonly accessible, each beauty brand could follow an alternative way, such as using printable objects for product testing that does not involve the exploitation of animals. Moreover, it can be estimated that very soon drug research facilities will use bioprinted tissue to replace human test subjects, which will promote health and safety. Hence, 3D bioprinting could become the safest scheme for testing newly developed drugs before their release.[86]

6.4. Biocompatibility

The suitability of biomaterials is based first and foremost on their biocompatibility, thereby limiting the number of materials that can be used to fabricate scaffolds. He *et al.* bioprinted a hydrogel for tissue engineering with highly bio-compatible features as shown in Fig. 6.[87] As previously stated, the 3D material must be biocompatible and cell cytotoxicity must be avoided. Maxson *et al.* reported an *in vivo* study that used

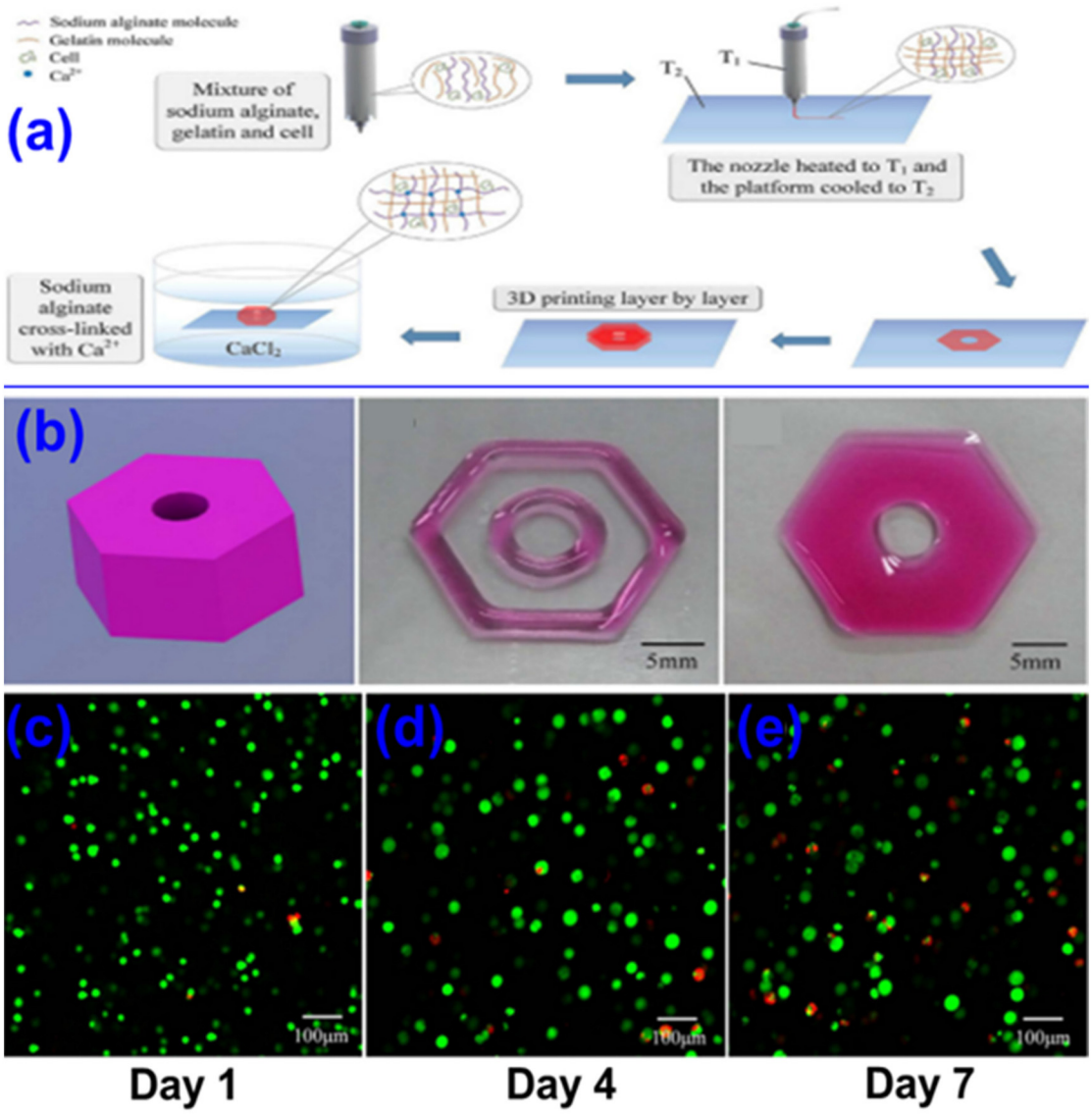

Fig. 6 (a) Illustration of the schematic of the bioprinting process, (b) image of the 3D printed hydrogel scaffold and laser scanning confocal fluorescence microscopy images showing the viability of cells after (c) one day, (d) four days, and (e) seven days. Live and dead cells are represented by the fluorescent green and fluorescent red spots, respectively. Reprinted from ref. 87 under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright (2016) Springer Nature.

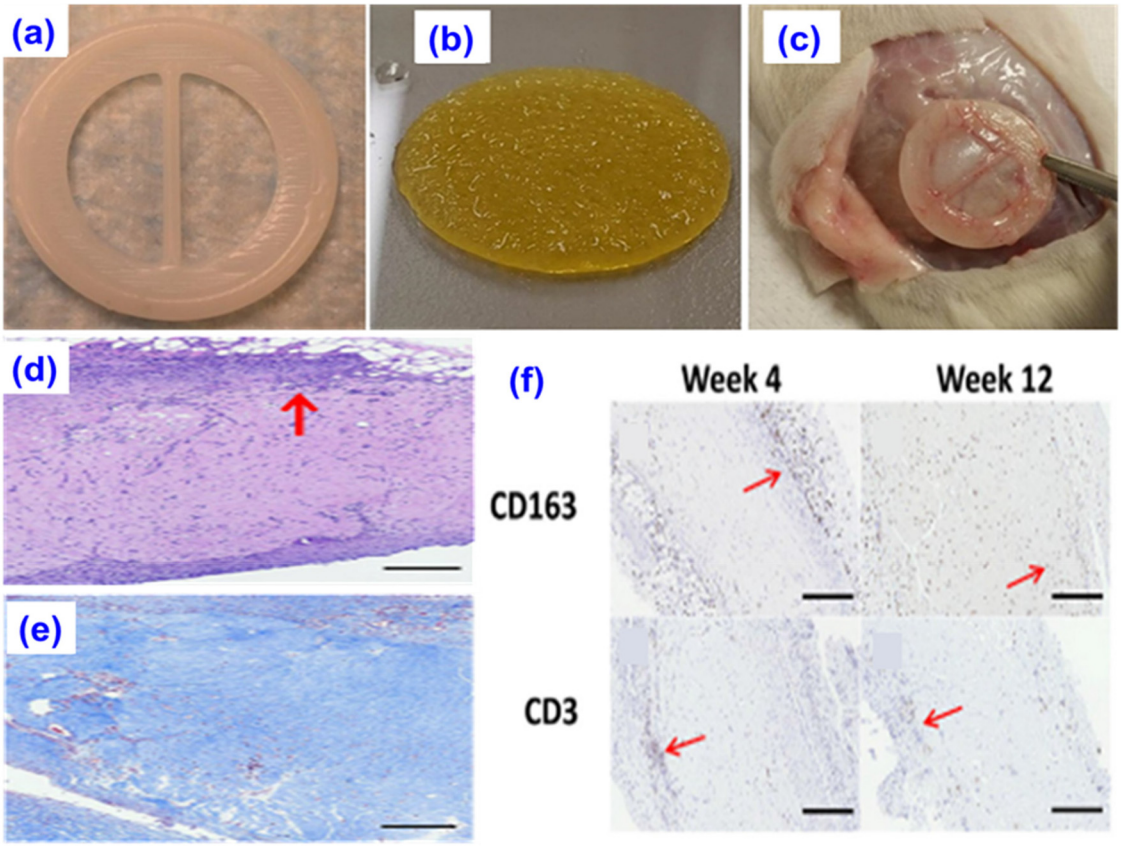

Fig. 7 Images of (a) the polycaprolactone support frame, (b) the bioprinted sample, and (c) the 3D-printed heart valve scaffold explanted at 12 weeks *in vivo*. Immunohistochemical staining of explanted scaffold (d) hematoxylin and eosin visualized using a slide scanner. The red arrow indicates an increase in host cellular concentration found at the periphery. Masson's trichrome (e) presents a diffuse blue expression representative of collagen within the 3D bioprinted disk scaffold. The scale bars in figures (d) and (e) represent 300 μm. (f) CD163 and CD3 immunohistochemical staining for the heart valve scaffold printed with rMSCs observed at 4 and 12 weeks, scale bar = 300 μm. Reprinted from ref. 88 under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright (2019) Elsevier.

collagen-based bio-ink as a substitute for an artificial heart valve (Fig. 7).[88] The obtained results indicated elevated host cellularisation potential, biocompatibility, and biomechanical behavior. Rat mesenchymal stem cells (MSCs) were successfully printed in bio-ink, which showed transformation.[89] Scaffold components should facilitate entrapped cell lines and the receiver body. As a result, the implant must be cytocompatible and encourage cellular growth, adhesion, proliferation, and migration while being suitable for the host and causing little irritation or immunological refusal. *In vivo* tests were carried out by Bejleri *et al.* using bioengineered cardiac patches made of native sub-dermal ECM and human cardiac haematopoietic cells (hCPCs). The cell viability of this particular combination of bio-inks was greater than 75%.[90]

7. Applications of 3D bioprinting

Much is being expected of the 3D-bioprinting process by scientists, who believe that it has promising potential in tissue engineering due to its adaptability and excellent resolution.[91] Several tissue types, including skin, bone, liver, cardiovascular, and neuronal tissues, have already been created using bioprinting techniques. Researchers demonstrated the capabilities of 3D printing for wearables and consumer electronics, but other applications are also a possibility.[92] The technology could, for example, be utilized to enhance robotic systems, power generation, tactile sensing, and smart architecture. It is also possible to customize the 3D-printed piezoelectric material as a monitoring tool for detecting collisions, vibrations, and other motions.[93] Microfluidic technology can be employed to build organs-on-chips by combining it with organ-printing technology. These organs-on-chips offer a multitude of uses, from disease models and drug development to the testing of thousands of compounds in a short period. The reaction of organs-on-chips to medications is realistic because they mimic the native extracellular matrix by using a 3D model.[94] Until now, research has focused on the heart and liver, but a full body-on-a-chip model may be created.

Body-on-a-chip systems, which use 3D-printed organs, can be created by merging several systems. Researchers have already utilized a heart-on-a-chip model to see how doxo-

rubicin and other medications that impact the heart rate might affect individuals.[95] The liver, heart, lungs, and kidney-on-a-chip are all included in the new body-on-a-chip system. Organs-on-a-chip are printed or built independently and assembled afterward. The use of this technology expedites drug discovery by enabling high-throughput toxicity assessments.[96]

7.1. Bioprinting of artificial skin

Skin plays a crucial part in offering protection from the surroundings as well as in the growth and repair of the human body.[97] Although many types of products have been developed that are considered substitutes and are currently used in clinical practice, these are not suitable for the treatment of individualized skin conditions. Such marketing of skin alternatives should be adjusted throughout therapeutic interventions, which enhances the total cost and complexity of wound care.[98] A cutting-edge approach to biological manufacturing is three-dimensional (3D) bioprinting. To produce intricate biological tissues, it precisely deposits bioinks into 3D structures that have already been developed.[99] The steps for the fabrication of 3D bioprinted skin tissue and the main factors affecting skin bioprinting are summarised in Fig. 8a and b.

Multicellular 3D constructs could be created using laser-assisted 3D bioprinting, according to Guillotin *et al.* and team.[60] These constructs are made up of fibroblasts that are integrated into an extracellular matrix and have epithelial tissue that is used as bio-ink. Skin tissue printing was traditionally accomplished through the use of colloidal suspension bioprinting. As an alternative to collagen-based biomaterials, a recent modification of chitosan-based biomaterials, which have antimicrobial properties, was described by Z. Deng *et al.* that is suitable for functional skin bioprinting applications. Since there is no sustained collagen crosslinking time in the chitosan-based method, it can overcome poor printability while also speeding up the process.[100] A vital component of successful skin grafting is the ability of the tissue graft to maintain tissue viability through the vascularisation of the grafted area.[101]

Cells can be printed on gels using inkjet devices, and they have high cell viability, indicating that they are viable cells. Once endothelial cells, keratinocytes, and fibroblasts were co-printed into a collagen matrix to encourage vascularization of skin implants, Baltazar *et al.* reported that the mixture favoured cell survival while also increasing wound contraction.[102] However, the generation of new hair follicles or sweat gland growth in skin grafts continues to be a significant challenge that will require additional research in the future for a better understanding.[103] Thankfully, there is hope that hair follicle growth is dependent on hair neogenesis between the dermal papillae and epidermal cells. Abaci *et al.* developed a biomimetic approach that led to keratinocyte (KC) differentiation into specific hair lineages and generated human hair follicles (HFs) within human skin constructs (HSCs) in an entirely *ex vivo* context by 3D printing technology.[104] It is possible that within a short period, skin-mimicking abstractions with vasculature, nerves, hair follicles, and sweat glands will be able to be created by 3D-bioprinting technology.[105] Recently, Weng *et al.* provided a detailed review focusing on 3D bioprinting focusing on skin tissue engineering, specifically on hair follicles, sweat glands, and vascularization.[106]

7.2. Bioprinting of artificial liver

Liver fibrosis is indeed a critical issue that impacts a significant portion of the population. The disease is the final outcome of a series of intricate and progressive exchanges among hepatocellular and non-parenchymal cells.[107] Experts have found replication challenging due to the intricacy of the

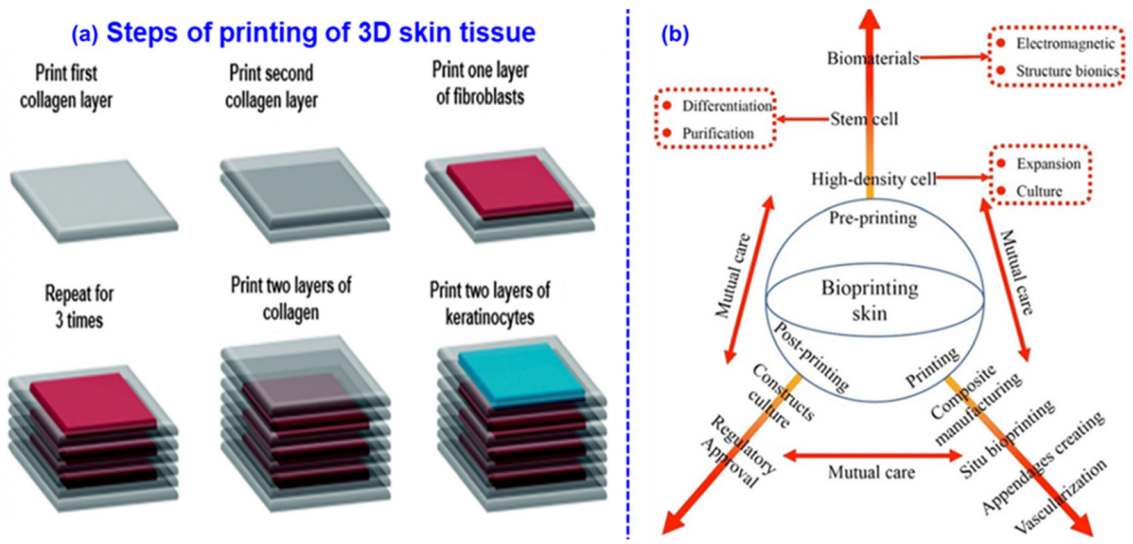

Fig. 8 (a) Steps for the 3D bioprinting of skin tissue and (b) a scheme presenting the relationships between major factors crucial for the development of bioprinted skin. Reprinted from ref. 99 with permission from Elsevier. Copyright (2021) Elsevier.

subject. In researching liver diseases, one of the initial bioprinted liver tissue models is made of *ex vivo* hepatocytes, endothelial cells, and Kupffer cells.[108]

Cheng *et al.* developed a prototype using 3D printing in which 30 layers of hepatocyte/gelatine mixture were laminated and enclosed in a high spatial structure. For more than two months, the 3D hepatocyte/gelatin continued to function well and conducted physiological activity in the structure.[109] 3D printing of human liver tissues was carried out by Organovo *et al.* by employing a syringe-based extrusion printer. They achieved operational reliability for 28 days in an attempt to develop individualized tissues and organs for targeted therapy.[2] Hepatocytes, hepatic stellate, and endothelial cells were used to demonstrate a multicellular liver structure such as endothelial cells (ECs). In 3D liver tissues, albumin formation, cholesterol biosynthesis, fibrinogen and transferrin production, and inducible cytochrome CYP 1A2 and CYP 3A4 activities, were all found. Such 3D-vascularised liver *in vitro* models could potentially be used to replace damaged livers in people.[110]

To assess clinical drug-induced toxicity *in vitro*, Nguyen *et al.* created a unique bioprinted human micro liver tissue from a co-culture of primary human hepatocytes, hepatic stellate cells (HSC), and human umbilical vein endothelial cells (HUVECs) using an inkjet 3D bioprinter[111] (Fig. 9a–h). At the tissue level, a histological investigation revealed the presence of discrete interstitial hepatocyte junctions, CD31+ endothelial networks, and desmin-positive, smooth muscle actin-negative quiescent stellate, which resembled the *in vivo* human drug response. Primary hepatocyte proliferation, long-term culture, and *ex vivo* preservation of hepatocyte function are fundamental hurdles in liver tissue engineering.[112] Arai *et al.* employed an inkjet 3D bioprinter to build a 3D growing medium with a synthetic scaffold to investigate the liver-specific functions of hepatocytes. The printed liver tissue produced liver-specific proteins and receptors like MPR2, albumin, and asialoglycoprotein receptor (ASGPR).[113] Recently, Taymour *et al.* used core–shell 3D bioprinting to build a viable model of hepatocytes with individually configurable compartments for distinct cell types. The scaffold was made of matrigel, alginate, and methylcellulose-based bioink (algMC). This serves as the foundation for more complicated *in vitro* models, enabling the co-culture of hepatocytes with other cell types specific to the liver to closely imitate the microenvironment of the liver. Additionally, matrix functionalization improved the adhesion, viability, proliferation, and function of both cell types in their respective compartments.[114] This 3D bioprinting technology not only helps to build an artificial liver for transplant but also helps in research to advance drug studies without harming animals.

7.3. Bioprinting of cardiac tissues

After the development of noninvasive and surgical treatments, cardiologists and cardiovascular surgeons are now able to spatially distinguish complicated cardiovascular anatomic interconnections.[115] Along with most of the advances, portable 3D printed models of cardiovascular structures provide a straightforward and unambiguous pathway for procedural and surgical planning in addition to traditional imaging techniques. Furthermore, 3D printed models are useful as teaching and communication systems for the medical practitioner.[116,117]

By utilizing a 3D bioprinting approach, Wang *et al.* constructed contractile heart tissue with cellular organization, homogeneity, and scalability. To test the efficiency of cardiac tissue engineering, primary cardiomyocytes were removed from newborn rat hearts and embedded in a fibrin-based bioink. Through a 300 μm nozzle, pressurized air was used to successively print this cell-filled hydrogel along with a disposable hydrogel and a sustaining polymeric frame. The spontaneous simultaneous contraction of bioprinted cardiac tissue constructions in the culture suggests the growth and maturation of heart tissue *in vitro*. Immunostaining for actinin and connexin 43 corroborated the progressive development of heart tissue, demonstrating that cardiac tissues were generated with dense, electromechanically connected, consistently aligned cardiac cells and could be further used in pharmaceutical and regenerative medicine applications[118] (Fig. 10a–c). A 3D bioprinted micro channelled aligned gelatin hydrogel, which improves the contractile capability of native cardiomyocytes (CMs) and stimulates human mesenchymal stem cell (hMSC) cardiac commitment, was developed by Tijore *et al.* using mature cardiac markers. It could be ascertained that the matched stem cells had myocardial lineage commitment. According to fluorescence-activated cell sorting analysis, the commitment to cardiac tissue lineage increased significantly. Additionally, it was discovered that seeded CMs on micro channelled hydrogel were more aligned than those on the unpatterned hydrogel. Thus, it was demonstrated that a microchannel hydrogel scaffold created by 3D bioprinting encouraged stem cells to differentiate into the myocardium and supported CM development and contractility[119] (Fig. 10d and e).

Zhu *et al.* prepared a gelatin methacryloyl (GelMA)-based bioink with gold nanorod (GNR) integration for printing 3D functional cardiac tissue constructions. The nanocomposite bioink has a low viscosity at optimal GNR concentrations, comparable to pristine inks, which makes it simple to integrate cells at high densities. The encapsulated cells experience less shear stress as a result, allowing for the rapid deposition of fibers that are packed with cells at a high resolution. Cardiac cells exhibit better cell adhesion and organization in comparing the printed GNR constructions to those lacking GNRs.[120] A 3D bioprinted cardiac patch without biomaterials was constructed by Ong *et al.* Cardiomyocytes produced from human induced pluripotent stem cells (hiPSC-CMs), fibroblasts, and endothelial cells (EC) were combined to form mixed cell spheroids. Using a 3D bioprinter, cardiac patches were fabricated from spheroids. Cx43, the primary cardiac gap junction protein, was localized to cell–cell boundaries as evidenced by immunofluorescence. The engraftment of a 3D bioprinted cardiac patch into the native rat myocardium is suggested by *in vivo* implantation of the patch. This represents an important step in developing a new class of stem cell-based heart failure

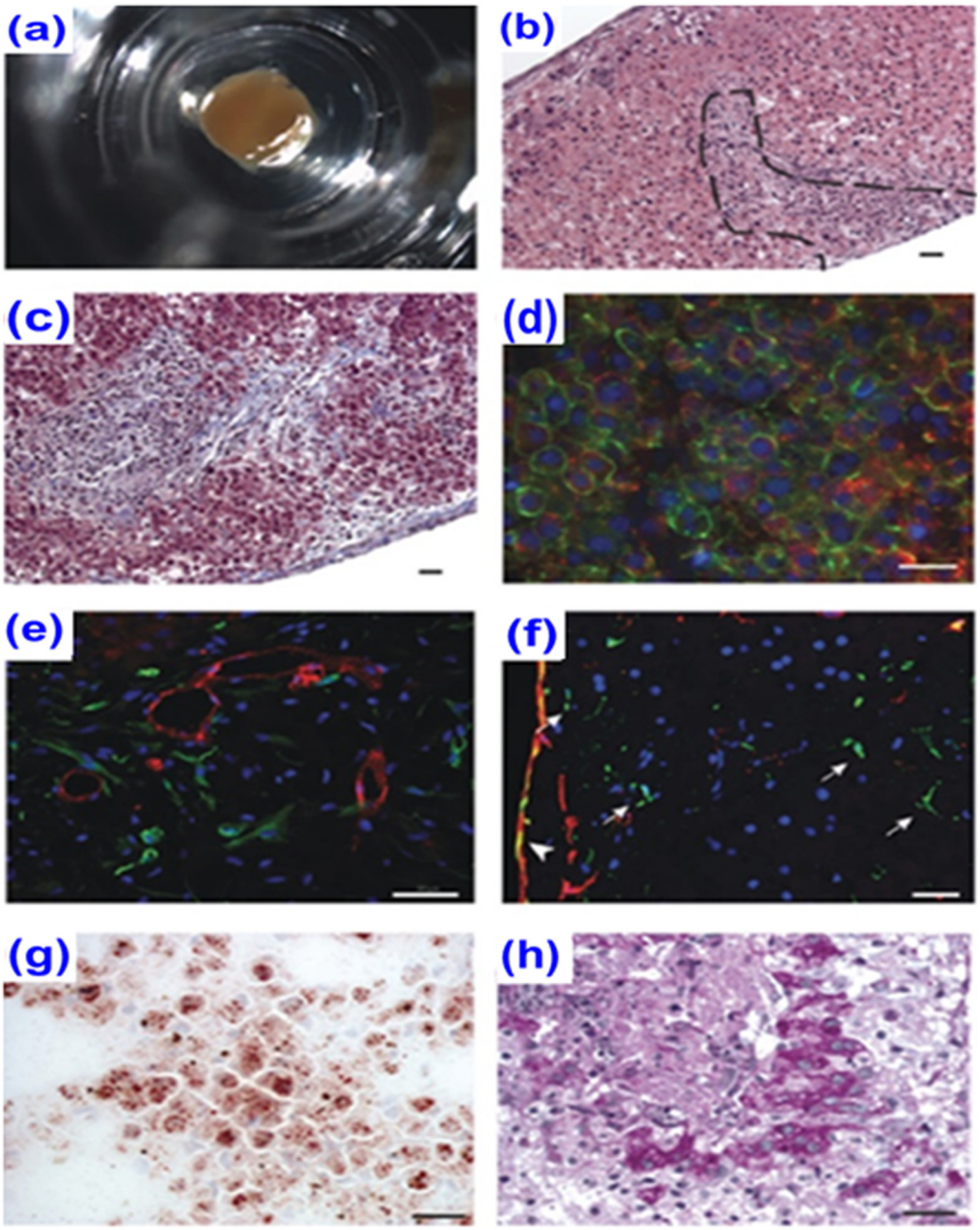

Fig. 9 A histological examination of 3D bioprinted liver tissues. (a) An image of 3D liver tissue housed in a 24-well transwell. (b) Hematoxylin and eosin staining of a tissue cross-section. The black dashed line shows compartmentalization between the parenchymal and non-parenchymal fractions. (c) ECM deposition was investigated with Masson's trichrome staining. (d) The immunohistochemical (IHC) staining of the parenchymal compartment for E-cadherin (green) and albumin (red). (e) IHC staining for CD31 (red) and desmin (green) to assess the organization of the endothelial cells and the presence of quiescent hepatic stellates in the non-parenchymal compartment. (f) IHC staining for desmin (green) and α-SMA (red) to assess stellate cell activation. The white arrows show the quiescent stellates in the tissue interior that stain positive for desmin and negative for α-SMA. (g) Oil-red O staining of 3D liver tissue cryosections to measure lipid storage. (h) PAS staining to identify glycogen granules. DAPI was utilized to stain the nuclei of the cells in all of the IHC staining samples (blue). The scale bars in figures (b–d, g–h) and (e and f) represent distances of 25 μm and 50 μm, respectively. Reprinted from ref. 111 under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright (2016) PLOS.

therapies.[121] Yang *et al.* provided a brief review of the fabrication of a heart-on-chip by 3D bioprinting technology and its application for *in vitro* culture, implants, and drug screening.[122]

7.4. Bioprinting of vascular grafts

Angioplasty, stent implantation in the clogged artery, and heart surgery are just a few of the vascular repair procedures

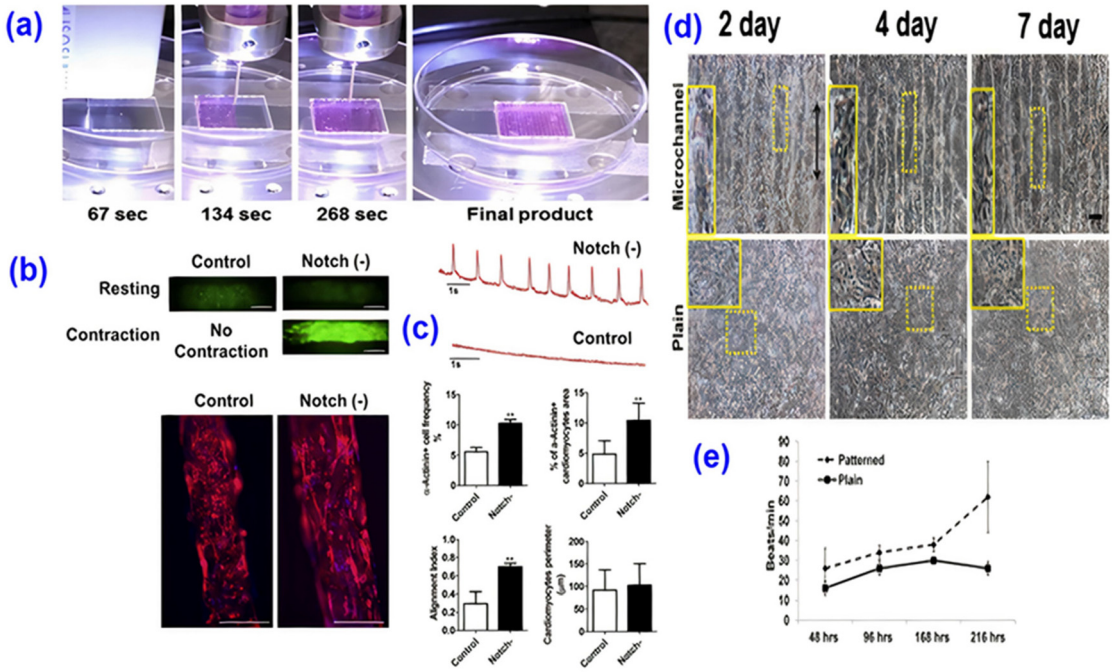

Fig. 10 (a) Time-lapse image sequence of cardiac tissue printing. Notch signaling blockade on bio-printed cardiac tissues. (b) Calcium images on a synchronous contraction of bio-printed cardiac tissues with and without DAPT treatment at 1 week in culture. Notch signaling blockade (DAPT) resulted in the early formation of synchronous contraction, while there was no synchronous contraction in the control (non-treated). Scale bar = 100 μm. Immunofluorescent analyses of bio-printed cardiac tissues with and without DAPT treatment for 1 week in culture: a-actinin (red) and cell nuclei (blue). Scale bar = 100 μm. (c) Plotting of the beating frequency of bio-printed cardiac tissues from Notch signaling blockade and control groups after 1 week in culture and quantification of cardiac tissue development by measuring the frequency of a-actinin positive cells, cardiomyocyte area, cardiac muscle cell alignment, and cardiomyocyte perimeters in Notch blockade and control groups (n = 3). $^{**}P < 0.05$ compared with the control. Reprinted from ref. 118 with permission from Elsevier. Copyright (2018) Elsevier. (d and e) Cardiomyocytes were seeded on hydrogel with and without bio-printed microchannels for 2, 4, and 7 days and observed under bright field microscopy for aligned and elongated morphology. Scale bar = 200 μm and visibly beating regions, as well as the number of beating contractions per minute, were recorded, (n = 3). Reprinted from ref. 119 under the Creative Commons Attribution 3.0 license.

used to treat cardiovascular disease-bearing patients. Tissue engineering is also currently constrained by the vascularization contest, which creates difficulties with nutrient perfusion, oxygen diffusion, and mass transportation in *in vivo* systems. Because they are directly integrated with the solution of the vascularization issue, vascular grafts are quite good models for 3D bioprinting technologies. It provides a desirable method for fabricating vascular grafts from various cell types.

For clinical arterial replacement, it is crucial to use small-diameter tissue-engineered vascular grafts. Huang *et al.* created a novel triple-layer poly(-caprolactone) (PCL) fibrous vascular graft by combining electrospinning and E-jet 3D printing methods to mimic the shapes and functionalities of natural blood veins. Results showed that the longitudinally-aligned fibers within the graft's lumen could promote the multiplication and migration of endothelial cells while maintaining the graft's good mechanical qualities when the biocompatible triple-layer graft was used for *in vivo* implantation. After implantation, the outer layer created a channel that allowed cells to move into the scaffold. The low porosity and poor cell penetration of routinely electrospun vascular grafts were overcome by this experimental graft.[123] Using a revolutionary rotary 3D bioprinter, Freeman *et al.* spawned a new method for biofabricating fibrin-based vascular grafts. The researchers created a novel bioink by mixing gelatin and fibrinogen to obtain the needed shear-thinning property for rotational bioprinting. By utilizing the advantageous rheological characteristics of gelatin, heat-treated fibrinogen was converted into a printable biomaterial for bioprinting of the graft. Notably, the printability and tissue volumetric changes of the printed vessel constructions during culturing were also influenced by the cell density present in the bioinks. The vessel creations' burst pressure was 1110 mmHg, and around 52% of the significance of a human saphenous vein.[124] This work reveals crucial factors for bioink formulation for constructing vascular graft models by 3D bioprinting.

Commercially viable vascular alternatives present a significant difficulty due to their hydrophobic surface restrictions, which are toxic to cell proliferation.[125] A cell-free structurally enhanced biodegradable vascular graft that recapitulated the anisotropic property of a native vascular graft was conceptualized by MSc *et al.* Vascular endothelial growth factor, an immobilized bioactive chemical, facilitates the nanofibrous scaffold (VEGF). The researchers examined the new graft's

mechanical analysis, compression test, burst pressure, histology, and hemocompatibility. As early as two weeks after implantation, the graft in a pig model's carotid artery showed an excellent patency rate. When used in vascular tissue engineering, this graft-enhanced design technique may significantly impact regenerative medicine.[126] Chiu *et al.* designed a 3D printed vascular graft using an amino-resin-based photosensitive, biocompatible material with excellent cellular adhesion and cell proliferation for tissue regeneration.[127]

7.5. Bioprinting of artificial lungs

3D bioprinters in lung and airway tissue engineering can promote the printing of numerous layers of different cells and materials and hollow structures.[128] In tissue engineering, the lung replicates an entire respiratory tree with a branching series of tubes, while surgically it is considered as a solid organ.[129] As a result, progress and problems revealed in the bioprinting of other tubular organs can be used to influence future lung bioprinting research. The gastrointestinal and urinary systems' organs have a multi-layered structure analogous to the lungs' big airways. Therefore, researchers have focused on engineering lungs and trachea to create implantable tissues.[130]

An inner coating of epithelium and concentric layers of supporting fibrous and muscle tissue are common features. End-stage pulmonary failure treatment is still a major therapeutic need. Due to a scarcity of donor organs and the risk of catastrophic transplant-related complications, researchers have turned to bioengineering to build a clinically translatable lung graft.[131] A distal lung model including vascular and airway gaps was published by Grigoryan *et al.* in 2019. They constructed a "breathing model" including tidal air ventilation and blood flow using poly(ethylene glycol) diacrylate and a stereolithographic printer. The authors were able to show pulmonary transport using this model by monitoring blood oxygenation during inhalation and exhalation.[132] Park *et al.* developed a grid structure of polycaprolactone (PCL) using a melt extrusion 3D printer. The scaffold was then layered with fibrin, thrombin, and rabbit mesenchymal stem/stromal cell solution. After coating, the scaffolds were sutured into a 5–10 mm surgical defect in the oesophagus of New Zealand white rabbits as an allogeneic implant.[133]

Chung *et al.* employed PCL to build a circumferential esophageal prosthesis later. The objective was to improve on an acellular graft that could keep the lumen open. Multiple rings were created by melting PCL onto a spinning mandrel using a 3D printer. PCL was electrospun over the rings while still on the mandrel, yielding a structure with a length of 5 mm and an interior diameter of 1.6 mm.[134] Berg *et al.* developed a bioprinted lung from monocytic THP-1 cells and primary human lung fibroblasts, then imprinted alveolar epithelial A549 cells on top of the base. The cells were embedded using alginate, gelatin, and collagen hydrogel. When the models were tested with the bacterial toxins LPS and ATP, there was a release of the proinflammatory cytokines IL-1 and IL-8, demonstrating the model's ability to elicit an immune response. The printed artificial lung design provides an alveolar model for studying the biology of respiratory pathogens and developing novel viral disease therapies.[135]

7.6. Bioprinting of artificial blood vessels

3D bioprinting holds enormous promise for the development of a highly bioavailable and operationally active organ for patients who need a substitute organ for their lost or damaged body parts.[136] In recent years, 3D-bioprinting has emerged as a powerful technique for fabricating micro-sized blood vessel channels in tissue engineering applications.

Pulmonary circulation is critical for the survival of various organs. Researchers have tried several approaches to create functional bioprinted vascular systems, with varying degrees of success for *in vivo* and *in vitro* blood vessels.[137] Skardal *et al.* and coworkers proposed another way, using an extrusion-based process to print cellularized tubular tissue structures built from hyaluronan hydrogels highly cross-linked with polyethylene glycol.[138] New biomaterials were designed and employed to fabricate structures that resembled a basic artery in their research. Furthermore, the manufactured cell constructs were demonstrated to have a month of high vitality in culture conditions. Hydrogels are appealing bio-inks for creating artificial blood vessels, but they typically have poor mechanical properties. Liu *et al.* developed a printable human umbilical vein endothelial cell (HUVEC)-laden polyrotaxane–alginate (PR-Alg) double-network (DN) hydrogel to overcome the poor mechanical properties of hydrogel bio-inks. The team significantly improved on the mechanical properties of hydrogels by incorporating special hydrogel structures of slide-ring (SR) and double network (DN). Furthermore, because of biocompatible materials and the delicate 3D-bio-printing procedure, the 3D-bio-printed channels demonstrated exceptional biocompatibility, particularly in cell cycle progression.[139] This study broadened the use of biomaterials with enhanced mechanical properties in biomedicine, specifically for artificial blood vessels.

Centola *et al.* devised a method for fabricating a hybrid vascular transplant. They utilized a combination of electrospinning and fused deposition modeling approaches to create a poly-L-lactide (PLLA)/polycaprolactone (PCL) scaffold that released heparin.[140] A study used a common inkjet bioprinter to print a micro-vascular structure with microvascular endothelial cells and fibrin bio-ink.[2] Progress in vascular bioprinting continues to be bogged down by the issue of cell survival. For the best possible printing efficiency while ensuring cell viability, thermal inkjet bioprinters are the obvious choice. For tissue viability, physical qualities, and printing speed, in addition to viability, a successful crosslinking approach is needed. While promoting cell survival, the hydrogel also boosts cell propagation and proliferation through improved cell growth and dissemination. Researchers are currently developing new kinds of filaments (such as Pluronic F127) to make fluidic channels (*e.g.*, Pluronic F127). It is not just the design of vascular patterns that can be made easier with these filaments, but printing itself may also be expedited.[141]

7.7. Bioprinting of artificial bone

Some of the many causes of bone injuries in older adults include old age, infection, trauma, and malignancy. The most common method of bone repair is either an allograft or a xenograft, although these techniques are both limited in that tissue supply may be scarce, there is a high likelihood of additional surgery being required, and infections are a risk.[142] 3D bone printing and other tissue engineering approaches could serve as a much-needed option to help overcome the limits of traditional bone repair.[3] Any biomaterial suitable for bone tissue printing should have available and appropriate cell types.

New research suggests that hydrogels could potentially help in bone regeneration.[143] Strong new bone tissue was formed after the application of poly(ethylene glycol) di-methacrylate (PEGDMA) hydrogel with acrylate RGD and matrix metalloproteinase (MMP) peptides. Bioactive glass nanoparticles can dramatically increase the osteogenic differentiation of mesenchymal stem cells if added to certain hydrogels (MSCs).[144] Shim *et al.*[145] replaced the hydrogel with a composite of polycaprolactone/poly(lactic-*co*-glycolic acid)/β-tricalcium phosphate (PCL/PLGA/β-TCP) membrane to improve osteogenic differentiation when used to stimulate bone regeneration (Fig. 11a–f). To test an appropriate ECM for *in vivo* healing of an alveolar bone defect using 3D printing technology, Ma *et al.* produced an encapsulated hydrogel made of gelatin methacrylate (GelMA) and poly(ethylene glycol) dimethacrylate (PEGDA).[146] Mechanical tensile and *in vitro* cell proliferation testing was carried out for PCL/PLGA/β-TCP membranes prepared by extrusion-based 3D bio-printing. Implant surgery and guided bone regeneration were carried out in three groups at random (n = 8 per group): no membrane, titanium membrane, and PCL/PLGA/β-TCP membrane.[145] Fig. 11 presents (a) a schematic of the open buccal defect model and (b) operation procedures of the implants in the edentulous mandibular alveolar ridge. Fig. 11(c–f) depicts that new bone formation was observed around implants in opened buccal defect regions in the PCL/PLGA/β-TCP group at 8 weeks after surgery. The membranes were fully or partially absorbed, whereas the remaining membranes remained structurally intact. The bone tissues partially surrounded the bone graft materials.

Calcium phosphate scaffolds (CPSs) have been created using inkjet-based 3D printing, with the calcium phosphate powder being temporarily bound by an adhesive polymer and subsequently irreversibly bound by the sintering of the printed structure.[147] Recently, Inzana *et al.* used a phosphoric acid binder to build a CaP and collagen composite 3D scaffold to improve the cytocompatibility and material characteristics of 3DP ceramics.[148] The McGrath mineralization process was tested in the development of 3D chitosan–calcium carbonate composites by Kurian *et al.* The McGrath approach was used to mineralize the as-printed chitosan hydrogel-based scaffolds with/without crystal growth modifiers such as polyacrylic acid (PAA). The final composite mineralization was improved by macropores and the layer-by-layer construction of the 3D chitosan scaffolds.[149] Igawa *et al.* constructed novel tailor-made bone implants (TIs) and tricalcium phosphate powder using an RP inkjet printer based on computed tomography (CT) data

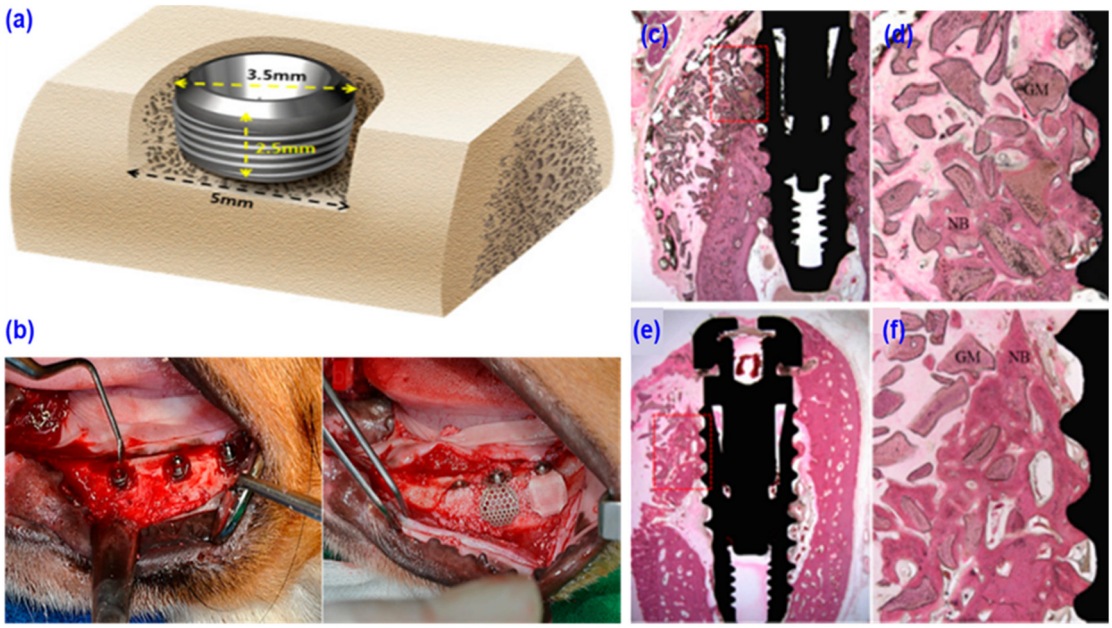

Fig. 11 (a) Schematic of the open buccal defect model and (b) operation procedures of the implants in the edentulous mandibular alveolar ridge. Histological analysis showing the effects of the 3D-printed resorbable PCL/PLGA/β-TCP membrane on bone regeneration ability and osseointegration in areas surrounding implants at 8 weeks after surgery. The images (c and d) and (e and f) were taken with magnifications of 12.5× and 40×, respectively. The specimens were stained with hematoxylin and eosin (H&E). The abbreviation "GM" refers to the bone grafting material and "NB" indicates new bone. Reprinted from ref. 145 under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright (2015) MDPI.

and investigated their safety and efficacy. CT scans of seven beagle dog skulls were collected and translated to CAD data, and bone abnormalities in the skulls were virtually created bilaterally. The TIs were adjusted to resemble the abnormalities and produced using a 3D ink-jet printer, having six horizontal cylindrical holes extending through the implants to aid vascular invasion and bone repair.[150]

7.8. Bioprinting of pancreatic tissues

3D bioprinting has recently become a viable alternative for constructing an artificial pancreas.[151] It can be used to place living cells at the exact scale of a human organ at the desired location. Additionally, 3D bioprinting is used to create the vascularization of the artificial pancreas. Therefore, a true pancreas-like artificial organ could be developed for therapeutic use.[152] The death of insulin-producing beta cells in the islet of the endocrine pancreas by the immune system causes type 1 diabetes mellitus.[153] At the time of islet transplantation, the host immune system rejects the transplanted islets. Islet encapsulation technology with biocompatible materials has emerged as an immuno-barrier to protect them immunologically; this is possible using 3D bioprinting systems.[154]

Pancreatic islet transplantation is a good potential treatment option for patients with type 1 diabetes who have unstable blood glucose control.[155] Duin *et al.* combined islet encapsulation with 3D extrusion bioprinting. Using a plottable hydrogel blend of clinically approved ultrapure alginate and methylcellulose (Alg/MC), they encapsulated pancreatic islets in macroporous 3D hydrogel constructs. Diffusion of glucose and insulin in the Alg/MC hydrogel is equivalent to dispersion in simple alginate. The integrated islets produce insulin and glucagon consistently throughout the observation period and respond to glucose stimulation, albeit to a smaller extent than control islets[156] (Fig. 12a). This study proved the preparation of a functionalized pancreas by 3D bioprinting and its application in regenerative medicine. Idaszek *et al.* developed innovative bioinks that could be used for the multi-material biofabrication of 3D porous pancreatic and vascular structures with microfluidic assistance. Alginate was mixed with either fibrinogen (A FBR) or pancreatic decellularized extracellular matrix powder (A ECM) to provide tissue-specific bioactivity. Despite having varying rheological characteristics, the prepared bioinks were 3D printed with good shape fidelity utilizing a multichannel microfluidic platform and a co-axial needle system. To test the bioactivity, high viability 3D-bioprinted bioinks were loaded with swine pancreatic islets and a combination of vessel-forming cells (HUVEC and HMSC)[157] (Fig. 12b and c). Finally, the successful 3D printing of three different configurations of heterogeneous 3D scaffolds showed that this strategy might be a possible step towards the bio-fabrication of a vascularized pancreas.

New *in vitro* models are urgently required since 2D cell culture models fail to simulate the 3D complexity of the pancreatic tissue. By employing laser-assisted bioprinting to create 3D pancreatic cell spheroid arrays, Hakobyan *et al.* were able to track the phenotypic development of these arrays over time.

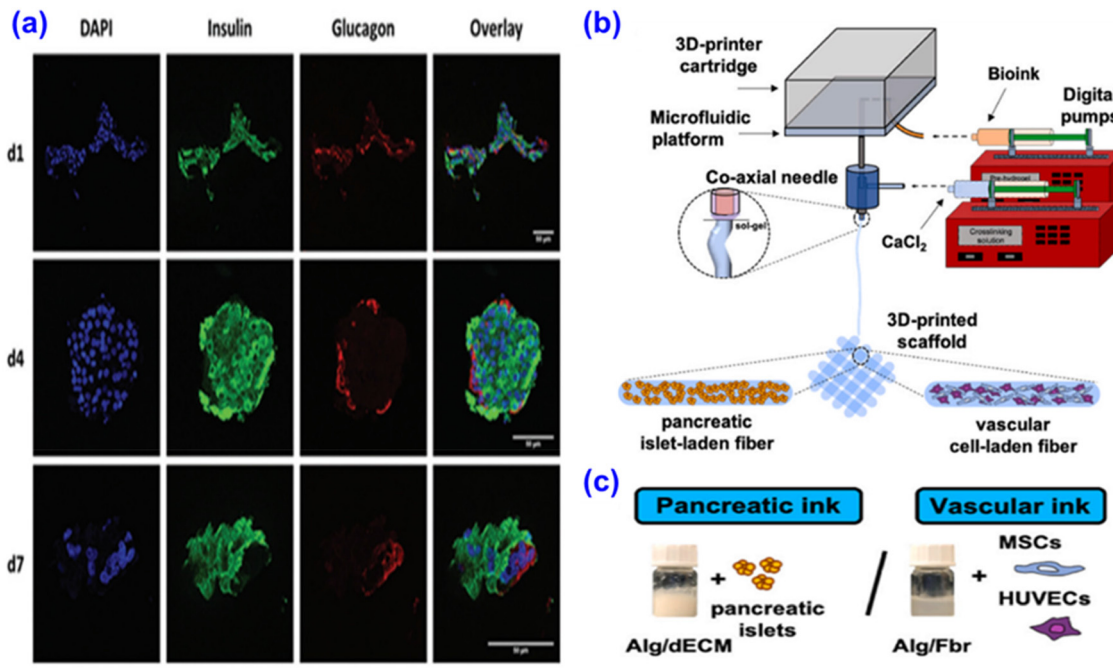

Fig. 12 Images of islets immunofluorescently stained for nuclei (DAPI), insulin, and glucagon. (a) Pancreatic islets from rats incubated for 1, 4, or 7 days (d1, d4, d7) under cell culture conditions. Scale bars depict 50 μm. Reprinted from ref. 156 with permission from Wiley. Copyright (2019) Wiley. (b) Bio-printing process showing that the 3D printed fiber undergoes gelation as it is extruded from the needle tip. (c) Alginate-based tissue-specific pre-hydrogel formulations of cell-laden bio-inks. Reprinted from ref. 157 under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

The model demonstrated the ability of these bioprinted spheroids, made up of acinar and ductal cells, to mimic the early stages of pancreatic tumor progression.[158] This study contributes to the diagnosis of metastatic disease, and the treatment of cancer should be possible using this bioprinted miniature spheroid-based array model, which may also provide insights into potential cancer treatment plans in the future.

7.9. Bioprinting of tumor models for cancer therapy and drug screening

Cancer research can be aided by 3D bio-printed tumor models.[159] The histopathological results for tumors are important to shed light on cancer progression. Cancer therapeutics can be better achieved by new 3D bioprinted models as, in the past, testing with mice led to disadvantages such as the surgical implantation of cancer causing a poorer mimic of human diseases. Some reports also suggest that deadly cancer cells of the brain, skin, and kidney cannot be established with mice models.[160,161] Hence research groups came up with the fascinating idea of 3D printing tumor models to analyze and stop cancer progression.[162] 3D printed tumor models have several benefits as they maintain phenotypic and genotypic heterogeneity.[163] The tumor models for cancer therapy can also act as a screening method for drug testing. Biopsy or monolayer cell culture analysis are commonly undertaken by clinicians to carry out preclinical research, which is costly and time-consuming.[164] Hence, 3D bio-printed tumor models can be potential candidates for understanding drug action toward tumors. The 3D models can be broadly classified as (1) cultured as multicellular aggregates, (2) cultured on inserts, and (3) embedded in extracellular matrices. The spheroid culture model is the easiest 3D printed model.[165–167] Fig. 13(a–c)

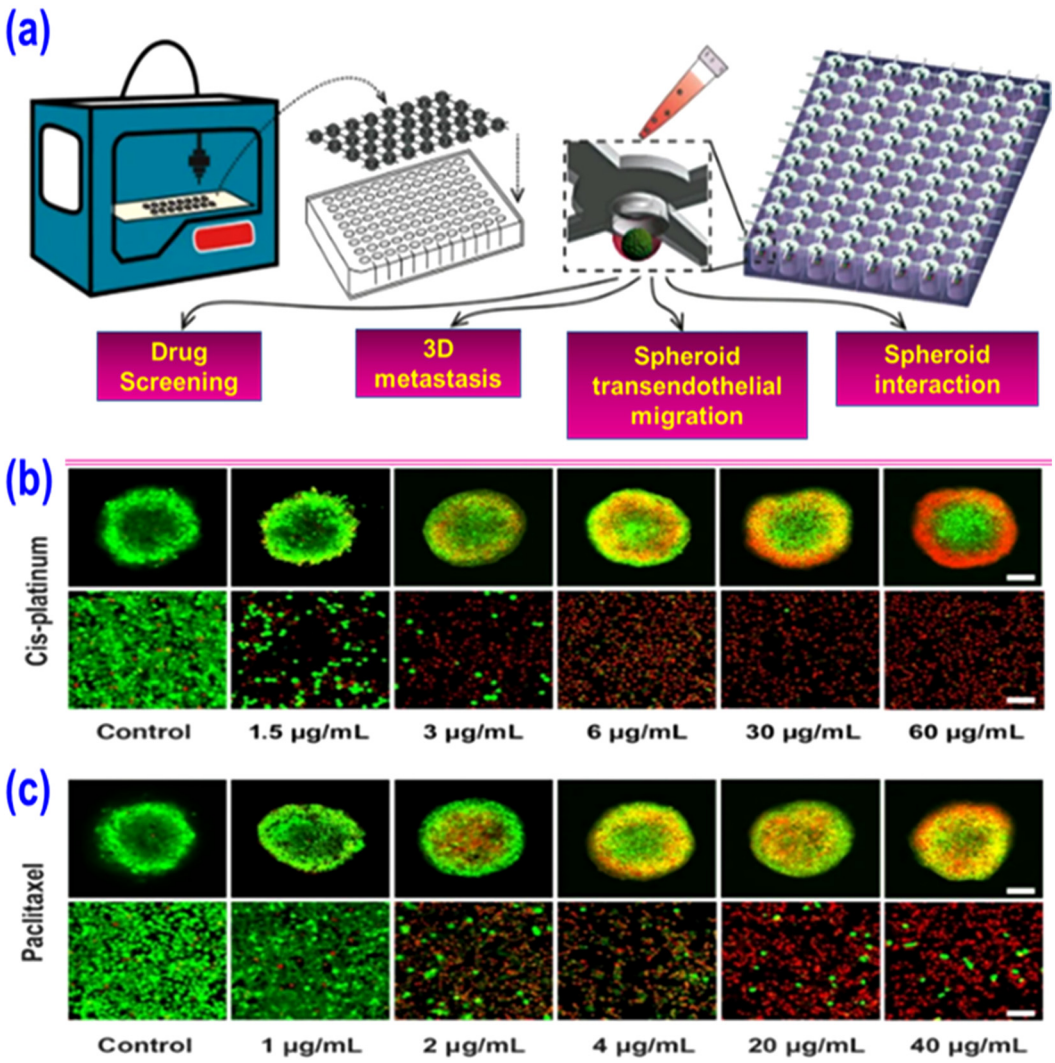

Fig. 13 (a) The workflow of a 3D-printed hanging drop dripper for studying tumor spheroid generation, drug-induced cell death, and metastasis in extracellular matrix gel, (b) confocal images (live/dead double staining) for dose-dependent drug screening of MCF-7 spheroids and 2D monolayer with cisplatin treated for 48 h on the 3D-printed hanging drop dripper and (c) confocal images for live/dead double staining of different concentrations of paclitaxel in 3D-phd formed spheroids *versus* conventional monolayer culture. Reprinted from ref. 167 under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Copyright (2019) Springer Nature.

shows the printing process of a 3D printed hanging drop dripper (3D-phd) and the effect of cisplatin and paclitaxel with 3D-phd for drug resistance analysis. Napolitano *et al.* used a micropatterning method to create microwells for controlling the growth of spheroids.[30] Hsiao *et al.* used a hanging droplet culture to stabilize the droplet using a microring template, which offered high reproducibility, as it emerged from primitive tumor tissues and a less costly procedure.[168] Soranzo and coworkers reported that the cytotoxicity of anthracyclines was low in the case of 3D printed spheroids rather than on monolayers. They found that the drug penetrated inside the core of the tumor in the case of the 3D spheroid structures.[168] 3D printed poly L-lactic acid implants with tunable morphologies and programmable micropore architectures were developed by Wang *et al.* as excellent carriers for anticancer drugs for osteosarcoma.[169] Tumor cells and extracted malignant cells from blood samples were trapped using a 3D inkjet-printed microfluidic device by Chen *et al.* A comparable microfluidic device was created to isolate circulating tumor cells by utilizing 3D inkjet printing technology; this led to 90% decrease of breast, ovarian, and prostate cancer cells.[170] Motaghi *et al.* fabricated a 3D printed microchannel with a closed bipolar electrode system and electrochemiluminescence detection for sensitive detection of human breast cancer cells (MCF-7).[171] A 3D colon cancer model was developed by Mohanty *et al.* to establish high throughput drug screening.[172] Rebelo *et al.* introduced a 3D-3-culture tool for understanding cell–cell and cell–matrix interactions during cancer growth.[173] Table 4 shows a comparison of the characteristics of the 3D test models for anticancer drug screening.

8. Challenges and future perspectives of 3D bioprinting

Even though 3D-bioprinting is progressing at a commendable rate, with researchers working hard to develop new printing methods, while also improving existing modalities, there are still challenges that must be overcome.[174] Only a few bio-inks are currently available that are both bioprintable and adequately reflect the tissue architecture required to regain organ function.[35] The fact that most stem cell research has been done in 2D environments means that there are a lot of unknowns when it comes to 3D stem cultured cells.[175] Another major challenge is the vascularisation of bioprinted structures to ensure suitable nutrient communication, as well as the assimilation of the printed microcirculation with the host vasculature after organ implantation.

In the medical field, 3D printing is a new and demanding technology with many intriguing potential applications but it has yet to prove itself. This must be regarded as a breakthrough in the medical industry, as bioprinting organs can alleviate the current scarcity of organs for transplants and the associated burden.[176] Additionally, because the organs will be available on time, it will assist in reducing the amount of money spent on healthcare and associated costs. Solving the problem of the scarcity of organs may aid in the reduction of the death rate associated with certain life threatening diseases. The likelihood of growth will also increase as the rate of refusal decreases. This will also lower the associated costs because the patient will be hospitalized for fewer days and will not require anti-rejection medications.

3D printed organ models still suffer from low production and applications in a specific area. The challenges may be high costs and complicated organ model creation routes. Fewer simulation characteristics make it difficult to mimic soft tissues. The selection of biomaterials used for bioprinting organs is not abundantly available. The resolution of printing is low, and the available printing space is smaller. These challenges need to be overcome shortly to extend the potential of the bioprinting of organs in various medical applications more widely.

However, most critically, 3D-printed organs will usher in an age of personalized medicine, where transplanted tissues would be created specifically for each patient. Overall, 3D organ fabrication will be a game-changer in regenerative medi-

Table 4 Comparative characteristics of 3D bio-printed test models for anticancer drug screening

Tumor model	Advantages	Disadvantages	Application	Cell type	Ref.
Three-dimensional spheroids	3D architecture and hypoxic conditions in spheroid centers	Difficult to standardize	Anticancer drug screening, invasion studies	One type of cell (liver cancer cells, breast cancer cells, neck cell carcinoma)	192
Three-dimensional organoids	Accurately reproduce *in vitro* tumor architecture	Difficult to create a large number of homogeneous organoids for drug screening	Anticancer drug screening, invasion, and extravasation studies	Organoids derived from lung cancer/ prostate cancer/ bladder cancer tissues	193
Three-dimensional scaffolds	Reproducing complex 3D tissue architecture and facilitating interactions between ECM and cells	Poor reproducibility and mimic of *in vitro* tumor architecture	Anticancer drug screening, invasion studies, cell infiltration studies	Co-culture of NSCLS cells + fibroblasts + immune cells on matrigel	194
Three-dimensional bio-printing	Reproducing complex 3D tissue architecture, mimicking tumor microenvironments, and producing cellular structures for drug screening	Low precision cell positioning	Anticancer drug screening, cell invasion studies	Co-culture of A549 lung carcinoma cells + HUVEC	195

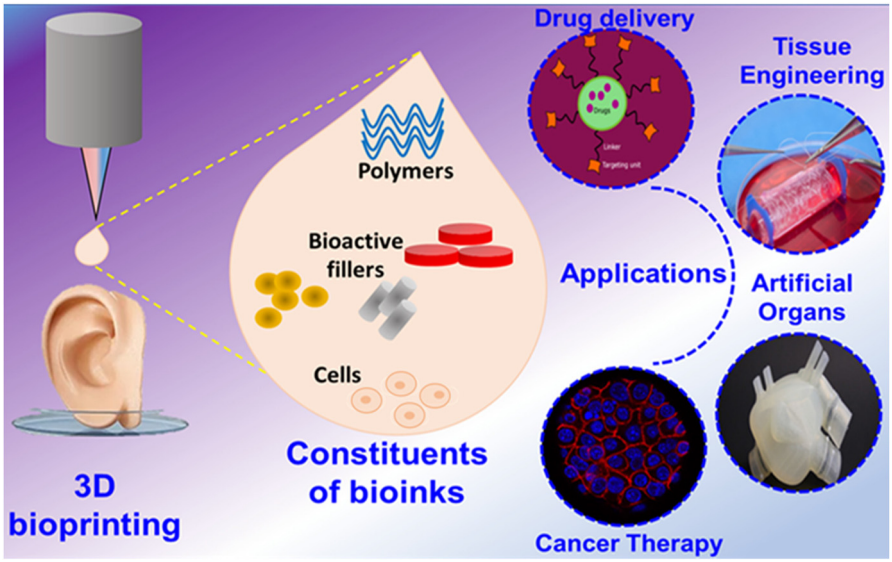

Fig. 14 Overview of 3D bio-printing toward promising medical applications.

cine.[177] Although tissue engineering appears to be a promising field, it is still in its infancy, and there is a long way to go before it can be considered a comprehensive and reliable technique on its own. Despite its limitations, such as the high cost of research and production and a lack of adequate infrastructure, the growing demands for organ transplants and the 3D cell culture industry are driven by technological advances and increases in graft refusal rates. Significant innovations in intelligent huge, cultured cells, bioprocess engineering and the integration of interdisciplinary methodologies, have been made in biotechnology (for example, the use of biological access for direct cell death). It is exciting to see these advances.[178]

Due to these improvements, researchers will produce cheap, extremely precise 3D structures; this is currently not conceivable in the medical field. Obtaining sick tissue or cells from the patient and subjecting such specimens to gene editing is another sensible option.[179] The gene-altered cells may be employed to attain a predetermined objective or set of endpoints. To achieve a wider endpoint, an array of several pairs of biomarker tissues can be synthesized together. When combined with transdisciplinary techniques, such as gene editing, typical bioprinting techniques have the potential to make significant advances in the fields of regenerative tissue medicine.[180] Researchers nowadays focus on microfluidic techniques to print 3D electroactive scaffolds for tissue regeneration and drug screening applications.[181] Fig. 14 gives an overview of 3D bioprinting as a promising tool for several medical applications.

9. Summary

3D bioprinting is increasingly being employed in pharmaceutical development and medical validation and will be used in clinical settings in the future. Bioprinting research currently focuses on 3D-printed skin grafts, bone grafts, implants, biomedical equipment, and even whole 3D-printed organs. Bioprinting completely functional complex internal organs, such as hearts, kidneys, and livers, is still at least ten years away, but rapid progress is being made following recent clinical research achievements. A network of cells, tissues, nerves, and structures must be precisely positioned for a human organ to operate properly. 3D bioprinting can do everything from organizing hundreds of tiny capillaries in a liver to printing a beating heart, which allows one to tailor artificial organs specifically for a person. The correct materials, cell kinds, and bio-inks must be chosen with the same precision as the blueprint. The idea of biomimicry could help in the selection process to maintain cell functions and ease of implementation in the real natural environment. Furthermore, navigating all of this complexity necessitates integrating and using numerous modern technologies from various domains, including engineering, biomaterials science, cell biology, physics, and cancer therapy. Despite these complications, 3D-bioprinting is advancing at a breakneck pace, making advancements in both the technology and in the understanding of how it might be applied. Bioprinting offers several merits, making it a strong tool for fabrication, high throughput, and cell deposition. Even with the progress made in recent years, bioprinting has the potential to serve as an emerging technology and a base for diverse applications.

Author contributions

Swati Panda: Conceptualization, writing – original draft, Sugato Hajra: Validation, writing – review and editing, Krystian Mistewicz: Funding acquisition, visualization, writing – review and editing, Bartłomiej Nowacki: visualization, Pichaya In-na:

Writing – review and editing, Anastasiia Krushynska: Supervision, writing – review and editing, Yogendra Kumar Mishra: Funding acquisition, validation, writing – review and editing, Hoe Joon Kim: Funding acquisition, writing – review and editing, supervision.

Conflicts of interest

There are no conflicts to declare.

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