Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

The Role of 3D Bioprinting in Drug Discovery; A review

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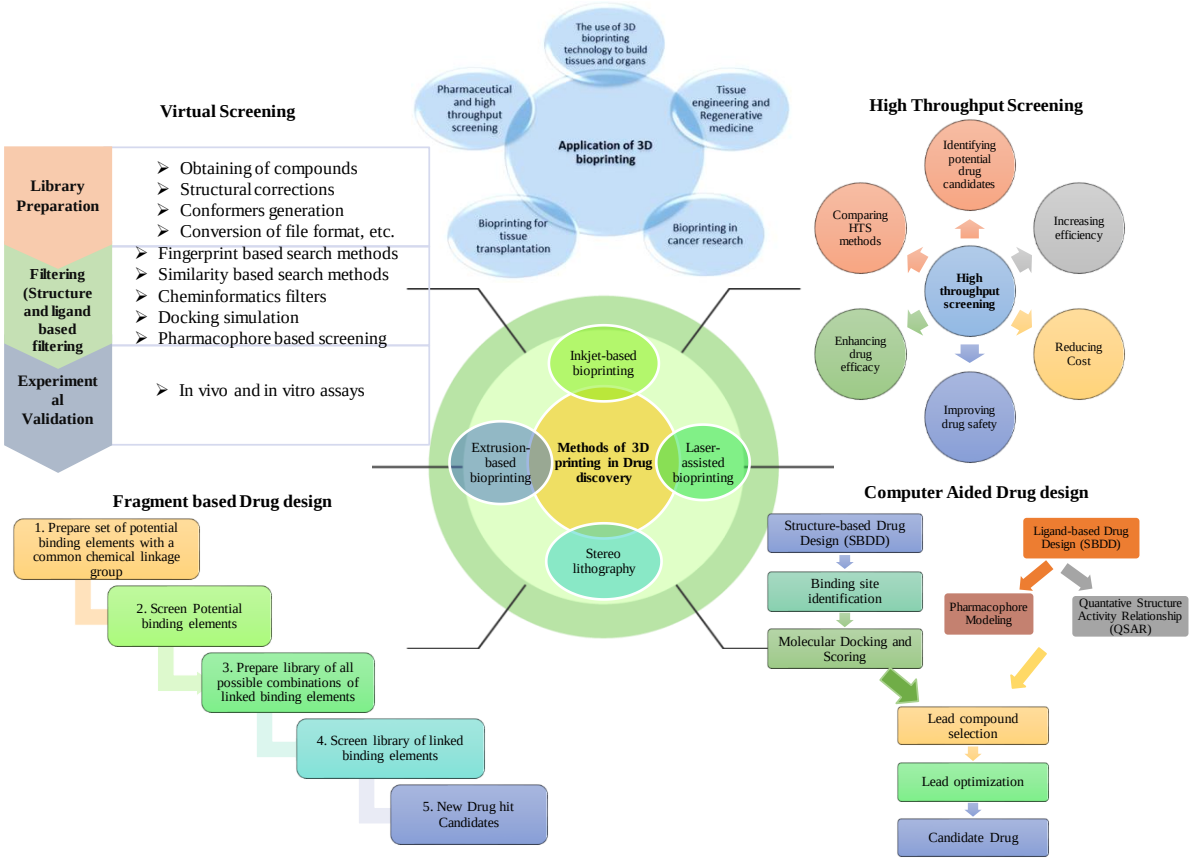
Received: January 23, 2024; revised: March 26, 2024; accepted: April 7, 2024

Abstract

The emerging area of 3D bioprinting is enhanced rapidly. The 3D bioprinting idea was formulated in the 1970s since this technology has been used extensively. In this technology, a digital file is used to produce a living organ-like structure in three dimensions. Being extensively used in tissue regeneration, medical devices, sensors, tissue scaffolds, etc. it plays a major role in drug design. In the last few decades, drug discovery in its traditional ways spans along with huge cost. These earlier techniques like high-throughput screening, computer-aided drug designing, and fragment-based designing, etc. have various limitations. Meanwhile, the 3D bioprinting technology offers the advantages of fastness, ease, and cost-effectiveness. Several reviews and experimental data are available that cover the application of 3D bioprinting in various industries, but comprehensive literature focusing on the role of 3D bioprinting in the drug was scarce. Hence, this review aimed to document the role of 3D bioprinting in drug discovery, methods of 3D bioprinting, and exploit 3D bioprinting technology in the drug development and discovery process. The critically evaluated literature shows the positive usage of 3D bioprinting technology in the development of drug discovery and provides benefits i.e. fast nature, low cost, and ease handling.

Keywords

Bioprinting, Drug Discovery, 3D printing, Virtual screening, Regenerative Medicine



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1. Introduction

1.1. 3D Bioprinting

Bio-printing is a new and emerging technology that uses a digital file to create a living organ-like structure. Due to its sophisticated and automated nature, researchers from various disciplines like pharmaceutical and tissue engineering were attracted to overcome the limitations in their field [1, 2]. Bioprinting falls within the realm of additive manufacturing (AM), which is also referred to as three-dimensional (3D) printing. Mechanistically, the bioprinting entails the fabrication of structures by utilizing live cells, biomaterials, and biological molecules by creating microarchitecture scaffold structures that offer mechanical stability and facilitate cell growth [2, 3]. The notion of 3D printing was initially outlined in the 1970s and subsequently refined in the 1980s [4]. Various techniques, like stereolithography, which entailed solidifying layers of materials to form solid structures, also emerged. Through progressive advancements, 3D printing evolved into 3D bioprinting, a specialized method that involves the precise arrangement of biomaterials, biochemicals, and living cells to fabricate structures that resemble human tissue. Distinct from conventional 3D printing, 3D bioprinting necessitates specific strategies to construct architectures suitable for cell deposition and tissue regeneration [5]. 3D bioprinting is an ever-evolving technology that facilitates the accurate replication of tissue structures. This is accomplished through the utilization of custom-developed bioinks and specialized 3D printers, which are specifically tailored for the bioprinting process [1, 2].

The bioinks utilized in 3D bioprinting can undergo cross-linking or stabilization either during or immediately after the bioprinting process. This transformation enables the bioinks to acquire the intended shape, structure, and architecture of the designed construct [1, 7, 8]. The extrusion of soft hydrogels, commonly referred to as bioinks, often results in inadequate accuracy and fidelity when printed in ambient air conditions [8]. The progress and adaptability of bioprinting are contingent upon the advancement of innovative printable hydrogels, a common bioink. Various properties of these bio-inks bioinks, like mechanical robustness, incorporation of cell adhesion sites, and facilitation of the functioning of the printed tissues, are of utmost importance for their usage in 3D bioprinting [7]. 3D bioprinting can be perceived from two distinct perspectives: a broad viewpoint and a narrow viewpoint. from a broad viewpoint, the establishment of 3D bioprinting occurred when it was used in the biomedical field. This encompassed the printing of various medical aids as well as scaffolds made of polymers, ceramics, or metals [9]. On the other hand, the narrow viewpoint focuses specifically on the concept of 3D cell assembly through printing, which is generally known as cell printing or organ printing [10]. Through the meticulous placement of diverse materials and cells, 3D bioprinting can fabricate tailored biological structures. The process of 3D bioprinting can be delineated into three primary stages: pre-bioprinting, bioprinting, and post-bioprinting [7, 12], as summarized (**Figure 1**). The foundation of 3D bioprinting technology rests upon three fundamental pillars: the bioinks, the bioprinter, and the corresponding bioprinting procedure. These elements collectively contribute to various aspects, ranging from the precise design of specific tissues to the deposition of bioinks during the development of the target tissue [6].

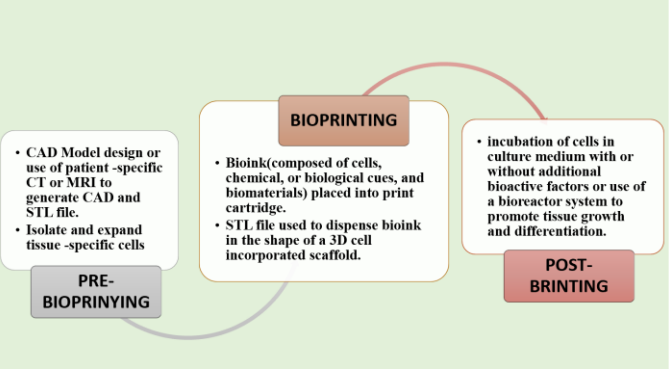

Figure 1. The three primary stages of 3D bioprinting technology.

Several studies have been published on the topic of 3D bioprinting, but there remains a dearth of comprehensive overviews encompassing techniques, materials, and applications, including emerging hybrid technologies. This review aims to fill the gap by presenting a recent advancement in 3D bioprinting, specifically within the context of drug discovery. It provides a thorough examination of various aspects, including the introduction, methods, mechanism of bioprinting, drug screening, and properties of drug discovery in the realm of 3D bioprinting. Additionally, this review also focused on the medical applications of 3D bioprinting, the advantages offered by 3D bioprinting in drug discovery, as well as the limitations and future prospects of this technology in drug discovery. By encompassing these crucial aspects, this comprehensive review can briefly explain the background, importance, and some well-known applications of this technology.

1.2. Drug discovery methods and their pros and cons

Drug discovery is a meticulous and iterative process that involves the identification of potential new medicines through a series of critical research activities. It draws upon various scientific disciplines, including pharmacology, medicinal chemistry, biochemistry, and biology [24, 25]. For decades, drug discovery centers and industries have played a vital role in providing life-saving medications, thereby offering novel treatment opportunities for a wide range of life-threatening diseases. The primary objective of drug discovery initiatives is to uncover new molecular entities that have the potential to address the unmet needs associated with certain diseases [25]. These conditions currently lack effective treatments and may pose significant risks to individuals' health and well-being. The discovery of new drugs has led to improved therapeutic options for conditions such as heart disease, metabolic disorders, osteoporosis, mental health disorders, oncology, gastrointestinal disorders, reproductive healthcare, viral infections, etc. These discoveries have greatly contributed to enhancing overall health and improving the quality of life for individuals affected by these conditions [25, 27].

Currently, there are multiple methods that are used in drug discovery processes these are summarized in **Table 1**.

1.3. Challenges Associated with Traditional Drug Discovery Methods

Presently, the field of medicinal chemistry heavily relies on conventional approaches that employ a trial-and-error strategy and high-throughput screening methods. These methods involve the screening of a vast number of candidate drug compounds to identify those exhibiting the desired properties. However, these approaches suffer from drawbacks such as time-consuming processes, substantial costs, and limited accuracy of results, as shown (**Figure 2)** . Furthermore, they are often constrained by the availability of appropriate test compounds and the challenges associated with accurately predicting their behavior within the human body [43, 44].

Figure 2. Illustration indicating the existing challenges associated with current drug discovery methods.

1.4. The importance of bioprinting

In the last decade, 3D printing has exhibited significant contributions to the manufacturing of medical devices, sensors, tissue scaffolds, and microfluidic chips [12]. These applications have been involved in various fields like tissue engineering, sensing, mixing chemicals, generating gradients, and Organ-On-a-Chip (OOC) systems [13]. However, cells are not incorporated into 3D printing, thus the utilization of cytocompatible biomaterials capable of 3D bioprinter to directly print with cells. Cells can be bioprinted, except for additional biomaterials. Furthermore, the collective advantages of microfluidic chips (including gas permeability, perfusion, and single-cell analysis) with 3D printing can edge to automated bioprinting of multicellular cultures [5, 10]. These cultures can be precisely positioned and perfused, possessing customized structures and features, such as specific pore sizes and morphologies [14]. Such systems have extensive applications in physiological studies, as well as organ-level drug analysis. The later included on-chip models simulating organs such as kidneys, liver, brain, heart and vasculature, bone and cartilage, cancer or tumor, lungs, etc. [15]. This technology also permits the control of the external size and geometry of the structures and has the potential to design anatomical structures [6]. Currently, 3D bioprinting is also used in the repair of damaged skin tissues [4]. The overall spectrum of 3D bioprinting is summarized (**Figure 3)**.

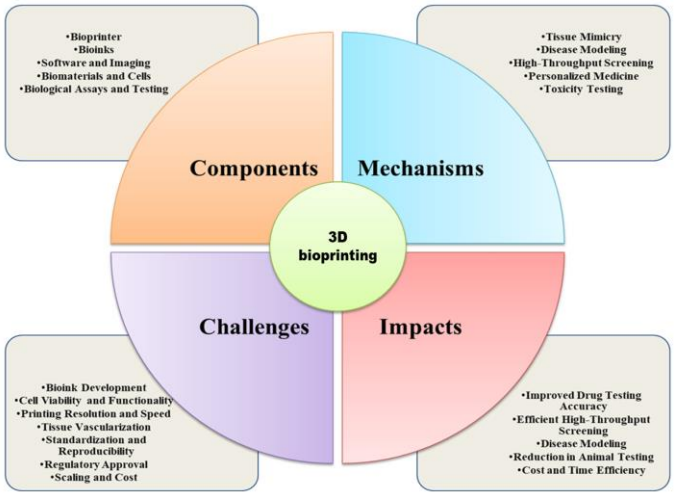

Figure 3. Illustration showing the components, mechanism of action, impacts, and challenges associated with this technology.

2. Applications of 3D bioprinting

There are several examples of bioprinting that have tackled biological questions, particularly using extrusion bioprinting, but are still many opportunities to be explored. These studies have been largely motivated by tissue development or tissue repair processes and have involved printing constructs with spatially patterned cell populations and/or biochemical factors. Here, the most prominent applications of 3D bioprinting were highlighted, while the focus remains on the drug discovery process. The various modalities in 3D bioprinting and their applications are described in **Table 2**. Each of these 3D bioprinting techniques has its own unique capabilities and limitations. Researchers choose the appropriate technique based on the desired properties of the printed constructs, the specific research objectives, and the types of cells and biomaterials involved.

2.1. Application in tissue development and repairing

The most basic and well-studied application of 3D bioprinting is related to the development and repair of tissues [6]. Before 3D bioprinting, the complexity of tissue development becomes a challenge in the morphogenesis and cell differentiation processes [3]. Now, 3D bioprinting offers a solution for such a process by using some recognized patterns. For instance, in studying vascular angiogenesis bioprinting has been employed to create vascular channels within cell-degradable hydrogels [20]. Tissue engineering and regenerative medicine encompass interdisciplinary domains that aim to restore tissue functionality [10, 22]. Tissue regeneration involves the implantation of cells and biomaterials into the body to rebuild tissues and facilitate their growth. Stem cell technology is often employed to stimulate cell growth alongside biomaterials. Tissue defects may result from genetic factors, aging, accidents, or diseases ranging from localized damage to organ failure [9]. The utilization of 3D bioprinting in preclinical tissue models, both in laboratory settings and in living organisms, is being explored to advance tissue engineering and enhance the field of regenerative medicine. This involves a focused investigation of specific materials and techniques that are employed in 3D bio-printing [6, 10]. Various studies were performed that successfully integrated cells associated with skin diseases into biomaterials, allowing for the construction of skin tissue through 3D bio-printing [5, 23].

This technique enables the examination of the pathophysiology of skin diseases by printing skin tissue with pathogenic cells [23].

2.2. Application of 3D bioprinting in drug discovery

Besides other vast applications of 3D bioprinting, researchers are also using this technology in the field of drug discovery, which reflects its potential and importance in various biological fields [40]. 3D bioprinting represents a promising and innovative approach to the design of drug screening systems. In comparison to conventional manual screening techniques, bioprinting enables the uniform distribution of cells onto microdevice surfaces, a crucial aspect for testing and evaluating the interactions between cells and drugs under investigation.

Scientists utilized a pneumatically-driven, extrusion-based bio-printer to construct a drug testing platform for the liver and applied alginate-encapsulated immortalized hepatocytes, which mimicked the *in vivo* microenvironments of various mammalian tissues [12]. The platform demonstrated the capacity to differentiate drug metabolism and make it valuable for screening the efficacy and toxicity of specific agents. Bioprinting has the potential for cell seeding during the fabrication of organ-on-a-chip devices showing the physiological functions of organs and thus helping in the investigation of potential drug effects on tissues [6, 16]. 3D bio-printing technology offers several advantages over traditional methods, including precise cell distribution, high-resolution deposition, scalability, and cost-effectiveness [38]. However, certain challenges need to be addressed for wider adoption in fields like medicine. These challenges include the limited availability of printable biomaterials, the requirement for faster printing speeds, improved scalability, and the desire for higher printing resolution to achieve specific biological functions while maintaining mechanical properties [41].

2.3. The emerging role of 3D bioprinting and its mechanisms in drug discovery

As above-mentioned, 3D bioprinting has wide applications in various fields, including drug discovery. There are a growing number of publications exploring the adoption of bio-printed models in various applications, including disease modeling, drug development, pharmaceutical manufacturing, and the engineering of complex tissue constructs. Nevertheless, the application of bioprinting in drug design and development across a variety of tissues has not yet been extensively addressed in the literature, which is a promising area for further investigation [3]. Additionally, the current drug discovery and development process typically spans 10-15 years and costs billions of dollars to successfully advance a new chemical entity from experimental stages to commercial use [42]. The bio-printing technology can be harnessed to advance capabilities and drive innovation in the field of drug discovery and development. The mechanism by which this technology is applied in drug discovery processes is described.

2.4. The creation of *in vitro* models

In vitro studies are crucial for drug development. They provide the basic and preliminary selection criteria for a molecule for its therapeutic potential. The man-made models also help in the understanding of the pathology, mechanisms, and effects of the desired drug molecule. However, traditional two-dimensional cell cultures often fall short of capturing the intricate three-dimensional architecture and cellular interactions found in native tissues. To overcome this limitation, researchers have turned to 3D bioprinting to recreate these complex structures, allowing for the development of more physiologically relevant models for drug screening and toxicity testing. These 3D models offer a more accurate and predictive assessment of drug efficacy, metabolism, and toxicity, potentially reducing the reliance on animal models and expediting the drug discovery process [17, 31, 47].

2.5. The importance and unique properties of 3D bioprinting in the field of drug discovery

The 3D bioprinting process can produce digitally designed, three-dimensional objects from the deposited materials [19]. Firstly, it effectively reduces the risk of cross-contamination when generating precisely organized co-culture models, which is particularly important while working with limited cell handling spaces. Secondly, it allows precise control over the delivery of genes, growth factors, and drugs, enabling targeted interventions. Additionally, it enables the high-throughput production of constructs with customized pore sizes, accommodating the heterogeneity of specific tissue architectures [16]. Despite some limitations in reproducing highly vascularized tumor constructs, bio-printing remains a prominent method in comparison to other techniques. The importance of 3D bioprinting and its unique characteristics are described (**Figure 4)**.

Notably, it allows the fabrication of constructs under physiologically relevant conditions, including different pH levels, temperatures, and hydration degrees. Moreover, bio-printing facilitates the incorporation of cells, genetic material, and proteins, which can effectively regulate cellular functions [18, 20, 48]. These advantages have propelled the rapid progress of the bioprinted entities, transitioning them from initial prototype stages to commercial applications. However, there are some challenges, like reaching fully reliable high-throughput capabilities and creating an error-free process, which are overcome by utilizing magnetic levitation (maglev) technologies in the bioprinting [4, 47]. The properties of 3D bioprinting in drug discovery play a crucial role in advancing the field and enabling the development of more effective and personalized therapies [16]. These properties contribute to the unique capabilities of 3D bioprinting for fabricating complex biological constructs and studying drug responses.

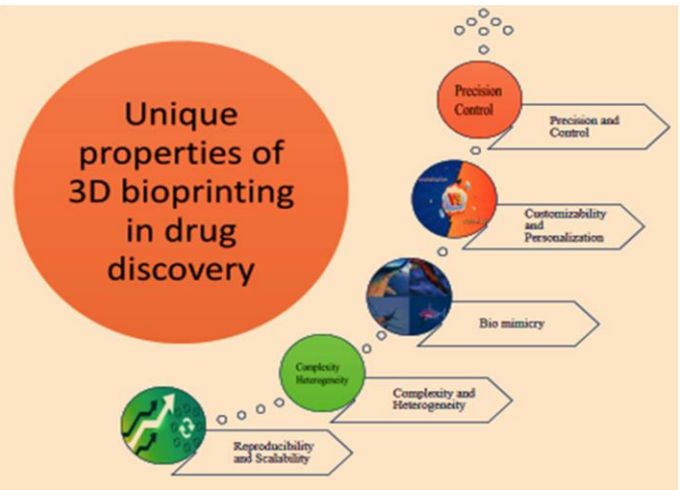

Figure 4. Figure illustrating the unique characteristics of 3D bioprinting in association with drug discovery

3. Future perspectives for 3D bioprinting

The emerging role of 3D bioprinting technology highlights its bright future. Their application is increasing rapidly and covers a wide area. Some of the new, emerging, and futuristic applications of this technology are listed here.

3.1. Tissue engineering and regenerative medicine

The bioprinting of functional organs poses significant challenges and remains a formidable task in this field. However, despite these difficulties, considerable progress has been made in the bioprinting of various tissues. Tissues, such as skin, blood vessels, and certain hollow organs, lend themselves more readily to bioprinting because of their relatively simpler architecture. Nonetheless, the bioprinting of more complex organs with intricate internal structures and vascularization remains a significant scientific and technical challenge [2, 4].

Table 1. The different existing drug discovery methods and its pros and cons.

Drug discovery process	Properties and advantages	Disadvantages	References
High-Throughput Screening (HTS)	HTS has predominantly emphasized the utilization of biochemical and cell-based assay formats. It is a new approach in which a larger number of biomolecules are screened for their therapeutic potential. In HTS, different types of libraries, i.e., combinatorial chemistry, genomics, protein, peptide libraries, etc., are screened. This technique accelerates the drug discovery process by screening large compound libraries at a rate that may exceed a few thousand compounds per day or week.	The disadvantages include failures to identify the target sites properly. A large number of compounds are screened and mostly performed in the industry	[24, 28-31]
Computer-Aided Drug Design (CADD)	CADD is comprised of a collection of diverse *in silico* techniques and has proven instrumental in minimizing research time and expenses, effectively accelerating the development process of new drugs. CADD can be integrated with wet-lab methods and can elucidate the drug mechanism of action. This technique has become a vital tool in current drug discovery, and several drugs have been identified while using this method	The biggest drawback is the target flexibility, as CADD mostly provides flexibility. Hence, providing a completely flexible ligand is a risky process that can even lead to negative results	[29, 33, 34].
Structure-Based Drug Design (SBDD)	Recently, there has been a notable surge in the utilization of machine learning (ML) techniques to propel the field of structural biology forward, particularly in the area of structure prediction. A significant milestone in this domain is the creation of Alpha Fold, a neural network (NN)-based tool developed by Deep Mind. The presence of 3D structures can provide the binding cavities of SBDD. This technique is a more specific and fast process that deals with the 3D structure of target proteins.	The major limitation of virtual screening and SBDD is the ignorance of the protonation and ionization of the compounds when the libraries are prepared.	[35-38]
Fragment-Based Drug Design (FBDD)	Fragment-based drug discovery (FBDD) got the researcher's attention due to the biophysical and biochemical methods used in the identification of small molecules called regiments. In this method, a relatively small library of low-molecular-weight compounds is screened to find the particular target. Different strategies are used to grow these hit compounds into drug-like molecules.	In this approach, small molecules are screened. The crystal structure of the molecules must be available.	[23, 34, 40, 41]
Virtual Screening (VS)	Virtual screening is a computational method in drug discovery in which large databases of molecular structures are screened. VS does not rely on brute-force search and is based on the starting information of the receptor under inspection or its active ligands. VS methods can be divided into two different categories: structure-based and ligand-based. It required less time and cost.	It misses the tautomerism and ionization of the compounds and, hence, hits fewer compounds.	[38, 42-44]

3.2. Transplantation and clinics

The bioprinting of living tissue and organ constructs has been extensively investigated. Different types of bioprinted tissues, such as nerves, cardiac, blood vessels, bone, and skin, have been implanted at their respective anatomical sites in animal models to assess their functionality, the formation of new blood vessels (neovascularization), the establishment of connections with the host tissues (anastomosis), and the integration of the bioprinted constructs with the surrounding host tissues (engraftment) [19, 49].

3.3. Bio-printing in organ-on-a-chip

Organ-on-a-chip systems, also known as organ chips, are microfluidic devices that incorporate living human cells or tissues. These devices have gained significant attention in the fields of drug development, disease modeling, and personalized medicine. They provide a more realistic representation of the three-dimensional (3D) environment compared to traditional 2D cell plating systems and hold promise as an alternative to animal testing. Each organ chip consists of small, hollow microfluidic channels that enable the integration of human organ-specific cells with a human artificial vasculature, allowing for the replication of physiological conditions and interactions within the organ chip [50,,52].

Table 2. Different modalities in 3D bioprinting and their applications

Methods	Characteristics	Applications	References
Inkjet-based bioprinting	This technique utilizes thermal, piezoelectric, or acoustic forces to create droplets of bio-ink that are jetted onto a substrate to form complex patterns. It offers high-resolution printing capabilities and precise control over droplet size, allowing for fine-scale spatial patterning	It offers high-resolution printing capabilities and precise control over droplet size, allowing for fine-scale spatial patterning.	[17, 19]
Laser-assisted bioprinting	This technique uses laser energy to selectively deposit cells or biomaterials onto a substrate by transferring them from a donor material to the target location. It enables high-resolution printing, cell viability preservation, and the deposition of delicate biomaterials.	Laser-assisted bioprinting is employed in drug discovery for creating tissue models, micro scale structures, and studying cell behavior in response to specific environmental cues.	[16, 18, 20]
Stereo lithography	This technique employs a laser or light source to polymerize photosensitive resins layer by layer, forming intricate three-dimensional structures. It enables high-resolution printing with excellent structural fidelity and the use of various biocompatible materials	Stereo lithography is utilized for creating tissue models, scaffolds for tissue engineering, and drug delivery systems.	[17, 19]
Extrusion-based bioprinting	This technique employs a laser or light source to polymerize photosensitive resins layer by layer, forming intricate three-dimensional structures. It allows for the deposition of a wide range of biomaterials and cells, including hydrogels, living cells, and growth factors	Extrusion-based bioprinting is widely used in drug discovery for creating tissue models, drug screening platforms, and personalized medicine applications.	[17, 18, 20]
Light-assisted bioprinting	Light-assisted bioprinting methods have gained popularity in recent years due to their high cell viability in post-printing and their higher printing speed and resolution. This system have many variations, where each type has the potential to modulate different parameters of the printed constructs, including mechanical properties, chemical compositions, cell and material distributions.	light-assisted bioprinting are mainly applied in tissue engineering and discussed in detail below - digital light processing (DLP)-based bioprinting and the two-photon polymerization (TPP)- based bioprinting.	[16]

3.4. Current limitations and challenges of 3D bioprinting

3D bioprinting, a technology rooted in the concept of three-dimensional printing of custom-designed biological materials, has seen widespread utilization in recent times [53]. With advancements in printing technology, there will be further creation of biomimetic, tissue-engineered organs. However, before Bioprinting of organs can be implemented in clinical settings; it is essential to address reductions in both reestablishment time and cost [54]. Significant progress has been made in the realm of 3D bioprinting, yielding numerous advancements in tissue engineering. From a technological perspective, there may be a need for increased printing speed to effectively replicate sizes, relevant for clinical applications [55]. Despite the high precision and reproducibility of 3D printers, the fabrication of organs and functional tissues with complete structures still necessitate layer-by-layer assembly using "bio-glue" [54]. Presently, the industrialization of bioprinting primarily emphasizes the production of 3D bioprinters, the development of bioinks, and the generation of bioengineered tissues and disease models for applications in tissue engineering and drug development [56]. The advent of personalized 3D printing technology is expected to introduce a series of regulatory challenges related to overseeing specific printed products. Consequently, there is an urgent need for the establishment and refinement of pertinent laws and regulations to ensure the sustainable advancement of 3D printing technology. Future studies are anticipated to make significant strides in printing micro-organs, such as pancreatic islet tissues capable of functioning independently of the complete pancreas structure, thereby benefiting millions of diabetic patients worldwide.

Conclusion

In conclusion, 3D bioprinting is an emerging and powerful tool used in various biological applications. Its role in drug discovery is evaluated to cope with the high cost and time of traditional drug developmental process. It enables the creation of complex, patient-specific tissues and organs and facilitates personalized medicine approaches. By accurately modeling human physiology, this technology enhances drug efficacy and toxicity predictions. Moreover, it offers a more efficient and cost-effective alternative to traditional preclinical testing methods, reducing reliance on animal models and accelerating the drug development process. Overall, 3D bioprinting holds great promise in advancing drug discovery and improving patient outcomes.

Author contribution

The initial manuscript was written by RT, while MR review and add material. AH formulate the full manuscript, formatted and reviewed.

Funding

This review has no funding

Conflict of interest

The authors have no conflict of interest.

Ethics

Ethical consideration is not applicable.

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