BIOINK CLASSIFICATION AND ITS RECENT TRENDS IN 3D BIOPRINTING FOR TISSUE AND ORGAN REGENERATION

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ABSTRACT

Three-dimensional bioprinting is a cutting-edge development in the rapidly expanding fields of contemporary scientific research, bringing together the fields of biotechnology, material research, and regenerative medicine. The reconstruction of biological structures with living cells and biomaterials is known as 3D bioprinting. The bioink, which is classified according to its ingredients, is a crucial part of the bioprinting process. The following characteristics—bio printability, biodegradability, biocompatibility, mechanical and structural qualities—must be present in a perfect bioink. The biological and functional characteristics of the intended tissue will determine which bioink is best for 3D bioprinting. Recent developments in bioprinting technologies include extrusion, laser-based bioprinting, stereolithography, and inkjet. This study aims to offer a rigorous examination of the current state of 3D bioprinting, the various uses such as the biofabrication of a variety of tissues and organs such as liver, near, skin, bone, cartilage, etc., and its significant impact on the scientific fields (e.g., drug delivery and screening). This review is an essential tool for researchers seeking to effectively use 3D bioprinting to address vital medical issues and extend the boundaries of science and medicine.

KEYWORDS: 3D bioprinting, biomaterials, bioink, biofabrication, biotechnology.

INTRODUCTION:

Since tissue regeneration is the primary process involved in cell creation and organ restoration, it is currently the subject of intense investigation. Depending on the circumstance and the extent of the injury, patients with damaged organs may be candidates for transplantation of organs, replacement, or repair. A novel method for creating complicated tissues and for the transplantation of organs is 3D bioprinting, which involves precisely depositing, localising, or joining cells and/or their underlying scaffold in customised geometries and dimensions. The integration of diverse cells, biomaterials, along with other compounds with medicinal and/or functional qualities is made possible by a biofabrication technique, which makes it possible to create sophisticated but complex therapeutic solutions quickly and easily. A combination of substances of cells &

biomaterials is widely used as the printing precursor for the 3D bio-printing of scaffolds[1]. The state-of-the-art technology of three-dimensional bio-printing, which merges biology and 3D printing, involves an accumulation of biomaterials on strata layer by layer (LBL), where they are introduced into appropriate biomaterials.

The most recent advancement in equipment used in tissue engineering and the field of regenerative medicine is bioink, the main component of 3D bioprinting. The use of bio inks, biomaterials, and cell types in 3D cultures is crucial for 3D bioprinting. The bioink can be categorised based on whether a synthetic or natural polymer was used to produce it. In comparison to synthetic polymers, natural polymers provide more options for the manufacturing of bioink. The biodegradability, mechanical properties, bio printability, modifiable functional groups on the surface, and last but not least, post-bioprinting maturation are important elements of the biomaterial requirements[2]. The choice of the ideal bioink with extremely particular qualities for very exact applications is in fact a difficult undertaking because there are so many variants in bioinks and even more factors involved in the process.

3D bioprinting encompasses several technologies that enable the fabrication of biological tissues and organs. Each of these 3D bioprinting technologies has its own strengths and weaknesses, making them suitable for different applications and research purposes[3]. The choice of technology depends on factors such as the specific tissue or organ being printed, the desired resolution, the type of cells and biomaterials used, and the overall goals of the bioprinting project. Researchers continue to refine and combine these technologies to advance the field of 3D bioprinting and its potential in regenerative medicine and tissue engineering. The use of 3D bioprinting reduces the need for organ donors, minimises the possibility of rejection of organs, and provides a more accurate method of engineering tissues. It is also applicable to create therapies and treatments tailored to individual patients[2].

The ability to "print" cells, tissues, and organs on demand thanks to three-dimensional bio-printing has significantly revolutionised the medical field. In general, 3D bioprinting offers the ability to advance the engineering of vaccines and treatments for improved management of infectious diseases, as well as in vitro models[1]. The potential for 3D bioprinting to address important healthcare needs increases as technology develops. The efficiency, accuracy, and biocompatibility of bioprinting methods are currently being improved. In this review article, first, we briefly described the various bioinks available followed by the different bio printing technologies. The main aim is to outline the basic concepts of 3D bioprinting and its applications in the research field which helps the readers to understand the significance of the 3D bioprinting process and its use.

BIOINK:

3D-bioprinted structures made of a mixture of living cells and biomaterials having the same properties as the extracellular matrix environment are being generated using bioinks, which may facilitate cell adhesion, proliferation, and differentiation[4,5]. Biomaterials are often referred to as organic or synthetic materials which are used in biological devices to fix or even transplant any organ of the body. These materials also provide essential physical and chemical signals and might have a significant impact on cell activities like adhesion, metabolism, proliferation, differentiation, and movement[4,6]. The bio ink acts as a geometrical support for the 3D structure that is primarily used as a biocompatible hydrogel to preserve the cells from harm that might occur from printing[1,7].

Bioinks, in contrast to conventional materials, must satisfy the following specifications:(a) The physiological temperatures needed for bioprinting must be maintained. (b) Their crosslinking should be minimal, and their gelation parameters should be moderate. (c) The biologically active components of the bioink should not be potentially dangerous to other cells or tissues or to the cells present within the bioink (d) The bioactive materials should be modifiable by the cells following the bioprinting process[2,3,5,8]. Several bioinks which are being used currently rely on the cross-linking or gelation process to transform liquid bio ink into a semisolid or gel like structure[5]. Therefore, the biomaterials utilised for in vitro tissue reconstruction via the use of 3D printing must be carefully chosen according to the tissue of interest as well as the study's goal and point of inquiry, in addition to taking into consideration the printing technique[9].

CLASSIFICATION OF BIOINKS:

Based on their chemical composition, biomaterials used in biological systems for organ repair or replacement have been classified into four categories: ceramics, metals, polymers, and composites. Although metals and composites have a higher mechanical strength than other types of materials, ceramics and composites have a

greater corrosion resistance when compared to other materials. Polymers are distinguished from other materials by being biodegradable and biocompatible. However, they are categorised into two primary groups for 3D bioprinting: synthetic polymers and natural polymers[2,10-15].

Synthetic polymer-based bioink:

Synthetic polymers stand out as highly applicable components for 3D bioprinting due to the characteristics they provide, such as high robustness, a predominant microstructure, and measurable degradability[16]. They are generated via chemical synthesis and are capable of being precisely intended with certain mechanical and chemical properties suitable for a wide range of bioprinting applications[17]. The properties, uses and drawbacks of some synthetic polymers used as bioinks is given in table 1.

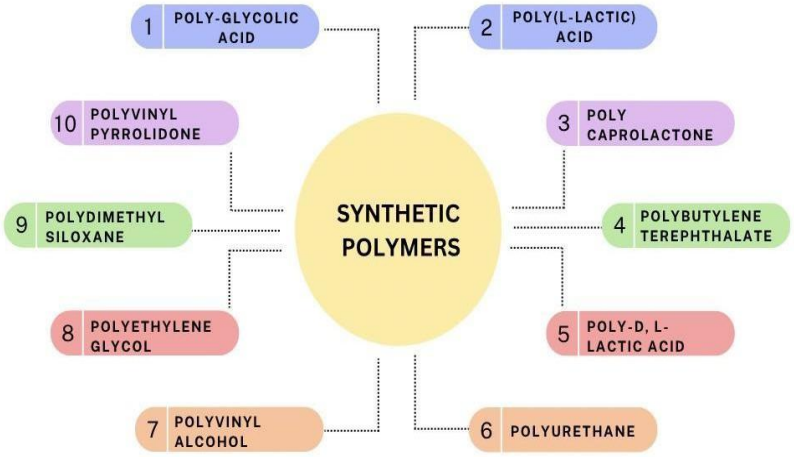


Fig. 1. Some synthetic polymers used in 3D bioprinting.

Table 1. Some synthetic polymers used in 3D bioprinting (Adapted from S. raaes. 2023).

No.	Synthetic Polymer	Properties	Uses	Drawbacks	References
1.	Poly-glycolic Acid (PGA)	Biocompatible, Biological characteristics. Processing simplicity, Chemical flexibility,	Internal devices for bone fixation	Mechanical strength, Prone to bulk erosion, Surface shape.	17,18
2.	Poly(L-Lactic) Acid (PLA)	Biocompatible, Hydrophilic Aliphatic ester, Printing capability.	Musculoskeletal tissue regeneration	Brittle in nature, Tissue inflammation and cell death upon decomposition.	17,18
3.	Polycaprolactone (PCL)	Rigid, Biocompatible, Biodegradable, Half-life of 3 years, Cost effective	Bone regeneration and cell ingrowth, Drug delivery carrier in sutures.	Prolonged half-life, Increased hydrophobicity results in decreased bioactivity.	17-19
4.	Polybutylene terephthalate (PBT)	Highly flexible, Resilient Simple processing,	Biomedical area, Tissue regeneration, Canine trabecular bone scaffolds, Orthopaedic surgery.	high melting point, non-biodegradable essence, Disintegration in aqueous medium.	17

Table 1. Some synthetic polymers used in 3D bioprinting (continued) (Adapted from S. raaes. 2023).

No.	Synthetic Polymer	Properties	Uses	Drawbacks	References
5.	Poly-D, L-lactic Acid (PDLLA)	Amorphous, Biocompatible, Hydrophobic.	Creates porous BC scaffolds, tissue exposition. engineering and Orthopaedic rehabilitation.	prolonged adsorbability, Membrane	17
6. Polyurethane (PU)	Great biocompatibility, Mechanical strength. Hugh printing resolution.	Cartilage tissue engineering, Skin and muscle Regeneration.	Hydrophilicity, Shorter half-life.		17,20
7. Polyvinyl Alcohol (PVA)	Semi crystalline, Hydrophilic, Chemically stable, Great	Bone tissue engineering, Repair	Lack of functional groups, Improper cell adhesion.		17,18
	tensile strength.	Of craniofacial			
		Defects.			
8. Polyethylene Glycol (PEG)	Biocompatible, non-immunogenic degradation, High tunability & affinity	Drug delivery,	non-biodegradable,		18,19,21
		Tissue engineering.	Absence of mechanical strength.		
	For biomolecules.				

Natural polymer-based bioinks:
Natural polymers, often referred to as bio-derived substances, are generated by living organisms and can be physically or chemically eliminated from their natural environments[22]. Gelatin, fibrinogen, alginate, collagen, hyaluronic acid, and agarose are examples of naturally produced ECM hydrogels that have been adopted in 3D cell culture models due to their history of excellent biocompatibility with various kinds of cell[20,22,23].

In order to describe the mechanical strength of hydrogels that are considered special viscoelastic materials, their modulus of elasticity and viscosity are frequently measured[24]. Fibres in solutions can become physically or chemically cross-linked during the sol-gel transition process in response to environmental factors including temperature, light source, or ion concentration. The absence of harmful chemical agents makes physical cross-linking very strong. On the other hand, covalent bond formation results in chemical cross-linking. As a result, the hydrogel that was created had good mechanical qualities. In contrast, hydrogels that have been chemically cross-linked tend to have larger volume variations than hydrogels that are physically cross-linked[25].

Natural gums are another form of natural polymer that have gained an immense amount of interest due to the multiple side chain groups that improve their bio adhesive capabilities[26]. A relatively small proportion of natural polymers are capable of being printed in films at cell-friendly temperature (for instance, room temperature) without the aid of the physical, chemical, or biological cross-linking of the corresponding chain of polymers. This is because only a few naturally existing polymers are capable of fulfilling the criteria for the 3D bioprinting of the cells, tissues, and organs[17]. Some of the commonly used natural polymers in 3D bioprinting are shown in fig. 2.

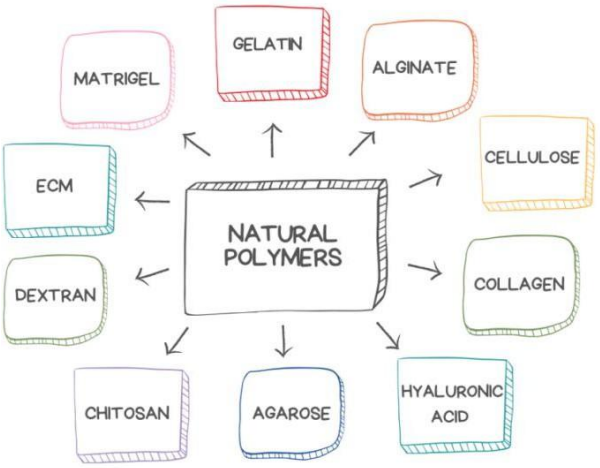


Fig. 2. Some natural polymers used in 3D bioprinting.

Gelatin: Gelatin is a protein that occurs naturally and is formed when collagen is hydrolyzed. It is used in the manufacturing of bioink because it possesses desirable characteristics such as biocompatibility, relatively low immunogenicity, non-cytotoxicity, ability to dissolve in water, cellular adhesion and growth, biodegradability, moderate translucency, viscoelasticity, and cost-effectiveness[17,20,27,28]. In order to produce bioinks, researchers have paired gelatin with several kinds of other substances that are either synthetic or natural[29]. Gelatin methacryloyl (GelMA), a common gelatin hydrogel, is used particularly for bioprinting load-bearing components such vascular networks, cartilage, bone, and skin[27,30,31]. Poor mechanical efficiency and organisational instability at physiological temperatures, such as 37°C, are the two primary challenges in the practical application of 3D organ printing of natural gelatin-based hydrogels[32].

Alginate: One of the most frequently utilised materials in 3D bioprinting is anionic, hydrophilic, natural polymer termed alginate, also referred to as algin, which is extracted from brown algae. Alginate refers to the salts of alginic acid, a compound which consists of the "-D-mannuronic acid" (M block) and "-L-glucuronic acid" (G block) building blocks, and can refer to both the acid and all of its derivatives[21,33,34]. Due to their excellent biocompatibility, rapid degradability, low toxicity, non-immunogenicity, elasticity, process flexibility, great printability, affordability, and chelating capacity, alginate and composite alginate hydrogels have been widely used as cell-laden bio-inks in numerous 3D-bioprinting procedures[33,35-37]. Alginate, however, has limited structural integrity and mechanical characteristics, yet they can be minimised by integrating with other materials such as fibrinogen, chitosan, gelatin, etc.,[38-43]. It is often used in biomedical applications such as articular cartilage formation, delivery of drugs, tissue engineering, enhancement of the tumour stem cell phenotype in breast, prostate, and hepatocellular carcinomas, and cancer stem cell enrichment[44,45].Various processes have been developed to further improve and stabilise alginate and expand the range of potential uses for it.

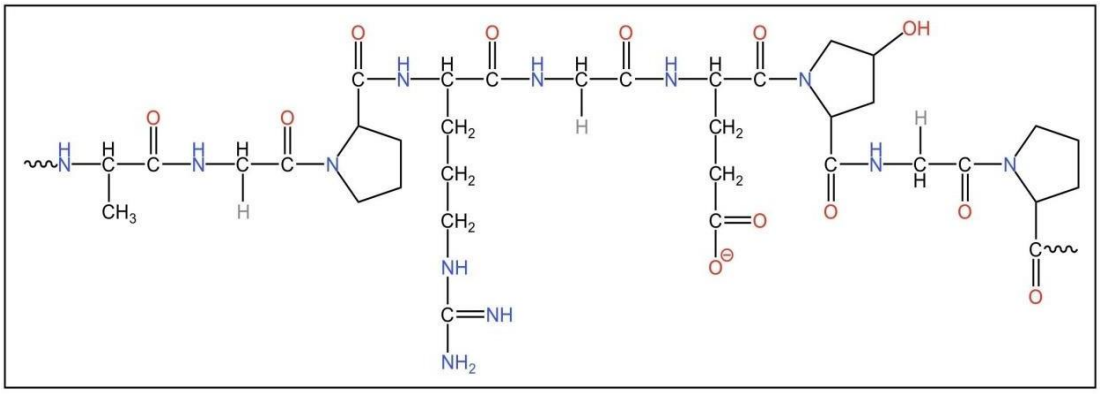
Collagen: Collagen is the most prevalent ECM protein in the mammalian body systems, which is the primary component of the musculoskeletal system and makes up the majority of tissues ECM[46]. Collagens are well-known for their biocompatibility, immunogenicity, structural support, and integrity-binding domains, all of which promote an increase in the adhesion, multiplication, and development capacities of osteoblasts, chondroblasts, and mesenchymal stem cells both in vivo and in vitro[23,47,48]. Collagens, however, may acquire immunogenic properties and result in inflammation or disease when present with other cell remnants or proteins. While collagen I is widely used in 3D bioprinting, it has poor mechanical strength. Its crosslinking abilities or concentration can be directly altered to improve the mechanical properties. In order to increase the bioink's bio printability, integrity, and bioactivity, collagen is often mixed with other biomaterials[49,50]. Alginate, for instance, may enhance cell survival, mechanically strengthen collagen I-based bioink, reduce unwanted differentiation in the bioprinted structures, and enable continuous drug delivery[51].

Cellulose: A biologically obtained polysaccharide known as cellulose, which is composed of 1-4 linked -D-glucopyranosyl repeating units, is an essential structural constituent of plant cell walls[52,53]. Different cellulose derivatives, such as methyl cellulose (MC), carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), and hydroxyethyl methylcellulose (HEMC), ethyl cellulose (EC) can be generated with varying physio-chemical properties[54-57]. Crystallinity, specific chain lengths, and intra- and intermolecular hydrogen bonds are all generated naturally by the physics-chemical characteristics of cellulose. Thus, this material can be used as a drug carrier to administer pharmaceutical agents, contact lenses, wound healing agents, and cartilage tissue engineering[19,58].

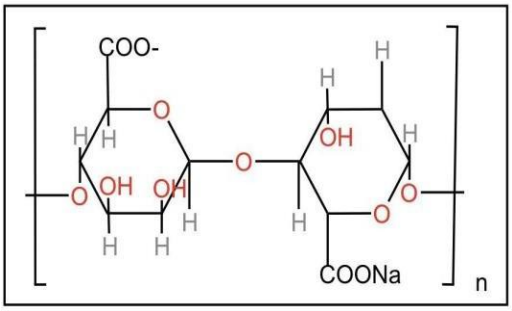
Hyaluronic acid: The extracellular membrane of some tissues and organs in mammals, such as the soft connective tissues, the eyeball, and the central nervous system, contains hyaluronic acid, a non-sulphated glycosaminoglycan constituted of the amino acid N-acetyl-D-glucosamine and D-glucuronic acid units which possess numerous desirable properties such as excellent biocompatibility and biodegradability, a high capacity to retain water, non-immunogenicity, and anti-inflammatory, mucoadhesive and viscoelastic properties that are suitable for cell encapsulation and transport of bioactive materials and interactions with receptors[21,59-63]. Hyaluronic acid cannot function as a bioink alone as it lacks significant mechanical strength and has low stability due to its high-water solubility. Hyaluronic acid is mixed with other substances to improve cellular adhesions since cells are unable to adhere to its surface. The hydrogel, which has a 3:1 ratio of collagen I to hyaluronic acid, is appropriate for bioprinting the liver due to its excellent mechanical properties, bio printability, and cellular viability of the bioink[34,64].

Decellularized extracellular matrix: The decellularized extracellular matrix is formed by decellularizing tissues through a number of physical and chemical methods, which include freeze-thaw cycles, the use of enzyme reagents, or detergents[65]. Decellularization is the process of eliminating the residing cells from tissues and organs while retaining the ECM in place[19]. The dECM maintains the natural biochemical signalling molecules of the host tissues in addition to being made of biopolymers including collagen, fibrin, and glycosaminoglycans as a supporting framework[21]. Every cell, tissue, and organ have a distinct composition that can be influenced by interactions between its cells and the extracellular matrix[66]. For bioprinting various tissues,

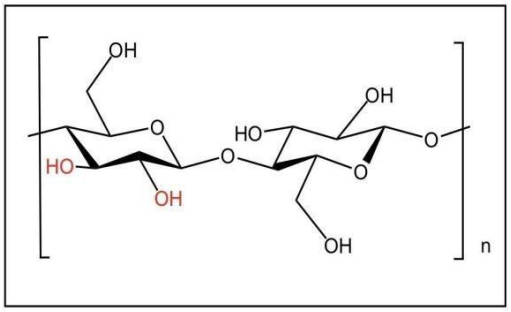
such as the skin, heart, intestinal tract, liver, bones, cornea, and tendons, dECM is naturally employed as a bioink material[11].



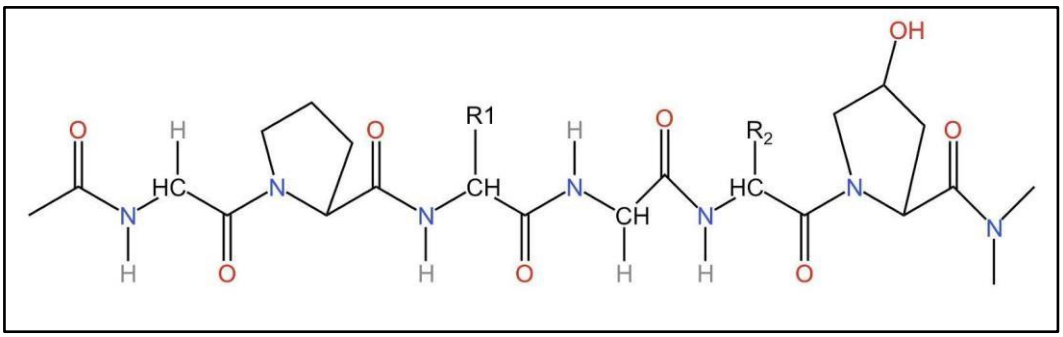
Gelatin



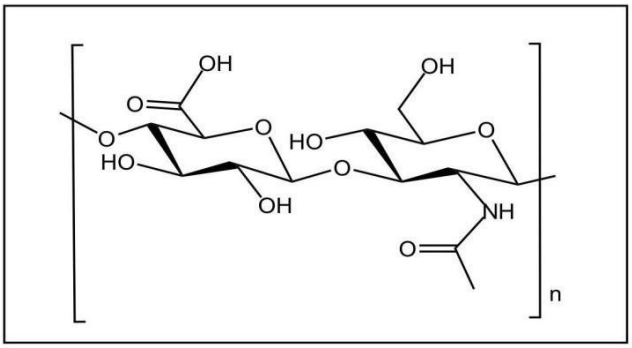
Alginate



Cellulose



Collagen



Hyaluronic acid

Fig. 3. Chemical structures of some natural polymers used as bioinks.

Although natural polymers and hydrogels offer the required surrounding environment for cellular adhesion and proliferation by closely resembling the original ECM, the adjustable properties of these substances such as mechanical strength, etc., are limited. Synthetic polymers may not trigger cell adhesion or promotion as effectively as natural polymers, but there are intriguing options for tuning the characteristics to enhance their mechanical characteristics, printability, cross-linking, etc. Therefore, in order to produce more stable structures with tunable properties for 3D bioprinting, these natural polymers have been paired with either synthetic or other natural polymers[67].

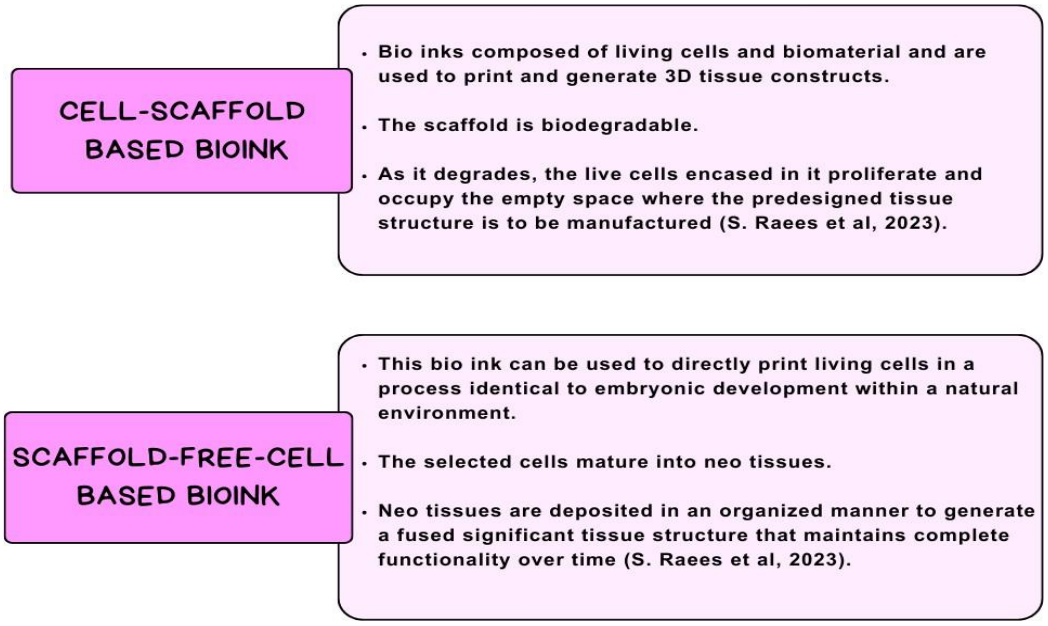


Fig. 4. Scaffold-based classification of bioinks.

While generating tissue or organ structures via 3D bioprinting, two key groups of bioink material are utilised. One is a cell-scaffold-based technique, and the other is a scaffold-free cell-based approach[68]. In the first technique, live cells and biomaterial are incorporated to create bioink that is printed to construct 3D tissue structures. Here, the scaffold biomaterials biodegrade as the encapsulated live cells grow and fill the space to create tissue forms that have been pre-planned. However, the second method directly prints living cells in a manner that replicates the development of an embryo. The selected set of live cells creates the neo tissues, which are later on placed in a particular arrangement to gradually form fused, enormously functional tissue structures (fig. 4)[69].

IMPORTANT PROPERTIES OF BIOINK:

When it comes to the selection of bioinks, the most important factors are the physiological and functional aspects of the desired tissue[7]. Some tissues, like cartilage and bone tissue, depend significantly on their mechanical characteristics to function[70], whereas other tissues, such as liver tissue, greatly relies on their sophisticated and comprehensive vascular network[71]. As a result, it's essential to consider the target tissue carefully while constructing a bioink and to improve its properties appropriately[72]. Additionally, the bioink needs to be able retain its original form after bioprinting[8]. To bioprint a desired construct, the bioink characteristics prior to, during, and after bioprinting should be optimised[7].

Due to the advancement of material science, a wide range of materials are currently ideal for 3D bioprinting. It's necessary to take into consideration biocompatibility, mechanical qualities, hydrophilic nature, porousness, pH values neutrality, and biodegradability while selecting the materials for bioink[73]. Therefore, the following criteria must be considered while selecting an ideal bioink (fig. 5).

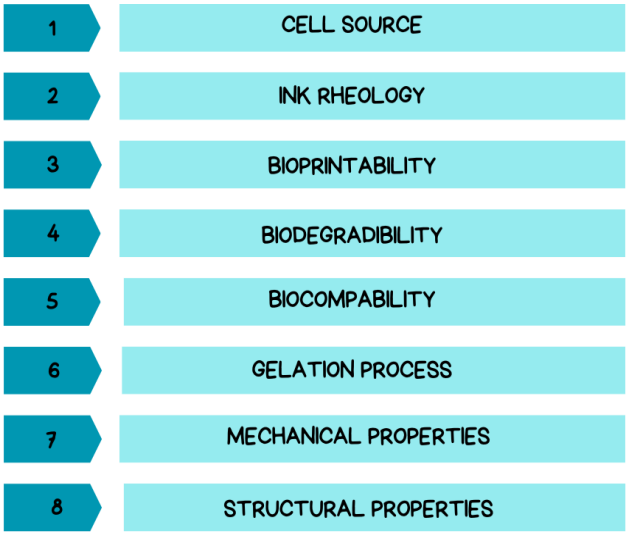


Fig. 5. Basic requirements of an ideal bioink for 3D bioprinting.

Cell source: The essential component of bioink are cells. For bioinks, as compared to various polymers, the type of cell and the source of the selected cells are important[74]. Several cell types, such as supportive, parenchymal, and non-parenchymal cells, constitute the human body[3]. During the bio-fabrication process, selected cells must retain their functionality, resemble their physiological state, grow appropriately, and survive[76]. In order to develop bioink, care should be taken in selecting the source, kind, and density of the cells. The cell source impacts cellular efficiency and function[77].

Ink rheology: When bioink passes through the nozzle, the pressure it experiences might affect its characteristics. A perfect bioink needs to maintain this pressure by functioning as a shear-thinning fluid for it to function appropriately[78]. Additionally, bioink has to demonstrate a viscoelastic response after being extruded from the nozzle in order to retain its structural integrity[79]. All of these features are included under the term "bioink rheology." The effectiveness of bioprinting bioink into well-structured 3D models depends on the rheology of the bioink[8]. The resolution, bio printability, structural stability, and biocompatibility of bioink are all affected by the consistency, extrusion rate, and shear stress that encapsulated cells experience as a result of a polymer's structure and rheological properties[80]. Desired rheological properties may differ depending on the biofabrication process. Polymers have the ability to increase or decrease their level of viscosity in response to changes in the shear rate, accordingly. By rearranging their chains to create more or less entanglement, polymers can alter their viscosity[79]. Shear thinning is crucial for gelation because viscosity should improve immediately following extrusion. It is essential to select a bio-ink with rheological characteristics that work with the preferred bioprinting method[72].

Bio printability: A biomaterial's capacity to be deposited in a controlled manner within a particular duration of time is known as bio printability[81]. Cell bioprinting is a prerequisite for tissue and organ bioprinting, however the limited options for bioink result from difficult bioprinting conditions[78]. A variety of factors, including, ink composition, its viscosity, gelation kinetics, and surface tension, can affect printability. Extrusion pressure, printing speed, nozzle diameter, printed/ambient temperature, and printing path are printing parameters that could influence the outcomes. Whatever parameter combinations are used, the structural result must be defined through an evaluation technique called printability. Utilising well-established methods including qualitative description, quantitative evaluation, and computer simulation, it is possible to determine the printability of bio-ink[67,82]. The structural and dimensional precision of the 3D bioprinted construction is related to a biomaterial's bio printability[79]. The prerequisite for bio printability differs depending on the desired bioprinting process. For instance, inkjet printing has a limit on the viscosity of the materials it can print, whereas extrusion-based printing may print materials with very high viscosities. However, the material must possess specific inter-layer cross-linking processes or shear-thinning properties in order to be printed using an extrusion-based technique[83]. Thus, certain shear thinning characteristics or crosslinking techniques are crucial when selecting a material for bioink[18].

Biodegradability: The secretion of proteases by the embedded cells and the subsequent synthesis of ECM proteins occurs as a result of the material scaffold degrading. The new tissue is characterised by these proteins. In the ideal situation, the ECM is created at the same rate that scaffolds degrade and its byproducts have no negative impact on the host[84]. The biocompatibility of a material includes a definition of the biological compatibility of the degradation byproducts. The byproducts should be rapidly digested, non-toxic, and eliminated from the body[72]. Proteins and other tiny molecules, variations in non-physiological conditions such as pH, temperature, and other elements that may have an impact on cellular function and viability are a few examples of degradation byproducts[34]. During the entire process, which involves both physical and chemical changes that begin with the bio-ink in its liquid form and end with the formed scaffold and functional implant, these attributes are related to each other and may have an impact on one another[17]. As a result, while selecting materials for specific purposes, it is critical to understand how the material interacts with the host tissue both during and following bioprinting[18].

Biocompatibility: The cells in bioink preserve the potential to proliferate and differentiate in vitro after bioprinting, which is critical in a mature tissue construct. Since they can reflect a natural cellular environment, bioinks made from natural materials provide a non-toxic biofabrication technique, although they have poor mechanical properties[10]. Synthetically developed bioinks, on the other hand, may not be as biocompatible, but may have greater mechanical properties[85]. Furthermore, naturally produced bioinks exhibit batch-to-batch variability, which can be prevented with synthetic bioinks[5]. As a result, the choice of bioink material could differ among both synthetic and natural sources depending on the desired function, target tissue, and the objective of the experiment[75]. The main objective of achieving biocompatibility has evolved over time, from requiring the implantation component to coexist with the host cell without having any harmful local or global consequences to permitting or actively promoting positive passive benefits in the host[17]. Various material science parameters, such as the chemical constitution, mechanical and surface characteristics, and structural morphology of the bioink, impact its biocompatibility. Surface modifications, integration of multiple materials, and generating biomimetic bioink are all approaches for enhancing biocompatibility of bioink.

Gelation process: Gelation is a procedure by which the gel form of bioink solidifies following extrusion. Since gelation may impact both viability as well as bioprint resolution, it should be rapid, biomimetic, and safe to the cells[78]. The gelation process might potentially affect compatibility with a particular bioprinting system. Depending on the chemical composition and material qualities of the bioink, the gelation process provides a broad range of options[86]. Due to its dynamic and mechanically unstable nature, physical gelation is unsuitable as a single cross-linking approach for the solidification of bio-printed constructs. As a result, subsequent chemical cross-linking is often used with physical gelation[5]. Chemical cross-linking, as compared with physically cross-linked gels, inserts covalent links into the network, which are thought to improve mechanical properties and structural fidelity. Enzyme-mediated cross-linking, photopolymerization, & click chemistry (such as Michael addition and Schiff base development) are often employed for producing covalently cross-linked inks[17]. As no gelation procedure can provide optimal outcomes in all domains of concern, it is recommended that the gelation process be chosen based on the experimental targets and material availability[18].

Mechanical properties: Since no gelation approach can produce ideal results in all areas of concern, the gelation process should be chosen depending on the experimental goal and material availability[87]. In addition, due to their low mechanical properties, natural polymers fail to provide adequate sacrificial support throughout the bioprinting process[88]. Synthetic polymers, on the contrary, are preferred because they have superior mechanical qualities and can be removed easily after bioprinting[85]. Moreover, the sacrificial scaffolds and its byproducts should have no negative impact on the bioprinted structure's properties[89]. Another significant feature of a bioprinted material is its sterilizability. The ability of a material to lose some features or keep a specific degree of performance within a permissible range after sterilisation is referred to as sterilisation compatibility[90]. Although every technique of sterilisation has pros and cons, the bioink material used must be suitable with at least one of the methods.

Structural properties: Bioink should be able to resist pressures, produce specific stresses, and give mechanical leverages during and after bioprinting to maintain the 3D form of the bioprinted construct[91]. In contrast to conventional procedures such as solvent casting, separation of phases, and melt moulding, 3D bioprinted objects include interlinked pores which enable intercellular communication. The size, volume, and shape of the pores can influence the formation of the extracellular matrix and the behaviour of cells after adhesion to the scaffold[51]. Porosity promotes interconnectivity, allowing nutrients and oxygen to migrate into the cell's tissues as well as the elimination of cellular waste. This connection is required for adjacent tissue ingrowth. Pore size and shape,

in addition to adhesion, have the potential to alter the survival and proliferation of cells. Furthermore, the surface structure of the constructs can be modified to control their loading & release potential[91]. As a result, materials must be carefully selected and designed based on the intended purpose and target tissue.

BIOPRINTING:

The additive fabrication of 3D functioning organs and tissues in three dimensions using biomaterials is accomplished with the help of automated machinery known as bioprinters or 3D bioprinters. Bioprinters are autonomous robotic devices that operate via several kinds of mechanisms[92]. There are several types of bioprinters based on the manner of bioprinting utilised by the machines: laser-based, extrusion-based, and inkjet bioprinters. Depending on the biomaterials used, these bioprinters operate through different mechanisms and perform a variety of function[18].3D bioprinting is a kind of additive manufacturing, and it is particularly a layer-by-layer fabrication method that initially emerged out of a desire for rapid prototype development and has since evolved into an efficient, customizable fabrication method in many fields which includes the agricultural sector, education, healthcare, energy, industry, transportation, communication, aviation, military operations, and national defense[21,93]. The purpose of 3D bioprinting is to reconstruct human body components in their natural form and functionality[94]. The majority of materials utilised in 3D bioprinting are either synthetic or natural biomaterials, each having advantages and limitations[95]. The ultimate goal of 3D bioprinting is to create and offer an appropriate, accessible, and cost-effective alternative to tissue implants, donation of organs, and animal testing techniques.

Steps involved in 3D bioprinting:

The bioprinting process involves 3 important steps, namely, pre-bioprinting, bioprinting and post-bioprinting (fig.6).

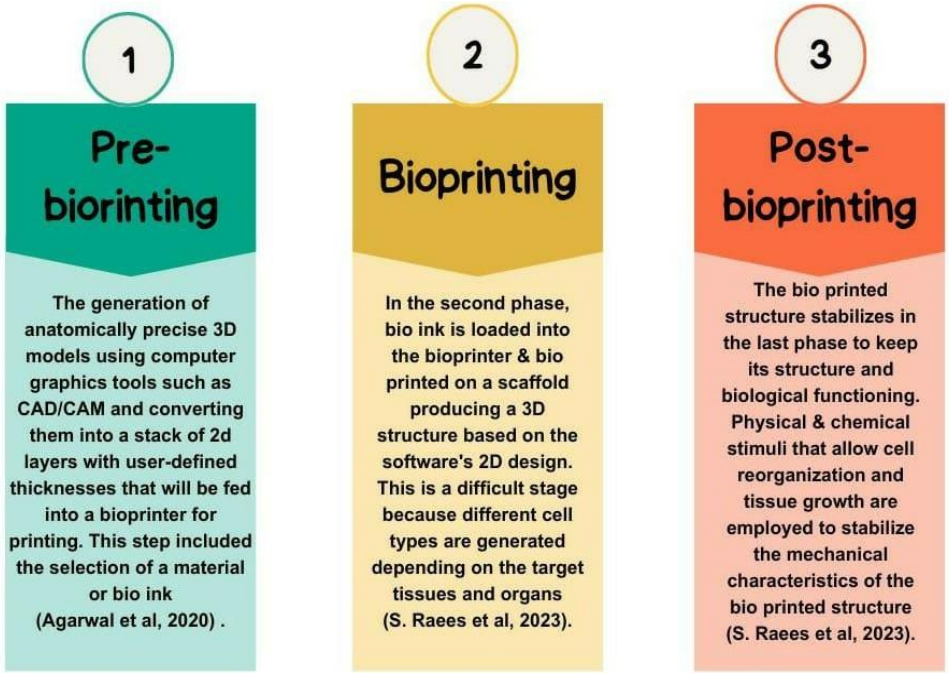


Fig. 6. Steps involved in 3D bioprinting.

TYPES OF 3D BIOPRINTING TECHNOLOGIES

Several additive manufacturing methods, comprising inkjet-based, extrusion-based, stereolithographic-based, laser-based, and droplet-based printing technologies, have been developed and utilised for manufacturing highly functional 3D structures. For printing each layer, each bioprinting technology has a varying resolution and dimensions.

Inkjet-based bioprinting:

Inkjet-based bioprinting is a non-contact printing process that uses a digitally controlled design to print[96]. The ejection of drops on the substrate through thermal or acoustic forces is the basis of this method[92]. Thermal inkjet

bioprinting is accomplished via electrically heating the print head in order to generate pressure, which causes droplets to be released from the nozzle[73]. A piezoelectric crystal is utilised in acoustic inkjet bioprinting to create an acoustic wave within the print head, which breaks the bio ink into droplets[97]. When a voltage is given to the piezoelectric substance, it undergoes a fast change in shape. As a result, the pressure that is required to drive droplets out of the nozzle is generated[73]. Inkjet printing is performed in two ways: continuous printing or drop-on-demand printing. Droplets are produced much faster by continuous inkjet printing systems than by drop-on-demand inkjet printing systems; nevertheless, due to the prerequisites for conductive fluid ink along with the potential of contamination in the procedure, drop-on-demand inkjet printing is extensively utilised for 3D tissue structure construction. The size of droplets and deposition rate are determined by the viscosity & tensile strength of the bioink, and the printing direction and droplet deposition process can be controlled by varying the voltage & pulse duration[19,96]. Because of its low cost and non-contact nature, this technology has been widely employed for mammalian cells printing & sequencing along with DNA and proteins[96]. However, one disadvantage of inkjet printing is that the bioinks' cell densities are limited by the narrow nozzle apertures—higher cell densities can induce nozzle clogging. Furthermore, the relatively low viscosity required for printing may result in a final printed structure with limited mechanical strength[21].

Extrusion-based bioprinting:

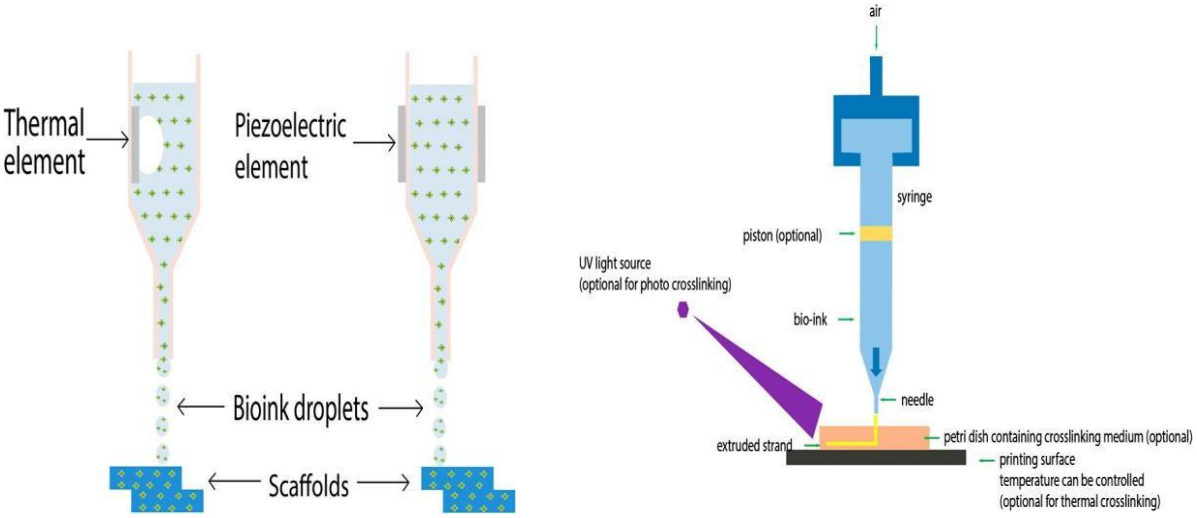
Extrusion-based Bioprinting is used in tissue and organ research by a variety of research facilities[92]. Bioink gets placed on the cylindrical deposit, and the biomaterial is launched onto the substance using pneumatic or mechanical pressure exerted by a piston, either continuously or in pulses[18]. During extrusion, pneumatic force is supplied using a valve-free or valve-based compressed air system[98]. A sterile air pump is connected to a syringe containing bioink. As shear stress is created during the pneumatic extrusion of this bio-ink, only shear-thinning bio-inks can maintain filamentous structure after extrusion. Extrusion without a valve is a rather simple technique. However, valve-based extrusion is preferred for high-precision performance. This constitutes one of the most feasible technologies for printing living cell bio-ink[99,100]. Mechanically powered extrusion, on the other hand, is suited for extremely viscid bioinks like synthetic & natural polymers. One common mechanical micro-extrusion approach is piston-based extrusion, which employs a piston connected to an electric motor. An electrical pulse drives the motor to revolve, propelling the piston forward and forcing the bioink out through the outlet.Extrusion-based printing resolution can be adjusted by altering the nozzle's size, flow rate (printing rate), and pressure. The print resolution is about 200 μm, which is low in comparison to other bioprinting processes[101]. Shear stress may arise as the bioink goes through the nozzle because this process is based on extrusion. Since shear stress may cause damage to cells & death, significant cell viability can be attained by carefully managing the inducible shear stress parameters (pressure, bioink viscosity, nozzle diameter, and shape). The total fabrication time will differ depending on the intricacy of the 3D construction[102,103]. The limited number of compounds that can be employed as bioinks for the bioprinting process is the primary disadvantage[18]. Despite their low resolution, extrusion-based bioprinting cells have an overall survival rate of more than 90%[104,105].

Laser-based bioprinting:A pulsed laser beam is used in this process for deposition of bio-ink including cells onto a substrate. Utilisation of laser for deposition of materials provides a non-contact direct writing process for 3D printing[106]. The LIFT (Laser induced forward transfer) bioprinting equipment is made up of three parts: (1) a pulsed laser beam source, (2) a substrate for donation that serves as a base to the bioink, and (3) a collecting substrate for the bioink. The energy source is ultraviolet (UV) or near UV wavelength laser with nanosecond pulse wavelength. Cell-containing bioinks are created by distributing them on the surface of a donor substrate. Droplets of bioink form whenever laser pulses are applied to the donor substrate and settle onto the collection substrate. The collector substrate may be moved along both the X and Y axes, & the bioink drops are continually layered on it to form a 3D shape[107]. Because the bio-ink is volatile, when a laser pulse is applied, a high-speed jet of cell-laden bioink is driven on the substrate. Many factors influence the resolving capacity of laser-assisted bioprinting, including laser energy density, or the amount of energy delivered per unit area, surface tension, substrate wetness, the dimension of the air gap that separates the substrate's surface and the ribbon, and the level of viscosity and width of the biological layer[73].Laser-assisted bioprinting can be used to make cellularised skin constructs, indicating bio-printing's potential for medicinally effective cell densities in multilayered tissue constructs[108]. LIFT bioprinting eliminates the need for nozzle and photocurable bioinks, so 3D models can be precisely created with low viscosity bioinks (1-300 mPa s). However, the disadvantage of the LIFT bioprinting method includes covering a donor substrate with a 1 $m^{2.}$ bioink layer when generating a 1 cm^3 construct[107].

Stereolithography:

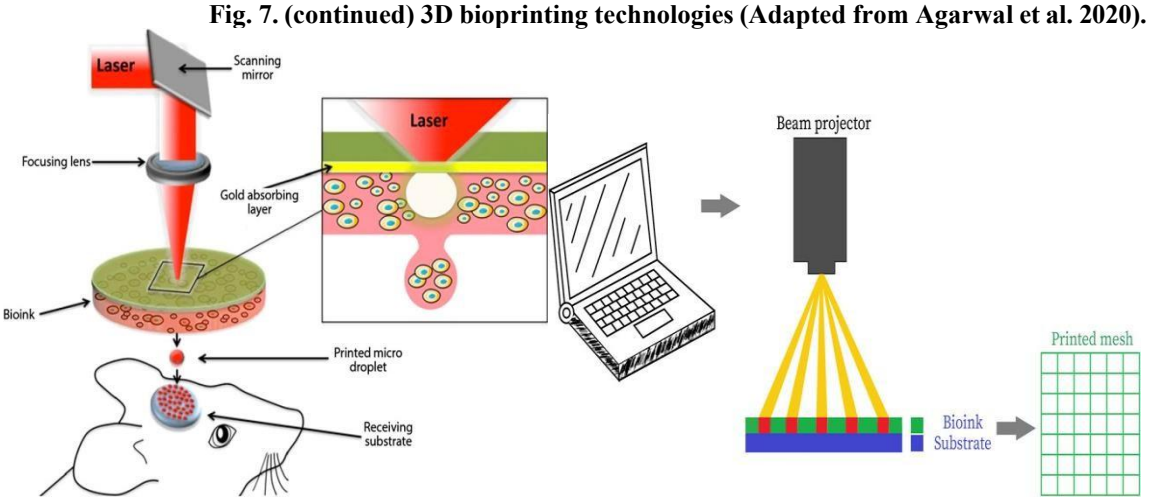
During the stereolithography (STL) technique, also known as vat photopolymerization, a light-emitting source (UV laser processor) is used to illuminate and cure the liquid photopolymer resin, which is a thermosetting

plastic, layer by layer[87]. The laser penetrates a 2D design point-by-point while scanning it for each stratum deposition, and the precise beam collaborates with the bioink material to polymerize it in accordance with a predetermined pattern. To allow the newest unpolymerized-ink ingredient to enter into position for the subsequent strata, the printing pedestal must be shifted up or down away from the laser source[109]. PEG dimethacrylate (PEGDMA) & PEG diacrylate (PEGDA) are two commonly used acrylate compounds of Polyethylene Glycol (PEG) for the photo polymerization of tissue-engineered scaffolds. Stereolithography has been used in conjunction with therapeutic imaging techniques which include CT scan/MRI to improve diagnostic techniques, the quality and design of devices and implants, and the useful completion of complex surgeries. These printing processes make it possible to create cell patterns with complicated geometries and sub-micrometer resolution. Because of these benefits, they are now commonly employed in tissue creation that necessitates the incorporation of microstructures, like perfusable blood veins and capillaries[110,111]. In addition to its advantages, the SLT approach has numerous disadvantages, including an expensive price label, a moderately slow printing pace, and a restricted selection of biocompatible polymers that are suitable for SLT processing. Other problems related to medical and rigid tissue engineering uses of the SLT approach include insufficient mechanical properties of printed scales and the possible cytotoxicity of the untreated resin & residual photo-initiator[112].



(a). **Inkjet-based bioprinting** **(b). Extrusion-based bioprinting**

Fig. 7. (continued) 3D bioprinting technologies (Adapted from Agarwal et al. 2020).



(c). Laser-based bioprinting **(d). Stereolithography**

Fig. 7. 3D bioprinting technologies (Adapted from Agarwal et al. 2020).

APPLICATIONS OF 3D BIOPRINTING:

Numerous potential applications of 3D bioprinting have rapidly evolved into a big industry. Several studies have lately discovered the potential of 3D bioprinting in healthcare fields to be exciting, and many firms across the world have contributed to the advancement of this technology in medicine by supporting studies in their labs. The applications of 3D bioprinting are classified into two groups: (1) tissue regeneration, involving printing blood arteries, cardiac valves, musculoskeletal tissue, liver, nerves, & skin, and (2) biomedical uses, which include medication discovery and drug screening, are the two broad categories of bioprinting applications[17].

Tissue and organ regeneration

Tissue engineering is a regenerative medicine application that uses in vitro and in situ procedures to regenerate particular tissues and reestablish normal biological functions[113]. Apart from drug testing, utilising just the previously existing tissue-engineering techniques is not possible. Organ transplantation accomplishment is also limited due to a scarcity of donors and immunological reactions. In the case of 3D bioprinting, it may be difficult to find a balance between these biological traits and the need for maximum printability[114]. An ideal tissue engineering framework should have high porosity with the appropriate size of pore, mechanical stability equal to the target tissue, changeable form, and customisable biodegradability for the regeneration of tissue[115]. Gradients, ECM organisation, & the varied architecture of natural tissues all have a substantial impact on cell movement, growth, and differentiation[114]. The printed structures may facilitate the establishment of vascular networks and provide cells with the necessary behavioural cues[116]. Overall, the application of 3D bioprinting in tissue regeneration will improve the integrity with respect to the native anatomy and physiology porosity, and numerous other features of the regenerated tissue.

Bone: The vertebrate skeleton is composed of bone tissue, an effective organ capable of producing blood cells, preserving minerals, and protecting the body's numerous organs[117]. To rebuild the injured bone, an appropriate porous scaffold with characteristics (biocompatibility, compressive rigidity, etc.) that are reminiscent of natural bone is required because it can mechanically support osteoblasts as they divide, grow, and develop a layer of extracellular matrix[118,119]. As a result, the scaffold's properties should be analogous to those of local bone. A resorbable scaffold must possess the following ideal properties: 70-80% porosity, pore size of 300 m, a compressive strength of 5-10 MPa, and a modulus of elasticity of 20 GPa. Furthermore, the ECM[120] is made up of 30% organic & 70% inorganic components. The organic component is 95% collagen of type I and 5% non-collagenous protein molecules, whereas the inorganic component is constructed of HAP nanoparticles (50 nm) connected by collagen fibres (5 m), giving the bone a greater capacity for bending (150 MPa for cortical bone)[121]. Bioresorbable materials must have osteo-conductivity, bio-inertness, and degradability, but they must also be biocompatible and shouldn't damage the structure or strength of the surrounding bone.The mineralized matrix cannot be formed by hydrogels utilised in bone Tissue Engineering. To address these challenges, 3D bioprinting for bone TE has various advantages over traditional TE approaches, such as providing enough mechanical support during tissue regeneration. Other benefits include the ability to modify shape and chemistry through linked porosity. Because no tissue either donors or other regions of the body is required for these Tissue Engineering creations, there does not exist any chance of rejection of tissue or disease transfer. Another advantage of employing 3D printing is that it uses anatomically accurate representations based on patient specific data gathered from clinical imaging via computer graphics such as CAD/CAM to imitate complicated bone shape[122]. To ensure the critical guidance and assistance of the creation of the new bone, the shape and size of the bone transplant must resemble that of the restored bone[123]. Micro-extrusion is the most extensively utilised bioprinting technology for producing bioprinted bone scaffolds[124]. Micro-extrusion enables bioprinting of a wide range of biomaterials, including both synthetic and natural polymers and composites[125] as well as quite dense cell deposition[126,127]. One study proposes a gelatin-based ink formulation cross-linked via Hydroxyapatite that can represent bone's natural composition by dramatically boosting the bioink's viscosity[96].

Cartilage: The development of cartilaginous tissues is another field of tissue engineering which has been receiving a lot of attention[17]. Articular cartilage, a distinct smooth and white connective tissue that covers the ends of bones, has a complicated structure composed of numerous biomolecules such as collagen, proteoglycans, and non-collagenous proteins. Since cartilage tissue is avascular and lacks a lymphatic and nervous system, any damage triggered by trauma or prolonged stress cannot be repaired, resulting in several degenerative diseases such as osteoarthritis (OA) and ultimately lowering the quality of life[128].Droplet-based, extrusion-based, and stereolithography are three popular approaches for cartilage bioprinting. The selection of acceptable bioink based on composition & mechanical properties is critical for the development of viable cartilage substitutes. These are made from natural polymers like collagen and fibrin, as well as synthetic polymers like polyethylene glycol (PEG)[129]. Hyaluronic acid, a major cartilage component, is capable of being

co-printed using poly-lactic acid (PLA) to create a unique bioink for cartilage the bioprinting process[130]. Cross-linker-free bioink is sometimes created by combining the self-gelling ability of silk fibroin with gelatin as a bulking component[131]. Cell-laden hydrogels & biodegradable polymers connected to sacrificial hydrogels were printed in integrated patterns, leaving tiny pores in the tissue scaffolds to aid in nutrition absorption. This procedure was used to repair muscles, cartilage, & bone in the jaw and calvarium[17].

Skin: Skin is the human body's biggest tissue and serves as the body's outermost protective barrier against external hazards[132,133]. Keratinocytes are organised in keratinized squamous epithelium that has been stratified in the uppermost layer of epidermis. The epidermis grows from the inside out, with mature skin cells at the top and growing keratinocytes at the bottom, within the basal layer[96]. Nevertheless, the skin is vulnerable to injury from surgical procedures, accidents, and burns, which might not heal on their own and require skin tissue replacement[134]. Among the various biofabrication methods, 3D bioprinting technologies have received special attention as an evolutionary strategy in straightforward designing functioning 3D tissue-like scaffolds for tissue engineering of skin[135].Extrusion and inkjet-based bioprinting are the most frequently utilised techniques for biofabrication of skin tissue. Bioprinting can be performed directly at the site of injury, known as in situ bioprinting, or in vitro, where the construct is allowed to grow in a bioreactor prior to transplantation. In situ bioprinting is preferred to in vitro processes because it enables greater accuracy in cellular deposition onto the wound and avoids the need for costly polymers as well as the time needed for in vitro development[136]. Because of the strength and vitality of skin, developing a skin fabrication in the lab is critical. One of the first innovations in this sector was the development of artificial skin graft, which may be used as a bandage for wound & burn healing[96]. Bio-ink must be biocompatible and help preserve the form and function of the skin tissue construct. It should be capable of facilitating cell differentiation in accordance with the functionality demanded of it. The biomaterials used in bio-inks can range from natural polymers like alginate, gelatin, collagen, and hyaluronic acid to synthetically produced polymers such as Polyethylene Glycol (PEG), Polycaprolactone (PCL), poly(lactic-co-glycolic acid) (PLGA), and others, or it can be a hybrid blend of natural and synthetic biomaterials. Researchers worked on a bio-ink suspension consisting of amniotic fluid-derived stem cells (AFSCs) & bone marrow-derived progenitor cells suspended in fibrin-collagen and crosslinked with thrombin that could be printed directly on a wound site[96]. Despite the fact that 3D bioprinting possesses the ability to construct skin, more research is required. Some of the issues that must be addressed are resolution, vascularity, optimum cellular and scaffold combination, and the cost of bioprinted skin[17].

Cardiac tissue: Cardiovascular diseases (CVDs), particularly within developed countries, are one of the leading causes of death globally. An estimate of overall cases of myocardial infarction each year comes to around eight million. A key issue with all of these heart disorders is the deterioration of essential cardiomyocytes, since such cells lack any form of healing or auto-regeneration mechanism. Instead, the depletion of cardiomyocytes is dealt with by the creation of non-functional scar tissue, which dramatically raises the possibility of acute cardiomyopathy. Tissue engineering helps to alleviate the challenges associated with the healing of injured blood arteries, heart valves, and so on. The traditional techniques for cardiac tissue engineering involve the development, maturation, and proliferation of stem cells on functional biomaterial scaffolds to enable stem cell differentiation. Due to their biocompatibility & similarity to the cells' native tissue matrix, scaffold construction for vascular tissue engineering is now being explored using decellularized tissue substrates along with synthetic & natural hydrogels. Because of lower risks of immune-mediated rejection of grafts and their ubiquitous availability, self-generated and allogenic embryonic stem cells are the cells preferred for cardiovascular tissue engineering[96]. Some of these strategies include cell self-assembly to generate vascular structures, endothelial-cell inkjet bioprinting, or thrombotic growth-factor operations in bio-printed frameworks, among others. Some ink properties are also required for cardiac bioprinting, such as spatial regulation of hydrogel deposition via the production of persistent filaments with moderate cross-linking mechanisms. The capacity of bio-inks for cardiovascular bioprinting to maintain cell viability is a critical requirement. For bio-ink creation, both synthetic and natural polymers capable of forming hydrogels, such as gelatin, collagen, and hyaluronic acid, are chosen[137]. Until date, 3D bioprinting is being utilised to bioprint fragile vascular tissue that can only survive in vitro for a limited period, but research is continuing to scale up this possible tissue regeneration strategy for bioprinting vascular grafts[138].

Liver: The liver is the largest and most important organ in the human body, and it also serves as the principal site for urea generation and metabolism, as well as the process of detoxification erythrocyte production and coagulation[18]. Human induced pluripotent stem cells (hiPSCs) were employed, and they were later developed into hepatocyte-like cells (HLCs). In addition, they joined generated HLCs using human embryonic stem cells (hESCs) by alginate hydrogels and ran protein assays on them, proving that the construct was composed of a liver. This study's findings demonstrated that hESCs & hiPSCs may be bioprinted while keeping pluripotency

and differentiation potential, and that these cells could also be used for individualised and animal-free medication discovery. A variety of biomaterials, including gelatin, dECM, hyaluronic acid, alginate, GelMA, & collagen in various combinations, as well as various cell sources, including primary hepatic cells, liver tumour cell lines, and stem cell-derived hepatocytes, have been used in stereolithography to create biomimetic structures of the liver[139]. Engineered human liver models are increasingly being used in the pharmaceutical business due to their superior functionality, maturation, and consistent metabolism as compared with 2D-cultured hepatocytes[140].

Table 2. Applications of 3D bioprinting in tissue engineering.

Tissue/Organ	Biomaterial	Technique	Reference
Bone	gelatin	Extrusion-based bioprinting	96
Cartilage	collagen, Fibrin, Polyethylene glycol	Droplet-based bioprinting129 Extrusion-based bioprinting, Stereolithography	
Skin	alginate, collagen, Hyaluronic acid, PEG, PCL, PLGA	Extrusion and Inkjet-based bioprinting	96
Heart	alginate, collagen, Hyaluronic acid	Inkjet-based bioprinting	137
Liver	gelatin, alginate, Collagen, dECM, Hyaluronic acid	Stereolithography	21

Cancer: In addition to the tumour size, the tumour microenvironment influences pathology and treatment resistance. Tumours have a high level of complexity and heterogeneity[141]. The heterogeneous and complicated microenvironment in which cancer cells originate consists of an extracellular matrix and many cell types. Recently, scaffolds or cancer-on-chip technology has been used to construct an array of 3D cancer cell models, most notably in the form of spheroids or organoids. These models, particularly spheroids, have the disadvantage that they are hard to replicate in production on occasion and of becoming unable to effectively handle the organisation of several cell types in a sophisticated architecture[142]. Complex, multicellular, and repeatable 3D bioprinted constructions may possess their matrix constitution and stiffness tailored to the xenograft models that are being studied on a local or global scale. As a result of these considerations, 3D bioprinting seems to be the preferred technology for accurately mimicking the in vivo microenvironment of tumours[143]. 3D bioprinting, which represents a very user-friendly technique, can be used to add high-complexity tissue modelling. In comparison to the current organoid approach, 3D bioprinting allows for the automated construction of complex 3D models with reproducible and exact cell and matrix deposition[142].For GelMA, laser-assisted bioprinting (LAB) technique is used to create pancreatic ductal adenocarcinoma spheroid arrays composed of both acinar and ductal cells. Extrusion-based bioprinting method & a sodium alginate-gelatin hydrogel were used by researchers to create NSCLC (non-small cell lung cancer) co-culture structures using patient-derived xenograft cells & tumour-associated fibroblasts. The approaches revealed excellent printability and cell viability[144]. Using 3D printing, researchers created a multicellular scaffold-free tumour tissue that represented subtypes of breast cancer and pancreatic cancer. The printedstructure's numerous cell types might organise themselves into biomimetic patterns and secrete their own ECMs to repair the tissues. Incorporating patient-derived cells into models provides a translational tool for examining therapy responses, potential carcinogenic endpoints, and interplay between different cell types significant to specific patients[145].

Drug delivery and screening

Three-dimensional (3D) tissue designs, as opposed to traditional two-dimensional (2D) designs, have now been demonstrated to produce better drug screening findings due to their ability to mimic the spatial and chemical properties of genuine tissues. However, the difficulty to generate living tissues using in vitro methods has stopped 3D models from attaining their maximum potential[146]. Recent advances in bioprinting provide a practical way for producing biomimetic structures that can be exploited at various stages of the development and

discovery of drugs[147]. A variety of techniques have been used to construct in vitro 3D tissue models, including the hanging drop method, microwell-based methods, micro-patterned matrix structures, hydrogel culture, bioprinting, microfluidics, acoustic approaches, and magnetic force. Spheroids are the most basic and frequently utilised 3D tissue model. Surface modification, revolving wall vessels, the hanging drop method, and spontaneous creation can all be used to make spheroids. High-throughput microarrays can also be used to micro-biofabricate 3D models using techniques including cellular bioprinting, micro-wells, surface patterns, and microfluidics. Many microarrays of physiological organs or 3D constructs, such as the heart, liver, kidneys, lungs, and skin, as well as disease models, such as pulmonary edema and cancers, have been developed to date for acute as well as chronic testing for drugs along with high-throughput screening (HTS) of metallic substances for pharmaceutical and cosmetic development[18].

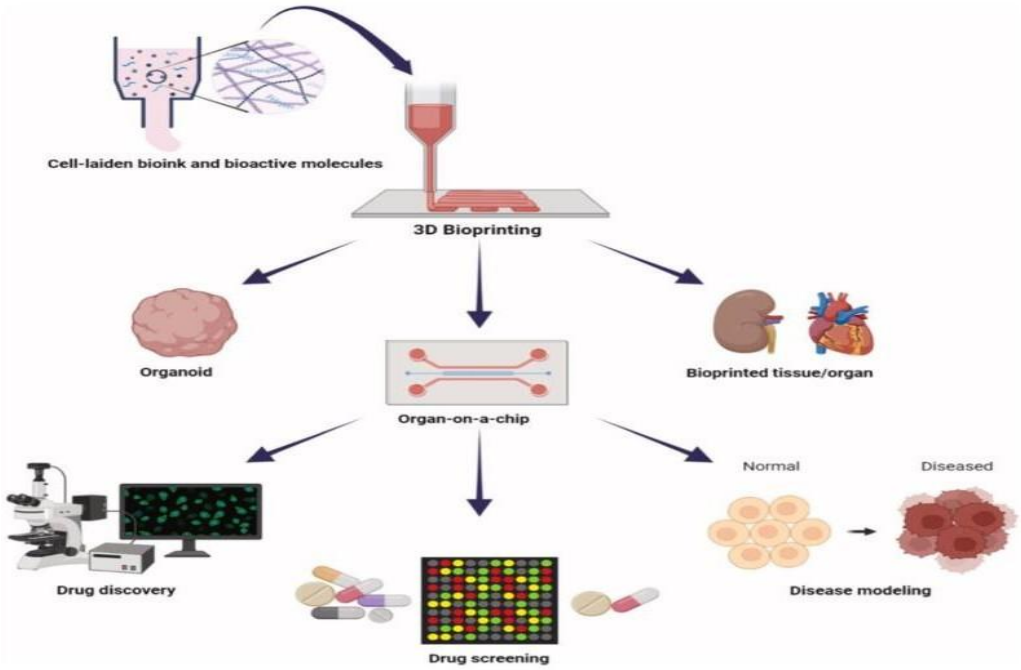


Fig. 8. Drug delivery and screening (Adapted from S. raees et al, 2023).

Bio-printing might be able to effectively and efficiently distribute growth hormones, medicines, and gene therapy. Organs-on-a-chip devices that mimic the pathways of normal organ activities can be created using bioprinting to evaluate potential pharmaceutical effects on tissues. 3D bioprinting allows for the manufacture of pharmaceuticals through drug screening and toxicity inspection in bio-printed tissue models, making it ideal for imitating human tissues in the most natural way possible. To produce an acceptable structure as well as the environment in the tissue model, cell types and source, bio materials and hydrogels, along with printing processes must be carefully chosen that correspond with the initial place of administration of drugs[17]. Thus, current research into the development of drug delivery methods for novel, physiologically relevant pharmaceutical innovations is likely to yield an abundant amount of data.

CONCLUSION

In conclusion, 3D bioprinting has evolved as a ground-breaking technology at the interface of biology, engineering, and medicine owing to its versatile bioinks and innovative printing procedures. We studied the development of printing techniques, the emergence of bioink materials, and a wider range of applications throughout this review. Bioinks have developed from simple hydrogels to complex, bioactive mixtures that improve cell viability and activity during printing. Additionally, a number of printing methods, including extrusion-based, inkjet, and stereolithography, have been improved to satisfy the particular requirements of various tissue and organ constructs. Applications for 3D bioprinting range from disease modelling and pharmaceutical testing to tissue engineering and regenerative medicine, with enormous potential for modified medicine and patient-specific treatments. The future of 3D bioprinting and its associated technologies appears exceptionally promising, poised to redefine the possibilities in healthcare and usher in an era of unprecedented medical advancements.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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