Journal of Medical Devices Technology
jmeditec.utm.my

Review Article

Current Applications and Future Potential of 3D Bioprinting in Tissue Engineering

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ARTICLE INFO

Article History:
Received 27 November 2024
Accepted 29 December 2024
Available online 30 December 2024

Keywords:
3D bioprinting,
bioinks,
cartilage tissue engineering,
cancer modelling,
regenerative medicine

ABSTRACT

This review explores the current applications and future potential of 3D bioprinting in tissue engineering. Key bioprinting techniques including inkjet, extrusion, and laser-assisted methods are discussed, highlighting their advantages and limitations. The review examines various bioink materials, categorized into natural polymers, synthetic polymers, and decellularized extracellular matrix, evaluating their properties and suitability for different tissue types. Current applications in vascular, cartilage, and cancer tissue engineering are analyzed, showcasing the versatility of 3D bioprinting. Despite significant progress, challenges remain, including improving printing resolution, vascularization of larger constructs, and maintaining cell viability during the printing process. Future perspectives focus on enhancing the mechanical properties of bioprinted tissues, developing novel bioprinting methods, and incorporating vascularization-promoting factors. This review provides a comprehensive overview of the state-of-the-art in 3D bioprinting for tissue engineering, evolution to regenerative medicine and drug discovery.

INTRODUCTION

Tissue engineering aims to develop biological substitutes that restore, maintain, or improve tissue function (Langer et al., 2000). Traditional approaches to tissue engineering have been limited in their ability to recreate the complex architecture and heterogeneity of native tissues. The advent of 3D bioprinting has revolutionized the field by enabling the precise deposition of cells, biomaterials, and growth factors in a spatially controlled manner (Murphy et al., 2014).

3D bioprinting combines additive manufacturing technologies with biological materials to fabricate tissue-like structures (Groll et al., 2018). This approach offers several advantages over conventional tissue engineering methods, including enhanced control over the spatial distribution of cells and materials, improved scalability, and the ability to create patient-specific constructs (Kang et al., 2016). 3D bioprinting has witnessed rapid growth and innovation in recent years. Advances in printing technologies, biomaterials, and cell biology have expanded the range of tissues that can be bioprinted and improved the functionality of engineered constructs (Moroni et al., 2018). Current applications span a wide range of tissue types, from simple structures like skin to more complex organs like the heart and liver (Miri et al., 2019). Despite significant progress, numerous challenges remain in translating 3D bioprinting technologies from the laboratory to clinical applications. These include improving the resolution and speed of bioprinting processes, developing more sophisticated bioinks, and addressing issues of vascularization and innervation in larger tissue constructs (Chimene et al., 2016).

This review aims to provide a comprehensive overview of the current state of 3D bioprinting in tissue engineering and explore its future potential. We will discuss various bioprinting

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techniques, bioink formulations and cell sources advancements, and key applications across different tissue types. Finally, we will address the challenges faced in the field and explore emerging trends that may shape the future of 3D bioprinting in tissue engineering and regenerative medicine.

3D BIOPRINTING FOR TISSUE ENGINEERING

The 3D printing involves several key steps, including creating 3D models using computer-aided design (CAD), computer-aided manufacturing (CAM) tools, and mathematical modeling techniques. These models are based on imaging data from CT scans, X-rays, and MRIs. Next, 2D cross-sectional images are produced from these 3D models using tomographic reconstruction. The 3D structures are then built through a computer-controlled, layer-by-layer deposition process. Constructed objects may undergo post-processing modifications, such as surface treatments for nano-architectures, to meet specific requirements. Variations in 3D printing techniques can influence the design of 3D models, especially during the reconstruction of 2D slices into 3D scaffolds (Bishop et al., 2017).

3D printing techniques are categorized into non-biological 3D printing and 3D bioprinting. Non-biological 3D printing methods include fused deposition modeling (FDM), stereolithography (SLA), selective laser sintering (SLS), selective laser or electron beam melting (SLM or EBM), and laminated object manufacturing (LOM). For 3D bioprinting, there are three primary methods: inkjet, laser-assisted, and extrusion bioprinting. Some bioprinting techniques can also be used for non-biological purposes, although the specific characteristics of non-biological methods limit their applications in biological 3D printing.

Table 1 Advantages and disadvantages of 3D-bioprinting techniques.

Type of 3D Bioprinting	Advantages	Disadvantages
Laser assisted	Non-contact, high cell viability	Complex operation, time consuming
Inkjet	Low cost, fast printing, widely accessible	Nozzle clogging
Extrusion	Deposition of high density cells	Low cell viability

Inkjet-based 3D bioprinting

This non-contact printing technique uses a digitally controlled pattern and includes two primary methods: continuous inkjet (CIJ) and drop-on-demand (DOD) printing. In CIJ, a continuous jet of droplets is created by applying pressure to the bioink, then deflected by an electric field onto the substrate, with excess droplets collected for reuse. DOD printing, ideal for bioprinting, creates droplets only as needed using a pressure pulse, reducing contamination risk. DOD is further categorized into thermal and piezoelectric methods. Thermal DOD uses a pulsed electric current to vaporize ink droplets in a microfluidic chamber, pushing them onto the substrate. Piezoelectric DOD uses a piezoelectric transducer to generate the pressure needed for droplet formation. The printability of bioink depends on its rheological properties, such as viscosity, typically around 30 mPa/s. This technique enables complex multicellular patterns and is cost-effective and low risk for contamination, making it practical for printing mammalian cells, DNA, and proteins (Agarwal et al., 2020).

Extrusion-based 3D bioprinting

The continuous extrusion of material from a nozzle creates 3D structures layer-by-layer, achievable through direct ink writing (DIW) or pressure-assisted methods. For smooth extrusion and shape retention, materials must have specific rheological properties, such as shear thinning and shear yield stress. To achieve these properties, fillers like silica particles or nano-clay are often added to polymer resins. The material solidifies through UV curing, and thermal curing, or is extruded into a support bath, known as freeform reversible embedding (FRE) or embedded 3D printing (e-3D printing). The printing resolution, ranging from hundreds to sub-microns, depends on the nozzle dimensions. This technique is ideal for creating self-standing structures and is widely used for printing viscous polymer resins (Yu et al., 2020).

Laser-assisted 3D bioprinting (LAB)

The pulsed laser beam deposits bioink, including cells, onto a substrate in a non-contact direct writing process involving three main components: a pulsed laser source, a bioink-coated ribbon, and a receiving substrate. UV or near-UV lasers create a high-speed jet of cell-laden bioink by vaporizing the bioink on the ribbon. A layer- often made of metal or hydrogel- is placed between the bioink and the ribbon to protect cells from the laser. This layer's rapid thermal expansion propels the bioink onto the substrate with minimal cell damage. Variants like absorbing film-assisted laser-induced forward transfer (AFA-LIFT) and biological laser processing (BioLP) use different materials and laser powers to optimize cell viability. The computer-controlled process allows for precise cell patterning and the creation of complex tissue structures. Factors affecting cell viability include ECM thickness, laser pulse energy, and bioink viscosity, with higher energy increasing cell damage. This method enables the fabrication of soft tissues with high cell density and precise spatial resolution (Xie et al., 2020).

A current research trend is combining additive manufacturing (AM) with conventional manufacturing (CM) to leverage both technologies' advantages. Hybrid AM is effective in metal manufacturing for creating products with complex structures and precise surface finishes. In tissue engineering, hybrid AM presenting a promising direction for 3D bioprinting in medicine, allowing structures with good mechanical and biological properties. However, these strategies involve more complex fabrication processes and require advanced software and hardware, making them challenging for researchers to implement.

BIOINKS FOR 3D BIOPRINTING

Bioinks are specialized materials used in 3D bioprinting that mimic the natural extracellular matrix (ECM) of tissues. They are designed to support cell viability, proliferation, and differentiation while maintaining the structural integrity required for printing complex tissue constructs.

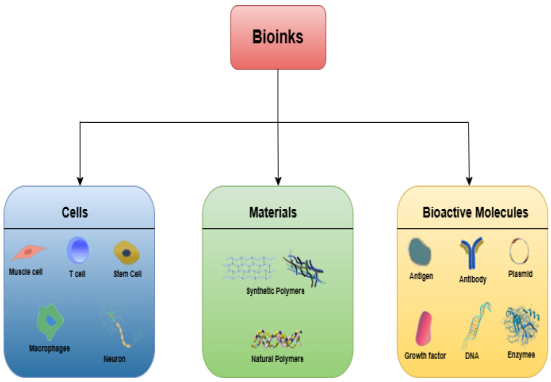

Fig. 1 Types of bioink in 3D printing.

With the advancements in 3D bioprinting technology, bioinks—also called printable hydrogels—play a crucial role in creating functional tissue constructs. The biomaterials used to produce bioinks must be biocompatible, suitable for bioprinting, and capable of degrading inside the human body without generating toxic byproducts (Yu et al., 2020). Bioinks can be divided into natural polymers, synthetic polymers and other recent bioink such as decellularized ECM.

Natural polymers

Natural polymers are a popular choice for bioinks in 3D bioprinting due to their biocompatibility and ability to support cell growth and proliferation. Some of the key natural polymers used in bioinks include alginate, chitosan and gelatin (Yu et al., 2020). Alginate, for instance, is popular for its rapid ionic crosslinking capabilities, while gelatin mimics the fibrous structure of natural tissues. These natural polymers can be used alone or combined in multicomponent bioinks to create biomimetic constructs for tissue engineering and regenerative medicine applications (Benwood et al., 2021). However, depending on their source, natural polymer bioinks can sometimes suffer from batch-to-batch variability, limited mechanical strength, and potential immunogenicity.

Synthetic polymers

Synthetic polymers are also widely used as bioink materials in 3D bioprinting, in addition to natural polymers. Synthetic polymer-based bioinks, such as those made from polyethylene glycol (PEG), polycaprolactone (PCL), polyvinyl alcohol (PCL) and many more, offer greater control over mechanical properties and printability, making them suitable for creating more complex 3D structures (Gungor-Ozkerim et al., 2018). PEG, for example, can be easily modified with various functional groups to promote cell adhesion or to incorporate growth factors (Yu et al., 2020). However, they often lack the biological cues present in natural materials and may require additional modifications to support cell attachment and function.

Other recent bioinks

Decellularized extracellular matrix (dECM) bioinks represent a more recent and promising approach in bioink development. These bioinks are created by removing cellular components from native tissues while preserving the complex mixture of structural and functional molecules of the extracellular matrix (Benwood et al., 2021). dECM bioinks can be derived from various tissue types, such as cardiac, adipose, or cartilage tissues, and maintain much of the biochemical composition and architecture of the original tissue (Gungor-Ozkerim et al., 2018). This bioink offers a unique advantage in mimicking the natural microenvironment of specific tissues, potentially leading to more physiologically relevant engineered constructs (Yu et al., 2020). However, challenges remain in standardizing dECM production processes, ensuring consistent properties, and optimizing their printability. Despite these challenges, dECM bioinks show great promise in creating more biomimetic tissue constructs and are an active area of research in bioprinting. Table 2 below effectively summarises the sources and potential applications of different bioink materials, highlighting the diversity of options available for tissue engineering and 3D bioprinting research.

Table 2 Source and applications of natural, synthetic polymers based bioink and other recent bioink

Type of Bioinks	*Source*	*Applications*	*References*
	Natural Polymers		
Alginate	Brown seaweed	Skin, cartilage bone	*(Mallakpour et al., 2021)*
Chitosan	crustaceans	Bone, cartilage, skin, liver	*(Jayashankar et al., 2022)*
Gelatin	Denatured collagen	Skin, cartilage	*(Das et al., 2024)*
	Synthetic polymers		
Polyethylene Glycol (PEG)	Synthetic	Drug delivery	*(Arif et al., 2022)*
Polycaprolactone (PCL)		Bone, cartilage, liver	*(Cardoso & Araújo, 2024)*
Polyvinyl Alcohol (PVA)		Cartilage, bone	*(Aitchison et al., 2024)*
	Other recent bioink		
Decellularized ECM (dECM)	Tissue sources	Various tissues	*(Golebiowska et al., 2024)*

Each type of bioink has its key strengths and limitations. Table 3 below provides a concise overview of the advantages and disadvantages of various bioink types used in 3D bioprinting, categorised into natural polymers, synthetic polymers, and other recent bioinks. This overview helps to quickly assess the strengths and limitations of different bioink materials for specific tissue engineering applications, facilitating informed choices in material selection for 3D bioprinting.

Table 3 Advantages and disadvantages of bioinks

Type of Bioinks	*Advantages*	*Disadvantages*	*References*
	Natural Polymers		
Alginate	Biocompatible, low-toxicity, low price	Does not provide binding sites for cell attachments	*(Datta, 2023)*
Chitosan	Biocompatible, biodegradable, non-allergen, antimicrobial activity	Weak mechanical integrity	*(Willson et al., 2021)*
Gelatin	High cell affinity	Rapid degradation, poor mechanical strength	*(Asim et al., 2023)*
	Synthetic polymers		
Polyethylene Glycol (PEG)	Biocompatible, non-immunogenicity	Low cell adhesion	*(Fang et al., 2023)*
Polycaprolactone (PCL)	Low melting point, High stability	Not suitable for cell encapsulation	*(Chen et al., 2023)*
Polyvinyl Alcohol (PVA)	Biocompatible, Biodegradable,	Low cell affinity	*(Jose et al., 2024)*
	Other recent bioink		
Decellularized ECM (dECM)	Promotes cell growth and differentiation, Biocompatible	Low viscosity, costly, complicated	*(Dzobo et al., 2019)*

CURRENT APPLICATIONS OF TISSUE ENGINEERING BASED ON 3D BIOPRINTING

Mimicking of Native Tissues

Three-dimensional (3D) printing is a promising technique for fabricating scaffolds in tissue engineering, allowing for complex geometries and integrating various cell types. Recent advancements have introduced novel biomaterials such as biopolymers, ceramic powders, living cells, and composite materials, enhancing 3D bioprinting methods for tissue engineering (Barua et al., 2022). Ceramics improve printability, while polymer composites offer versatile material properties, aiding the development of tissue engineering solutions (Jammalamadaka & Tappa, 2018).

Traditional methods for replacing defective blood vessels often rely on autologous or allogeneic vascular membranes but face limitations due to donor scarcity, compromising treatment effectiveness. The advancement of 3D bioprinting enables the creation of vascular tissues using various biomaterials and specialised cells, providing flexible and donor-independent solutions (Zhang et al., 2021). For vascular tissue engineering, creating hierarchical, perfusable channels is critical to replicate natural vascular systems and ensure proper nutrient delivery and waste removal (Novosel et al., 2021).

Innovative methods like scaffold-free tubular structures with lumens as small as 1.5 mm address challenges in fabricating functional blood vessels and avoid issues with scaffold material interference (Itoh et al., 2015). Techniques like freeform reversible embedding of suspended hydrogels (FRESH) allow for the precise placement of bioinks and cells, supporting complex vascular structures (Lee et al., 2019).

In cartilage tissue engineering, 3D bioprinting enables the creation of structures that closely mimic natural cartilage properties. Ideal bioprinted cartilage must be biocompatible, controllably biodegradable, and mechanically strong, with natural bioinks often using a combination of alginate, chitosan, hyaluronic acid, collagen, and gelatin (Ramasamy et al., 2021; McGivern et al., 2021). Synthetic bioinks, using materials like PCL, PGA, and PLA, provide additional mechanical strength and structural integrity (McGivern et al., 2021).

Evaluation criteria for 3D bioprinted constructs include fabrication, architectural, mechanical, and surface properties. Constructs are designed using CAD, printed with 3D bioprinters, and undergo post-processing for enhanced properties. The architecture affects cell adhesion and response, while mechanical properties ensure strength post-implantation. Surface properties, including energy, topology, and chemistry, are crucial for hydrophilicity and may require bioactive adhesives to improve cell interaction (Ramasamy et al., 2021; McGivern et al., 2021).

Cancer diagnosis and treatments

3D bioprinting allows precise control over the placement of cells, biomolecules, and extracellular matrix components. It also enables the fabrication of complex tissue models that closely mimic the natural environment of tissues. 3D bioprinting can create complex tissue and organ models for studying the behaviour of biomolecules, such as growth factors and cytokines and other signalling molecules on tissue development and functions. Besides, 3D bioprinting can print tissues that replicate disease conditions, like cancer, to understand the disease's

molecular mechanisms and identify potential therapeutic targets (Murphy et al., 2014).

Cancer is the cause of death worldwide, accounting for about 12% of all deaths, with 90% of these due to metastatic spread. This spread starts when tumour cells degrade their basement membrane, invade surrounding tissue, and enter the lymphatic and blood vessels to grow in distant organs. This process is known as Epithelial-Mesenchymal Transition (EMT). It involves changes in cell architecture and functions, caused by the tumour microenvironment. A significant challenge in cancer research is developing in vitro models that accurately recreate tumour progression, especially migration and invasion. 3D bioprinting has better tumour capture architecture by providing conditions that control tumour growth. Biomaterials like collagen and matrigel have been used extensively (Datta et al., 2020).

Matrix stiffness plays a significant role in the metastatic behaviour of cancer cells and can be incorporated into 3D bioprinted tumour models. For example, 3D constructs of polyethylene glycol (PEG) with varying stiffness were created to study cell migration, showing that HeLa cell migration speed decreases with increasing vessel diameter. Additionally, a hydrogel matrix gradient was developed in 3D bioprinted cancer models to facilitate directional cell migration by mimicking the cancer cell environment (Murphy et al., 2014). Beyond stiffness, the spatial distribution of biochemical factors can also mimic the native tumour microenvironment. The Freeform reversible embedding of suspended hydrogels (FRESH) technique, a recent method, was used to create a neuroblastoma model with sodium alginate and 3D-printed capsules. With a biomolecule core and polymer shell, these capsules released their contents upon specific irradiation, allowing precise control over their location in the hydrogel (Datta et al., 2020).

To achieve spatial control of matrix properties, scaffold-based bioprinting must optimise material properties like hydrogel density, biocompatibility, cell-material interactions, and minimise toxicity from scaffold degradation. Multimaterial bioprinting and gradient-based material deposition approaches are necessary to study cancer metastasis across different tissue interfaces, such as breast cancer spreading to bones, lungs, or the brain (Yu et al., 2020). The bioprinted 3D scaffolds are employed in various applications, including drug delivery systems, cell-free therapeutic products for cartilage repair, regenerative medicine studies and creating biomolecules. These applications demonstrate the versatility and potential of 3D bioprinting technology in addressing the complexities of tissue engineering and paving the way for innovative therapeutic solutions.

LIMITATIONS OF 3D BIOPRINTING IN TISSUE ENGINEERING

The limitations of 3D bioprinting in tissue engineering can be categorised into different categories which is the biomanufacturing process and integration process involving post-implantation functionality. Like the typical 3D printing process, the nozzle clogging issue also happens in 3D bioprinting, mostly in nozzle-based fabrication methods. It is crucial to prevent the occurrence of nozzle clogging in the bioprinting process as it could result in severe accidents or profit loss. To avoid clogging nozzle, bioink used must be homogeneous and of proper viscosity, also it should exhibit shear thinning properties (Derakhshanfar et al., 2018). While extrusion-based bioprinting techniques have low survivability due to shear stress, methods that use photopolymerization to harden bioink such as SLA encounters issues due to the damage inflicted by UV radiation and the cytotoxicity of the photoinitiators used (Kačarević et al., 2018).

Furthermore, researchers have faced difficulties in selecting materials that are both biocompatible and mechanically strong for human applications (Zhang et al., 2023). In order to prevent undesired cellular interactions, researchers are moving towards novel biopolymers and hydrogel, which are more biocompatible and mimic the tissue environment due to their similar properties (Bishop et al., 2017). However, despite the superior mimicry and biocompatibility, these materials have relatively weak structural integrity and could often collapse due to their softness. The structural integrity of the bioprinted construct or scaffold is a crucial characteristic to ensure successful transplantation. An example given by Derakhshanfar et al., shows that in the case of hard tissue repair, elastic modulus plays a critical to maintain the designed structure and porosity during the process of implantation. To overcome this issue, several techniques are utilized such as crosslinking technique, physical blending and multi-material printing, which involves the combination of different types of material to achieve a biocompatible and excellent structural integrity scaffold (Grigoryan et al., 2021; Agarwal et al., 2022).

The greatest challenge to bioprint functional tissue in the lab involves the printing of vascular networks. Vascularity is vital to ensure the functionality of bioprinted tissues as it plays the role of nutrient delivery and waste disposal. According to the diffusion limit of oxygen, vascularity is required for tissue to grow over 100-200 µm (Bishop et al., 2017). Engineered tissues might not receive balanced nutrients and necessities to survive without proper vascularity. To 3D print functional tissue, a vascular network must be present at the earliest stage of development to prevent tissue death while allowing attachments of tissues. Current challenges faced for bioprinting vasculature are mainly due to mechanical limitations in printing resolution and speed (Bishop et al., 2017). With latest technology, the highest resolution laser-based bioprinters utilise a droplet size of 20µm while the diameter of a capillary is about 3µm. If the printing accuracy could improve until 3µm, the 3D printer will require a long period to complete, which could affect the cell's viability (Bishop et al., 2017).

Moreover, it is also mentioned that some cells cannot maintain its biological activity after experiencing the 3D bioprinting process (Tan et al., 2021). As discussed before, the 3D bioprinting process involves shear stress and UV radiation, which could be harmful to the cells. Instead of using thermal or mechanical bioprinting, it is possible to use safer methods such as piezoelectric or acoustic waves. Nevertheless, although piezoelectricity could control the bioink droplet size without exposing it to thermal or mechanical stress, a frequency of 15 - 25 Hz could damage the cell membranes and cause cell lysis (Tan et al., 2021).

Future Perspectives and Advancements

One of the future advancements of 3D bioprinting in tissue engineering is strengthening the mechanical properties of the 3D bioprinted tissues. This can be realized by researching on the suitable bioinks with optimal mechanical properties (tensile strength, Young's modulus, and shear stress) for different parts

of body tissues or organs. Furthermore, it is possible to research the most optimum material to act as the main bioink while mixing other materials to construct different tissues. The result of this research could boost the possibility of generalising the market's bioink while focusing more on tissue design. Besides, the mechanical properties of the 3D bioprinted tissues must be easily tunable on the degradation rate and hence can better increase the controllability of scaffold degradation rate, growth factor releasing rate, and drug releasing rate. This is due to the vast application of the 3D bioprinting industry. Depending on the application, with different customised rates, it is possible for researchers to better visualize the effect of drugs on human tissues.

Studies have highlighted several limitations in current 3D bioprinting techniques, underscoring the need to explore alternative methods to enhance productivity. Simultaneously, advancing existing techniques remains critical, as it contributes to developing more stable and reliable bioprinting processes. While achieving vascularization with current technologies remains challenging, research into incorporating vascularization-promoting factors shows promise. For instance, Bishop et al. demonstrated the inclusion of angiogenic growth factors in bioink to promote vascularization. Although their approach yielded encouraging results, further refinement is necessary to develop a functional tissue bioprinting technique.

CONCLUSION

The current application of 3D bioprinting in tissue engineering utilizes three types of 3D bioprinting methods which are inkjet-based, extrusion-based, and laser-assisted 3D bioprinting. The bioinks involved in current 3D bioprinting in tissue engineering are classified into either natural polymer, synthetic polymer, or decellularized ECM. Each 3D bioprinting method and bioink has its advantages and disadvantages. The current applications of 3D bioprinting in tissue engineering vary from skin, bone, cartilage, cardiovascular, and liver tissue engineering. It shows the high customization and exponential growth of 3D bioprinted cells into various body tissues, ranging from soft to hard tissue. The advancement of 3D bioprinting plays a significant role in enhancing the research fields of replacing worn-out organs and therapeutic research of current diseases or cancers.

ACKNOWLEDGEMENT

We acknowledge the support and guidance provided by the Department of Biomedical Engineering and Health Sciences, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, in conducting this review.

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