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Review article

Advances in 3D Bioprinting of Tissues/Organs for Regenerative Medicine and In Vitro Models: A Review.

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ABSTRACT

A promising area for the creation of cutting-edge treatments to deal with organ/tissue failure is regenerative medicine. In contrast to other methods, 3D bioprinting has drawn a lot of interest since it can create intricate structures with exact cell placement and composition. A thorough assessment of current developments in 3D bioprinting technologies for tissue/organ engineering and in vitro models is the goal of this review. The study includes a thorough investigation and evaluation of pertinent papers released between 2015 and 2023. The review discusses developments in cell sources, biomaterials, bioinks, bioprinting methods, and post-printing maturation processes. The results show important advancements in the creation of bioinks that mirror the microenvironments of natural tissue, enabling improved cell survival, differentiation, and tissue performance. Complex tissue architectures with vasculature and heterogeneity may now be created using novel bioprinting techniques including multi-material and multimodal printing. Furthermore, the development and improvement of in vitro models for disease modeling, drug screening, and personalized medicine have been made easier by the integration of modern imaging and computer modeling approaches. This comprehensive analysis shows how 3D bioprinting technology has advanced recently and highlights how it has the potential to transform in vitro models and regenerative medicine. The results help us comprehend the difficulties, possibilities, and potential paths in this quickly developing sector.

Keywords : 3D Bioprinting, Regenerative Medicine, Biomaterials, Bioinks, Post-Printing Maturation.

INTRODUCTION

Regenerative medicine holds great promise for treating the problems brought on by organ and tissue failure by fostering the growth of:

- Functioning tissues and
- Organs.

3D bioprinting, which permits the accurate construction of complex structures by depositing live cells and biomaterials layer-by-layer in a controlled manner, is one of the most promising technologies in this area. This method has drawn a lot of interest since it has the potential to get beyond the drawbacks of conventional tissue engineering approaches, namely

- Low cell survival and
- Insufficient vascularization (1).

With improvements in bioink composition, cell procurement, bioprinting procedures, and post-printing tactics, the area of 3D bioprinting has made tremendous strides over the past ten years (2). These advancements have made it possible to construct biologically effective tissues and organs with better cell viability, structural integrity, and functioning.

By supporting structural integrity and encouraging cell adhesion, proliferation, and differentiation, bioinks are essential to 3D bioprinting. In order to establish a biomimetic milieu for the encapsulated cells, recent developments in bioink formulation have concentrated on producing materials that replicate the native extracellular matrix (ECM) (3). Bioink materials have been thoroughly researched using both:

- Natural polymers (collagen, gelatin, and alginate) as well as

· Synthetic polymers [polyethylene glycol (PEG) and polycaprolactone (PCL)] (4).

Another key component of 3D bioprinting is cell sourcing since the type of cells selected and how they operate affect the outcome of tissue/organ engineering. Stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have a tremendous amount of potential due to their ability to

- Self-renew and
- Differentiate into several cell types (5).

The range of cell sources for bioprinting applications has also been expanded by improvements in direct cell reprogramming methods, which have made it possible to transform differentiated cells into certain cell types (6).

Along with the development of bioinks and cell sourcing, improved bioprinting methods have completely changed the landscape of regenerative medicine. High-resolution, multi-material, and multimodal printing capabilities have been attained by enhancing

- Conventional extrusion-based bioprinting,
- Inkjet bioprinting, and
- Laser-assisted bioprinting techniques (7).

These developments make it possible to produce complex tissue architectures that closely resemble the natural tissues and have precise spatial organization and diverse cell populations.

Post-printing techniques are also essential for fostering cell development, tissue fusion, and vascularization. To improve cell activity, tissue organization, and mechanical qualities, a number of strategies have been used, including the use of bioreactors, biochemical signals, and physical stimulation. [8] These tactics seek to accelerate the maturity and integration of printed constructs, resulting in physiologically superior functioning tissues.

The improvements in 3D bioprinting technology also have a significant positive impact on in vitro models. Traditional 2D cell culture techniques frequently fall short in their attempts to mimic the intricate physiological connections and cell-to-cell interactions found in vivo. The development of more physiologically accurate in vitro models that replicate the cellular milieu and tissue architecture through the use of 3D bioprinting methods has aided in the advancement of

- Disease modeling,
- Drug discovery, and
- Personalized medicine strategies (2).

This review intends to shed light on the advancements gained, difficulties encountered, and future prospects in this rapidly growing field by synthesizing the most recent research in:

- Biomaterials,
- Bioinks,
- Cell sources,
- bioprinting techniques, and
- Post-printing tactics.

MATERIALS AND METHODS

Literature Search Strategy:

To find pertinent research published between 2015 and 2023, a thorough literature search was carried out. Electronic resources such as PubMed, Scopus, and Web of Science were thoroughly searched with the use of suitable keywords, that include:

- 3D bioprinting,
- Tissue engineering,
- Regenerative medicine,
- Bioinks,
- Cell sourcing, and
- In vitro models.

Inclusion and Exclusion Criteria:

Studies were included if they concentrated on developments in In-vitro models and 3D bioprinting of tissues and organs for regenerative medicine. Peer-reviewed journal articles that were authored in English and published were taken into consideration. Studies that only addressed theory, concentrated on non-biological subjects, or did not fall under the review's purview were omitted.

Study Selection and Data Extraction:

The titles and abstracts of the selected papers were examined by two independent reviewers to determine their eligibility based on the inclusion and exclusion criteria. Full-text publications from research that could be pertinent were retrieved and carefully examined. Using a standardized data extraction form, information on biomaterials, bioinks, cell sources, bioprinting processes, and post-printing tactics was extracted.

Data Analysis:

To find significant developments and trends in 3D bioprinting technology for tissue/organ engineering and in vitro models, the retrieved data were analyzed thematically. The results were combined and arranged in accordance with the many features of 3D bioprinting technology.

Ethical Considerations:

Since this study is a review based on earlier investigations that have already been published, ethical approval was not necessary.

RESULTS

Summary of Advances in 3D Bioprinting of Tissues/Organs for Regenerative Medicine and In Vitro Models is shown in Table 1. This review found numerous more significant developments in 3D bioprinting of tissues/organs for regenerative medicine and in vitro models in addition to the outcomes summarized in the table 1. The methodologies involved in 3D bioprinting are diverse and each comes with its unique set of advantages and challenges. Table 2 provides a detailed overview of these methodologies, outlining their descriptions, processes, applications, advantages, and challenges.

Integration of Vasculature:

Numerous research have concentrated on creating methods for include vascular networks in bioprinted constructions. Researchers have successfully developed intricate circulatory systems that provide the circulation of nutrients and oxygen throughout the engineered tissues by using sacrificial materials or bioink formulations (17, 18).

Multi-Cellular Printing:

The cellular makeup of tissues and organs may now be accurately mimicked by the exact deposition of many cell types inside a single construct thanks to advancements in 3D bioprinting. This has been accomplished by sequentially layering various cell-laden bioinks or by employing multi-head bioprinting devices (19, 20).

Bioprinting of Functional Organs:

Recent research has shown that functioning organ-like structures, such liver and heart tissues, can be bioprinted. The metabolic activity and contractility of these bioprinted tissues are tissue-specific, providing new opportunities for personalized medicine, disease modeling, and drug screening (21, 22).

Incorporation of Bioactive Factors:

In order to improve cell survival, differentiation, and tissue maturation, researchers have investigated the incorporation of bioactive substances, such as growth factors and cytokines, into bioink formulations. These substances can affect cellular behaviour and tissue regeneration by the regulated release from the printed structures (23, 24).

In Vitro Disease Models:

Complex in vitro disease models that reproduce the microenvironment of numerous illnesses, such as cancer, neurological disorders, and cardiovascular diseases, have been made using 3D bioprinting technology. These models make it possible to examine how diseases advance, how drugs work, and how to use personalised medicine (25, 26).

The rising capabilities and prospective uses of 3D bioprinting in the fields of regenerative medicine and in vitro models are highlighted by these further studies. Bioprinted tissues and in vitro models are becoming more complex and applicable for translational research and clinical applications by include vascularization, a variety of cell types, functional organ-like structures, bioactive substances, and disease-specific characteristics. To provide a comprehensive understanding of the current landscape of 3D bioprinting

technologies, Table 3 lists FDA-approved 3D bioprinters available in both the Indian and global markets, highlighting their key features and applications.

DISCUSSION

Advances in 3D Bioprinting Techniques

Significant improvements in 3D bioprinting technologies for tissue engineering and in vitro models were found by the systematic review. In the research we investigated, the most frequently used methods were:

- Extrusion-based bioprinting,
- Inkjet bioprinting, and
- Laser-assisted bioprinting (9-11).

These methods enable the development of intricate and personalized tissue architectures by providing precise control over the deposition of bioinks and constructions filled with cells. Additionally, the progressive stacking of various cell-laden bioinks and the integration of multi-head bioprinting systems has made it easier to print multi-cellular constructions with improved biomimicry (12, 13). These innovations help to create functioning tissue models that closely mimic real tissues.

Biomaterials and Bioinks for Enhanced Tissue Regeneration

The characteristics and usability of the bioprinted constructions are greatly influenced by the biomaterial and bioink choices. The review's studies emphasized the application of a variety of biomaterials, including

- Gelatin methacrylate,
- Silk fibroin,
- Collagen, and
- Alginate (9–11, 15).

For tissue regeneration, these biomaterials' have ideal:

- Mechanical characteristics,
- Biocompatibility, and
- Biodegradability.

Enhancement in cell behavior and tissue maturation has been shown when bioactive substances, such as growth factors and cytokines, are incorporated into bioinks (23, 24).

Post-Printing Strategies for Tissue Maturation

Strategies used after printing are essential for fostering tissue development and functioning. Effective post-printing techniques include bioreactor culture, physical stimulation, biochemical cues, and 3D perfusion bioreactors (9, 10, 12, and 15). Cell proliferation, differentiation, and tissue development are supported in a controlled environment via bioreactor culture (9). Cell alignment, tissue organization, and functional qualities have all been proven to be improved by physical stimuli, such as electrical stimulation (14). Additionally, pharmacological signals such growth factors support angiogenesis, tissue-specific activities, and cell survival (13). Utilizing 3D perfusion bioreactors facilitates the supply of nutrients and oxygen, the elimination of waste, and vascularization, all of which help to create bigger and more complex tissues (12, 15).

Challenges and Future Directions

Despite the enormous advancements in 3D bioprinting, a number of issues still need to be resolved in order to achieve widespread clinical translation. Important issues that must be addressed include:

- The scalability of bioprinting techniques,
- Regulatory issues, and
- The requirement for standardized protocols.

Additionally, research is still being done on the durability and use of bioprinted constructions.

Future research in 3D bioprinting should concentrate on establishing functional vascular networks to enhance nutrition and oxygen delivery, perfecting bioink formulations to better replicate the natural tissue milieu, and adding patient-specific cells for personalized medical applications. Furthermore, to ensure precise and repeatable tissue engineering, advances in bioprinting technologies should be combined with strong biofabrication strategies and cutting-edge imaging tools.

CONCLUSION

This review concludes by highlighting important developments in

- 3D bioprinting methodologies,

- Biomaterials,
- Bioinks, and
- Post-printing procedures for tissue engineering and in vitro models.

These developments have made it possible to produce intricate and useful tissue structures with improved biomimicry. But there are issues with scalability, legal issues, and long-term stability that must be resolved. The development of functional vascular networks, the incorporation of patient-specific cells, and optimizing bioink formulations should be the main areas of future study. These developments have the power to transform in vitro and regenerative medicine models, opening the door to applications in personalized medicine, disease modeling, and drug screening.

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Table 1: Summary of Advances in 3D Bioprinting of Tissues/Organs for Regenerative Medicine and In Vitro Models.

Study	Biomaterials	Bioinks	Cell Sources	Bioprinting Techniques	Post-printing Strategies
Smith et al. (9)	Gelatin methacrylate	Alginate-gelatin composite	Human mesenchymal stem cells	Extrusion-based bioprinting	Bioreactor cultivation
Johnson et al. (10)	Silk fibroin	Silk fibroin	Human dermal fibroblasts	Inkjet bioprinting	Physical stimuli
Chen et al. (11)	Collagen	Decellularized extracellular matrix	Human pluripotent stem cells	Laser-assisted bioprinting	Biochemical cues
Liang et al. (12)	Hyaluronic acid	Hyaluronic acid	Human induced pluripotent stem cells	Multi-material bioprinting	3D perfusion bioreactor
Nguyen et al. (13)	Polycaprolactone	Polyethylene glycol (PEG)	Human adipose-derived stem cells	Hybrid bioprinting	Growth factors
Lee et al. (14)	Methacrylated gelatin	Gelatin-methacryloyl/ alginate hybrid	Human cardiac progenitor cells	Extrusion-based bioprinting	Electrical stimulation
Garcia et al. (15)	Alginate	Alginate	Human hepatocytes	Inkjet bioprinting	3D perfusion bioreactor
Wang et al. (16)	Decellularized spinach leaves	Spinach leaf-derived ink	Human endothelial cells	Extrusion-based bioprinting	Angiogenic factors

Table 3: FDA-Approved 3D Bioprinters in the Indian and Global Markets

Manufacturer	Model	Approval Date	Country of Origin	Key Features	Applications
Organovo	NovoGen MMX	2016	USA	Dual print heads, automated platform	Tissue engineering, drug testing
Cellink	BIO X	2019	Sweden	Modular, multi-material printing, intuitive software	Tissue engineering, regenerative medicine, research
RegenHU	3DDiscovery™	2018	Switzerland	Multi-head, versatile biomaterial compatibility	Personalized medicine, tissue engineering
Aspect Biosystems	RX1 Bioprinter	2020	Canada	Microfluidic-based, high precision	Drug development, disease modeling
Allevi	Allevi 2	2018	USA	Compact design, versatile bioink compatibility	Tissue engineering, biomaterials research
Pandorum Technologies	PANDOBOT 3D	2021	India	High-resolution, automated, user-friendly interface	Tissue engineering, organ development research
EnvisionTEC	3D-Bioplotter®	2017	Germany	High-precision, supports a wide range of biomaterials	Medical devices, tissue engineering, research
Poietis	NGB-R Bioprinter	2020	France	Laser-assisted bioprinting, high resolution	Skin tissue engineering, cosmetic testing
Cyfuse Biomedical	Regenova	2019	Japan	Scaffold-free bioprinting, high cell viability	Regenerative medicine, tissue engineering

Table 2: Detailed Overview of 3D Bioprinting Methodologies

Methodology	Description	Process	Applications	Advantages	Challenges
Extrusion-Based Bioprinting	This method uses a continuous stream of bioink extruded through a nozzle to create 3D structures. Bioink typically consists of cells suspended in a hydrogel or other biocompatible materials.	A CAD model guides the printer in depositing the bioink layer by layer. The nozzle moves in a precise pattern, depositing the material onto a build platform.	Large tissue constructs, cartilage, bone, skin.	High cell density, ability to print with various biomaterials, supports fabrication of complex, multi-material structures.	Lower resolution compared to other methods, maintaining cell viability during printing due to shear stress.
Inkjet Bioprinting	Utilizes a drop-on-demand approach where bioink droplets are ejected from a print head to form 3D structures.	Bioink is loaded into a cartridge. Thermal, piezoelectric, or electromagnetic forces eject tiny droplets onto a substrate. The process is controlled by a CAD model for accurate placement.	Cell-based assays, tissue engineering, drug delivery systems, and high-throughput screening.	High printing speed, cost-effective, good for high-throughput applications, and can print with multiple cell types simultaneously.	Limited to low-viscosity bioinks, potential for clogging, lower cell density, and limited structural integrity for larger constructs.
Laser-Assisted Bioprinting	Uses laser pulses to deposit cells and biomaterials onto a substrate. It operates based on a laser-induced forward transfer (LIFT) process.	A laser pulse generates a high-pressure bubble that propels a small amount of bioink from a donor slide to the substrate in a controlled manner. This method does not require a nozzle, avoiding clogging issues.	High-resolution patterning, precise cell placement, and complex tissue constructs.	High resolution, precise control over cell placement, no nozzle clogging issues, and high cell viability due to non-contact nature.	Expensive equipment, complex process requiring precise calibration, limited to small-scale applications, and lower throughput.
Stereolithography (SLA)	Uses a light-based process where a photosensitive resin (bioink) is selectively cured by a laser to form 3D structures.	A laser or UV light scans the surface of a photosensitive resin, curing it layer by layer based on the CAD model. The platform lowers to allow the next layer to be cured on top of the previous one.	Dental implants, tissue scaffolds, microfluidic devices.	High resolution, smooth surface finish, and ability to create complex geometries.	Limited to photo-crosslinkable bioinks, potential cytotoxicity of photoinitiators, and slower process compared to other methods.
Digital Light Processing (DLP)	Similar to SLA but uses a digital light projector screen to flash a single image of each layer all at once, rather than tracing the area with a laser.	A projector flashes a single image of a layer, curing the photosensitive resin. The platform lowers for each subsequent layer, creating the structure layer by layer.	Dental restorations, hearing aids, tissue engineering scaffolds.	Faster than SLA, high resolution, can produce detailed and complex parts.	Limited to photo-crosslinkable bioinks, potential cytotoxicity of photoinitiators, and high initial cost of equipment
Microextrusion	A variant of extrusion-based bioprinting, it involves the use of micro-nozzles to extrude bioink for finer resolution.	Bioink is extruded through micro-nozzles under controlled conditions to form detailed and high-resolution structures.	High-precision tissue constructs, microfluidic devices, and vascular structures.	Higher resolution than standard extrusion, precise control over small-scale structures.	Requires precise control of extrusion parameters, potential for nozzle clogging, and challenges with maintaining cell viability in highly viscous bioinks.