Article

Advanced 3D Bioprinted Tissue Models: Revolutionizing Research, Drug Discovery, and Toxicology

Md. Sajedul Islam Sagar [1], Md. Alal Hossain [1], Kawsar Sheikh [1], Khan Rajib Hossain [2,3*]

[1]Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi 6205, Bangladesh.
[2]State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China.
[3]Department of Natural Sciences, BGMEA University of Fashion and Technology, Dhaka 1230, Bangladesh.
***E-mail:** apexlabbd2@outlook.com

*Corresponding author: State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China.

Abstract:

The advent of 3D bioprinting has ushered in a new era in biomedical research by providing previously unattainable chances to replicate complex tissue models that replicate natural physiological circumstances. An overview of the effects of sophisticated 3D bioprinted tissue models on toxicology, research, and drug development is given in this study. With 3D bioprinted tissue models, researchers can study tissue behavior in controlled environments to better understand human physiology. These models greatly enhance drug development by providing more physiologically appropriate platforms for evaluating the toxicity and efficacy of medicines by mimicking the microarchitecture of tissues. Additionally, the technique may lessen the need for conventional animal models. 3D bioprinted tissues contribute substantially to toxicity research by providing a scalable and realistic platform for assessing the adverse effects of different chemicals. Translational potential is increased by including patient-specific cells, which promotes personalized treatment and closes the gap between preclinical and clinical research. 3D bioprinting processes are briefly covered in this overview, with a focus on bioink formulation, scaffold design, and cellular inclusion. Standardization and scalability are recognized as challenges, and integration with high-throughput screening is one of the potential opportunities examined. In conclusion, the application of 3D bioprinting in toxicology, research, and drug development has the potential to influence biomedical research in the future by encouraging the creation of safer and more efficient treatment procedures.

Keywords: 3D bioprinting, Tissue models, Bioink, Drug discovery, Toxicology.

1. Introduction

Bio-3D printing is an additive manufacturing technique that uses biological materials, extracellular matrix, biological factors, and living cells as raw materials to create biological things that can be inanimate or alive. The primary distinction between bio-3D printing and traditional 3D printing of metals, ceramics, polymers, and other materials is the ability to process living materials such as

cells and other biologically functional components and produce living objects. Ordinary printing uses ink, but bio-3D printing also uses ink, but it is bio-ink [1]. It combines biological materials (hydrogel) and biological units (cells, DNA, and proteins) according to bionic morphology and biological functions. Personalized biological functional structures are created using 3D printing in the cell development microenvironment and other necessary conditions. By meticulously layering biological components, biochemicals, and living cells while also controlling where functional parts are located in space, 3D bioprinting creates three-dimensional models. Several techniques for 3D bioprinting include biomimicry, autonomous self-assembly, and mini-tissue building blocks. Biotechnologists strive to close the gap between cell culture and actual tissue. This objective can be accomplished using cell cultures that have been 3D printed. Within a 3D bioprinting culture, cells grow in a small space with the same shape, mechanics, and biochemistry as real tissues. It creates physiological connections between cells and between cells and substrates, which control proliferation and differentiation in space and time. Cell biology and proteomic research have shown that this maintains the original tissue function and homeostasis [2]. Recently, 2D monolayer cultures and preclinical animal models have been used to assess the processes underlying human disease and conduct drug screening. On the other hand, traditional 2D cultures do not fully mimic the complex environment of human tissue, and treatment effects and side effects seen in experiments on animals do not always reflect those seen in humans. This means that animal models are not always useful for developing new treatments for human diseases. Between 2D cell culture and in vivo animal models, 3D bioprinted in vitro models are becoming more and more important [3]. Many cells that can handle the stresses of bioprinting are needed to make a lot of bioinks that are full of cells. Adipose-derived mesenchymal stem cells (MSCs) are an appealing cell type for tissue engineering. They can be acquired from patients in large quantities and endure the shear stress of 3D bioprinting due to their robust qualities [4]. A useful method for creating trustworthy, high-throughput simulations of biological function for drug development is 3D bioprinting [5]. While creating a new medicine, a scientist seeks to enhance or inhibit a specific biological process by treating a specific condition. According to the rules of pharmacodynamics and pharmacokinetics, xenobiotics (new chemicals) that contain any active moiety in certain biochemical pathways should be safe to eat. ADME-Tox is the study of how a drug is distributed, broken down, absorbed, and excreted inside the body, as well as its toxic effects on other living things [6]. The absorption, distribution, metabolism, excretion, and toxicology (ADME-Tox) processes cover many mechanisms. People who work on drugs and other related fields need to test ADME-Tox because it can help with pharmacodynamic, pharmacokinetic, and toxicokinetic studies. Drug safety assessment and development are the two main stages of the drug's creation, such as drug discovery in the preclinical phase and drug development in the clinical phase [7-8]. The current way of making drugs takes a long time and costs a lot of money. It involves finding leads and making them work better, often on targets not fully tested in animal models of cellular illness. Complex in vitro models (CIVMs) can be utilized to identify the proper target, the right tissue, exemplary safety, and the suitable patient. Note that this viewpoint article discusses just the following topics: multi-organ systems, spheroids, organoids, 3D bioprinted tissue, organs-on-a-chip, microphysiological systems (MPS), and the human body on a chip [9-10]. One great thing about this method is that it lets you put live cells, growth nutrients, and biomaterial scaffolds in the exact spot where you want them to be, so the structure and growth process are like those in natural tissue. It is easy and quick to use 3D bioprinting to make small human tissue models or organoids that can be used to study drugs, model diseases, and replace damaged patient tissues with ones that work perfectly [11]. In this situation, in

vitro 3D tissue models effectively replace conventional 2D cell cultures and animal testing. These models are more suitable for testing and comprehending the mechanisms of drug action. It can imitate the extracellular matrix's (ECM) physiological environment, the interactions that occur between cells and the ECM, and the spatial layout of the cells. In recent years, cellular spheroids have become very important for creating 3D tissue-like conditions in vitro, and they have been used a lot to create many native organs and tissues [12-13]. Extrusion-based bioprinting is a new tool for better drug screening, and it is the most frequently used method after inkjet-based, extrusion-based, and laser-assisted bioprinting. Numerous benefits of 3D bioprinting include the ability to co-culture, high throughput, and customized microarchitecture [14]. This protocol provides a standardized approach for evaluating the 3D bioprinted tissue model, drug discovery, and toxicology. In this study, the use of 3D bioprinting techniques to generate in vitro tissue models. First review the available biomaterials, widely used cell sources, and state-of-the-art 3D bioprinting methods for producing functional tissue models that could be used for disease modeling and tailored drug screening. Finally review the potential applications of 3D bioprinted liver models, cardiac tissues, vascularized structures, mesenchymal stem cells (MSCs), and cancer models in preclinical research and toxicology.

1.1 Overview of 3D bioprinting technologies

The three main categories of 3D bioprinting technologies are light-assisted printing, microextrusion, and inkjet printing. All 3D bioprinting processes share the ability to construct scaffolds for cell seeding and directly enclose cells within scaffolds to create tissue architectures. Nevertheless, there are differences across these platforms in terms of printing procedures, print quality, turnaround times, and available materials. The most widely used method is extrusion-based printing [15], then light-assisted [16], inkjet-based [17], and other methods. The benefit of 3D bioprinting is its ability to build complex structures using data such as computed tomography (CT) or magnetic resonance imaging (MRI) and to produce accurate structures from predefined digital blueprints, such as computer-aided design (CAD) models. A combination of appropriate cells, biomaterials, and 3D bioprinting technology is needed to create functional tissue models [18–20]. Nowadays, the three primary methods of 3D bioprinting are inkjet bioprinting, microextrusion bioprinting, and laser-assisted bioprinting. These strategies have all been widely applied, each with many benefits and disadvantages (**Table 1)**. More evaluation and contrast of these types of platforms are given below.

Table 1. Various bioprinting processes are compared. [1]

Category	Inkjet	Microextrusion	Laser/Light
Material viscosity (mPa.s)	Low (3–12)	High (30–6×10^7)	Medium (1–300)
Cross-linking methods	Chemical or photo-cross-linking	Chemical or photo-cross-linking	Chemical or photo-cross-linking
Print speed	1–10,000 droplets/s	10–50 µm/s	200–1600 mm/s
Resolution	50–300 µm wide droplets	100 µm to 1 mm wide	50 µm
Accuracy	Medium	Medium-low	High
Biomaterials used	Hydrogels, fibrin, agar, collagen, alginate	Hyaluronic acid, gelatin, alginate, collagen, fibrin	Hydrogels, nano-hydroxyapatite
Fabrication time	Medium	Short	Long
Preparation time	Low	Low-medium	Medium-high
Cost	Low	Medium	High
Example applications	Skin, vascular, cartilage	Trachea, cardiac valve	Skin

The most popular form of printer for both non-biological and biological purposes is the inkjet printer, also referred to as a drop-on-demand (DoD) printer. Liquid is transported to designated areas in controlled volumes. Modified versions of 2D ink-based commercial printers were the first inkjet printers utilized for bioprinting applications [21]. The printing stage of inkjet-based bioprinting systems uses precise picoliter droplets of bio-ink (material solution or cell-material mixture) dispensed by customized desktop inkjet printers [22]. Thermal, piezoelectric, and electromagnetic inkjet printing methods are all available. The most popular of these approaches is the thermal one because of its affordable total cost, easy-to-use design, and comparatively good cell viability following printing. For thermal inkjet printing, localized heating causes the temperature to rise to 300°C briefly, inflating an air bubble that forces droplets out of the nozzle head [23]. Although flexible and low-cost, the inkjet-based method has drawbacks that need to be resolved before its use in creating more intricate tissue models can be increased. These include limited material availability, frequent nozzle clogging, and slow printing speed because of point-by-point deposition, and potential cell damage from shear or thermal stress. In contrast to inkjet-based bioprinter's discrete droplets, extrusion-based 3D bioprinting devices lay down continuous filaments **(Figure 1)** [22]. With this technique, the dispensing system drops bio-ink at a precise time and location under the digital supervision of a computer. The printer nozzle, or stage, is managed by automated motors. There are several ways to operate the dispensing system, including mechanical, solenoid, and constant pressure-based controls. In this situation, acellular or cell-rich bioinks can be layer-by-layer printed onto a receiving substrate. A 3D structure is eventually built as the stage or microextrusion head is moved down the z-axis, with the deposited layer acting as a foundation for the subsequent layer. These include cell spheroid suspension and decellularized extracellular matrix (ECM) solutions, as well as hydrogels with a broader range of viscosities, like gelatin, hyaluronic acid (HA), alginate, and PEG-based hydrogels [24–25]. With reported printing speeds ranging from 10 to 50 µm/s, extrusion-based bioprinting has the slowest printing speed among the three printing processes [1]. "Laser-induced forward transfer technology" [26] is a method that uses a donor slide that has a layer of bioink that contains cells and a layer that absorbs laser energy. The focused laser pulses evaporate the absorbent layer, creating a high gas pressure that moves the bioink compound toward the collection slide. This technique allows the precise deposition of constituents and high densities of cells in reasonably compact 3D structures without compromising viability or cellular function [27]. Since there are no nozzles involved, clogging problems are not a concern. It has been utilized successfully with bioinks that range in viscosity from 1 to 300 mPa.s. Since creating more significant, more therapeutically applicable 3D constructions takes longer, the successful translation to widespread application needs to be improved. [28]

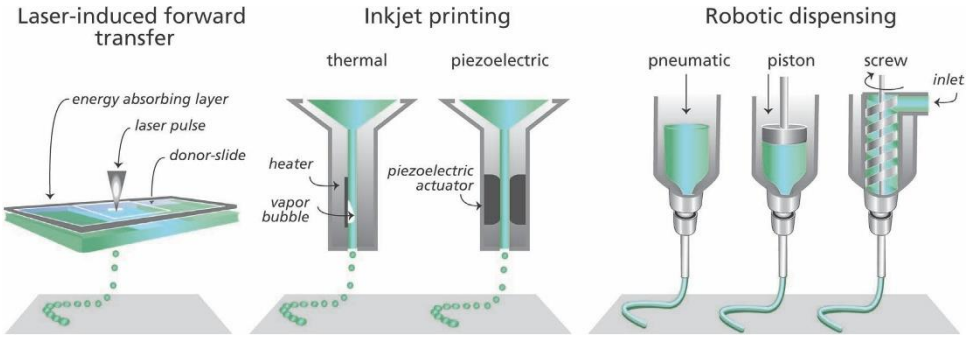

Figure 1. Diagram showing the three 3D bioprinting technologies: There are three types of bioprinting: Laser-induced forward transfer bioprinting, Inkjet bioprinting, and Robotic dispensing/microextrusion bioprinting. [22]

2. Designing of 3D bioprinting for fabrication of tissue models

The process of designing 3D bioprinting, a revolutionary approach to the design and engineering of human organs and tissues, is complex and multidisciplinary, using ideas from biology, materials science, engineering, and 3D printing technologies to fabricate tissue models [29]. The development of 3D bioprinting has made it possible to create intricate, high-resolution 3D tissue models, such as the intricate microphysiological conditions inside the human body. Extrusion-based technology has received substantial recognition and adoption for producing 3D hydrogel tissue models. This method enables accurate control and placement of bioink materials, establishing it as a favored option for shaping these tissue models [30]. The photopolymerization process must start with digital light sources such as blue light, ultraviolet (UV) light [31], visible light, or near-infrared (NIR) light for digital light processing (DLP)-based 3D bioprinting to work. With remarkable accuracy and intricate structural detail, this process turns liquid polymer ingredients into solid, three-dimensional structures. The structure of tissues, particularly those of the liver, lung, eye, brain, blood vessels, and pancreatic tissue, can be studied for drug screening, disease modeling, tissue repair, and regenerative medicine. Scaffold production relied heavily on electrospinning, freeze-drying, gas forming, and injection molding. However, modern approaches, specifically additive manufacturing (AM) techniques, have risen in importance within the field of tissue engineering (TE) because of their ability to construct complex scaffold structures to ensure effective scaffold manufacturing. Sodium alginate (SA) hydrogel exposed to divalent cations like Ca^{2+}, Ba^{2+}, and Mg^{2+} undergoes a remarkable transformation that provides crucial support for cell growth, movement, and specialization and also facilitates the flow of oxygen and nutrients while creating a three-dimensional highly hydrated environment reminiscent of natural soft tissue (**Figures 2a-b**) [32]. Due to its excellent biocompatibility and the ability to gel under mild physiological conditions, SA has emerged as a promising choice for 3D bioprinting applications, particularly in creating cartilage scaffolds.

Nevertheless, it is essential to recognize that SA's inherent low toughness poses a significant challenge to its broader clinical use as a material for cartilage scaffolds. In response to this challenge, nanoparticles, including graphene, bioglass, silica, and additional biopolymers, have been introduced into or combined with SA hydrogel to enhance its toughness. Polyvinyl alcohol (PVA), a water-soluble synthetic polymer, stands out due to its favorable properties, encompassing hydrophilicity, biocompatibility, and innate toughness. By physical crosslinking through a straightforward process of repeated freezing and thawing, eliminating crosslinking agents. This characteristic makes it highly suitable for crafting hydrogel implants, providing a promising avenue for advancements in tissue engineering and regenerative medicine in **Figure 2c** [33].

Hyaluronic acid (HA) is a water-soluble polymer that exists in vertebrate tissues, comprises unbranched chains of negatively charged glycosaminoglycans (GAGs) without sulfation, distributed across various tissue types, including epithelial, soft connective, and nerve tissues. HA actively participates in numerous critical biological processes, including cell adhesion, migration, proliferation, differentiation, angiogenesis, regulation of inflammation, support for wound healing, promotion of tissue repair, influence on morphogenesis, and even involvement in tumor growth and metastasis. The three-dimensional network of cross-linked HA hydrogels closely resembles the structure of the native extracellular matrix. HA is essential to tissue engineering and regenerative medicine because it encourages cell attachment [34-35]. **Figure 2d** shows the design of bioprinting for the 3D printing of tissue models.

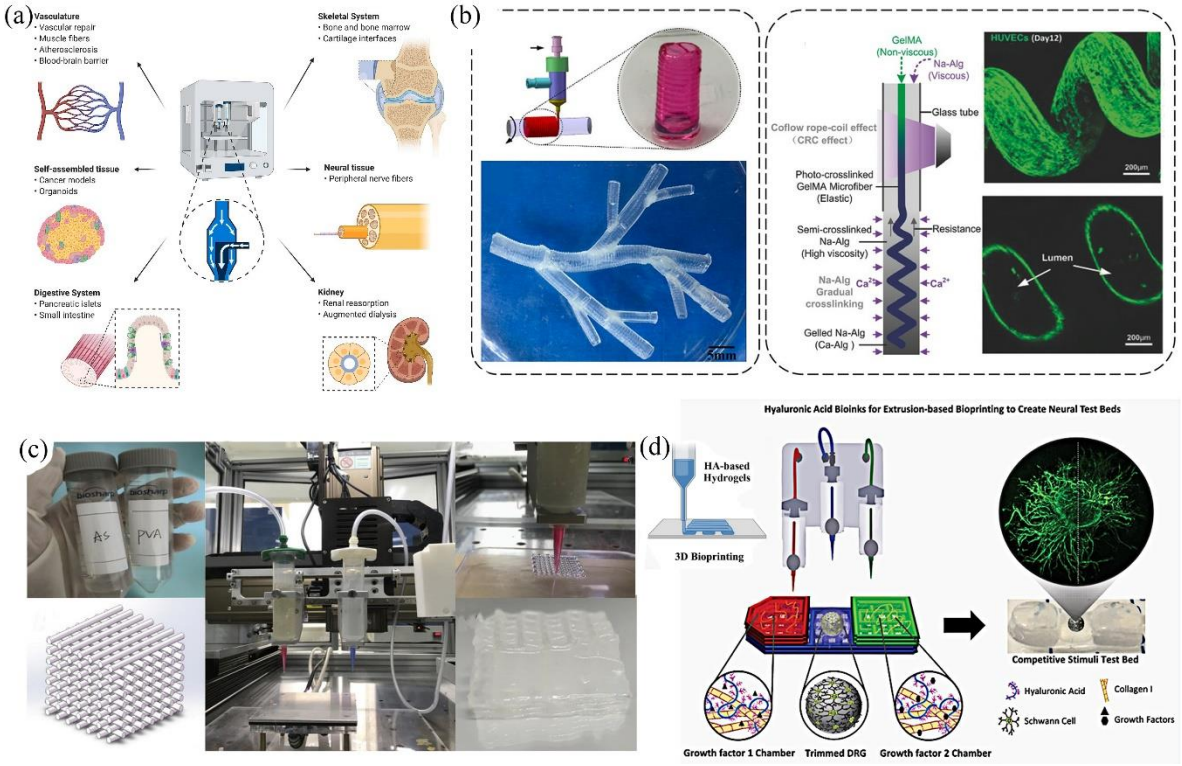

Figure 2. (a) Diagram illustrating the main structural components and tissue applications enhanced by coaxial bioprinting. Coaxial printing techniques can be used to generate a variety of tissues, such as the kidney, digestive system, self-assembled tissue, skeletal muscle, and vasculature, (b) applications of coaxial printing in the vasculature. Sodium alginate (SA) was extruded onto a revolving rod to form tubular vessels, crosslinking inside a glass tube and adjusting the viscosity of the core and sheath fluids (middle), resulting in coiled-rope constructions (upper right), and allowing space for the lumen development of endothelial cells [32], (c) The combination of the hydrogel scaffold printing process. SA and PVA hydrogels; a porous scaffold model; an independently constructed bioprinter; the printing procedure; and a printed SA/PVA hydrogel scaffold [33], and (d) an illustration of the three-dimensional bioprinted in vitro test bed concept, which includes two chambers that may be filled with various growth factors and a base printing with the primary bioink. [34]

2.1 Bioink selection

Bioinks are an essential component of 3D bioprinting. They contain living cells and biomaterials that simulate the extracellular matrix microenvironment. They are cross-linked or stabilized during or immediately after the bioprinting process to form a final structure that simulates the expected tissue structure shape. An ideal bio ink's characteristics include printability, adjustable gelation, fluidity to ensure high resolution and rapid printing prototyping, modifiable chemical structure to

achieve printing of specific tissue structures, and a high degree of biological stability [36]. Compatibility to accommodate living cells, support cell attachment, proliferation, and differentiation, and sufficient mechanical strength and stability to maintain structural morphology. The printability of bioinks depends on the viscosity, surface tension, and cross-linking ability of the bioink itself and the printer nozzle's surface characteristics [37]. The hydrophilicity and viscosity of the bioink affect printing reliability and living cell encapsulation. The key factor. The tissue and organ target for printing, the cell type, and the 3D bioprinter model determine the selection and modification of matching bioinks. Biomaterials are used to formulate bioinks, which can be roughly divided into natural biomaterials and synthetic materials. [38]

Compared with synthetic materials, natural materials have more advantages in composition or structure close to the extracellular matrix (ECM), self-assembly ability, biocompatibility, and biodegradation. Collagen and hyaluronic acid (HA) are derived from natural ECM and biomaterials commonly used for bioprinting 3D structures [39]. Agarose is a natural polysaccharide extracted from seaweed. It has good gelling properties, mechanical properties, and biocompatibility, but its ability to support cell growth is limited [40]. Alginate is a natural biopolymer extracted from brown algae. Its advantages include a low price and high flexibility [41]. Natural materials include fibrin, cellulose, and silk (silk fibroin). Although natural polymers can provide the desired microenvironment for cell attachment and proliferation that mimics the native ECM, natural polymers have less tunable properties. Consequently, natural polymers are frequently mixed with synthetic materials or other natural polymers to achieve more stable structures and improve the tunability of 3D bioprinting. Polyethylene glycol (PEG), polycaprolactone (PCL), and block copolymers (pluronic) are common polymers utilized in 3D bioprinting (**Figure 3**). [42]

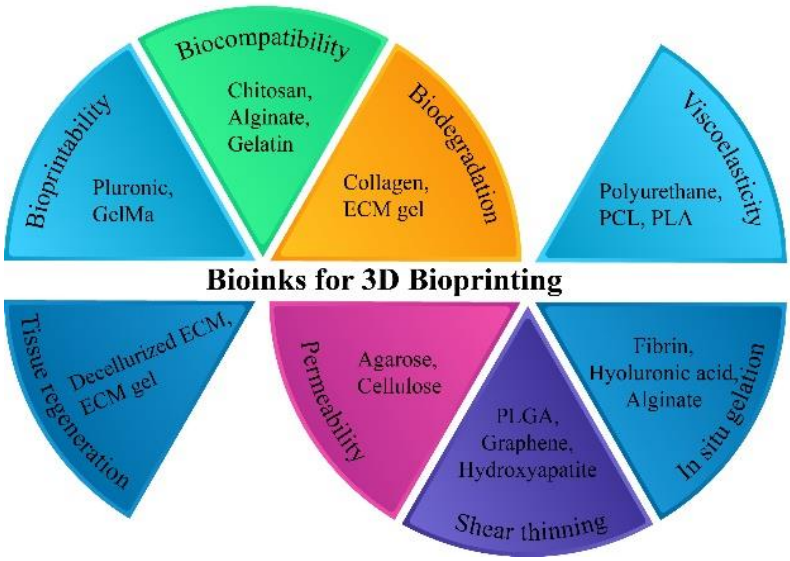

Figure 3. Significant specifications for choosing a bioink in biomaterial characteristics for 3D printing.

2.2 3D Bioprinting process selection

Choosing the suitable 3D bioprinting method hinges on many variables, from the specific tissue or organ under development to the materials in the project's arsenal to the exacting demands of

resolution and precision. An array of 3D bioprinting techniques is available, and here are some critical considerations for making an informed selection.

2.2.1 Extrusion-based bioprinting

Extrusion-based bioprinting (EBB), which uses viscous gels/pastes and does not need a melting step, stands out as the preferred choice among bioprinting methods [43]. The extensive adoption of EBB can be attributed to its ability to 3D print hydrogels with a wide range of viscosities, spanning from 30 mPa.s to over $6x10^7$ mPa.s, and to produce substantial, three-dimensional constructs at a large scale, often in the centimeter range, while maintaining high cell densities exceeding 10^8 cells/mL. The process's resolution is limited, with the most petite achievable feature sizes falling within the 200-1000 µm range. This constraint poses a significant challenge when replicating intricate features resembling biological tissues. Some biomaterials that work well for printing and keeping cells alive are alginate, pluronic, polyethylene glycol (PEG), and natural biomaterials such as collagen, gelatin, chitosan, fibrin, and decellularized extracellular matrix (dECM) [44]. Signaling proteins, primary cell morphologies, and bio-inks (hydrogels) are used in extrusion platforms for skin applications. Moreover, because it can mimic cartilage tissue's complex structural and functional features, it has proven far more reliable for tissue engineering applications [45]. The primary strengths of EBB are rooted in several key advantages. It is the capacity to extrude a diverse array of materials, encompassing viscosities from 10^{-3} to 10^4 Pa.s. The potential to deposit biomaterial inks, often called bio-inks, contains a high concentration of living cells with maintained viability. Its cost-effectiveness and straightforward hardware can be tailored to suit the specific requirements of researchers. [46]

2.2.2 Inkjet-based bioprinting

Inkjet printing, also known as droplet-based bioprinting (DBB), represents a 'bottom-up' methodology for constructing structures, in contrast to the conventional 'top-down' approach. The ink is propelled through the nozzle of a jetting device in the form of droplets, typically within the nano-to-pico-liter volume range (**Figure 4**) [47]. Inkjet printing can be categorized into two primary variants, thermal and piezoelectric [48], based on the actuation technique or jetting mode employed and either continuous or drop-on-demand on a deposition basis. Inkjet bioprinting requires lower viscosity fluids and higher dropping velocity. Generally, inkjet bio-inks are fibrin, alginate, gelatin methacrylate (GelMA), synthetic polymers, and composite. The several vital advantages are yield high-resolution (spot size resolution of around 50–75 µm), bottom-up deposition of cells, cell viability higher than 85%, and speed in two- dimensional (2D) or 3D patterns (1–10,000 droplets/s). [49]

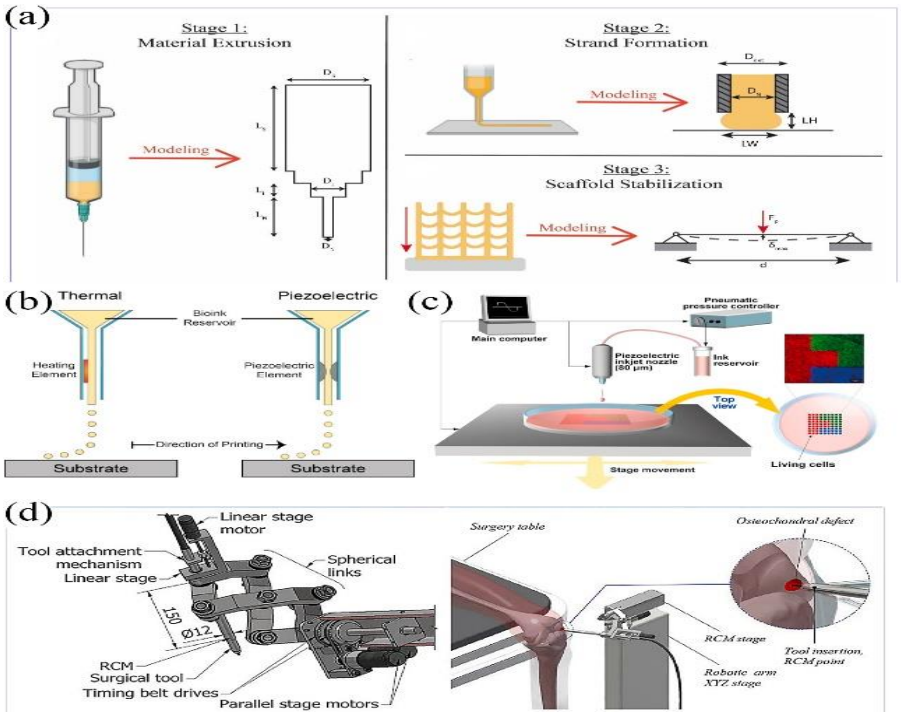

Figure 4. (a) Illustrates the three primary actions that comprise the EBB. Stage 1 involves material extrusion through the syringe needle; Stage 2 involves strand formation during the initial layer's deposition; and Stage 3 involves strand stabilization over time [46], (b) diagrammatic representation of the inkjet printing processes using piezoelectric and thermal technology, (c) diagrams showing how to print cells on a liquid substrate to produce cellular micropatterns, (d) illustration of a schematic design for a robotic arm that has a remote center of motion (RCM) and six degrees of freedom (x, y, z, yaw, pitch, and roll). It also shows a suggested setup for in vivo cell printing and surgery on a damaged or diseased knee joint. [47]

2.2.3 Laser-assisted bioprinting

Laser-assisted bioprinting (LAB) is characterized by its remarkable precision and reproducibility. This innovative approach is at the forefront of bioengineering, enabling the creation of intricate scaffolds that closely resemble the architecture of natural tissues and organs. Through LAB, researchers can print sophisticated structural frameworks by seamlessly integrating various biomaterials and living cells, a pivotal step in fostering the interaction between cells and scaffolds. This dynamic process catalyzes the regeneration of tissues and organs, surpassing the limitations of traditional scaffold fabrication methods [50]. The foundation of this printing technique relies on the laser-induced forward transfer (LIFT) phenomenon (**Figure 5a**). LAB, which employs LIFT, is constructed around three principal components: (1) a pulsing laser source, (2) a target or ribbon serving as the foundational structure for dispensing the biological material, and (3) a receiving substrate that gathers and captures the printed material [51].LAB, or laser-assisted bioprinting, prints at high throughput and up to 5 kHz using a high-frequency pulse laser with different wavelengths ranging from 94 to 1064 nm. Concerning cell viability, resolution, and accuracy, this method permits the accurate deposition of five thousand droplets per second. LAB can separate individual cells using a nozzle-free method and apply thick bio-ink. Bioprinting applications can benefit from its versatility as it has been effectively used in various tissues, including stem cells, colon cancer cells, and osteosarcoma cells. [52]

2.2.4 Stereolithography bioprinting

Stereolithography (SLA) 3D bioprinting is highly effective for producing complex tissues because of its remarkable resolution, excellent cell survival, biocompatibility, and multi-scalability. It is perfect for clinical applications because it can quickly build customizable and anatomically accurate scaffolds. Sliced biomedical images from MRI or CT scans are used as input by SLA to produce tissue scaffolds. After adding bioink to a petri dish to the appropriate thickness, an image from the input stack is projected onto the bioink to start the crosslinking process, which allows for the formation of incredibly intricate and useful tissue structures [51]. The SLA bioprinting technology frequently used UV or visible light, depending on which photoinitiators were needed. These two steps, bio-ink deposition, and image projection, are iterated until all patterned images have been projected to construct a 3D model. Finally, the uncrosslinked portion of the bioink is eliminated, resulting in the 3D-printed scaffold, complete with encapsulated cells. In stereolithographic (SLA) bioprinting, the bio-inks must exhibit crosslinking capabilities when exposed to various types of light within this nozzle-free and time-independent approach (**Figure 5bc**). [53]

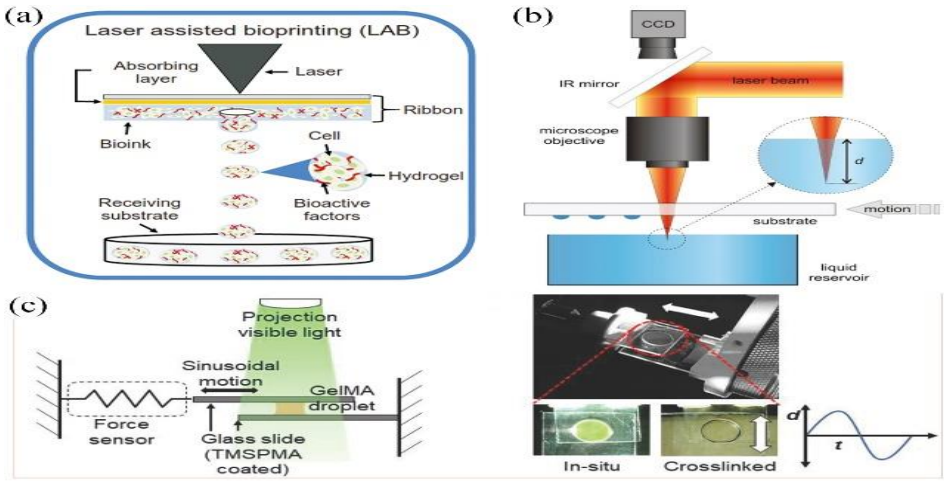

Figure 5. (a) Schematic illustration showing the various components of laser-assisted bioprinting [50], (b) laser-induced backward transfer [52], and (c) photocrosslinking kinetics of GelMA. A diagram shows the curing kinetics measurement with an altered probe, and a modified probe is designed to shear GelMA droplets sinusoidally. [53]

2.2.5 Direct Ink Writing Bioprinting

A modern facility technique in 3D bioprinting and direct ink writing (DIW) has great potential to advance tissue engineering and regenerative medicine. Using this innovative technique, scientists and researchers may carefully apply bioinks—a mixture of biomaterials and living cells—layer by layer to produce complex three-dimensional structures. DIW allows the creation of intricate and personalized biological structures by precisely extruding bio-ink through a nozzle using a computer-controlled 3D printer. With the great degree of control this approach allows over the spatial arrangement of cells, tissues that closely resemble the natural architecture of organs can be created. By modifying the composition of bioinks to correspond with particular tissue types, researchers can guarantee compatibility and facilitate effective integration after implantation. DIW 3D bioprinting has many uses, from building in vitro models for disease research and medication testing to fabricating artificial organs for transplantation [54]. The ability to replicate the intricate cellular and circulatory networks seen in natural tissues can help create functional and viable

structures. Even with the tremendous advancements, issues, including increasing printing speed, boosting cell viability, and fine-tuning biomaterial compositions, still need to be addressed. Realizing the full potential of 3D bioprinting will require ongoing research and technological developments in direct ink writing, ultimately opening the door for ground-breaking discoveries in regenerative medicine and customized healthcare. *Tay et al.* [55] studied a significant increase in wearable and implantable bioelectronics due to the rising need for individualized health monitoring. Diverse bioelectronic devices can be fabricated thanks to advanced 3D printing and straightforward ink writing (DIW), which provide design flexibility and material adaptability. The methods, materials, and uses of DIW 3D printing are examined in this paper, focusing on the benefits of its quick prototyping and efficient manufacturing for healthcare applications (**Figure 6**).

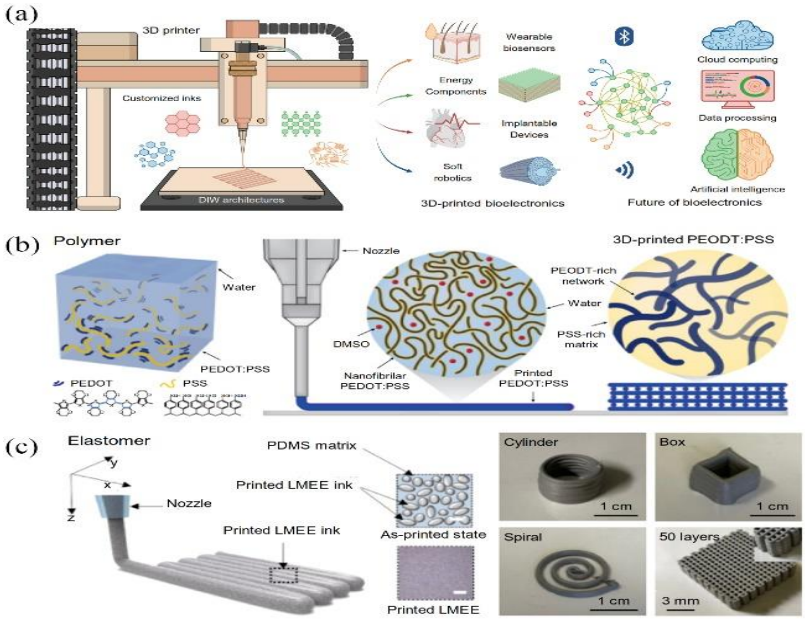

Figure 6. (a) A schematic of DIW 3D printing for bioelectronics using specially formulated inks, (b) Bioelectronic materials made with DIW 3D-printed materials. A method for printing conductive polymers using cryogenic lyophilization and solvent re-dispersion, and (c) Liquid metal injected elastomers for soft materials printed in three dimensions. [55]

3. 3D bio-printed toxicity testing

Combined with life sciences and tissue engineering, using human cells as 3D printing materials to cultivate transplantable human organs is the application and development direction of 3D printing technology in the medical industry. Although 3D printing cannot "manufacture" fully functional liver and kidney organs at this stage, 3D-printed liver, skin, and other tissues have been used to research and develop drugs and cosmetics. Because 3D-printed, three-dimensional human tissues are very close to human organs, drug developers can understand the effects of drugs on human organs without volunteers taking the drugs. Bio-3D-printed three-dimensional human tissues are also gradually being used in drug trials. Unlike two-dimensional cytology experiments, 3D-printed three-dimensional human tissues can survive longer and replicate complex cell-cell interactions and the human tissue environment. Using 3D-printed, three-dimensional tissues for drug testing will yield more reliable results and help reduce the risk of potential drug failure during clinical trials (**Figure 7**). [56-57]

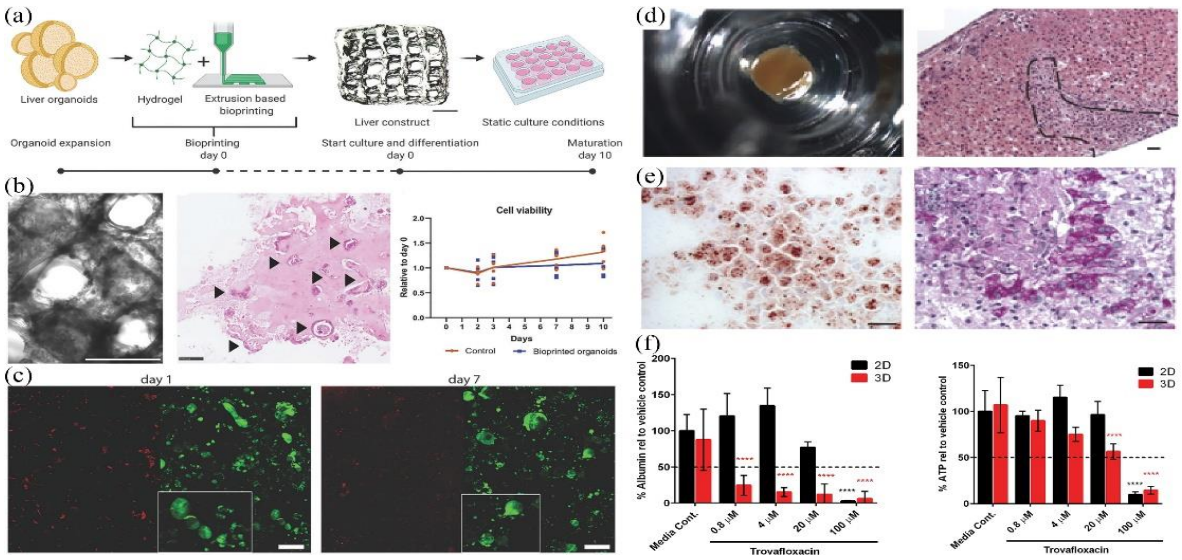

Figure 7. (a) Liver structures can be 3D bioprinted. A schematic illustration of the liver-build bioprinting experimental process. Liver organoids (ICOs) are enlarged and then encased in hydrogel (GelMA), (b) 3D bioprinted liver construct's brightfield picture. Bar scale: 1,000 µm. (c) The 3D bioprinted liver construct stained with HE. Liver organoid cell viability in GelMA (five donors) following plating (control; orange) and printing (blue), (c) An illustration of the liver organoids' live/dead staining at days 1 and 7 following printing [56], (d) A macroscopic picture of a 24-well transwell filled with 3D liver tissue. A tissue cross-section stained with H&E, which makes it easy to see how the parenchymal and non-parenchymal fractions are separated (dashed line), (e) Reddish-oil O staining of cryosections of 3D liver tissue to quantify the amount of fat stored. Glycogen granule identification using PAS staining. All of the IHC staining samples' cell nuclei were stained with DAPI (Blue), (f) Trovafloxacin-treated 3D liver tissues' albumin (ALB), and ATP levels. [57]

Lawlor et al. [58] loaded "bio-ink" based on human stem cells into a particular bio-3D printer and then squeezed out the "bio-ink" through a computer-controlled pipette and placed it in a petri dish. "Print" living kidney tissue. This technology can print about 200 micro-kidney organoids no more significant than the size of a fingernail in about 10 minutes. These organs have the basic unit of kidney structure and function—nephrons. They have filtering functions and can be used for detection. Drug toxicity to the kidneys or being used to test the efficacy of new kidney disease treatments could help develop personalized treatments for patients with different kidney diseases. The authors tested the toxicity of aminoglycosides, a class of antibiotics that often damage the kidneys, and found increased death of specific cell types in the kidneys treated with aminoglycosides (**Figure 8**).

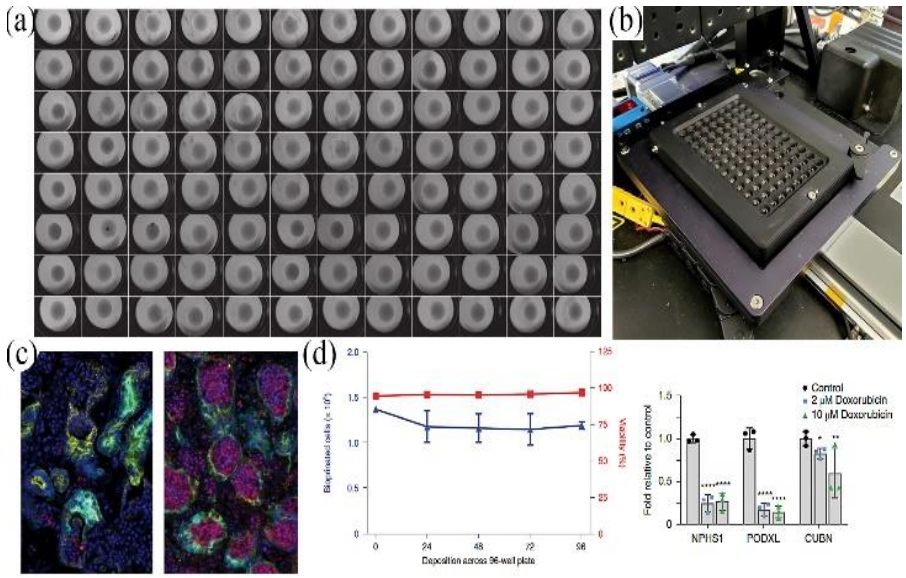

Figure 8. (a) Integrating bioprinted organoids in 96-well format for compound testing: 1×10^5 cells per organoid of bioprinted day 7 + 18 organoids in a 96-well Transwell configuration, (b) On the print stage, a 96-well plate is contained in a plate holder, (c) Following 10 µM doxorubicin treatment, immunofluorescence was used to illustrate nuclei (4,6-diamidino-2-phenylindole (DAPI)), distal tubules (cytokeratin 8/18; CCK8/18), apoptotic cells (cleaved caspase-3 (CC3)), and podocytes (MAFb), and (d) Quality control evaluation of the vitality and number of cells per organoid in a 96-well plate, and measurements of cell numbers, error bars display the standard deviation. [58]

4. Applications of bioprinting in biomedical

The wide range of uses for 3D bio printing drives its quick growth into a substantial industry. Recently, 3D bioprinting has attracted the attention of numerous researchers for its potential uses in the medicinal field. Businesses worldwide have funded laboratory research studies that have advanced the application of this technology in medicine. This technology is advantageous for biomedical applications and devices because it may be manufactured to meet specific patient needs. There are two main areas in which bioprinting finds application. Among the biomedical applications are drug discovery, screening, and printing of blood vessels, heart valves, musculoskeletal tissues, liver, nerves, and skin for tissue regeneration. Consequently, this study's focus will be the several biomaterials at the vanguard of bioprinting technology.

4.1 Tissue and Organ Regeneration

The ability to regenerate tissue has become more critical to restoring the functioning components of damaged tissues and organs. Regenerative medicine's tissue engineering division employs in vitro techniques to regenerate particular tissues and restore them to their original biological capacities. Traditional tissue engineering methods involve the implantation of scaffolds on their own, cells cultivated in conjunction with additional bioactive substances, or a combination of cells implanted in or on scaffolds. These techniques function similarly to the organism's extracellular matrix (ECM) and aid in tissue engineering [59]. Tissue engineering's current challenge is to create biocompatible scaffolds that accurately mimic the in vivo environment. Natural scaffolds' essential features affect how cells behave and interact with biomaterials [60]. These include surface porosity and topology, fiber density, and network structure. Bio-printing-based regenerative medicine (RM) advancements

target skeletal, neuronal, chondronic, and epidermal tissue replacement, rejuvenation, and repair (**Figure 9**). In general, the new tissue created through 3D bioprinting for tissue regeneration will resemble the original in terms of its structure, porosity, and other characteristics. [61]

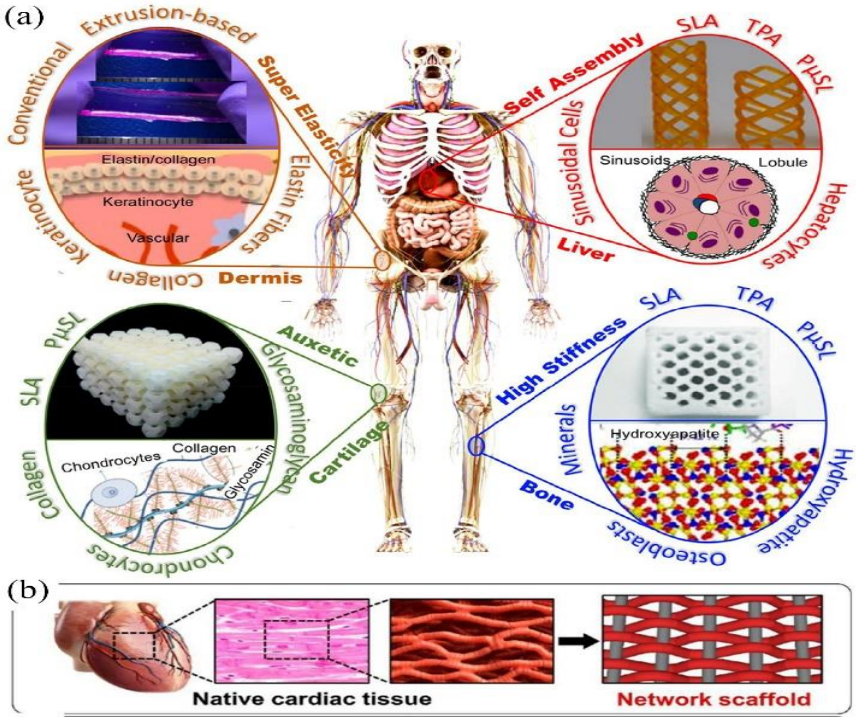

Figure 9. (a) An overview of mechanical meta-biomaterials that have been proposed for four different organs in tissue engineering and regenerative medicine provides high-stiff models for cortical bone tissue, auxetic models for articular cartilage, self-assembly models for the liver, and super elastic models for the dermis and skin tissue [62], and (b) A hybrid scaffold made of hydrogel and interlaced conductive nanofiber yarn that mimics the natural structure of heart tissue. [63]

4.2 Bone

A complicated mixture of mineral deposits and organic matrix with a preset structural order makes up bone. Despite its mending ability, the bone usually possesses somewhat limited regeneration potential. Maxillary, mandibular, and skull-bolt bones can all be quickly, often, and cheaply restored using bio-printing, which can accurately mimic the intricate architecture of bone tissue [64]. It is also essential that this porous scaffold works well with living things and is as strong as natural bone-built PEGDMA scaffolds made with a thermal inkjet bioprinter (**Figure 10a)**. Hydrogen-peroxide (H_2O_2) nanoparticles and bioactive glass were printed simultaneously on human mesenchymal stem cells (HMSCs). HMSCs could be spread out evenly with bioprinting, unlike when pipetted by hand, where they gathered near the bottom of the scaffold (**Figure 10b).** Scientists let it grow in a dish for 21 days. The bioprinted structures with hMSCs and HA inside had the most living cells, collagen production, alkaline phosphate activity, and compressive modulus [65]. Porous polypropylene fumarate (PPF) scaffolds were printed in a different study. These scaffolds demonstrated the degradation process for more than 224 days, demonstrating their feasibility in bone tissue engineering [66]. After that, immune-deficient mice were implanted with the construct subcutaneously. Pericytes in mice also keep the newly formed arteries alive. This shows that artificial bone tissue that has already been vascularized can cause normal bone and blood vessel formation after it is implanted (**Figure 10c**). [67]

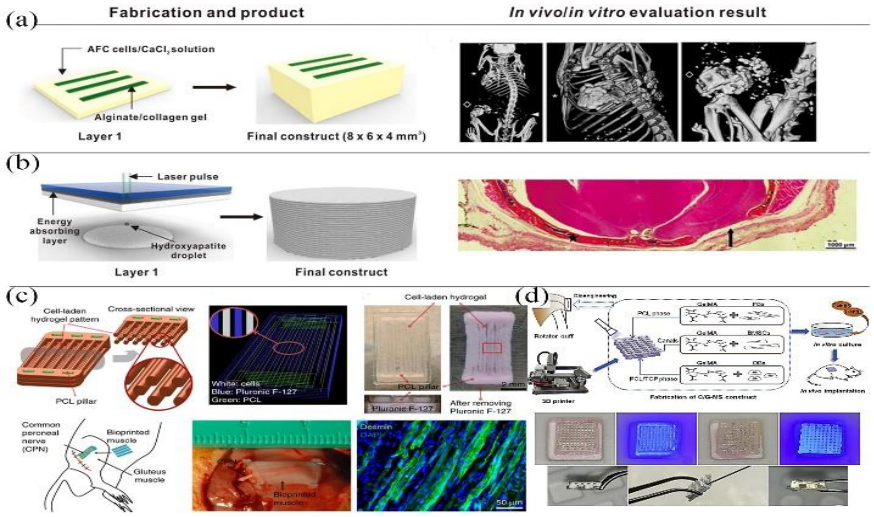

Figure 10. (a) An overview of the processes used to create bone tissue: AFS cells are printed in a $CaCl_2$ solution layer by layer to create collagen and alginate gel during bone tissue production. After eighteen weeks in mice, in vivo tests demonstrate the effective formation of bone tissue, (b) A layered construction diagram with 20 µm-thick 30 layers of nano-hydroxyapatite is used to treat mice with calvaria problems. In contrast to the non-reconstructed control site indicated by the arrow, decalcified histology shows effective defect healing at the star-marked site after three months [68], (c) Mice implanted with 3D bioprinted skeletal muscle constructions demonstrated well-organized muscle fibers, innervation, and vascularization, demonstrating good cell survival and muscle-like properties, and (d) Using 3D bioprinting, multiphasic tendon-bone interface constructs showed improved multi-tissue growth, in vitro biocompatibility, and regeneration potential when implanted in mice. [69]

4.3 Skin

The ability to create or regenerate skin through bioprinting is becoming more and more crucial to human survival. Precise cell placement, cell-to-cell interactions, and interactions with the matrix are necessary to print the skin utilizing 3D bioprinting. Scaffolds commonly employed in skin tissue engineering include fibrin, synthetic cellular allogeneic dermis, and type I collagen. Keratinocytes, fibroblasts, and stem cells are the three main cell types used in skin printing. One of the body's most intricate, multilayered organs is the skin. In this instance, Kim and his associates combined the inkjet and extrusion features to make an entirely new version that generated a three-dimensional epidermis. The attempt proved to be efficient by combining two distinct bioprinting techniques. *Qulez et al.* [70] produced skin tissue by packing human fibroblasts and keratinocytes into a fibrin matrix from human plasma. The findings showed that, in contrast to the control groups, the implanted constructs hardly caused contractions and that bioprinted HMVECs developed microvessels. The research above-created tissue structures by in vitro bioprinting, which were inserted into a host.

However, because the AFS cells are not immunogenic, it was also possible to make stratified skin substitutes in situ. These were created by alternately layering fibrinogen, collagen, and thrombin-loaded thrombin. Skin substitutes 3D printed directly into full-thickness wounds on pigs using in situ bio-printing closely resembled the native skin compared to control groups that included bio-ink loaded with MSC and cellular hydrogel (**Figure 11**). Creating skin substitutes that closely mimic genuine skin is challenging since attempts to bio-print skin tissue have not been able to replicate the integration of sweat glands and hair follicles [71]. Further research is necessary even though 3D bioprinting can create skin. Resolving problems with bio-printed skin include vascularity, cost, and the best mixes of cells and scaffolds. This technology may eventually be employed in

reconstructive surgery. Still, its initial application is anticipated to be in small-scale 3D skin-tissue models for tumor modeling and evaluating medication and cosmetic toxicity.

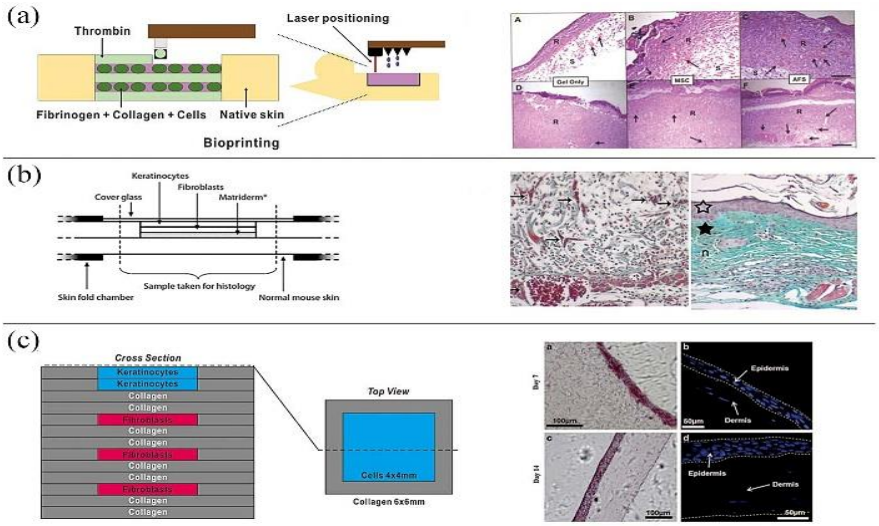

Figure 11. (a) An overview of the steps involved in creating skin tissue, along with a hypothetical representation of a fibrin/collagen gel covering a mouse wound using MSCs or AFS cells. Bio-printed structures in vivo exhibit improved neovascularization and tissue regeneration, (b) Mouse dorsal skin fold chamber containing Matriderm, 20 layers of fibroblasts, and keratinocytes apiece. After 11 days, a histological study reveals a rich epidermal layer and neovascularization, and (c) a diagram illustrating layer-by-layer construction of collagen matrix, KCs, and FBs in the skin. Histological study on days 7 and 14 shows evolving epidermal layers. [68]

4.4 Cartilage

The development of cartilaginous tissues is another aspect of tissue engineering that is heavily studied. Most of the connective tissue that makes up cartilage comprises collagen and proteoglycans. Also, 3D bioprinting utilizing thermoplastics like polycaprolactone (PCL) and PLA was done on cartilage tissue. Chondrotissue regeneration is complex due to the low cell concentration and avascularity of cartilage, which prevents it from healing on its own [72]. The framework of an ear is made up of a single piece of cartilage with a complex geometry of ridges, which is printed by an extrusion printer that prints tissues and organs all at once (ITOP). This allows the ITOP to create complex tissue constructs, such as human-sized external ears, and is tested in this regard. **Figure 12** depicts an ear in CT transform. In the tissue constructs, microchannels were left by printing integrated patterns of sacrificial hydrogels packed with cells and biodegradable polymers connected to them [73]. In the mandible and calvaria, skeletal muscle, cartilage, and bone were repaired using this technique, which has also been applied to cartilage defect healing and cartilage tissue engineering. Human chondrocytes loaded in PEGDMA hydrogel were able to be printed using a modified HP desktop printer; according to Cui et al. [26], the bioprinted cartilage construct exhibited mechanical and biochemical characteristics that were comparable to those of natural cartilage. Also, when bioprinted cartilage structures were implanted into articular cartilage defects, they integrated better with the native tissue and had a more vital interface. This made the repaired cartilage much better.

4.5 Liver

Considering the liver's remarkable capacity for regeneration and repair, liver tissue engineering has grown in popularity. Liver failure is a significant cause of morbidity and death, especially when it happens in conjunction with other organ failures. Engineering hepatic tissues could assist in

satisfying the future need for organ donations because liver tissue is prone to medication toxicity. Bio-printed liver tissue models are also up-and-coming for drug testing and high-throughput screening [74]. HepG2 immortal cells from liver cancer were also used to print larger tissue models. Using a modified NovoGen MMX Bioprinter, fibroblasts, and HepG2 cells were printed onto agarose and gelatin-methacrylamide (GelMA) hydrogel strands in addition to printing liver tissue structures. The agarose solidified instantly after printing due to a fast drop in temperature, and the GelMA precursor was photo-crosslinked for up to a minute using 6.9 mW/cm^2 of UV light (360–480 nm) [75-76]. The agarose was removed after GelMA had fully gelled to make perfusable channels. According to the study, cells could work for up to eight days. Later, different concentrations of other hydrogels, including PEGDMA, PEGDA, and star poly(ethylene glycol-co-lactide) acrylate (SPELA), were added to the study [65]. Only attempts to print liver tissue for tissue engineering are covered in this section; parts after this one will address hepatic tissue printing models for high-throughput screening and pharmacological testing. Liang Ma et al. [77] highlighted the potential of 3D bioprinting to produce in vitro liver tissue models, highlighting tactics, bioinks, benefits, constraints, difficulties, and opportunities for furthering liver tissue modeling in the future (**Figures 12c-d**).

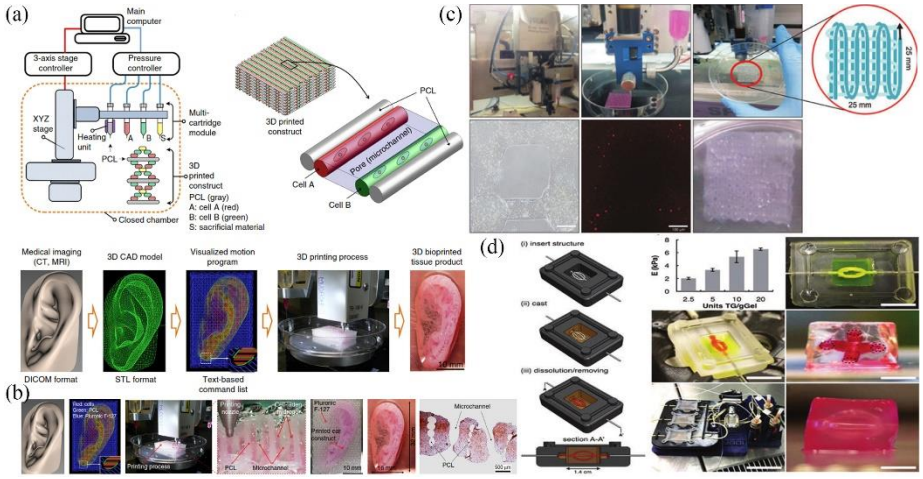

Figure 12. (a) A dispensing module for 3D printing, a 3-axis stage/controller, and a closed acrylic chamber comprise the Integrated Tissue-Organ Printer (ITOP) program. The system automatically prints cell-filled hydrogels and PCL polymer support, simulating target tissues or organs using CAD/CAM technology. A motion program for accurate XYZ stage movements and pneumatic pressure management is generated using a 3D CAD model created from medical imaging data, (b) a combination of PCL, Pluronic F-127, and cell-rich hydrogel, a human ear structure, is bioprinted during the ear cartilage regeneration procedure using a 3D CAD model and motion program [73], (c) the creation of 3D-bioprinted hepatic grid structures with liver tissue models fabricated from alginate, and (d) 3D bioprinted liver-on-chips and a 3D vascular network created via printing sacrificial layers. [77]

4.6 Cardiac and Other Tissues

In the early stages of basic research, more work is being done in addition to the tissue types mentioned above for the bioprinting of brain and retinal tissues. Piezoelectric inkjet printing was used to print retinal ganglion cells (RGCs) and glia. It also investigated how the bioprinting variables affected the cells' ability to increase and remain viable [78]. Researchers have combined traditional tissue-engineering techniques with 3D bioprinting tools to illustrate in vitro circulatory networks. These tactics include endothelial-cell inkjet bio-printing, the incorporation of angiogenic growth factors in bio-printed frameworks, and the self-assembly of cells to form vascular structures. To encourage cardiac tissue regeneration and maintain heart function, bio-printed

cardiac tissue architectures must be vascularized, electrophysiologically stable, flexible, but firm and responsive. Norotte et al. [79] developed a scaffold-free, cell-self-assembly technique for small-diameter vascular regeneration using pig aortic smooth muscle cells (PASMCs), human skin fibroblasts (HSFs), and human umbilical vein smooth muscle cells (HUVSMCs). Its two primary flaws are the investigation's spatial resolution and the thickness of the artery walls. It was feasible to demonstrate improved cell viability in alginate treated with RGD, bio-printed alginate, and EBB by using human fetal cardiomyocyte progenitor cells (HCMPCs). A unique simultaneous photo-cross-linking and 3D printing technique was proposed to fabricate complex and customizable aortic valve scaffolds. Using alginate-bound poly (ethylene glycol)-diacrylate (PEG-DA) hydrogels, native anatomic and axisymmetric aortic valve designs were 3D printed (**Figure 13**). The pig aortic valves' interstitial cell-seeded scaffolds held onto them for almost all of their lives for 21 days, proving that anatomically heterogeneous valve conduits can be quickly created using 3D hydrogel printing and strategically placed photocross-linking to encourage cell engraftment [80]. **Table 2** provides an overview of the research conducted on using bio-printing techniques to restore several additional types of soft tissues and organs. The skeletal muscles and tendons, which provide both physical integrity and movement assistance, exemplify this. Several research groups have created this kind of tissue using different components. Also, the generation of liver tissues, renal tubular tissues of the kidney, and gonad leydig cells has been studied using bioprinting [61]. A thorough analysis of the nanocomposite structures at various levels described the potential for creating three-dimensional, personalized scaffolds to regenerate mandibular deformities (such as the symphysis and ramus). Many organ-like tissue constructs, including the pancreas, neural cartilage, heart, lung, or muscular tissues, have been developed, given the previously discussed uses of bio-printing in TE and regeneration. These have all been bioprinted separately using 3D bioprinting techniques.

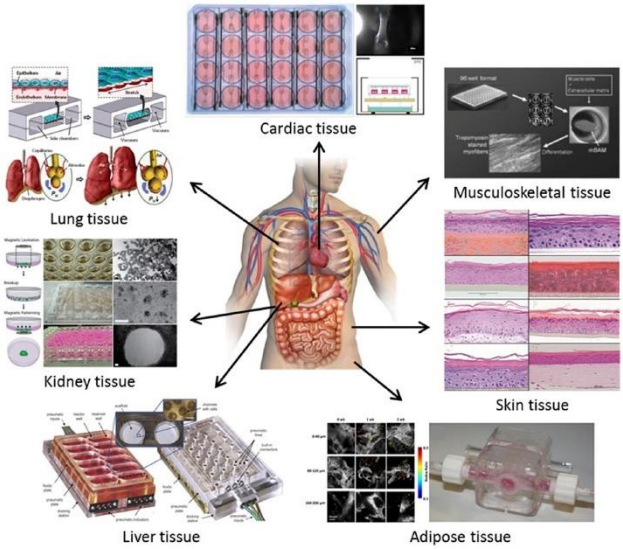

Figure 13. Illustration of high-throughput bioengineered tissue models, such as 3D in vitro rebuilt skin models and perfused multiwell bioreactors for the liver, lung-on-a-chip for lung tissue, ring closure assays using magnetic levitation for kidneys, perfusion bioreactors for adipose tissue, and bioartificial muscle [mBAMs] on posts for the skin. [81]

Table 2. An overview of the tissue engineering uses for 3D bioprinting [61, 65].

Tissue/Organ	Polymer	Technique	Cell Source	Outcome
Bone	Alginate/PVA, PEGDMA	Extrusion Bio-printing/ Thermal inkjet	Primary muscle-derived stem cells; bone marrow stem cells.	Bone tissue can be printed when polyvinyl alcohol and alginate bio-inks are used in insufficient quantities.
Cartilage	Cellulose/alginate PEGDMA; fibrin-collagen type.	Extrusion Bio-printing /Inkjet-based bioprinting	Human mesenchymal stem cells and nasal chondrocytes.	Creating cartilage in constructs with excellent fidelity and strong mechanical characteristics is essential for therapy.
Skin	Alginate, collagen/fibrin gen and thrombin	Extrusion Bio-printing; thermal inkjet	Mouse embryonic Fibroblasts, Human foreskin fibroblast, and HaCaT keratinocytes.	It has been found that the suggested skin dermis decellularized bio-ink is a good contender for tissue engineering applications and that the PSP-ink employed was non-toxic.
Heart	Alginate, gelatin, and Me-Gel	Extrusion Bio-printing	Aortic valvular interstitial cells, H9c2 cells, and human umbilical vein endothelial cells	Valentine-like structures with a self-defined height and appropriate mechanical qualities can be created by 3D bioprinting with sacrificial and hydrogel materials.
Vascular Grafts	poly(ethylene glycol) diacrylate, alginate with carbon nanotubes, and Alginate.	SLA Bio-printing; piezo inkjet; valve-based inkjet	Human red blood cells, human skin fibroblasts.	This study demonstrates the ability to control tissue structure and biomaterials simultaneously and in an orthogonal manner to produce regenerated tissues.
Neural Tissue	Gelatin Methacrylamide; polyurethane, cell pellet and agarose	SLA Bio-printing or Microvalve-based inkjet/ extrusion-based (mechanical)	BMSCs, Schwan cells, and mouse neural stem cells.	Neural stem cells within the printed construct demonstrated neurite extension and neuron differentiation after two weeks of culture, indicating the enormous potential of the 3D-bio printed neural construct for neural tissue repair.
Liver .	Alginate, hyaluronic acid, glycomyl methacrylate, and gelatin sodium methacrylate (GelMA).	SLA Bio-printing/ extrusion-based (Mechanical)	Hepatic progenitor cells produced from human pluripotent stem cells, human umbilical vein endothelial cells, and stem cells derived from adipose tissue	After weeks of in vitro development, hiPSC-HPCs show phenotypic and functional gains in the 3D triculture paradigm.

4.7 Drug Delivery and Screening

Three-dimensional items can be produced from a computer file using additive manufacturing techniques like 3D printing. 3D bioprinting has become an up-and-coming method for developing highly adaptable and repeatable tissue-based platforms through the exact alignment of biomaterials with cells and proteins. **Figure 14** shows how these aspects of 3D bioprinting, closely related to the needs of drug delivery and screening systems, make it possible to build more complex pharmaceutical applications. An analogous approach can be used to give drugs instead of the customary oral technique. 3D bio-printing is one innovative way drug-screening devices might be made [82]. Bio-printing provides a uniform cell distribution on micro-device surfaces for analyzing and evaluating drug-cell interactions, which is superior to manual screening methods. Adhering to a BM carrier allows for exact control over improving the security and efficiency of drug delivery. Alginate is an excellent shipper for the containment and condensing medications, bioactive chemicals, proteins, and cells because of its high biocompatibility and biodegradability [81]. The limited printability of medical polymers in FDM 3D printing was addressed in the current research by demonstrating the utilization of polymer mixes. The outcomes showed how the interplay between media solubility influenced the dispersions' capacity to release the medication, mix excipient miscibility, and interface creation between printed strips during FDM printing. As a result, research into the creation of novel, physiologically active pharmacological discoveries, and their drug delivery systems would provide a lot of data.

Microchannels were created using printable polymeric materials and bioprinting techniques based on extrusion and lithography. *Lee et al.* [70] say that a one-step building method uses 3D extrusion

of poly(ε-caprolactone) (PCL) to deposit different kinds of living cells into a chamber that has already been built.Cell seeding is required in standard organ-on-a-chip (OOC) systems; however, this approach uses PCL material, which reduces protein absorption. With its low resolution (>200 µm), extrusion-based 3D bioprinting has difficulty producing capillary-like channels. Lithography, conversely, provides microfluidic devices with higher-resolution printing, which benefits precise tissue modeling and medication monitoring. [83]

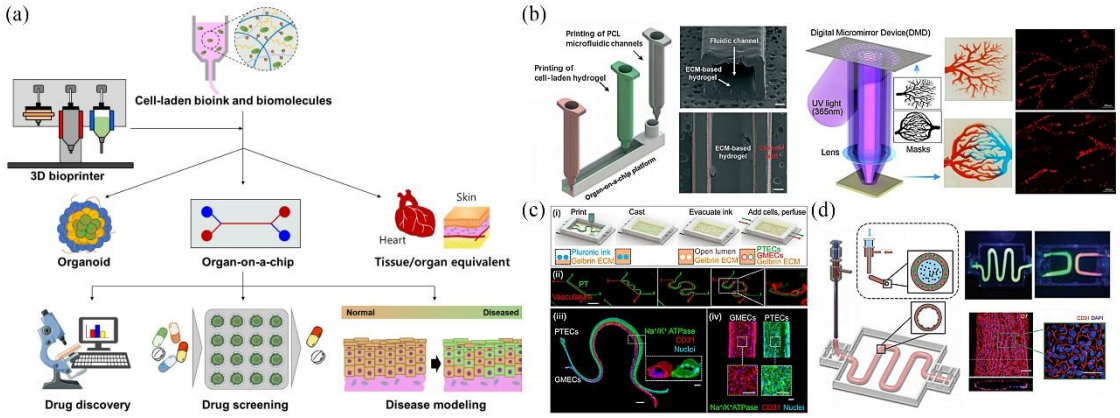

Figure 14. (a) A schematic representation of 3D bioprinting that makes it easier to make 3D cell culture instruments such as organoids, organ-on-a-chips, and tissue/organ equivalents. These developments improve the use of pharmaceuticals in illness modeling, screening, and medication discovery, (b) microfluidic chips created in three dimensions, a projection-based 3D printing platform, and visuals illustrating the capillary action of channels and cell dispersion in the scaffold resembling capillaries, (c) high-magnification pictures of the modeled tissues following staining and the creation of a 3D vascularized proximal tubule model, and (d) the development of printed vasculatures and the creation of freestanding functional vascular models with intricate patterns. [82]

5. Outlook and Conclusion

Although 3D bioprinted tissue models are up and coming, there are still some obstacles to their widespread use. Standardization of bioprinting materials and processes is still an obstacle that affects repeatability and prevents the development of global standards. Another challenge is striking a balance between scalability and complexity since complex tissue topologies frequently come at the expense of production efficiency. Furthermore, vascularization integration into bioprinted tissues is a significant challenge because long-term viability and usefulness depend on a functioning blood supply. It is essential to consider the ethical issues surrounding procuring and applying human cells for bioprinting. The appropriate growth of this technology requires striking a compromise between the need for individualized, patient-specific models and ethical standards.

Applying 3D-bioprinted tissue models to toxicology, medication development, and research signifies a paradigm change in the biomedical sciences. To resolve ethical concerns and develop uniform processes, researchers, engineers, and regulatory agencies must work together to overcome the current problems. Future developments in bio-ink compositions, scaffold materials, and bioprinting methodologies should alleviate existing constraints. Integrating immunological components and creating integrated multi-organ systems will improve the models' physiological relevance even more. Furthermore, bioprinting has the potential to advance faster and expand the use of 3D bioprinted tissues as it continues to converge with other cutting-edge technologies like artificial intelligence and microfluidics. Achieving the full potential of 3D bioprinted tissue models will require a concentrated effort toward scalability, reproducibility, and ethical principles as the

field develops. This will ultimately transform how we approach research, medication development, and toxicity assessment in the future.

Declaration of Competing Interests

The authors state that they are unaware of any financial or personal conflicts that would have affected the findings of this study.

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