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Review Article

3D Bioprinting: Potential Technology for Drug Delivery

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ABSTRACT

The advent of three-Dimensional (3D) bioprinting offers transformative opportunities in tissue engineering, enabling the precise fabrication of functional tissues and organs for medical and research applications. This study aims to explore the scientific advancements in 3D bioprinting techniques and bioinks while evaluating their potential to overcome current biomedical challenges. Using a comprehensive review of cutting-edge bioprinting methods, this work analyses the integration of diverse bioink formulations, printing strategies, and their applications in areas such as regenerative medicine and drug testing. The findings reveal significant progress in creating complex tissue structures and organ models, although limitations such as vascularization, regulatory hurdles, and ethical concerns persist. Conclusively, addressing these barriers is critical to realizing the full potential of bioprinting, paving the way for innovative solutions in organ transplantation, personalized medicine, and disease modelling.

Keywords: 3D bioprinting, Regenerative medicine, Personalized medicine, Bioinks.

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INTRODUCTION

Multilayered printing of 3 dimensions is a process where a layer by layer printing is done to create 3D structures, they are utilised in various ways in today's generation but the material used determines the objective of the task, if used resin it becomes resin based layer printing, if used bioinks it becomes bioprinting.[1] When bioprinting is being discussed it is unlikely to forget the makes and bakes of a biostructures which would help build an organ, which are tissues, and their further breakdown leads to cells. To put it in formal way constructing a cell or collection of cells or any organ retaining its said scientific build along with its function is bioprinting.[2]

Bioprinting is a specialized form of additive manufacturing, commonly referred to as 3D printing, that enables the creation of structures using living cells, biological materials, and biomolecules.[3] A critical aspect of bioprinting is the development of scaffolds with precise microstructures that not only maintain mechanical stability but also promote cell growth and integration. Moreover, the manufacturing process itself must be carefully managed to prevent negative effects on cell viability, such as chemical toxicity from solvents or cell death caused by excessive pressure during extrusion. A key benefit of bioprinting lies in its ability to integrate cells directly into the structure as it is being created, overcoming challenges related to uneven cell distribution that often occur with traditional post-printing cell placement.[4]

This kind of printing is one of the 3D fabrication methods that was initially launched more than thirty years after Charles Hull's 1986 invention of 3D lithography. Sachs *et al.*, developed a powder-based free-form production scheme in the early 1990s, that made a substantial advancement in 3D printing possible.[5] This approach employs a normal ink-jet printhead and adds binders to the powder bed in order to keep the wobbly particles composed. As a result, in its primitive phases, it was mostly used to build prototypes with hard materials, when the technology rapidly advanced, 3D printing is now widely used in the military, the automotive sector, electrical device engineering, and, more recently, bio fabrication.[6]

Although full or partial organs can also be developed using 3D bioprinting techniques, the primary benefit is the ability to print entire organs for use in transplantation. With a combination of cells-chemicals in scaffolds biological structures could be erected with exquisite micro level or nano level architectures depending on the ask of the task which are close duplicates to the structures in the body anatomically at the cell/tissue level using computer-aided design software. These abilities have piqued the dwellers of regen medicine or formally regenerative medicine although it is still not going to be totally perfect to be transplanted into a body as of now.[7]

Bioprinting will soon offer various advantages in operating rooms and will spring to bedside once licensed for use, assuming biomaterials, cell, transplantation technologies progress.

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Bioprinting does not require regulatory approval, and there is already a developing bioprinting business for printing of tissue in need of testing drugs along with high-throughput experiments, it has already made considerable strides in pharmaceutical application before moving into clinical practice. Bio-printed tissue models, such as liver models, have been employed in drug screening because they facilitate a complex heterocellular physiological milieu and include several kinds of cells. Furthermore, to research cancer pathophysiology, development as well as metastases in a physiologically realistic milieu. Recently, bioprinting has been used to study cancer.[8]

To complete a construct, 3D printing often necessitates technical steps such as using a CT or MRI scan to create the 3D geometry of the required anatomical site, optimising the printing file, selecting acceptable materials, printing, and assessing the manufactured structures.[9] When using 3D printing, the material selected for prototyping is crucial.

Bioprinting broadly happens in 4 steps:

CT or MRI scans for the whole configuration of the organ.

Optimise the 3D output to work with bioprinters.

Selecting the appropriate materials.

Check for the efficiency of the final product.[10]

Initially, 3D printing technologies were developed for non-biological purposes; metal, ceramic, and thermoplastic polymer deposition were the primary applications for these printers. The use of crosslinking agents, high temperatures, or organic solvents in printing processes cannot coexist with biological materials or living cells. One of the biggest challenges in 3D bioprinting is locating suitable printing materials with exceptional printability, biocompatibility, and the necessary mechanical and degradation properties for tissue formation.

In parallel bioprinting broadly happens to have its own challenges like:

Finding suitable materials that are biocompatible and do not crumble when put through the printing process.

Materials that facilitate required properties of tissue formation.

Because 3D bioprinting was not conceived as a concept for biological purposes so it is so hard to overcome these challenges.[11,12]

Based on the type of tissue and the intended use, a variety of bioprinting methods, including droplet-based, extrusion-based, laser-induced forward transfer, and integrated bioprinting, can be employed. Every printing method is predicated on different physical processes that specify the requirements (such as the photo reactivity, thermal stability, oxidative stability, and rheology profile) of an appropriate bioink. There are many disciplines like tissue engineering, multiple researches waiting for the advancement in 3D bioprinting because of its capability to print cells accurately with cell viability.[13] So this variant of additive manufacturing becomes discussion of the hour prompting us towards its anatomy.

MATERIALS AND METHODS

Techniques for 3D-Bioprinting

The fundamental ideas behind the various bioprinting methods-such as inkjet, Laser-Assisted-Bioprinting (LAB), Pressure-Assisted (extrusion) Bioprinting (PAB), acoustic Stereolithography (SLA), and magnetic bioprinting-are based on their ability to produce functional tissue constructs.[14] The techniques were represented in Figure 1.

Inkjet Based Bioprinting (IBP)

Originally introduced by Hewlett-Packard in the 70s as a 2D printing technology, inkjet printing evolved in 1992 when the addition of a chamber and an adjustable stage enabled control over the z-axis, paving the way for its adaptation into 3D printing techniques.[15] One of the first AM methods was introduced: inkjet printing. With assistance from our binary friend i.e., computer droplets of bioinks can be controlled effectively to mind the size and pattern of the print. For inkjet droplet squeezing, there are now four methods available: thermal, piezoelectric, acoustic, and electrostatic inkjet printing.[16] Any one into IBP would mostly come across Thermal and piezoelectric technologies in the fabrication of structure. When using live systems in a bioprinting process, the bioinks are often pre-polymers what would be composed of cells or not. The inkjet printing process has been effectively applied in various scientific and commercial domains due to its rapid manufacturing and cheap cost advancements.[17]

Two methodologies are employed to form the ink drops used in bioprinting namely Drop-on-Demand printing (DOD) and Continuous inkjet printing (CIJ). Conversely, DOD method works by the production of bioink drops over the base/substrate as fond. In contrast to DOD systems, CIJ-based bioprinters produce drops far more quickly.[18] This doesn't mean CIJ made DOD obsolete because the conductive fluid inks used by CIJ along with the danger of contamination while recycling fluids limits them while DOD are masters of finesse because of their ability to waste the bioink in minimal amounts while meticulously patterning as required with adequate material deposition.[19]

Thermal, piezoelectric, or acoustic methods can produce the DOD. The resistor receives a short electric pulse that causes a drop, which generates heat and a tiny bubble or vapour pocket. The bubble either grows or collapses the moment heat isn't applied. Such compressive-expanding forces push the droplets out of the nozzle at different volumes. The term "bubble jet bioprinters" is hence another name for thermal inkjet bioprinters.[20] Although thermal inkjet bioprinting is a cost-effective as well as efficient printing method, there are still a lot of obstacles to overcome.

Weak Hydrogels make it impossible to create perfect geometrical shapes as it is hard to determine the direction of the drop

Nozzle gets clogged for unwarranted reasons

Thermal and piezo electric methods work on different principles and any of them has a capability to damage the intrinsic structure of the bio ink.[21]

Laser Assisted Bioprinting (LAB)

Laser-Assisted Bioprinting/LAB in short has emerged through the integration of laser direct-write and laser-induced transfer technologies. The key component of this method is a ribbon-shaped donor layer, which includes an energy-absorbing material like titanium or gold, while the bioink solution is positioned beneath it. When a laser pulse hits this layer, it propels the bioink with precision to the desired surface. The foundation of Laser-Induced Forward Transfer (LIFT) forms foundation of Laser-Assisted Bioprinting (LAB). A high-energy laser pulse creates high-pressure bubbles that propel the thin biomaterial layer into the specified area. A LIFT system consists of a ribbon, a biomaterial layer, a pulsed laser beam, an energy-absorbing layer, and a focusing device. The thin energy-absorbing layer, which is often composed of metal, is supported by the transparent ribbon, and for the biomaterials to spread across the metal layer, they must be in the liquid or gel state. The layer that absorbs energy transforms into a layer that deposits energy to propel items outward. Any component of the system, including the biomaterial's viscosity, laser intensity, and laser frequency, can have an impact on the printed material's resolution. High-energy laser pulses rarely impact a cell's vitality or function, and different cell types can be selectively written. Small droplets of hydrogel precursors and biomaterials with any required viscosity can be printed thanks to LAB. This approach can precisely regulate the deposition of high-viscosity material because it does not require a nozzle.[22]

The resolution of the LAB system is decided by the energy of laser, substrate surface type, air gap between the absorbent layer and substrate, surface tension, and bioink viscosity. Because this printing technique does not require nozzles, bioink or cell blockage can be prevented. However, to create constructions with acceptable shape fidelity, this kind of printer needs bioink with quick gelation kinetics, which could impede print flow.[23]

The appeal of Laser-Assisted Bioprinting (LAB) lies in its ability to deliver automation, consistency, and high output, making it an efficient approach for producing 3D tissue structures. A pivotal consideration in LAB is the selection of appropriate biomaterials. These materials must possess fast gelation properties or rapid cross-linking capabilities and be compatible with the working laser wavelengths to maintain the precision and structural integrity of cells and biomaterials during printing-a notably complex aspect of the process. However, challenges such as prolonged production durations and the tendency of cells to settle under gravity during printing remain significant hurdles to address.[24]

Extrusion Based Bioprinting (EBP)

Extrusion-Based Printing (EBP) operates using pressurized systems to deposit materials. One of its standout advantages is the ability to print with exceptionally high cell densities, making it a versatile and effective technique. However, despite its adaptability, EBP has notable drawbacks compared to other bioprinting methods. Its resolution is relatively lower, as the smallest achievable feature size typically exceeds 100 µm, limiting its precision for applications requiring finer details.[25] This may restrict its use in some soft tissue applications where smaller pore sizes are necessary for better tissue response; it may nevertheless apply to hard tissues with pore sizes more than 10 mm. Although numerous studies have documented it, the pressure at which the material is extruded may change the morphology and function of the cells.[26]

For extrusion-based bioprinting to function effectively, materials such as bioinks must possess sufficient flow properties to pass through the nozzle. The geometry of the deposited filament is largely dictated by the cross-sectional shape of the nozzle opening. Upon extrusion, the material must quickly transition into a stable form to maintain the structural integrity of the printed filament and support the layer-by-layer assembly of a 3D structure. This critical transformation is inherently tied to the material's specific properties, which is why certain extrusion-based techniques are closely aligned with particular substances or material classes. While various specialized methods exist, most extrusion-based bioprinting processes involving hydrogels and bioinks fall under the broader category of "extrusion-based bioprinting," without further classification.[27]

In pneumatic extrusion systems, compressed air is used to drive bioink through the nozzle at a rate and volume pre-set by the manufacturer. The amount of pressure applied is determined by the specifications of the air-pressure mechanism. While pressure buildup can extend printing times, the simplicity of this system offers a distinct advantage over mechanically driven extrusion methods. This approach is particularly well-suited for rapid prototyping applications, especially when creating porous scaffolds.[28] According to Bernoulli's principle, the speed at which bioink is ejected can be regulated by adjusting the applied pressure. Mechanical dispensing systems, utilizing robotically controlled screws or pistons, provide greater spatial precision in biomaterial extrusion for extrusion-based bioprinters. However, these systems come with significant drawbacks, including a more complex design compared to pneumatic systems and a higher likelihood of mechanical failure.[29]

The extrusion bioprinter has good compatibility with a wide range of materials and can print materials with a wide range of viscosities. Extrusion bioprinters, like this one, are known for

having numerous printer heads that enable the simultaneous printing of various bioink kinds. Furthermore, it is also capable of precisely regulating the printed scaffolds' or prosthesis' pore sizes, porosities, and cell distribution for tissue engineering. Because of all these advantages, extrusion bioprinting has become the most popular commercial 3D printing technology in recent years.

Stereolithography

Stereolithography (SLA) bioprinting is another technological technique for producing micro- and nano-architecture scaffolds with 3D patterns. Typically, 3D scaffold constructions made with standard printing methods are not endowed with the characteristics that let the user manipulate the mechanical, porosity, and resolution.[30] Stereolithography (SLA) bioprinting uses light to harden bioinks that are sensitive to specific wavelengths, building structures layer by layer. A projector guides the light to solidify the bioink along flat planes, joining layers seamlessly into a cohesive form. Interestingly, the time required to cure each layer remains unchanged, no matter how large or complex the design.[31] By measuring the structure's thickness, one may estimate the overall printing time. SLA has been utilised to print 3D cell-enclosed structures in less than 30 min with very high cell viability (>90%) and resolution as low as 100 μm.[32] SLA printing has been successfully performed at an affordable cost, achieving a resolution of 50 microns and a cell survival rate of 85%. This process utilized fibroblast cells (NIH 3T3) and employed bioinks that are cross-linkable under visible light. The bioinks were a mix of Polyethene Glycol Diacrylate (PEG-DA) and Gelatin Methacrylate (GelMA) hydrogels, which provided the necessary support for cell viability and structural integrity.[33]

In the past, materials based on gelatin, polypropylene fumarate, and trimethylene carbonate were chemically altered to enable stereolithography printing of the materials to create implants that resembled bones. Moreover, scaffolds for tissue engineering have been built using a number of high molecular weight polymers, such as poly(propylene) fumarate and d,l-lactide, that hydrolyse both *in vitro* and *in vivo*. Stereolithography can also be used to print constructions loaded with cells on PEGDMA hydrogel and PEGDA. However, the use of live cell printing is limited because of the nature of this bioprinting technique.[34] Stereolithography (SLA) bioprinting relies on photopolymerization, a process where UV light or a laser is directed along a specific path to cure photopolymerizable liquid polymers, causing them to bond and form solid layers. Here's how it works:

***Photopolymerization process*:** A light source, typically UV or laser, activates the liquid polymer, turning it into a solid layer as it follows a programmed pattern.

***Layer-by-layer building*:** Once a layer is solidified, the printing platform is lowered into the polymer solution, allowing the next layer to be formed. This process repeats, gradually building a 3D structure.

***Multiple cycles*:** The process involves several cycles of curing and layering to achieve the final object.

This method allows for precise, controlled printing of complex 3D structures, layer by layer.[35] This method is especially effective when working with photopolymerizable materials like acrylics and epoxies, which offer higher production accuracy than other techniques. For example, stem cell-based molds for artificial heart valves have been successfully created using stereolithography. However, the main challenge of using SLA for biological applications is the need for intense UV light to initiate the polymerization process, which can be problematic for sensitive biological materials. Other limitations include the requirement for SLA-compatible materials and the long post-processing times required to finalize the printed structures.[36] When applied to bioprinting, stereolithography offers numerous advantages. Bioink is a preferred choice for incorporating cells into scaffolds due to its unlimited viscosity and lack of shear stress. SLA isn't immune to its own challenges like:

Danger of UV spectrum on DNA

Limited availability of materials to work with that is sensitive to light.

Extra cytotoxicity that comes with all of this.

Alternatives, like materials free of photo-initiators or photo-initiators that absorb visible light, have previously been the subject of certain research efforts.[37]

Acoustic bioprinting

The fields of bioprinting, surface acoustic wave technology, and single-cell manipulation are related. Cells can be manipulated in many directions by acoustic or sound waves to create complex 3D designs. Using an open pool of bioink and a mild acoustic field, acoustic bioprinters create and deposit cell-encapsulated picolitre droplets.[38] Acoustic bioprinting stands out for its nozzle less design, which not only avoids the common clogging issues but also protects the cells from harmful forces, heat, and pressure-challenges often faced in methods like Drop-on-Demand (DOD) printing. Over a decade ago, scientists developed an acoustic bioprinter that could print multiple types of cells, including cardiomyocytes, fibroblasts, hepatocytes, and stem cells, within biological fluids. Remarkably, this technology maintains a cell viability rate of over 85%, even when handling complex cell types. To ensure the bioinks stay in place during the printing process, the system utilizes one or more 2D microfluidic channels, offering greater precision in cell deposition.[39] The acoustic bioprinter relies on interdigitated gold rings placed over a piezoelectric substrate made of materials like quartz, Murata, and lithium niobate/tantalate. This setup enables the device to generate controlled surface sound waves, which create an acoustic focal point at the interface between the air and the fluid. When these sound waves exert enough force to overcome the surface

tension of the bioink, tiny droplets are ejected. The diameter of these droplets varies as the acoustic frequency is adjusted. The printer can eject droplets ranging from 1 to 104 picoliters per second, depending on the frequency. However, further research is required to explore whether it's possible to integrate multiple cell types and growth factors into this process, potentially creating scaffolds populated with cells that mimic the natural biological environment.[40]

Bio-inks

Bio-inks, which are biomaterial solutions containing living cells, are the basic components of bioprinting. During the printing process, the biomaterial solution's constituents must protect the cells from harm. The four main types of bio-ink materials are cell aggregates, hydrogels, microcarriers, and decellularized matrix components. Tissue spheroids, cell pellets, and tissue threads are three distinct subtypes of cell aggregation.[41] Droplet integrity is one important aspect of this that can be altered by the components of the bio-ink material. If the droplet's integrity is weakened, it may splash or spread, which could lead to structural collapse or shift the deposited cells from where they were supposed to be. A droplet may scatter across its surface area or fragment into smaller droplets (splashing) after colliding with the substrate. The size, density, and surface tension of the droplets determine the sort of collision.[42]

Natural Polymer-Based Bioinks

A bioink must have (i) physico-mechanical properties and (ii) biological attributes that are comparable to those of the targeted tissues in order to be considered biofunctional. The bioink needs to be printed in addition to maintaining cell viability. As such, bioinks can be altered based on the target tissue and the printer being used.[43] Typically, a combination of two or more biomaterials is needed to accomplish all these qualities, particularly for extrusion-based bioprinting. Additionally, multicomponent bioinks are frequently better than single-component bioinks since the former typically have low levels of biocompatibility and strong mechanical and functional requirements that prevent them from forming biomimicry tissues. Multicomponent bioinks can also work as a supplement to help produce more complicated tissue structures since their components can balance each other out, make up for any deficiencies in the other bio-ink material, and augment one another.[44] Additionally, nanoparticles are a desirable addition to bioinks because they can change the bioink's viscosity or, in certain cases, make it conductive, which improves signal transduction.[45] In the upcoming sections, we will explore the various properties of natural bioinks, such as agarose, alginate, gellan gum, dextran, Hyaluronic Acid (HA), silk, fibrin, collagen, Decellularized Extracellular Matrix (dECM), Matrigel, cellulose, gelatin, and chitosan. Additionally, we will delve into examples of commonly used multi-component bioinks and discuss the role of nanomaterials in enhancing bioprinting applications.[46]

Alginate

This naturally occurring polymer, which is generated from brown seaweed, is a well-liked option because of its affordability, low toxicity, and biocompatibility.[47] Alginate is commonly ionically crosslinked with Calcium Chloride ($CaCl_2$) to create printable hydrogels. In a variety of applications, it demonstrates excellent tissue engineering capability and viable cell encapsulation.[48] Its shortcomings, however, are that it lacks intrinsic cell-binding sites, degrades quickly under physiological settings, and has very low mechanical strength.[49]

Chitosan

Derived from the shells of crustaceans, chitosan possesses biocompatibility, biodegradability, and significant antibacterial/fungal characteristics.[50] Typically, genipin or glutaraldehyde are used to create crosslinks. It has shown potential in bone, cartilage, liver, and skin tissue engineering.[51] Chitosan's poor mechanical strength, quick dissociation in physiological conditions, and restricted cell attachment, however, present further difficulties.[52]

Gelatin

Made from the collagen of animals, gelatin has special thermo-reversible gelling qualities. At lower temperatures, it stays a gel, while at higher degrees, it turns into a liquid.[53] Type A (acid-treated) and Type B (alkaline-treated) gelatin are widely used in tissue engineering. Pure gelatin is not as viscous or strong enough mechanically at physiological temperature (37°C) to support cell growth, even though it mixes well with other bioinks.[54] While glutaraldehyde is a very powerful crosslinker, its cytotoxicity makes it necessary to investigate other crosslinkers, such as transglutaminase and Horseradish Peroxidase (HRP) in conjunction with Hydrogen peroxide (H_2O_2), carbodiimide, and genipin.[55]

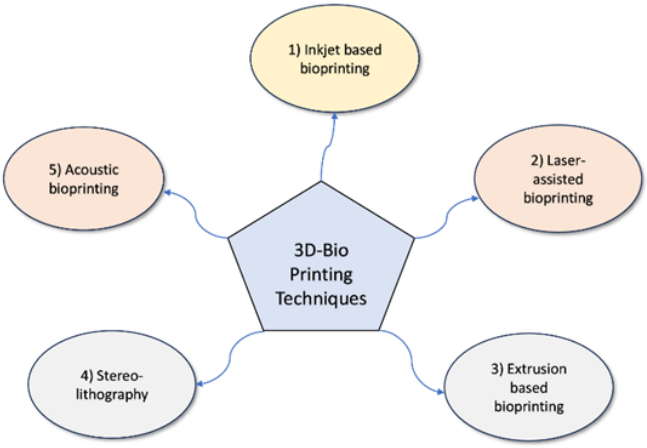

Figure 1: Techniques of 3D bioprinting.

Gelatin Methacrylamide (GelMA)

The GelMA, also known as gelatin methacrylamide, is a photo cross linkable bioink with adjustable mechanical properties that was created by modifying gelatin with methacryloyl groups.[56] It is a well-liked alternative for a range of tissue types because of its remarkable printability and ability to encourage cell adhesion and proliferation. Nonetheless, to reduce the possibility of UV-induced cell damage, photo crosslinking parameters must be properly optimised.[57]

Collagen

Collagen, the primary structural protein of the Extracellular Matrix (ECM), is highly biocompatible and provides the ideal conditions for cell attachment, proliferation, and activity.[58] However, there are significant printing problems since collagen has a low viscosity. It is frequently combined with other hydrogels or used in conjunction with supporting structures to get around this.[59]

Silk

The naturally occurring protein fibre known as silk, which is generated by silkworms and spiders, has several desirable qualities that make it a good choice for bioprinting, including its high viscosity, non-toxicity, biocompatibility, and progressive biodegradability.[60] Silk's propensity for beta-sheet crystallisation can cause nozzle blockage during printing, despite its potential in many applications. Furthermore, blending with other materials is frequently necessary to improve cell adhesion due to its low cell-binding capacity.[61]

Fibrinogen

A vital part of the blood clotting cascade, fibrinogen can be polymerised with thrombin to form fibrin. Its exceptional biocompatibility, ability to promote cell adhesion and proliferation, low immunogenicity, and minimal inflammatory response make it suitable for tissue engineering, particularly wound healing.[62] Unfortunately, its quick disintegration makes it unsuitable for use in *in vivo* or long-term cultures. Because of its low viscosity, printing quality is frequently improved by mixing it with other bioinks.[63]

Agarose

A polysaccharide that is derived from red seaweed, agarose exhibits thermo-reversible gelation, changing from a liquid to a gel state at about 37°C.[64] Although it is immunogenicity-free and biocompatible like other natural bioinks, it is weak cell adherence and brittle solid form restricts its use in sacrificial bioink applications like voids or channels within constructions.[65]

Hyaluronic Acid (HA)

Hyaluronic Acid (HA), a naturally occurring polysaccharide, is abundantly found in connective tissues, exhibits strong biocompatibility, and is easily altered to produce a variety of hydrogels. In tissue engineering, it is widely utilised, usually in combination with other bioinks.[66] HA hydrogels, however, have limited mechanical stability and are quickly degraded in physiological settings. Combining with different polymers is a typical tactic to get around these restrictions.[67]

Matrigel

It is a gelatinous protein mixture that was taken from mouse sarcoma cells. It is very supportive of cell development and differentiation since it contains a lot of growth factors and extracellular matrix components.[68] Although there are many possibilities for Matrigel in tissue engineering, it is costly, cannot be used in clinical settings because it comes from animals, and needs to be blended with other materials to be printable.[69]

Table 1: Patents with their names.

Sl. No.	Patent ID	Patent Name
1	US8,241,905B2 (2012).[95]	Self-assembling Cell Aggregates and Methods of Making Engineered Tissue.
2	UK2,478,801(2012), US20130345794A1(2012).[96]	Multilayered Vascular Tubes.
3	US 8,143,055 B2 (2009).[97]	Self-assembling Multicellular Bodies and Methods of Producing a Three-Dimensional Biological Structure.
4	US 11,046,001 (2021).[98]	Temperature-regulated print bed design for precise control of bioink gelation.
5	SE 543880 (2021).[99]	Temperature control of cartridge and nozzle to improve cell viability and printing consistency.
6	US 9,855,369 B2 (2018).[100]	Bioprinting of 3D Tissue Constructs.
7	US 11123456 B2 (2022).[101]	Bioprinting of Bone Tissue.
8	US 2020/0206385 (2014).[102]	Methods, Devices, and Systems for the Fabrication of Materials and Tissues using EMR.

Synthetic Polymer-Based Bioinks

Synthetic polymer-based biomaterials are often used to produce bioinks because of their ability to be both mechanically strong and precisely controlled during printing. However, they still pose significant challenges, such as poor biocompatibility and unpredictable degradation over time. In this section, we will provide a concise overview of the key properties of several widely used synthetic polymers, including Polylactic Acid (PLA), Polyethylene Glycol (PEG), Polyvinyl Alcohol (PVA), Pluronic F-127 (PF127), Polycaprolactone (PCL), and Polylactic-co-Glycolic Acid (PLGA).

Polycaprolactone (PCL)

This biodegradable polyester's biocompatibility, flexibility, simple melting points (~60ºC), exceptional resilience, along with slow disintegration make it a popular choice for a structural scaffold in hybrid bioinks.[70] It gives naturally occurring hydrogels with poor mechanical qualities. Unfortunately, high temperatures or organic solvents are usually needed for processing, which makes it unsuitable for direct cell encapsulation.[71]

Polyethylene Glycol (PEG)

PEG, a hydrophilic polymer known for its biocompatibility and non-immunogenicity, is commonly employed as a sacrificial bioink to create channels or vascular networks. Modifications using acrylate or methacrylate groups allow for photo crosslinking, which gives you more control over the characteristics. However, PEG frequently requires further changes to improve cell adherence.[72]

Pluronic F-127 (PF127)

This thermoresponsive copolymer, a liquid at low temperatures and gels at ambient temperature, is largely employed as a sacrificial bioink. Its capacity to construct complicated structures is valuable, but its inadequate cell support and mechanical qualities limit its potential use.[73]

Polyvinyl Alcohol (PVA)

This water-soluble, biodegradable polymer has already received approval from FDA, USA and is thermostable and biocompatible.[74] While PVA is widely utilised in tissue engineering, particularly in mixes with other bioinks, it has low cell affinity. Crosslinking with glutaraldehyde or mixing with other materials can increase the mechanical characteristics and cell interactions.[75] To create stable and desired composites, PVA is physically changed utilising a variety of techniques, including freeze-thaw and homogeneous blending with other hydrogels, due to its low cell affinity.[76]

Polylactic Acid (PLA) and Polylactic-co-Glycolic Acid (PLGA)

Polyester-based polymers like PLA and PLGA are widely recognized for their FDA approval, biodegradability, and biocompatibility. However, one of their major limitations is their inherent hydrophobic nature, which leads to poor cell adhesion.[77] To overcome this, plasma treatment and surface coatings have been used to enhance cell attachment and protein absorption When these types are bioprinted with such surface modifications, they can create an environment for cell culture that supports the development of a variety of tissues.

Emerging Bioink Concepts

Cell Spheroids/Aggregates

The utilisation of 3D cell clusters as bioinks shows promise for replicating tissue organisation and improving cell-cell interactions. However, concerns include the risk of necrosis in larger spheroids and the difficulty of putting them into bioprinters without clogging.[78,79]

Nanocomposite Bioinks

The incorporation of nanoparticles (e.g., nanocellulose) into bioink formulations presents a means to enhance various properties, including mechanical strength, printability, and even biological activity. However, careful characterisation and optimization are essential to ensure biocompatibility and avoid any cytotoxic effects.[80,81]

CHALLENGES AND POTENTIALS

3D bioprinting needs to go past a number of obstacles, such as limitations in material selection, cell viability, ethical concerns, and others before it can become therapeutically relevant or commercially appealing. The issues are categorised into areas such as biological, technical, ethical or legal, and regulatory in order to make comprehension easier.

Biological challenges

One of the major limitations in tissue-engineered structures is the lack of vasculature. These tissues rely on the porosity of the scaffolds to allow nutrients and waste to flow, but this only works until the host's own process of neovascularization-creating new blood vessels-takes over. Without this vascular network, the size and complexity of the tissue-engineered structures are severely restricted.[82] Preliminary studies suggest that increasing pore size or incorporating angiogenic growth factors, such as VEGF, can enhance natural angiogenesis and inosculation (the process of blood vessel connection). While this approach shows promise, it is still too slow to significantly support the growth of larger or more complex tissue structures in a timely manner.[83] While bioprinting of isolated cell-lined microfluidic channels has made significant progress, most studies have been limited to proof-of-concept

experiments conducted *in vitro*. The challenge remains in creating functional blood vessels and capillary networks *in vivo*, capable of supporting nutrient delivery to engineered tissues. In addition to these vascularization challenges, there are several other difficulties associated with printing complex composite tissues. These include:

Long manufacturing times: Extended biomanufacturing processes can lead to reduced cell viability.

Cellular dedifferentiation: Over time, cells may lose their specialized functions, diminishing their regenerative potential.

Acidic byproducts: Degradation of biomaterials can release acidic substances, harming the tissue.

Non-homogeneous matrix synthesis: Achieving uniformity in the matrix structure remains a challenge.

Lack of post-printing remodelling: After printing, tissues often fail to naturally remodel, impacting long-term functionality.

Poor mechanical strength: The structural integrity of printed tissues may deteriorate over time, limiting their durability.

These issues highlight the complexity of creating functional, long-lasting engineered tissues.[84]

Technical challenges

The precise and accurate deposition of bioink, or printability, is one technical challenge that needs to be resolved. In the 3D microenvironment, higher resolution will enable better control and interaction. Faster printing and process scalability are essential for commercial success. It is important to assess the 3D scaffold's mechanical and structural qualities in relation to the native tissue.[85] To increase mechanical qualities, sacrificial material must be used during printing or incorporated into the construction. With the current availability of bioinks, it is still challenging to retain the required tissue's mechanical strength. Vascularization is a challenge that can be solved by a variety of tries and errors. One method is to use synthetic or biodegradable polymers to create vasculature during bioprinting, which ultimately results in vascularised tissue. Adding angiogenic factors to the bioink is an additional technique that will draw cells to the construct and promote the growth of the vasculature. During the print of any tissue or organ, researchers face a few additional technical constraints.[86] The intricate complexity and cellular diversity make designing a tissue or organ blueprint extremely difficult. Matching the mechanical characteristics of 3D-printed organs, such as bone, articular cartilage, and meniscus, to physiologically relevant tissues while maintaining biological activity is the next hurdle.[87]

Ethical Challenges

Further challenges will arise in the design of clinical trials: it would be unethical to test tissue-engineered organ transplantation on healthy volunteers, and employing patient-specific cell populations would necessitate the patients acting as controls, introducing a high degree of heterogeneity in the evaluation of treatment efficacy. This could be especially troublesome when evaluating positive outcomes from patients in clinical trials: how much of the benefit is due to the bio-printed product itself, and how much is due to the patient's natural response to treatment?[88] Before beginning any major clinical investigations in this area, a thorough and trustworthy methodology for evaluating the effects of bio-printed medicines must be established. The few tissue-engineered building trials that have been conducted so far have involved patients who had terminal illnesses when these "last resort" options are usually seen as "more ethical," even though the hazards remain unknown. Skeletalized trachea from cadaveric sources implanted with patient mesenchymal stem cells for surgical use is one example. Getting ethical consent in many situations requires proving the patient's clinical urgency. Getting ethical consent to perform the tissue-engineered trachea experiment on patients was made easier by describing it as a last-resort option and a last chance for a life-saving intervention. Although this approach has significant limitations, it was successful in quickening a translational bioengineering step.[89]

Regulatory challenges

When it comes to 3D-printed devices, the regulatory landscape is fraught with challenges, largely due to the unique and evolving nature of the automated production process. The very technology that enables 3D printing-its intricate, layer-by-layer fabrication method-can lead to surface imperfections, edge flaws, and inconsistencies between layers, all of which need careful attention and regulation.[90] On top of that, because digital tools like CAD software are central to these advanced manufacturing techniques, it's not just the physical printing process that needs oversight-there's also a need for regulations that encompass the entire software ecosystem supporting these machines.[91] Some argue that current regulations may be enough to handle 3D-printed objects, but with the rise of personalizing and decentralizing production, these technologies are poised to shake up the regulatory status quo, possibly creating new waves of uncertainty and instability.[92] Further complicating matters is the question of legal liability-who is responsible when a 3D-printed product fails? With no established legal precedent, it's unclear how the courts will handle cases involving defective 3D-printed goods, which leaves a significant gray area in terms of accountability. In the world of biomanufacturing, things get even more complicated. Just like any tissue-engineered product, bioprinted tissues must comply with stringent Good Manufacturing Practice (GMP) regulations and secure FDA or EMA approval. Bio-printed tissues that incorporate cells are considered "combination products"

by the FDA and "advanced therapy medicinal products" by the EMA, meaning they must undergo extensive clinical trials before they can be approved for regular use.[93] The real challenge lies in standardizing, validating, and constantly monitoring these 3D bioprinting processes-an incredibly complex task for a process that is inherently customizable and constantly changing.[94] The information on patents is tabulated in Table 1.

APPLICATIONS

Applications of tissue regeneration

3D bioprinting has advanced significantly over the past ten years, opening the door for potential uses in numerous clinical medical domains and perhaps all of the body's major systems. The main therapeutic option is surgical repair or artificial restoration because some tissues cannot regenerate on their own.[103] Therefore, when organ transplantation is difficult or impossible, bioprinting has proven to be quite beneficial. 3D-bioprinted tissue implantation has shown promising results in major body tissues, including the skin, blood vessels, and heart.[104]

Cardiovascular

Bioprinting blood vessels in many layers and integrating endothelial cells into the vessel walls. Developing bio-printed cardiac patches infused with stem cells to improve angiogenesis and heart tissue recovery following a myocardial infarction.

Bioprinting Multi-Layered Blood Vessels Researchers have successfully bio printed multi-layered blood arteries using gelatin hydrogel. Endothelial cells inserted into vessel walls grew and matured normally within three to five days. This technique, developed by *Hasan et al.*, 2015, shows promise for creating functioning blood arteries for a variety of applications.[105]

Bio printed cardiac patches, composed of mesenchymal stem cells and endothelial cells, have shown great potential in treating myocardial infarction (heart attacks). In a study by *Gaebel et al.*, (2011), these bio-printed patches were found to enhance angiogenesis-the formation of new blood vessels-highlighting their promising role in supporting the healing of cardiac tissue after an infarction.[106]

Integumentary (Skin)

Directly bioprinting skin cells and hydrogels onto wounds to accelerate healing. Bioprinting bilayer skin constructs *in vitro* for later transplantation, potentially incorporating sweat glands, hair follicles, and melanocytes.[107]

The effectiveness of direct bioprinting for promoting skin wound healing has been demonstrated by Binder *et al.*, 2010. They used a cartridge-based delivery system to print hydrogels containing fibroblasts and keratinocytes directly onto mouse wounds. This innovative approach successfully accelerated wound healing and supported skin endothelialization, with full recovery observed after eight weeks.[108]

Bioprinting Bilayer Skin Constructs, Cubo *et al.*, 2016 bioprinted bilayer skin constructs using human plasma as the bioink. These constructs, containing various skin cell types, showed remarkable similarity to natural human skin in terms of structure and function, highlighting their potential as skin grafts for transplantation.[109]

Musculoskeletal

Bioactive glass scaffolds can be printed for bone regeneration. Using laser-assisted bioprinting to manufacture bone structures including mesenchymal stromal cells. Bioprinting cartilage constructions using chondrocytes to address cartilage breakdown and replacement.

Bioprinting bone tissue using bioactive glass, Qi *et al.*, 2017 investigated the utilisation of bioactive glass scaffolds containing calcium sulphate hydrate for bone regeneration. Their findings revealed that human mesenchymal stem cells flourished on these scaffolds, and *in vivo* implantation in rats resulted in dramatically increased bone growth compared to controls.[110]

Laser-Assisted Bioprinting for Bone Regeneration Keriquel *et al.*, 2017 repaired defects in mice's bones using LAB. They have printed a bone using collagen, hydroxyapatites, and mesenchymal stromal cells and precisely implanted it in the trouble spots. The bio printed structures produced promising findings, including increased cell viability and bone repair.[111]

Bioprinting Cartilage with Enhanced Properties, *Cui et al.*, 2012 used inkjet bioprinting to create 3D cartilage structures using chondrocytes and PEGDMA. After six weeks of cultivation in a bioreactor, the resulting cartilage-like tissue had a higher collagen composition than natural cartilage, demonstrating the technique's potential for cartilage regeneration.[112]

Bioprinting Bone and Cartilage Tissues

Using an oxidised methacrylated alginate microgel support bath, researchers successfully bio printed human Mesenchymal Stem Cells (hMSCs). Following printing, the hMSCs developed into functional bone and cartilage tissues. This approach shows promise for rebuilding these tissues, particularly in applications requiring elaborate forms and complicated structures.[113]

Bioprinting Macroscale Intestinal Tubes

Scientists developed a method called Bioprinting-Assisted Tissue Emergence (BATE) to create tubes that could function as intestines on a macroscale. They bioprinted cell aggregates within a supportive matrix of Matrigel and collagen. These aggregates are self-organized into tubular structures, mimicking the intestinal tissue's architecture and offering a potential platform for studying intestinal function and diseases.[114]

Bioprinting Dense Tissues-Osteogenic and Chondrogenic

Researchers have explored the use of cell-only bioinks, specifically in the form of spheroids, suspended in a hyaluronic acid bath, to bio print dense, functional tissues. This method allowed for the creation of tissues that could effectively differentiate into bone (osteogenic) and cartilage (chondrogenic) cell lines. By achieving high cell density in these bio printed tissues, this approach holds great promise for enhancing the functionality and integration of bio printed structures, making them more viable for therapeutic applications.[115,116]

Bioprinting Cardiac Tissue Models

In an innovative approach, researchers used cardiac cell spheroids as a cell-only bioink, embedding them in a supportive hyaluronic acid bath to fabricate 3D cardiac tissue models. These models closely mimic the structure and function of native heart tissue, offering a promising platform for investigating heart function, studying cardiovascular diseases, and testing new drug therapies.[117]

Bioprinting Complex Channels within Cell-Laden Hydrogels

To address the challenges of traditional embedded printing techniques, researchers developed an innovative method to create complex channels within cell-laden hydrogels. Instead of using the typical support bath, they used a layer-by-layer printed support matrix made from photocurable Methacrylated Alginate (MeAlg) and Methacrylated Hyaluronic acid (MeHA) hydrogels. By incorporating a sacrificial ink (Pluronic F-127), they were able to form channels within the hydrogel structure, which could later be seeded with endothelial cells to build a functional vascular network. This breakthrough paves the way for creating vascularized tissues that can better support nutrient delivery, opening up new avenues in tissue engineering.[118]

Volumetric Bioprinting of Cellular Meniscus Constructs

Scientists used computed axial lithography, a volumetric bioprinting technique, to create centimetre-scale meniscus constructions in minutes. Using a Methacrylated Gelatin (GelMA)-based ink containing chondroprogenitor cells, they formed meniscus-shaped structures with good cell viability and new tissue creation *in vitro*.[119] This quick and economical approach has enormous potential for producing larger and more complex tissues for regenerative medicine.[120]

Volumetric Bioprinting of Hepatic Organoids

Volumetric bioprinting has also been successfully applied to create hepatic organoids with complex, perfusable structures. In this approach, researchers bioprinted GelMA-based bioink containing hepatic organoids, resulting in structures that exhibited key liver-specific functions, including improved urea and albumin secretion. This advancement opens the door to the creation of bio printed liver tissues, which could be used for transplantation or drug testing, offering new possibilities in both medical treatments and pharmaceutical research.[119]

Organ-on-a-Chip Platforms for Multi-Tissue Interactions

3D bioprinting is being utilised to develop organ-on-a-chip platforms that contain several cell types and tissues, simulating the interactions between organs.[121] For example, scientists developed a liver, heart, and lung tissue organ-on-a-chip system. The system demonstrated that inflammatory cytokines released by the lung had an impact on the cardiac tissue when lung-specific damage was introduced, indicating the platform's capacity to replicate intricate inter-organ interactions.[122,123]

Four-Dimensional Printing for Shape-Morphing Structures

In order to produce pre-programmed structures that change shape over time in response to environmental inputs, researchers have developed 4D bioprinting processes.[124] For instance, Oxidised and Methacrylated Alginate (OMA), which supports cells, was combined with a gradient hydrogel layer of OMA and GelMA to create a shape-morphing bilayer hydrogel disc. This disc made it possible to use hMSCs-only bioinks to create scaffold-free structures that could be extracted after 21 days of growth with chondrogenic development and pre-programmed shapes.[125]

In situ Printing for Direct Tissue Repair

In situ bioprinting involves directly printing bioinks onto the defect site, eliminating the need for separate *in vitro* tissue fabrication. This approach utilizes automated robotic arms or handheld devices to deposit bioinks based on patient-specific medical images. Successful in situ bioprinting has been demonstrated for various tissues, including bone, cartilage, muscle, skin, brain, and dental pulp, highlighting its potential for personalized tissue regeneration.[126]

Cardiac Tissue

3D-bioprinted cardiac tissues using fibrin-based bioinks and rat cardiomyocytes successfully reproduced heartbeat. This model responded to cardiotoxic medications such as adrenaline and carbachol, exhibiting variations in beating frequency.[127] Another model used endothelial cells to generate vascularised heart tissue, allowing researchers to investigate the anti-cancerous effects medication doxorubicin on both cardiomyocytes and endothelial cells.[128]

Renal Proximal Tubules

Researchers bioprinted functional renal proximal tubules, first using a simple model and then a more complex vascularized

model. These models were used to study drug-induced kidney damage, specifically focusing on cyclosporine A's effects on epithelial barrier function and the impact of high glucose and dapagliflozin on glucose reabsorption.[129,130]

Liver Tissue

Multiple approaches were used to create 3D bioprinted liver models: a hepatic lobule model with hiPSC-derived hepatic cells, a model using primary human liver cells in NovoGel, a HepG2 cell-laden Matrigel model, and a liver-on-a-chip platform with HepG2/C3A spheroids. These models were used to investigate drug metabolism, hepatotoxicity, and anti-radiation drug efficacy.[131,132]

Intestinal Tissue

To conduct drug permeability studies, researchers bioprinted a bilayered intestinal tissue model using human intestinal myofibroblasts and epithelial cells. This model was created through a specialized extraction process. In addition, studies on the toxicity of indomethacin showed a decline highly correlated to the dose in the intestinal tissue's barrier function, highlighting the potential of bioprinted tissues for testing drug effects on human tissues.[133]

Tumour Model

Bioprinting has enabled the creation of various advanced models, including a mini-brain model made from glioblastoma cells and macrophages, designed to study the effects of medications and the interactions within the tumor microenvironment. Additionally, a glioma model with improved resistance to the chemotherapy drug temozolomide has been successfully bioprinted. In another study, HeLa cells encapsulated in gelatin, alginate, or fibrinogen were used to bioprint a 3D cervical cancer tumor model. These 3D models demonstrated significantly higher chemoresistance to paclitaxel compared to traditional planar cultures, showcasing the potential of bioprinting for cancer research.[134,135]

Ovarian Cancer: A high-throughput inkjet bioprinting method was used to create an ovarian cancer model on Matrigel, facilitating drug screening applications.[136]

Vascularized Tumour: A complex 3D-bio printed vascularized tumour model, incorporating lung tumour cells, vascular conduits, and growth factor-loaded capsules, was used to study tumour cell invasion, intravasation, and the effectiveness of targeted toxins.[137]

Ophthalmology: 3D bioprinting enables the fabrication of artificial corneal structures using bioinks composed of materials like collagen and corneal stromal cells. This approach aims to address the shortage of donor corneas and provides customized solutions for patients with corneal diseases or injuries. Studies have demonstrated the successful creation of corneal stromal equivalents with appropriate optical and structural properties.[138]

CONCLUSION

By expanding the realm of what is feasible at the nexus of biology and engineering, 3D bioprinting is a monument to human creativity. While challenges remain, the vision of a future where we can build functional organs, personalize medicine at the cellular level, and deepen our understanding of life itself is too compelling to ignore. This is a call to action for scientists, engineers, ethicists, and policymakers alike. Continued investment in research, fostering interdisciplinary collaboration and ensuring responsible innovation will be critical to transforming the promise of 3D bioprinting into a tangible reality. The journey ahead will be demanding, but the potential to alleviate suffering, extend human life, and reshape the future of medicine makes it a journey well worth taking.

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ABBREVIATIONS

3D: Three Dimensional; **CT:** Computed Tomography; **MRI:** Magnetic Resonance Imaging; **LAB:** Laser Assisted Bioprinting; **AM:** Additive manufacturing; **EBP:** Extrusion based Bioprinting; **VEGF:** Vascular Endothelial Growth Factor.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The review summarized the concept of 3D bioprinting and emphasized the techniques and challenges of 3D bioprinting It also detailed on applications of 3D bioprinting. The review Provided the information related to patents on 3D bioprinting.

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