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Hydrogel-based bio-inks: Current progress and future directions in 3D printing

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Hydrogel-based bio-inks have become a cornerstone in the advancement of 3D bioprinting, particularly for tissue engineering applications. This review explores the current progress and future directions in the development of hydrogel-based bio-inks for 3D printing. Hydrogels, with their high water content and biocompatibility, closely mimic the natural extracellular matrix, making them highly suitable for creating tissue scaffolds. Recent advancements include the enhancement of mechanical properties, printability, and bioactivity through the incorporation of nanomaterials and the combination of natural and synthetic polymers. These improvements have led to better structural integrity and functional capabilities of printed scaffolds. The integration of bioactive molecules and growth factors within hydrogel matrices has further promoted cell proliferation and differentiation, and enhancing tissue regeneration. However, challenges such as the need for standardized protocols, improved printing resolution and precision, and the development of bio-inks that can support complex cellular microenvironments are still exiting. Future research is likely to focus on developing multifunctional bio-inks capable of adapting to diverse biological and mechanical needs. This includes stimuli-responsive hydrogels and novel bioprinting techniques like microfluidic-based and laser-assisted printing. Addressing these challenges and exploring new directions will significantly advance the field of tissue engineering and regenerative medicine.

Keywords: 3D printing, Additive manufacturing, Biocompatibility, Bio-inks, Hydrogel

Introduction

The goal of the developing scientific discipline of tissue engineering is to replicate or create functional organs or tissues that mimic those found in humans. To create structures that resemble tissues and organs, additive manufacturing is a potent approach that is widely employed in regenerative medicine and tissue engineering. These structures can be used as in vitro diagnostic models, experimental models, transplant candidates, and diagnostic predictors. Bioprinting is a technology that combines biomaterials, living cells, and coordinated motor systems to form complex structures[1]. It builds functional tissues or organs by depositing biomaterials, including cells, layer by layer in a predetermined design.

By giving precise control over the structures produced, this technology enables the creation of intricate designs, including tissue models, biomedical products, and tissue engineering scaffolds with unique mechanical, permeability, and porosity characteristics. Since human body cells reside in a 3D matrix, our method replicates the original 3D forms and functions of biological tissues. Compared to traditional tissue seeding for tissue engineering, three-dimensional (3D) bioprinting has a number of benefits. It enables the precise arrangement of cells, biomolecules, and biomaterials in three dimensions within predetermined spatial parameters[2-5]. Furthermore, 3D bioprinting enables the creation of complex structures with high precision, speed, and affordability, as well as patient-specific designs. A computed tomographic scan of a patient's injured or damaged body part can be used to create bio-mimicked structures using CAD, which oversees the development of a patient-specific design. The primary goal of this technique is to convert a scanned 2D image into a CAD model[6-11].

Nowadays, there are numerous 3D printing methods available, including stereolithography and extrusion-based bioprinting, which use distinct mechanics and guiding principles to create 3D structures[12]. It is helpful for researchers and practitioners to compare and evaluate the benefits and drawbacks of each methodology in order to determine which is best for their particular use. Recent years have seen a huge surge in published work on bio-fabrication, with a focus on extrusion-based bioprinting to produce relatively large, centimeter-sized structures with resolutions down to the 10 µm scale. A "resolution/time of manufacturing" ratio can be used to assess this capacity, which is beneficial for a variety of TE applications because it allows for rapid fabrication times while resolving microscale characteristics.

These methods make it easier to create implants or other structures that are customized for each patient and that closely mimic the complicated and asymmetrical geometric features of real tissue. It is also possible to create devices with complex interior pore networks that can be adjusted for pore size and interconnectivity to promote nutrition transfer and preserve transplanted cells. The most widely used type of 3D printer for bioprinting is an extrusion machine, which deposits material layer by layer using screw, piston, or pneumatic syringes. This procedure makes it possible to put materials precisely and carefully, producing 3D models that are accurate and detailed. Similar to conventional material jet printers, some 3D bioprinters use lasers as a power source or photo-initiators to accelerate the polymer crosslinking process, while others deposit thermal bio-ink drop by drop[13-15].

The ability to successfully pick bio-inks that offer a distinct environment to support cellular growth is essential for the success of 3D bioprinting. Technological developments in 3D printing have made it possible to precisely control the composition of scaffolds used in bone tissue engineering. For improved degradation and biocompatibility, scaffolds reinforced with hydroxyapatite can be used in place of coral, and methods for printing calcium carbonate nanoparticles in its stead have been developed. The efficiency of these scaffolds in promoting bone growth and repair has been confirmed by characterisation techniques and in vivo investigations. Numerous biomaterials, such as synthetic hydrogels (polyethylene glycol, polyurethane, poly(vinyl alcohol), polylactide, and derivatives) and natural hydrogels (collagen, fibrin, silk, chitosan, cellulose, agarose, carrageenan, bacteria, and hyaluronic acid), have been researched in order to create physiologically complex heterogeneous human tissues.

3D bioprinting is predicted to be ushered to a new era of tissue engineering in which complex morphological changes or functional responses can be triggered to enable 3D printed scaffolds loaded with cells. However, two significant challenges in this research remain the abuse of smart hydrogels and the lack of cytocompatible stimuli. This study examines methods for using hydrogel-based bioinks to replicate the extracellular matrix environment of real tissue and provides a comprehensive examination of several 3D printing processes for producing tissue scaffolds. The rheology and printability of organic hydrogel-based bioinks are investigated. This article also discusses the potential, difficulties, and developments related to 3D bioprinting of organic and synthetic hydrogel bioinks. It also looks at the development of 3D printing in tissue engineering and its application to organic hydrogel bioinks.

Bioprinting Technology

The novel approach of 3D bioprinting to create bone tissue is made possible by developments in materials science, cell biology, and 3D printing technology. Layer by layer, 3D bioprinting creates tissue structures, just like conventional printing. There are three primary steps in this process. Preparing a bio-ink made of cells and a hydrogel for mechanical support and biocompatibility is the initial step, or pre-processing. The soft tissue structure is then formed during the processing phase by bioprinters depositing the bio-ink in accordance with a computerized model. To assure stability, this bio-ink is solidified using techniques like UV radiation or temperature adjustments. The last stage of post-processing is using mechanical or electrical impulses to stimulate the tissue's cells in order to promote development and differentiation[16, 17].

Understanding the geometry and structure of the biomimetic scaffold needed for the injured tissue is the first step in designing the target tissue or organ. MRIs and CT scans are examples of medical imaging methods that are useful in creating a strategy for the cell-loaded scaffold. Next, a mixed model is made to show how the cells and components are distributed throughout the scaffold. Digital scans or 3D modeling software are utilized to build these digital models, which are then employed in bioprinting to construct patient-specific bio-scaffolds. These models are transformed into STL files, which include surface geometry data, using CAD software. The STL file is then converted by a slicer program into gcode, a machine-readable format that the 3D printer can read. Additionally, this application improves the slicing data by adding machine-specific information[18]. The many stages, from data collection and modeling to bioprinting applications, are shown in Fig. 1.

3D Printing Methodologies

Bioprinting is the use of biomaterials and living cells along with various 3D printing processes to

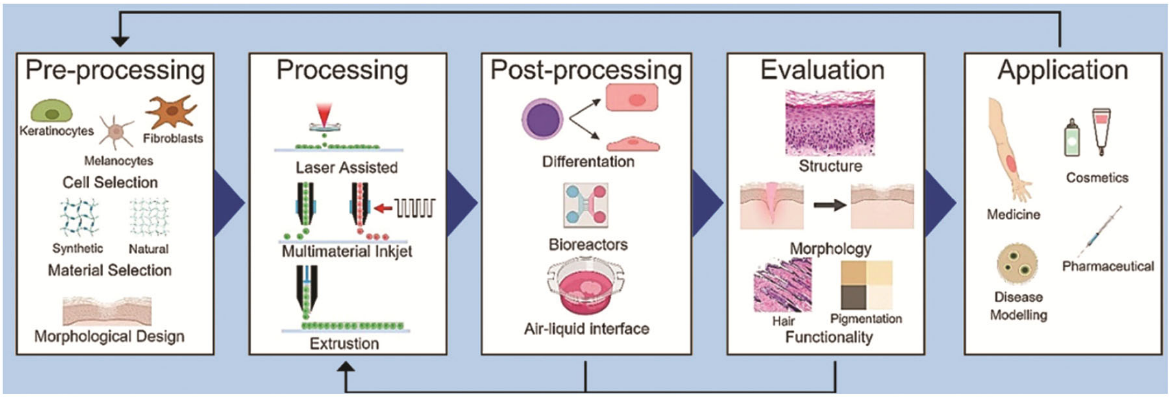

Fig. 1 — Bioprinting methodology

generate complex tissue architectures and organ-like creations. These methods can be generally divided into multiple types, each of which provides unique benefits and capabilities for applications involving bioprinting[19-21]. One category includes material extrusion bioprinters such as Laminated Object Manufacturing and Fused Deposition Modeling. These bioprinters create complex tissue topologies by layering bioinks using extrusion-based processes. Another category includes methods such as Stereolithography and Selective Laser Sintering, which solidify liquid or powdered biomaterials into precise three-dimensional structures, respectively, using UV-curable or laser-based resins. Heat-sensitive biomaterials can be printed using Ultrasonic Additive Manufacturing, a process that uses ultrasonic radiation to fuse thin material layers together. Binder jetting is the process of selectively depositing a liquid binding agent onto powder layers to create scaffold-like structures.

Additionally, by employing light projection to selectively cure photosensitive materials, High-resolution printing is made possible by PolyJet and Digital Light Processing technologies. Direct ink writing allows precise bioink deposition through controlled extrusion, whereas liquid deposition modeling for bioprinting applications uses liquid-based materials. By providing researchers and healthcare providers with a range of tools to create complex tissues and organs for regenerative medicine, drug discovery, and disease simulation, these 3D printing techniques are crucial to the advancement of bioprinting technology[22].

In bioprinting, fused deposition modeling, or FDM, is a popular additive manufacturing method for producing three-dimensional tissue constructions. With this process, a thermoplastic filament is extruded onto a build platform via a heated nozzle, where it solidifies layer by layer to create the desired structure. The procedure is controlled by computer-aided design (CAD) software, which makes it possible to generate intricate forms and deposit them precisely. Polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) are common biocompatible thermoplastics used in FDM due to their mechanical properties, biocompatibility, and ease of processing. The current focus of FDM-based bioprinting research is on generating new biomaterial formulations for tissue regeneration, enhancing cell viability and functionality within printed constructs, and optimizing printing settings. For instance, Wei *et al.* created a neural network model to forecast and maximize the specimens' tensile strength for tissue engineering that are printed using FDM. Furthermore, Luo *et al.* examined the mechanical qualities, energy absorption, and recovery of ethylene vinyl acetate (EVA) in FDM printing and reported mechanical characteristics and printing quality that were good[23-25].

Another lucrative manufacturing method used in bioprinting to produce three-dimensional tissue structures is called laminated object manufacture (LOM). Using heat or glue, thin sheets of material, such as paper or polymers, are assembled layer by layer to create the desired product. Scaffolds in bioprinting are frequently constructed from biocompatible materials such biodegradable polymers or paper based on cellulose[26]. The first step in the process is to digitally slice a CAD model. The material sheets are then either cut or laser-cut to fit the contour of each layer. The final 3D structure is subsequently formed by stacking and bonding these layers together. LOM has advantages including quick build times, affordable prices, and the capacity to create large-scale structures. The focus of LOM-based bioprinting research is on improving cell integration and viability, exploring new materials for tissue engineering applications, and fine-tuning process parameters for exact control over scaffold features. The goal of recent research has been to replicate the natural tissue milieu by creating scaffolds with regulated porosity and hierarchical designs through the use of LOM. Furthermore, multi-material printing and the incorporation of sacrificial elements are two LOM technological breakthroughs that show promise for creating intricate, functional tissue structures for drug discovery and regenerative medicine[27-28].

A method of additive manufacturing called Selective Deposition Lamination (SDL) combines elements of 3D printing and conventional lamination. To create a three-dimensional item in SDL, layers of solid material—typically in the shape of sheets or rolls—are laminated and selectively deposited. Unlike other 3D printing methods, which frequently employ powdered or semi-liquid ingredients, this approach is unique. Various biomaterials, including metals or polymers that are particularly helpful in bioprinting, can be employed in SDL. The required object is first represented digitally and then separated into thin cross-sectional layers. Using a cutting or stamping tool, each layer is removed from the material and placed onto the build platform[29]. Depending on the materials employed, heat, pressure, or glue is then used to adhere the layer to the preceding one. The thing is further layered until it is completely constructed. SDL is especially useful in tissue engineering since it makes it possible to create intricate structures with precise mechanical characteristics and great resolution. SDL-based bioprinting research is moving quickly forward, with an emphasis on generating new biomaterial formulations for tissue regeneration applications, improving cell viability and functionality, and optimizing process parameters[30].

Another method with special tissue engineering attributes is ultrasonic additive manufacturing (UAM), which offers a versatile platform for building intricate scaffolds and structures with qualities that support tissue regeneration. Hydrogels, bioactive ceramics, and biodegradable polymers are examples of biocompatible materials that can be linked using the ultrasonic bonding of thin metal layers, which is the foundation of UAM's operation[31–33]. One of UAM's many notable qualities is its ability to create complex structures without the need for melting or high temperatures, enabling the precise deposition of different materials. This enables the scaffold matrix to be supplemented with cells, growth agents, and biomolecules. Because UAM-produced scaffolds can closely resemble the extracellular matrix architecture of genuine tissues, their mechanics, porosity, and interconnectivity can all be regulated. This makes them advantageous.

The tissue regeneration is aided by these scaffolds, which promote cell adhesion, proliferation, and differentiation. Furthermore, UAM provides reproducibility and scalability, enabling the creation of organ models and implants customized for each patient's unique anatomical needs. The main goals of current UAM tissue engineering research include process parameter optimization, evaluation of the mechanical and biocompatibility characteristics of UAM-produced scaffolds, and investigation of novel applications in regenerative medicine. The viability of using UAM to create scaffolds with hierarchical structures as well as bioactive features that support tissue ingrowth and cell adhesion has been demonstrated by recent investigations[34-35]. Hybrid scaffolds with improved bioactivity alongside regenerative potential are being created by combining UAM with other bio-fabrication methods like electrospinning and bioprinting.

A novel additive manufacturing method called binder jetting has great promise for bioprinting applications such as tissue scaffolding and organ-like structures. A liquid binding agent is applied selectively onto a powder bed composed of biocompatible materials, like hydrogels or ceramics, in this procedure. Using a computer-aided design (CAD) model as a guide, the binding agent is applied layer by layer to create the object's desired shape [36]. Each layer of powder is deposited on top of the construction platform, and the procedure is repeated until the entire object is produced. Drying and curing are examples of post-processing procedures that harden the binder and eliminate surplus powder.

It may also be added that binder jetting also has several other benefits for tissue engineering, such as the capacity to create intricate, porous scaffolds that may be customized in terms of pore sizes, shapes, and distributions so as to replicate the extracellular matrix (ECM) architecture found in natural tissues. Additionally, it permits the scaffold matrix to be expanded to include cells, growth factors, and bioactive compounds, all of which improve cell adhesion, proliferation, along with differentiation.

Furthermore, Binder Jetting is an adaptable and scalable technique that can create organ models and implants customized to meet each patient's unique anatomical needs. The current focus of binder jetting bioprinting research is on investigating advanced tissue regeneration applications, evaluating the mechanical and biological properties of printed structures, and optimizing printing parameters. Zhou *et al*., for instance, showed that it is possible to create scaffolds with hydroxyapatite powder and a water-soluble glue utilizing Binder Jetting and yet achieve high geometric accuracy[37-38].

Another cutting-edge additive manufacturing method that has great promise for bioprinting is selective laser sintering (SLS), which can be used to create intricate tissue scaffolds and organ-like structures. Using a powerful laser and a computer model as a guide, SLS selectively fuses powdered materials—like ceramics or polymers—layer by layer. Bioprinting commonly uses biocompatible materials, such as natural or synthetic polymers like polycaprolactone (PCL) or poly(lactic-co-glycolic acid) (PLGA). The laser selectively sinters the powder particles, combining them to create solid structures that adhere to the specified design. SLS provides a number of benefits for tissue engineering, one of which is the capacity to create intricate, porous scaffolds that accurately replicate the pore size, shape, and distribution of natural tissue[39-40]. Additionally, SLS makes it easier for cells, growth hormones, and bioactive substances to be incorporated into the scaffold matrix, which encourages cell adhesion, proliferation, and tissue renewal. Furthermore, SLS makes it possible to create organ models and implants that are unique to each patient and their anatomical needs, offering individualized medical treatments. The current focus of SLS bioprinting research is on improving the mechanical and biological qualities of printed constructions, investigating new materials, and optimizing process parameters. Recently, Han *et al*. used SLS to create a tailored porous scaffold that showed good in vitro and in vivo biocompatibility by integrating Borate bioactive glass (BBG) into a Polycaprolactone (PCL) polymer matrix[41].

Stereolithography and Digital Lightning

Stereolithography (SLA), a cutting-edge additive manufacturing method, holds enormous promise for creating complex tissue architectures and organ models in bioprinting. The fundamental process of SLA involves layer-by-layer selective solidification of a liquid photopolymer resin using a computer-controlled UV laser directed by a digital model. Biocompatible hydrogels or bio-inks, which are printed with cells and growth factors, are frequently employed in bioprinting. The liquid resin is precisely cured by the UV laser, creating solid structures that follow the predetermined design. SLA has many advantages for bioprinting and tissue engineering[42]. It makes it possible to create elaborate, highly-resolution scaffolds that closely resemble the natural architecture of tissues and organs, complete with intricate features and complex geometries.

Further to note that for the purpose of cell infiltration, nutrition diffusion, and tissue regeneration, scaffold porosity, pore size, and distribution must be precisely controlled, which is what SLA offers. To further improve cell adhesion, proliferation, and differentiation, SLA facilitates the inclusion of cells, growth factors, and bioactive compounds inside the scaffold matrix. The development of novel biomaterial formulations, optimization of printing parameters, and enhancement of the mechanical and biological properties of printed constructions are the main areas of recent progress in SLA-based bioprinting[43-45]. For example, Hu *et al*. developed a novel 3D printing method that might be regarded as a sophisticated version of stereolithography. They made use of a supporting bath that is thermo-reversible and simple to remove by reducing the temperature. In order to construct the 3D item, this approach uses a vat of liquid resin that is cured layer by layer using a light source.

Similar to liquid resin, the thermo-reversible supporting bath can solidify or liquefy in response to temperature variations. This function makes it possible to print delicate structures using hydrated materials without the need of harsh chemicals or time-consuming support removal processes by providing temporary support that is readily removed by changing the temperature. In another study, Rstakyan *et al*. created a ceramic resin with silica and hydroxyapatite and utilized stereolithography to print tissue scaffolds with enhanced bioactivity. Their findings demonstrated reduced cell toxicity and enhanced mechanical properties. Similarly, Paulina *et al*. used masked stereolithography using a mixture of silicone resin and photocurable acrylates to create porous scaffolds[46]. They claimed that by employing stereolithography, the overall porosity of the scaffold and its geometric complexity were improved.

A new bioprinting technique called Digital Light Processing (DLP) has the ability to produce fast and high-resolution models of organs and intricate tissue constructions. In order to create three-dimensional structures, DLP projects light patterns onto a vat of photosensitive resin, where the resin solidifies layer by layer. This process is known as digital micro mirror technology (DMD). Biocompatible hydrogels or bioinks comprising of cells alongside growth factors are commonly utilized in bioprinting. Based on a computerized design, the DMD accurately regulates where the resin is cured by light. DLP has various advantages for bioprinting and tissue engineering. It makes it possible to quickly produce sophisticated scaffolds that closely resemble the natural milieu of tissues and have high resolution and detailed features. Additionally scalable, DLP boosts efficiency and throughput by generating numerous structures simultaneously within a similar size volume. DLP can also include a variety of materials and bioactive elements in the scaffold matrix to promote cell adhesion, growth, and differentiation[47-50]. According to recent investigations, DLP can be used to fabricate tissue scaffolds with complicated geometries that are appropriate for a variety of tissue regeneration applications. The materials printed by DLP also exhibit good biological properties[93]. Furthermore, Greant *et al.*'s recent work[94] employing DLP to create intricate thermoresponsive structures for tissue engineering applications further establishes the technology's usefulness in the creation of scaffolds for tissue engineering.

It is also to be noted that with the use of sophisticated bioprinting techniques like Direct Ink Writing (DIW), intricate three-dimensional tissue constructions with programmable designs may be made with exact control over the deposition of bio-inks. In order to create complex structures layer by layer, DIW works by extruding bioinks onto a substrate using a fine nozzle or syringe tip, adhering to patterns predetermined by computer-aided design (CAD) software. Bio-inks used in bioprinting are usually made of a viscous hydrogel matrix containing biomolecules, growth factors, and cells[51]. The bio-ink's rheological characteristics are precisely calibrated to guarantee regulated extrusion and preservation of shape throughout the printing procedure. DIW has many advantages for bioprinting and tissue engineering.

It makes it possible to deposit different kinds of cells and materials within the same construct, resulting in heterogeneous tissue architectures that can be precisely controlled in terms of cell distribution. Furthermore, DIW offers design flexibility because it may create scaffolds with customized pore sizes, mechanical qualities, and geometries to match the unique requirements of various tissues and organs. Moreover, DIW makes it possible to incorporate bioactive elements into the scaffold matrix, which improves tissue regeneration, cell adhesion, and proliferation. The DIW approach is being used in many investigations using various systems, including screw- and piston-based mechanisms. While a screw-driven system offers better spatial control and the capacity to dispense bio-ink at higher viscosities, a piston-driven system offers consistent control over the flow of bio-ink. Nozzle obstructions can be reduced by using bio-ink in its liquid phase; nozzle diameters are usually larger than 100 µm. Nevertheless, this might cause harm or even death to cells due to the increased shear stress it causes. Xu *et al*. created a biodegradable Fe scaffold having variable porosity, and Kachit *et al*. created a metallic tissue scaffold equipped to macropores using DIW. These instances highlight the emphasis of numerous research projects on creating innovative DIW-based tissue engineering materials.

Bio-inks and their Rheological Behaviour

The terms "bio-ink" and "bio paper" were first used in relation to organ printing in 2003. Bio-ink, a mixture of bioactive compounds, biomaterials, and cells used to create the desired printed scaffold, is a crucial component in 3D bioprinting. The initial idea was to use tissue spheroids or living cells as the "bio-ink" for bioprinting on hydrogel biopaper. Nevertheless, the word "bio-ink" originally referred to a biological material that was mixed with or added to three-dimensional hydrogels. In the beginning, 3D bioprinting studies commonly employed cells and cell clusters as the bio-ink. Some experts contend that in order to preserve cell viability, support cellular processes, maintain shape fidelity, match the mechanical characteristics of tissue, and assure biocompatibility and stability, an efficient bio-ink formulation needs to be modified "functionally and structurally" even at this early stage. Optimization is crucial for producing bioprinted tissues that are viable, functional, and reproducible for use in biomedical applications such as tissue engineering and regenerative medicine [52–55]. At the same time, numerous studies have been conducted on various types of bio-ink, and additional additive manufacturing techniques for bioprinting are now accessible.

The word "bio-ink" has been defined recently into four categories: bio-inks that function, bio-inks that flee, sustaining bio-inks, and structuring bio-inks. After administration, supporting bio-inks function as a synthetic extracellular matrix (ECM) to support cell growth and population maintenance. A 3D bioprinted scaffold may have fleeing bio-inks removed fast to form internal spaces or channels. The printed scaffolds are mechanically stabilized by the structural bio-inks. When 3D bioprinting is done, functional bio-inks provide mechanical, electrical, and biochemical signals to the scaffold that impact biological behaviour. These, together with polycaprolactone thermoplastics, may also serve as transient fleeing bio-inks. Since vital elements play a critical role in the ultimate functionality of a 3D bioprinted scaffold, this classification of bio-ink is based on component materials rather than the fabrication technology utilized [56].

The principal physicochemical criteria influencing hydrogel printability are their rheological properties. Rheology is the study of how substances move and change in response to forces. Bio-ink undergoes a transformation while passing through the nozzle in 3D extrusion-based procedures. It starts out in a bulk resting state, moves to a greater shear form, and finally settles into a new resting state. Shear stress, viscoelastic shear moduli, viscosity, and elastic recovery are the main rheological characteristics of these transitions. Fig. 2 lists a number of important considerations for choosing a bio-ink for 3D printing using biomaterials.

Further, viscosity, a measure of a fluid's resistance to flow under stress, has a major impact on the precision and efficacy of cell encapsulation in printing. In general, higher viscosities result in better printing quality. High viscosity, however, also causes more shear stress, which might have an effect on the cells suspended in the bio-ink. A polymer's concentration in solution and molecular weight are two important determinants of its viscosity. The ratio of shear stress to shear rate determines viscosity[57-58]. Shear stress and shear rate in Newtonian fluids have a linear relationship. Non-Newtonian fluids have a nonlinear connection, wherein the ratio experiences a decrease or an increase. Shear-thinning and shear-thickening fluids are examples of non-Newtonian fluids that can be categorized as either time-dependent or time-independent.

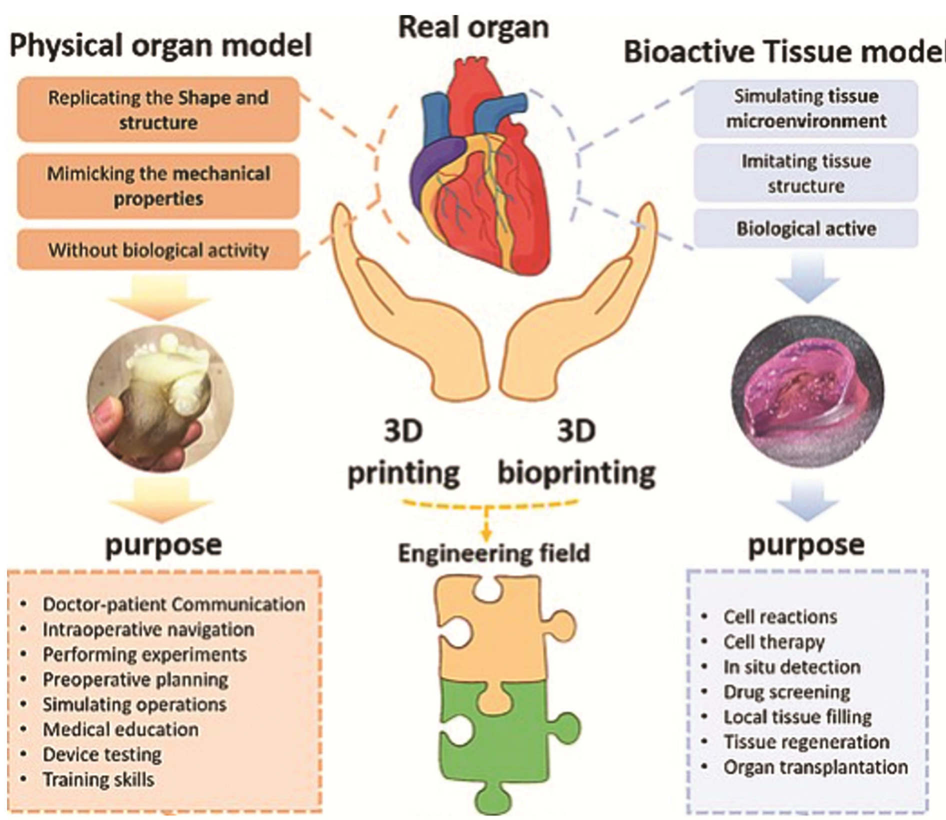

Fig. 2 — Bio-ink for 3D printing using biomaterials

Moreover, the most prevalent time-independent non-Newtonian fluid behaviour, known as shear-thinning, happens when viscosity decreases with increasing shear rates. Materials utilized for extrusion printing, such as polymer solutions, partly cross-linked hydrogels, polymer melts, and gels, generally have this feature. Because viscosity drops throughout the extrusion process when shear pressures increase, shear-thinning helps bio-inks to be extruded while retaining their original shape. Following extrusion, a drop in the shear rate leads to an increase in viscosity, which aids in maintaining the printed shape. In order to prevent the printed structure from collapsing before secondary crosslinking takes place, high zero-shear viscosity enables constant flow and mild distortion. For instance, shape maintenance is aided by the high zero-shear viscosity of calcium phosphate cement[59].

The molecular mechanisms of shear-thinning and physicochemical interactions that are responsible for shape preservation vary amongst bio-inks. Higher molecular weight polymer molecules are entangled and randomly orientated while they are at rest. Shearing reduces internal resistance and viscosity by untangling and aligning these polymer connections. These bio-inks' rapid transition from a liquid to a solid state ensures form retention. Shear-thinning also occurs in bio-inks based on colloidal solid suspensions, dispersions, and pastes because shear causes the connections between solid particles to break[60-62]. Shape stability is supported by the strong viscoelasticity at rest that arises from the reinforcing of connections amongst the suspended particles. Polymer solutions, such as dispersions of nano-silicates using biomaterial inks or bio-inks, and calcium phosphate cement are notable examples. Materials that shear-thicken are another class of fluids that behave in a time-independent manner. Non-Newtonian materials could have viscosity that is based on time as opposed to shear. When a substance is thixotropic, its viscosity gradually decreases at a fixed shear rate and then returns to its initial level after a resting period. On the other hand, rheopectic behaviour entails a gradual increase in viscosity at a steady shear rate.

Hydrogel Categories

It is to be noted that hydrogels are hydrophilic polymers that are primarily made of water. They are biocompatible, have mechanical and chemical characteristics that may be adjusted, and resemble the extracellular matrix (ECM) found in nature. As such, hydrogels are very promising for the creation of scaffolds in tissue engineering (TE). Hydrogels with exact three-dimensional dimensions and forms can be produced in the biomedical area using various additive manufacturing processes, especially for bone and tissue engineering applications. Hydrogel scaffolds are known to promote cell migration, proliferation, and organization in the context of wound healing and tissue regeneration[63].

Hydrogels are also perfect for encasing cells. Hydrogels can be categorized according to their origin or source, degradability, crosslinking techniques, and stimulus reactivity, as shown in Fig. 3. The idea of 3D printing hydrogels was born out of the need for smart materials, which are hydrogels that respond to outside stimuli. Hydrogel degradability is a crucial consideration for tissue engineering applications. Based on their origin, hydrogels can be divided into two groups: synthetic and naturally occurring. Numerous creatures, including humans, animals, plants, and microorganisms, can be used to create natural hydrogels.

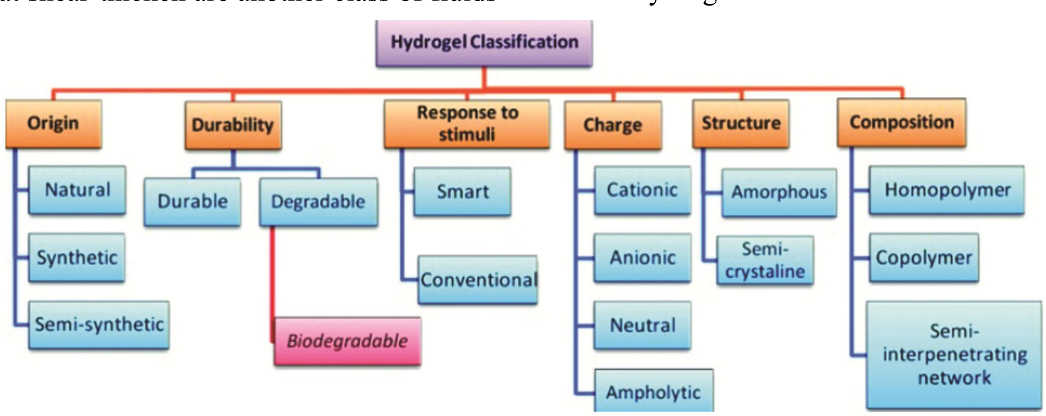

Fig. 3 — Classification of hydrogels

Natural Hydrogel and Chitosan

Hydrogel and bio-ink have important applications in the fields of tissue engineering and bioprinting. Hydrogels, which are three-dimensional networks of hydrophilic polymers that promote cell growth and multiplication, make comprise the structural matrix for bio-inks. Bio-inks, which are living cells suspended in a hydrogel matrix, are used to accurately deposit cellular structures layer by layer, mimicking the structure of real tissue [64-65]. Through the provision of a biocompatible milieu brimming with nutrients and growth factors, the hydrogel component of bio-inks maintains cell viability and function after printing while also facilitating the extrusion process during bioprinting. The combination of hydrogels and bio-inks is driving bioprinting technology, enabling the creation of intricate tissue structures with a high degree of resemblance to genuine tissues. This holds great potential for applications in regenerative medicine. Because hydrogels can react to physiological stimuli like pH, temperature, and particular enzymes, they can release drugs selectively and at specific sites. By ensuring that medications are selectively released at the desired location of action, this responsiveness maximizes therapeutic efficacy and reduces adverse effects[66]. Some naturally occurring hydrogels that may find use in tissue engineering are listed below.

After cellulose, the natural polymer chitin is the second most common organic substance worldwide. It is mostly found in the exoskeletons of arthropods, including insects and crustaceans, and the cell walls of fungi. Chitin can be partially deacetylated to produce chitosan, a cationic polymer known for its exceptional biocompatibility, biodegradability, and low immunogenicity. These characteristics make chitosan a great option for tissue engineering applications, where it can be used as a scaffold to encourage cell growth and tissue regeneration. Moreover, systems that respond to specific physiological states can be designed thanks to chitosan's pH sensitivity, which enhances targeted delivery [67]. While chitosan typically does not dissolve in water, it does dissolve in somewhat acidic ($pH < 6$) solutions.

The growth of hydrogen bonds, which are affected by pH, is strongly linked to the production of chitosan hydrogels. Chitosan interacts with polyanions to generate three-dimensional crosslinked composites because of its high charge density. Because of its adaptability, chitosan and its derivatives find extensive use in a variety of industries, including biomedicine, agriculture, and manufacturing. Some of the products that use it include hydrogels, films, scaffolds, drug-releasing bandages, wound dressings, suture lines, tissue engineering, drug delivery, cancer therapy, bone regeneration, and cartilage repair.

Chitosan is a preferred option for drug delivery systems because of its exceptional qualities, which include its mucoadhesive nature, biocompatibility, and capacity to modify drug release kinetics. For example, Yin *et al.* investigated a novel chitosan-based material for self-regulated insulin administration that released insulin in a controlled way in response to glucose levels. The in vitro investigations demonstrated the non-toxicity of this chitosan material, underscoring its potential uses in insulin delivery applications. Chitosan's distinct properties originate from its molecular makeup, which is comprised of glucosamine and N-acetylglucosamine repeating units[68-70]. By adjusting the degree of deacetylation during synthesis, one can modify the physicochemical properties of chitosan, including its solubility, charge density, and molecular weight. Deacetylation is a typical process that turns chitin into chitosan. It can be done chemically (with alkaline conditions) or enzymatically (with deacetylase enzymes). Through this procedure, acetyl groups in chitin are eliminated to create chitosan.

Alginate and Synthetic Hydrogels

In tissue engineering, alginate—a linear polymer derived from bacteria or brown algae—is frequently utilized to create hydrogel scaffolds. Alginate is a naturally occurring polysaccharide that provides biocompatibility and biodegradability. When multivalent cations are added, alginate gels. One obvious drawback of alginate is its lack of cell attachment sites, which may limit cell adhesion and activity. To overcome this limitation, alginate hydrogels can be enhanced with other biomaterials or cell-recognition peptides, like the tripeptide Arg-Gly-Asp (RGD)[71-72]. These alterations enhance cell adhesion and proliferation, which makes it easier to create tissues that function.

Polymers that are natural or synthetic can be used to make hydrogels. Hydrogels based on natural polymers offer advantages including biodegradability, biocompatibility, and the capacity to sustain cellular processes, but they also have drawbacks. These restrictions include the possibility of bacterial infection, allergic or inflammatory responses, and inadequate mechanical qualities. Conversely, for a variety of uses, synthetic hydrogels can be designed with precise forms and modifiable rates of deterioration. For instance, polyvinyl alcohol (PVA) hydrogels are extremely appropriate for biomedical applications because of their nontoxicity, biocompatibility, and biodegradability[73]. They are also known for their high mechanical strength and self-healing powers under some circumstances,. Commercial PVA comes in many forms with different stereoregular structures (i.e., atactic, syndiotactic, and isotactic), which affect its physical and chemical characteristics.

Further, as a result of its flexibility, polyethylene glycol (PEG), sometimes referred to as poly(ethylene oxide) or PEO, is another synthetic polymer that is frequently utilized in tissue engineering. PEG can be changed to create PEG-diacrylate or PEG-dimethacrylate (PEGDMA/PEGDA), which can then undergo photopolymerization to create crosslinks and hydrogels. Hydrogel scaffolds with regulated properties can be produced with this approach and used for a variety of purposes, including the encapsulation of cells and the production of three-dimensional structures.

PEGDA-based scaffolds have drawbacks as well, such as the inability of cells to adhere to and pass through scaffolds thicker than 1 mm in the absence of nutrient delivery systems. In order to improve functionality, researchers have created PEGDA-PCL scaffolds that include polycaprolactone (PCL) electrospun nanofiber membranes. Furthermore, Ma *et al*. examined how 3D-printed polymeric scaffolds tainted under various circumstances and found that micro-channel circulation had a major impact on the characteristics of the scaffolds, including weight loss, water absorption, and shape. The significance of managing degradation processes for efficient tissue regeneration is highlighted by this study. Many times, single-component hydrogels fall short of all the requirements for mechanical performance, biocompatibility, and biodegradability [74-76]. Composite hydrogels composed of several components or including nanoparticles have been created as solutions to these problems. These composite hydrogels perform better for a range of applications thanks to their remarkable mechanical, chemical, and physical characteristics.

Challenges to 3-D Printing

The field of bioprinting has a multitude of opportunities for the production of synthetic tissues; nevertheless, the use of hydrogel scaffolds and scaffolding techniques to emulate natural extracellular matrices presents unique problems. These hurdles include the creation of complex tissues that require a certain ECM composition and a range of cells, as well as problems with biocompatibility that can result in toxicity and cell death. Even though creating tissues with a single cell type has advanced significantly, challenges still exist when the extracellular matrix has to sustain several cell types, correct uneven cell seeding, and provide sufficient cell penetration through precise temporal and spatial control. Because engineered tissues lack complex microvasculature, they also have difficulty sustaining cell survival and function. Furthermore, certain materials have weak mechanical qualities at both the macroscopic and microscopic levels, which make them unsuitable for bioprinting soft or non-load-bearing tissues. Notwithstanding these difficulties, 3D bioprinting is still an innovative and promising technique for the biomedical industry. The creation of optimal bio-inks is still ongoing, despite significant global advances. This technology may eventually prove to be profitable. Using functionally sensitive materials that change momentarily in response to external stimuli is a promising strategy that could potentially overcome the present drawbacks of hydrogel-based bioprinting. Large-scale tissue construct fabrication has limitations, but 3D bioprinting provides a workable workaround.

While cell-laden bio-inks are widely used, other options such as decellularized bio-inks, bio-inks based on extracellular matrix, spheroids, and cell aggregates all exhibit considerable promise for 3D bioprinting-based tissue and organ creation. However, the applicability of these approaches to other tissues and organs may be limited due to the requirement for vast volumes of particular cells. The sector could be substantially improved by developments in bio-inks and high-resolution, low-cost bioprinters. The utilization of novel biomaterials with supramolecular characteristics, reversible cross-linking polymers and stimuli-responsive hydrogels as bio-inks, are promising future possibilities. These advancements could pave the way for the development of patient-specific devices and tissues, which would result in the construction of intelligent or programmable tissue structures.Smart polymers have the potential to enhance cell activity and interaction in printed tissues by modifying their characteristics in response to external stimuli including temperature, pH, and

Table 1 — Challenges in 3D printing

Challenge	Description
1. Material Limitations	Limited availability of suitable materials for various applications. Different materials have different properties, and not all can be used in 3-D printing.
2. Print Speed	3-D printing can be a slow process, especially for large or complex objects, making it less suitable for mass production compared to traditional manufacturing methods.
3. Cost	High costs of 3-D printers, materials, and maintenance can be a barrier, particularly for high-quality industrial-grade printers.
4. Quality and Consistency	Variability in print quality and potential for defects such as warping, layer shifting, or incomplete prints. Achieving consistent results can be challenging.
5. Post-Processing	Many 3-D printed objects require post-processing, such as sanding, painting or curing, to achieve the desired finish and properties, adding time and complexity to the process.
6. Design Constraints	Limitations in current 3-D printing technologies restrict the complexity and scale of designs that can be printed. Design rules must often be followed to ensure successful prints.
7. Intellectual Properties	Concerns about intellectual property rights and the potential for unauthorized reproduction of patented designs.

magnetic fields. The field of 3D bioprinting has great promise for advances in tissue engineering, stem cell therapies, and medical applications as the technology progresses. It is anticipated that functional tissue regeneration will surpass the mere replication of geometric structures. Furthermore, Table 1 represents the some of the additional challenges encountered during 3D printing.

Conclusion

The production of tissue and organ structures through 3D bioprinting has advanced quickly, but there are still a number of areas that require development, including bio-inks and the marketing of 3D-printed goods. With this printing technique, intricate, patient-specific 3D structures could be produced for vital medicinal uses. Its benefits include the ability to use specific cell lines, control over mechanical and biodegradation qualities, flexibility in design, and a wide range of printing capabilities. Utilizing hydrogels that are packed with cells is a popular method for producing these three-dimensional structures. This review examines the wide variety of bio-inks that are currently accessible, as well as different selection criteria and their attributes. While considerable global efforts are currently being made to develop optimal bio-inks, the use of this technology holds promise for the future. The study provides a thorough overview of composite bio-inks, their mechanical properties, and biological reactions in tabular format. It also analyzes the most recent advancements in ink-based 3D printing technologies and materials for tissue engineering application. Because natural hydrogels may be made to resemble the extracellular matrix for the creation of scaffolds, it emphasizes the great potential of these materials for TE applications. In order to improve printability, biocompatibility, and functionality, future research will concentrate on optimizing bio-ink compositions. Additionally, sophisticated bioprinting methods will be developed, and novel materials will be investigated in order to produce more adaptable and efficient scaffolds for tissue regeneration.

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