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3D Bioprinting- An Advanced Manufacturing Process for Healthcare Applications

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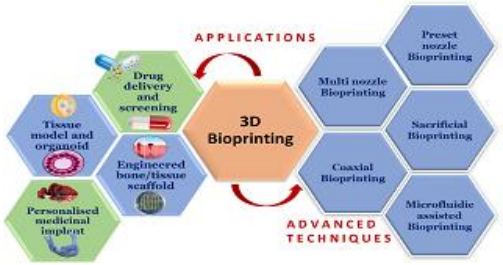
Abstract: 3D bioprinting is a cutting-edge technique used to create intricate mechanical and biological structures. It was developed to impart few advanced features to the process of biomanufacturing mainly for healthcare applications. This state-of-the-art technique is a viable alternative for the manufacturing of complex 3D biological scaffolds employing different bioinks/ biomaterial inks that improves the ability significantly to solve the shortcomings adhering to the traditional 2D biomanufacturing processes. Despite enormous advances of 3D bioprinting technology, the clinical translations of this technique are still constrained by several important issues such as restricted biocompatibility, fragile mechanical strength, and insufficient printability. Replicating native tissue architecture of an organ is challenging due to lack of suitable bioink resolving the above limitations. The present review briefly outlines the available polymeric hydrogels (as bioink) that could mimic the cell-ECM microenvironment using advanced 3D bioprinted scaffolds. Additionally, this review will also briefly present the recent advancements in material selection for successful bioprinting leading to futuristic applications in healthcare and medical research. It also explores the potential limitations of 3D bioprinting as future challenges to be addressed with advanced research strategies.



Keywords: *3D Bioprinting, bioink, hydrogels, tissue engineering, biomanufacturing*

Contents

1. Introduction

A computer-supported technology of assemble tissues through “layer-by-layer (L-b-L) precise positioning of biological materials, biochemical and living cells, with spatial control of the placement of functional components” is called Bioprinting.[1] It will be aimed for producing organ in a mechanized, engineered tissue or optimized manner and organized way. In an article by Groll *et al*. it is stated that bioprinting was restructured and distinguished as one of two main strategies within biofabrication.[2] The term ‘bioprinted’ is referred when bioactive molecules, living single cells, cell-aggregates or biomaterials are small enough and to be printed for

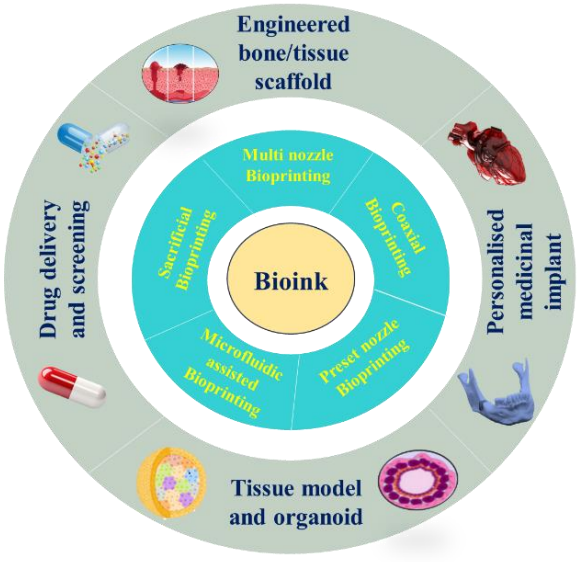


Figure 1. *Schematic diagram of the current review.*

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fabrication". Computer-assisted regenerative medicine and tissue engineering have been required the interplay of various technologies and disciplines like computing sciences, stem cell, developmental biology, and material sciences. To construct engineered tissue integration of different disciplines and comprehensive engineering strategies need to be required. However, it is always challenging to design the principle and strategy of functional three-dimensional (3D) bioprinting for different technologies and disciplines. There is a need to correlate the bioprinting process parameters and the printing strategies when someone will apply it for different tissue engineering applications. Many reviews article have highlighted the bio-printing for different applications, like, skin, heart valve, stem cell and cancer research etc. Additionally, these review article highlighted the common bioprinting techniques, material selection and cell sources etc. 3D printing is a process in which additive manufacturing can be done by successive layers of material are deposited or solidified to form a three-dimensional structure. This 3D printing technology has been applied in various fields, representing the large variety of applications, including the consumer of aerospace research, regenerative medicine goods industry, medical device development and the automotive industry. Using 3D printing we can manufacture drug which is an emerging field of research. The growing interest of 3D printed pharmaceutical products increases since 2015 as Food and Drug Administration (FDA) fist 3D printed drug on that year.[3] Since, several research over the methods and materials have been investigated and demonstrated in this area. Binder deposition, another powder-based method, where a bed of powder is taken and a liquid binding solution is printed onto it. For drug manufacturing Stereolithography, (in which selective solidification of a pool/bed of photosensitive material) may be used. High resolution alternative method of printing is Inkjet printing which offers 3D printing using both solid and viscous materials. Additionally, using polymer-based or hydrogels filaments drug products can be printed.[4] The growing interest in the research in 3D printing for drug formulation are very much important. Generally, there is a huge demand for adaptability, a feature that is not often observed in pharmaceuticals. This includes the ability to fabricate dosage forms with complex geometries and architectures, which is directly correlated to increased controlled release and complexity. 3D printing may also be applied using safe digital control for those drugs where precise and unique dosing is required. In addition, multiple doses or multiple drugs may be printed together in a singular dosage. Lastly, and most importantly, 3D printing is allowing to adapted an on-demand, prescription specific production for drug production. The ability of on-demand drug formulation will have a major application in emergency medicine and for short life medications. Moreover, 3D printing of drugs means that they can be produced for patients on an individual centric manner. This ability directly responds to the demand for individual centric medicine and health care product. Inkjet-based bioprinting is high speed and cost-effective techniques among mostly employed techniques which offers it for low viscous bioinks. However, the challenges appear while controlling droplet volume and cell density.

Most commonly used method is extrusion based bioprinting by which cell laden hydrogels can be printed using pneumatic or mechanical projection system. Although it is more compatible and versatile with different bioink but often limits of resolution. Laser assisted bioprinting is one of the complex and costly bioprinting method which can provide cell viability through laser induced forward transfer. Highly resolution 3D parts can be printed by stereolithography based bioprinting. These techniques are the main backbones of current bioprinting process. It serves as a framework for ongoing research and clinical purpose.[5-6]

Another definition of 3D printing, closely associated with additive manufacturing (AM), rapid prototyping (RP), or solid-freeform (SFF), a 'process of joining materials to make objects from 3D model data, usually layer by layer'[1], which had been described in 1986 by Charles Hull for the first time.[7] By adding materials, it will achieve satisfactory geometric accuracy to reduce waste. It starts with a meshed 3D computer model which can be create image data or structures in computer-aided design (CAD) software. A STL (Surface Tessellation Language) file is generally created. The mesh data will be processed into a build file of 2D layers and it have been sent to the 3D printing machine. Thermoplastic polymer materials, like polylactic acid, acrylonitrile butadiene styrene, polycarbonate, and polyamide as well as thermosetting polymer materials (epoxy resins) can be used by 3D printing technology.[8-11]

Tissue engineering is a vast interdisciplinary field which is aimed for development of functional tissues/organs as in vivo transplants to alleviate organ shortages or as in vitro models for studying disease mechanisms and invent drugs.[12] For the production of functional tissues/organs it is very much required to mimic the extracellular matrix (ECM) and cellular components of the human body. By understanding the tissue-specific microenvironment, like the ECM composition, cellular arrangement, and their natural physical properties, researchers can achieve the conditions that could affect neo-tissue formation. By understanding the human diseases various new drugs and therapeutic are now being developed, and they are mainly examined using 2D or 3D cell cultures or genetically modified transgenic animals. However, it has been shown by recent studies that the simplified in vitro models fail to execute physiological conditions as they have insufficient complexity. As an outbreaking alternative, 3D tissue models will represent better about the spatial and chemical complexity of living tissue than the 2D counterparts.

Even though various review articles have been published in this regard, however, here we highlight on the commonly used biomaterials and bioinks along with different printing approaches. Herein, the major purpose of our review article is to discuss mechanically stable, biocompatible bioinks specially for healthcare applications. In this review article (**Figure 1**), we have thoroughly discussed the 3D printing techniques, including the major processes utilizes, the materials used, their present status, and applicability in a variety of field. Additionally, this article also highlights the knowledge gaps and problems in implementation of this technology.

2. Cell-ECM Interaction Assisted by Biomimetic Scaffold

Humans possess specialized cells and are multicellular organisms. They communicated with other cells or the surrounding environment to carry out vital functions. The three-dimensional proteins and molecules that interact with cells by giving them structural and biological cues are known as extracellular matrices (ECM). Mechanical and chemical cues from the environment affect the cell. Shear, stretch, and compression are some of the physical forces that the mechanosensitive cells in the musculoskeletal system experience during movement or development. By adding ECM components, certain mechanical characteristics, such as stiffness or scaffold design, can be adjusted during tissue repair and regeneration.[12] The extracellular matrix (ECM) varies by organ, by area within an organ, and by physiological state. Their structure, signaling, and composition distinguish them from one another. ECM has a function in tissue formation and repair by providing mechanical support and biochemical signaling to the heart, dermis, or lungs. Various factors like aging, disease, and environmental variables can all have an impact on the extracellular matrix. This results in an excess of fibrotic tissue and organ failure. These perceptions draw

attention to the fact that cells and their extracellular matrix are intermingled. As the natural matrix is removed, tissue development may become dysfunctional.

Researchers have developed many ECM mimicking techniques to mimic the in vitro environment by using natural, synthetic, and hybrid materials. The spatiotemporal biochemical and mechanical stimuli are very hard to reproduce. These can be addressed by employing organ-derived extracellular matrices, bioinks, and functionalizing synthetic scaffolds in 3D printing processes. An endogenous-based strategy, in which cells create their own extracellular matrix (ECM) within a natural three-dimensional environment, shows great potential for simulating native tissue functions in vitro. This approach is especially useful for researching fibrosis, tumor microenvironment (TME) transformation, and other ECM-related disorders. It also better supports processes including morphogenesis, ECM remodeling, and response to injury.[13] In this regard, Samanta *et al*. have fabricated scaffolds that mimic the extracellular matrix (ECM) and stem cells for the restoration of brain tissue. This study highlights the importance of injectable 3D scaffolds that mimic the soft, native brain environment to promote neuronal maturation, neurite extension, and network regeneration. Furthermore, this scaffold aids in the restoration of neural tissue and serves as an in vitro functional model for cell-matrix interactions, disease mechanism comprehension, and medication screening and toxicity evaluation.[14]

3. Common Biomaterials in 3D Bioprinting

Biomaterials used for bioprinting are generally based on a "matrix", typically a natural or synthetic polymer, that interact with the biological system to repair, replace, or temporarily sustain tissues/organs for a time of period. Depending on their chemical composition, the biomaterials generally used in the 3D bioprinting field, are mainly classified into four groups such as metals, polymers, ceramics, and composites. While metals, metal alloys, composites and ceramics all have strong mechanical strengths, ceramics and composites are considered as corrosion resistant. On the other hand, in contrast to other materials, the polymers are highly biocompatible and biodegradable in nature. In order to replicate natural tissue, the ideal biomaterial for 3D printing should be biocompatible, biodegradable, and easily processed using 3D printing techniques.[15]

The choice of appropriate biomaterial for 3D printing purposes mainly depends on the intended use of the end products. For example, in the bone tissue engineering field, the biomaterials utilized should be mechanically rigid and biodegrade slowly. On the other hand, the biomaterial utilized for cutaneous or other visceral organ applications, the material needs to be flexible and degrade more quickly. Since most of the biomaterials like metals, hard polymers, ceramics, and composites employed in modern 3D printing biomedical technologies are rigid, therefore, they are commonly utilized in orthodontic applications. Hydrogels and other soft polymers are frequently utilized in bioprinting cells to create tissues and organs.[16]

3D bioprinting mostly uses thermoplastic biomaterials, including both natural and synthetic polymers. Synthetic biomaterials are frequently employed because of their regulated degradability, potential mechanical strength, and ease of production. They are used in biomedical applications because of their tunable characteristics as well as structural stability. In contrast, the higher molecular weight of natural polymers, causes a few drawbacks, such as their high viscosity and poor solubility. Therefore, in the realm of 3D bioprinting and tissue engineering, synthetic biopolymers are more preferable compared to the natural ones due to their optimized microstructure and construction flexibility. An overview of a few synthetic biomaterials is provided below:

3.1. Poly-vinyl Alcohol (PVA)

PVA is a semicrystalline, water-soluble, biocompatible, bioinert, and biodegradable polymer that is synthesized from the monomer vinyl alcohol and acetate. When the right adhesive is present, PVA's complex structure can create a matrix that aids in bone cell development.[17] Using the SLS printing technology, PVA material can be printed. PVA is utilized in biomedical applications to develop artificial articular cartilage because of its comparable tensile strength and load-bearing capacity to human articular cartilage. Furthermore, it has also been utilized as an articulation material in tissue engineering applications due to its load-bearing and lubricating qualities.[18,19] According to a few recent studies, PVA can be utilized in cartilage, craniofacial cartilage, dermal, and other tissue engineering applications when combined with other appropriate biomaterials and crosslinkers to create a blended hydrogel. For cartilage tissue engineering, Mohsen *et al.* have fabricated a norbornene-functionalized PVA bioink with thiol-ene crosslinker that exhibits good mechanical strength, cell survival, and printability.[20] We have developed a gelatine-PVA bioink for cutaneous fibroblasts that has been crosslinked with genipin, which is a repeatable, biocompatible bioink that can be applied to wound healing.[21] Muscolino, and coworkers have designed a noble bioink formulation for 3D bioprinting scaffolds by utilizing polyvinyl alcohol and kappa-carrageenan (kC). Interestingly, this bioink's quick gelation, excellent mechanical qualities, cell compatibility, and printability make it suitable for craniofacial cartilage tissue engineering application.[22]

3.2. Poly-caprolactone (PCL)

PCL is widely utilized in the scaffold production process for bone tissue synthesis as it exhibits broad rheological and viscoelastic behaviour. Through ring opening polymerization, it is produced from the monomer caprolactone. It is frequently used in biomedical applications due to its high solubility, reduced melting point, and blending compatibility. Furthermore, because of its drug-resisting properties, PCL can be used in controlled drug delivery and is completely eliminated from the body upon biological degradation. Its hydrophobic nature hinders its ability to readily adhere to cells and tissues.[18,23,24] Additionally, for tissue engineering applications involving cutaneous, adipose, bone, and muscle, it can serve as the primary component. In this regard, Cunniffe *et al*. have produced gene-activated bioinks based on polycaprolactone for bone tissue engineering applications. Here, the nanohydroxyapatite-plasmid DNA combination serves as a gene delivery vehicle, and polycaprolactone serves as a structural scaffold for alginate hydrogel. The difficulty of treating big bone deformities and complicated fractures has been addressed in this article. Interestingly, the bioink has facilitated the transfection of mesenchymal stem cells (MSCs) within the 3D-printed structure.[25]

In another article, Neufurth *et al*. used polycaprolactone and polyphosphate together to create regenerative bone repair implants. Polyphosphate is a high-energy, morphologically active inorganic polymer that is typically present in the human body. For load-bearing bone implants, the 3D-printed polyphosphate components are insufficiently robust. Polycaprolactone is therefore used to improve mechanical qualities in order to address this. Interestingly, the hybrid bioink promotes metabolic activity, cell motility, growth, and adhesion.[26] Nejad *et al*. have designed scaffolds based on polycaprolactone for the regeneration of pulp and dentin. Two distinct scaffolds were used to create a bilayer scaffold: polycaprolactone/hyaluronic acid and polycaprolactone/45S5 Bioglass. The bioglass improves the surface's mechanical strength and wettability, while the hyaluronic acid increases the

scaffold's hydrophilicity and provides a favorable cellular environment.[27]

3.3. Poly-glycolic Acid (PGA)

PGA is one of the synthetic polymers used in 3D scaffolding because of its biological properties, ease of processing, biocompatibility, and biodegradability. PGA's high porosity facilitates the diffusion of nutrients through tissue-scaffold contacts during implantation and, ultimately, neovascularization. When the ester group in the polymer undergoes hydrolysis, it produces natural metabolites that are non-toxic and are easily removed from the body as water and carbon dioxide. It is frequently utilized in bone fixation devices and resorbable structures. While surface functionalization through ester bond hydrolysis may impair the scaffold's structure, it can enhance cell adherence and dissemination.[28,29]

3.4. Poly- D, L-Lactic Acid

Polylactic acid (PLA), an aliphatic polyester, is well-known for its biocompatibility, biodegradability, and highly effective 3D printable bioink for tissue engineering. The FDM technique is generally utilized to fabricate the 3D scaffold of PLA. PLA-based filaments are particularly useful in musculoskeletal tissue engineering since they can be used to replace non-biodegradable fibers and ligaments. Long-term biocompatibility is compromised by the acidic byproducts that PLA produced during decomposition, which can cause local tissue irritation and even cell death. Furthermore, PLA's intrinsic brittleness limits its use in load-bearing situations by producing mechanical qualities that are subpar to those of genuine bone. In order to overcome these constraints, PLA is frequently mixed with inexpensive ceramic materials like calcium phosphate. This composite method helps neutralize the acidic breakdown products, which lowers cytotoxicity and improves scaffold performance overall. It also increases the mechanical strength of the resultant scaffold, making it more appropriate for bone tissue production.[19, 28] With these modifications, PLA-based scaffolds can provide safer and more efficient options for orthopedic and regenerative medicine applications.

Through such modifications PLA-based scaffolds can offer more effective and safer solutions for regenerative medicine and orthopedic applications. For example, Zamboni and coworkers have proposed injectable curcumin-encapsulated polylactic acid nanoparticles embedded in alginate/gelatin bioinks as a replacement for the intervertebral disc nucleus pulposus (NP). Such as, Zamboni *et al* have approached an injectable curcumin encapsulated polylactic acid nanoparticles embedded in alginate/gelatin bioinks to replace the nucleus pulposus (NP) of intervertebral discs. Interestingly, the PLA/curcumin hydrogel is demonstrating good mechanical, anti-inflammatory, and compressive strength characteristics.[30] In another article, Pant *et al*. have designed poly(L-lactic acid) (PLA)/β-tricalcium phosphate (β TCP)/mesoporous silica material (MSMs) composites to construct a scaffold for bone regeneration. This scaffold exhibits increased osteogenic gene expression, calcium mineralization, and increased ALP activity. In this study, the unique combination of PLA with β-TCP and different MSMs provided the desired mechanical, biological, and osteogenic qualities.[31]

3.5. Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) is a hydrophilic polymer synthesized through radical polymerization, typically forming either linear or branched chains with asymmetric or dissymmetric hydroxyl groups at the terminal ends. PEG is widely used in many biomedical applications, such as drug delivery systems, scaffolds for tissue engineering, and surface modifications to produce amphiphilic block copolymers and ionomers, because of its exceptional biocompatibility. One of PEG's noteworthy qualities is its resistance to cell adhesion and protein adsorption, which makes it perfect for fabricating hydrogels with anti-fouling features. Despite these benefits, PEG has drawbacks. Because of the carbon–carbon (C–C) polymer backbone, it is intrinsically nonbiodegradable. Its limited mechanical strength prevents it from being used in structural or load-bearing applications. Nevertheless, PEG may partially degrade in biological settings under specific circumstances due to hydrolytic and enzymatic activities. By copolymerization PEG with biodegradable components or altering it with cleavable links, these degradability improvements are frequently accomplished. However, in certain tissue engineering situations, PEG's non-adhesive properties might prevent cell adhesion. This feature is helpful in medication delivery, where biocompatibility and controlled release are important considerations. Overall, PEG is still a flexible polymer, particularly when structurally modified to serve certain biomedical purposes.[19,32,33] In a recent article, Bandyopadhyay *et al*. have developed a bioink based on photo-cross linkable silk methacrylate (SilMA) and polyethylene glycol diacrylate (PEGDA) for cartilage tissue engineering applications. Interestingly, the bioink has demonstrated favorable rheological and mechanical characteristics, cytocompatibility, and high printability. The printed structure promoted the development of neocartilage and cell proliferation.[34] According to Liu and coworkers, blended oligo(poly(ethylene glycol) fumarate with gelatine produces a durable, cell-rich UV crosslinking hydrogel for bone and nerve tissue engineering. This micro-structured hydrogel, which is cross-linkable and biodegradable, improves the transfer of nutrients and oxygen to cells that are encapsulated. Bioprinted scaffolds allowed PC12 nerve cells and MC3T3 pre-osteoblasts to proliferate and remain highly viable. This confirms that OPF-based bioink is appropriate and compatible for bone and nerve tissue.[35] Sometimes traditional drug delivery systems have occasionally failed to respond to each patient's needs. Biotechnology and genetics advancements are enabling patients to receive personalized treatment tailored to their specific needs. In this regard, Acosta-Vélez *et. al*. has developed a photocurable bioink based on polyethylene glycol (PEG) for printing hydrophobic drugs, which comprise a significant portion of pharmaceutical substances. Faster tablet manufacture and reliable active ingredient encapsulation are made possible by the UV-cured bioink. Overall, this approach highlights the potential for safe, customizable, and efficient fabrication of personalized oral dosage forms.[36]

4. Common Bio-ink in 3D Bioprinting

Bioinks are essential for creating scaffolds because they give tissue construction structural support. To preserve the intended shape and integrity after bioprinting, they are usually stabilized by crosslinking. Depending on the particular cell types and applications, different bioinks and bioprinters should be chosen.

An ideal bioink should have a well-balanced combination of physicochemical characteristics, such as mechanical strength, chemical stability, biocompatibility, and suitable rheological behaviour.

The primary function of bioink is to facilitate the creation of tissue constructions that closely resemble the mechanical characteristics of natural tissues while demonstrating enough mechanical strength and structural integrity. These mechanical properties should ideally be adjustable to accommodate the unique requirements of various tissue types. In order to produce bio printed objects with excellent shape fidelity and structural precision both during and after the printing process, the materials need to exhibit predictable gelation and stability behaviour. Apart from mechanical and processing qualities, biocompatibility is a crucial prerequisite to ensure that the

substance maintains cell viability and function without triggering unfavourable immunological reactions. Additionally, the material should be chemically modifiable so that bioactive cues or functional groups can be included to customize it for certain tissue applications. Lastly, for clinical and practical translation, the substance needs to be able to be produced on a wide scale with high batch consistency. For biomedical applications to be reliable, reproducible, and compliant with regulations, there must be very little variation from batch to batch.[37,38] Different applications of bioink in medical industries are illustrated in the **Figure 2**.

4.1. Collagen

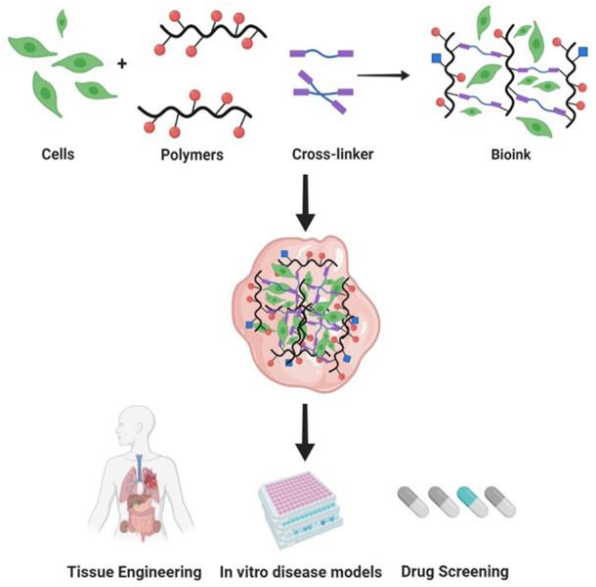

Figure 2. *State-of-the-art of the application of bioinks in different medical industries* (*reproduced from Ref. 19 under CC BY 4.0.).*

Collagen is a biocompatible natural polymer based on triple helix proteins that contains sulfur-containing amino acids and proline, hydroxyproline, and glycine amino acid residues. It is the major constituent of the extracellular matrix of skin and therefore has been extensively used in extrusion based bioprinting as bioink.[39] Collagen is considered as cell-friendly due to its excellent characteristics like cell binding capacity, hydrophobicity and enzymatic degradability. Furthermore, the collagen's ability to undergo gelation at different temperatures has improved its suitability as a bioink for tissue engineering applications. However, compared to other natural or synthetic hydrogels, it slowly undergoes gelation and has poorer mechanical qualities. These difficulties can be minimized by combining synthetic polymeric hydrogel with collagen to create hybrid collagen, boosting the concentration of collagen, or printing the hydrogel in sacrificial support gel.[40,41] In a recent approach, on an extrusion-based bioprinting technology, the differentiation potential of bio printed mesenchymal stem cells (MSCs) was investigated using bioinks based on collagen and collagen–agarose blends. Interestingly, MSCs in the stiffer collagen–agarose hybrid matrix preserved structural integrity but inhibited cell spreading, whereas those encapsulated in collagen-only bioink displayed spreading behaviour after printing. The findings also demonstrated that although the stiffer isotropic agarose-rich matrix promoted adipogenesis, the soft anisotropic collagen matrix promoted osteogenesis. In another approach, a composite bioink was made by combining collagen and alginate. To bio print 3D porous structures, pre osteoclasts and adipose-derived stem cells (ASCs) were cultivated on a collagen gel and subsequently combined with alginate. Compared to alginate alone, this collagen-alginate bioink showed increased osteogenic potential. Additionally, the bio-printed structures promoted the development of ASCs' hepatic lineage, indicating a wider range of tissue engineering applications. Collagen is also commonly utilized as a "bio paper" in bioprinting, acting as the substrate for the deposition of spheroids or bioinks loaded with cells. Similar to traditional printing media, bio paper offers the supporting hydrogel surface required to preserve structure and permit cell function in bio printed tissues, much like conventional printing medium. This demonstrates how collagen can be used in a variety of bio fabrication techniques, from fostering controlled cell differentiation to providing structural support.[42,43]

4.2. Hyaluronic Acid

Hyaluronic acid (HA) is an anionic, non-adhesive, non-thrombogenic viscoelastic biomaterial that contains *D*-*N*-acetylglucosamine and *D*-glucuronic acid repeating units in its structure. It plays a crucial role in the extracellular matrix (ECM) of various tissues, including cartilage, synovial fluid, cardiovascular, connective, and neurological tissues. Hyaluronic acid is essential for a number of tissue engineering processes, including angiogenesis, wound healing, cell proliferation, and interactions with cell surface receptors[44]. In this regard, Unal and coworkers suggested a method for creating a supramolecular hydrogel based on HA utilizing a 3D bioprinting technology based on extrusion. When mechanical deformation is applied, the hydrogel exhibits reduced viscosity because of the non-convent link and its associated characteristics[45].

4.3. Gelatin

Gelatin, a naturally occurring polymer based on fibrous proteins and produced from collagen, is water soluble, biocompatible, biodegradable, and non-immunogenic. The composition of gelatin varies depending on the source of collagen. For example, gelatin derived from human bodies has been used for decades in regenerative medicine, while gelatin derived from fish products has a lower melting point, viscosity, and gelling point. The behaviour of gelatin can be affected by temperature, concentration, and pH. Gelatin shows dual acid-base behaviour due to the presence of both acidic and amino acid groups. The gelatin molecules are bound together in the solution by nonspecific bonds such as hydrogen and static bonds. Furthermore, the hydrogel becomes thermosensitive when high temperatures cause that connection to break. Due to this feature, the hydrogel must be printable and stack up the specific CAD/CAM design.[46]

4.4. Chitosan

Chitosan is a naturally occurring polymer of *D*-glucosamine and *N*-acetyl-*D*-glucosamine that is *β*-(1-4)-linked. Chitosan is used in biological tissue engineering because of its non-toxic nature, antibacterial, biodegradable, and biocompatible properties. As chitosan is hydrophilic, it adheres to cells and promotes cell development.[47] It further encourages the production of collagen by fibroblasts. The benefits of chitosan in wound healing are further enhanced by its ability for medication transport and homeostatic activity. Chitosan and glycosaminoglycans share a similar structure and thus become an excellent option for bone cell colonization and chondrogenesis. However, the limited mechanical strength of chitosan itself prevents it from being used in several applications. In this regard, nanocellulose, hydroxyapatite, and other elements must be added to chitosan to prevent this kind of restriction.[48,49]

4.5. Cellulose

Cellulose and cellulose-based materials are being reintroduced as promising bioinks for 3D bioprinting applications due to their exceptional shear thinning, controlled

viscosity, and great shape-retention capabilities. Cellulose pulp is frequently broken down into nanoscale fibers by mechanical disintegration, which also improves the mechanical characteristics of nanocellulose by delaminating its fibrils. Typical methods include electrospinning, mixing, ball milling, and ultrasonication. High-pressure homogenization is especially effective because it breaks down cellulose slurry using shear and impact forces by forcing it through a tiny nozzle at high pressure. Mechanical techniques, however, use a lot of energy and could produce worse-quality products with lower yields. Pre-reducing cellulose size can help reduce nozzle clogging, a major problem in homogenization. Despite limitations, mechanical techniques provide an environmentally benign, chemical-free way to produce nanocellulose.[50,51,52]

4.6. Multi-material Bioinks

In order to develop a suitable bioink, optimizing material characteristics and improving ink printability should be necessary. With the goal of advancing the field and reaching important milestones in bioprinting technology, numerous studies have been carried out. Researchers have developed multi-material bioinks, including collagen-alginate, alginate-methylcellulose mix hydrogel, and gelatin methacrylate-based hydrogel, which are the most acceptable approach.[53,54] These works contribute to the advancement of multimaterial fabrication in 3D bioprinting. In addition to the development of multimateral bioinks, a novel concept has emerged for

constructing large anatomical structures—the self-assembly of bioinks. This approach offers a promising strategy for achieving complex tissue architectures with enhanced structural and functional integration.[55]

5. Different 3D Bioprinting Techniques

In tissue regeneration, scaffolds primarily give transplanted cells the mechanical strength and structural support they need to grow properly and readily perform their physiological functions. In order to facilitate cell adhesion, proliferation, differentiation, and extracellular matrix (ECM) secretion, the perfect scaffold should be biocompatible. Cell migration, vascularization, and tissue integration also depend on appropriate pore size and interconnectivity. Different cell types and tissues, each with specific mechanical and biological needs, must be supported by a good scaffold. However, some 3D fabrication methods-such as fiber deposition, electrospinning, gas foaming, electrospinning, and salt leaching-frequently lack the accuracy required to regulate internal architecture and spatial organization. These restrictions impede the creation of complicated, useful scaffolds particularly for the regeneration of complex tissues or organs and the healing of clinically relevant damage. Therefore, to solve these problems, advanced fabrication techniques are required. In this regard, additive manufacturing (AM) has emerged as a viable method for creating intricate scaffolds for interfacial tissue engineering.[56,57]

Additive manufacturing uses computer-aided design/computer-aided manufacture (CAD/CAM) technology to create 3D biocompatible structures layer by layer. One of the main facets of AM that focuses on printing bioactive materials with extreme precision is 3D bioprinting. It enables control over scaffold shape, size, porosity, and interconnectivity— all of which are essential for tissue growth, nutrition diffusion, and cell adhesion. This technology allows the fabrication of customized scaffolds that closely mimic natural tissue architecture, making it an essential tool in regenerative medicine and advanced tissue reconstruction.[58]

Presently, bioprinting technologies based on various technologies (extrusion-based, jetting-based, and laser-based bioprinting) have been widely adopted in the field of tissue engineering, resulting advanced progress in tissues engineering. Extrusion-based bioprinting comes with a printing nozzle to extrude biological materials and active cells along with other matrices into continuous filaments under the applied mechanical force, and deposit them layer by layer (LbyL) for a

Table 1. *Pros and cons of few commonly used biomaterial / bioink and their applications .*

Bioink material	Pros	Cons	Applications
Poly-vinyl alcohol[18-20]	Comparable tensile strength Great load-bearing capacity	Suboptimal biocompatibility, Limited structural stability, Non-biodegradable	Cartilage, craniofacial cartilage, Dermal tissue engineering, wound healing application
Poly-caprolactone[23-,26]	High solubility, Reduced melting point, Good blending compatibility Cell- tissue adhesion capability	Tends to brittle for thin structure, Grenerate cracks, Poor print resolution at low temperature	Cutaneous, adipose, bone, and muscle tissue engineering
Poly-glycolic acid[28,29]	Biocompatibility, Biodegradability	Release acidic degradation Less mechanical strength	Neovascularization, Tissue scaffold
Poly- D, L-Lactic Acid[19,28,31.30]	Good mechanical, Biocompatible	Release acidic byproducts while long term use	Musculoskeletal tissue engineering, Regenerative medicine, Orthopedic applications
Polyethylene Glycol[32-36]	Good flexibility, Exceptional biocompatibility	Nonbiodegradable, Limited mechanical strength	Drug delivery systems, Scaffolds for tissue engineering, Cartilage, bone and nerve tissue engineering,
Collagen [39-43]	Nano-fibrous architecture, Cell proliferation	Soluble in acid	Tissue engineering, extracellular matrix components for bone tissue
Chitosan[47-49]	Antimicrobial property, Mechanical strength, Biocompatible	Less structure stability	3D neural tissue construction, Personalized Medicine
Gelatin[46]	Highly water soluble, Biocompatible in nature	Low rigidity, Less shape stability	Tissue engineering, Living tissue constructs
Hyaluronic acid[44,45]	Cell proliferation, Capable of gelation	Difficulties to maintains structural integrity	Tissue engineering, Cartilage bioprinting, cartilage matrix development
Cellulose[50,51,53]	Shear thinning, Controlled viscosity, Great shape-retention capabilities	Less water solubility	Skin Regeneration, Bone Tissue Engineering, Organ-on-a-Chip model
Multimaterial bioink[19,55]	Higher structural and functional integration cell adhesion	Difficult to uniformly printing, Increase regulatory complexity	3D neural tissue construction, Skin Regeneration, Bone Tissue Engineering

three-dimensional structure. Extrusion based printing is widely in use due to its simple printing method and low requirements for materials and cells. Following sections consist of different types of bioprinting methods generally are being used in recent years.

5.1. Bioprinting with Multi Nozzle

Multi Nozzle Bioprinting is a sophisticated manufacturing technique in additive manufacturing with multiple printing heads and nozzles. Although there has been progress in scaffold-based tissue engineering, it is still challenging to precisely arrange various cell types at high densities inside a three-dimensional scaffold. This precision is essential for the fabrication of tissues which can function as good as the natural one.[59] One efficient method for accurately positioning several cell types within a three-dimensional scaffold is to use cell aggregates or spheroids. When cells come together, they naturally create these aggregates, which enable them to arrange into structures that mimic actual tissues. This technique is particularly beneficial since it preserves cell-to-cell connection and encourages improved functionality.[60] Furthermore, the higher cell density of these units makes them less vulnerable to cell damage during the deposition process. The spheroids must be held in place by a printed structure in a multi-material bioprinting system.

In the **Figure 3** a multi nozzle bioprinting system is demonstrated where the extrusion nozzles move parallel to the X-Y axis and the building platform is in the Z directions. The extrusion-based 3D printing system consists of three printing nozzles, two FDM liquefier nozzles to extrude ABS and TPU filaments, respectively, and a pneumatic dispensing nozzle to extrude the customized silicone ink.[61]

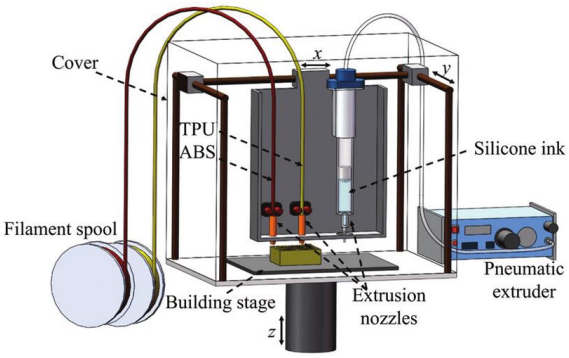

Figure 3. *Schematic of custom-made multi-nozzle 3D printing system (reproduced from Ref. 61 under CC BY 4.0.).*

5.2. Bioprinting Coaxial Nozzle

This is a newly developed bioprinting technique that uses coaxial nozzles on hollow alginate filaments. "Coaxial" describes the concentric configuration of two nozzles, one of which delivers a crosslinker and the other a bioink.

Extrusion based bioprinting is the most common bioprinting method but it limits higher resolution and is very sensitive to external condition. Coaxial nozzle based bioprinting is a special type of extrusion based bioprinting where coaxial nozzle is used instead of a single nozzle. By this method, tubular and vascularized structure can be printed easily (**Figure 4**).[62]

By imparting microchannels, the crosslinking nature can be sophistically controlled, implanting adhered hollow filaments to melt and form three-dimensional hydrogel structures. Moreover, they can function as pre-fabricated microchannels for nutrient delivery, facilitating cell growth within scaffold. Such approach offers a better and functionally more effective method compared to existing printing techniques.

As illustrated in **Figure 4**, a coaxial bioprinting system comprises a motorized XY stage that is connected to a coaxial nozzle, a Z stage that is connected to a Z-shaped printing bed platform, a container that contains a calcium chloride solution, a four-channel syringe pump, and a computer that controls the axes' movement and the pump's operation. For the printed structure to remain accurate and of high quality, the XY stage in this case makes sure that the nozzle is positioned precisely. This coordination enables the bioprinter to produce intricate and precisely defined three-dimensional structures. The Z stage as compared to others is equally critical, as it controls the printing speed for each layer, ensuring proper deposition and mechanical stability. By adjusting the Z stage appropriately, the fusion and gelation sequence of the material can be synchronized, enabling uniform layer adhesion and enhancing the integrity of the final construct. Added control of these parameters complements the fabrication of stable and functional tissue constructs, making this printing technique more effective for developing active biomaterials.[63]

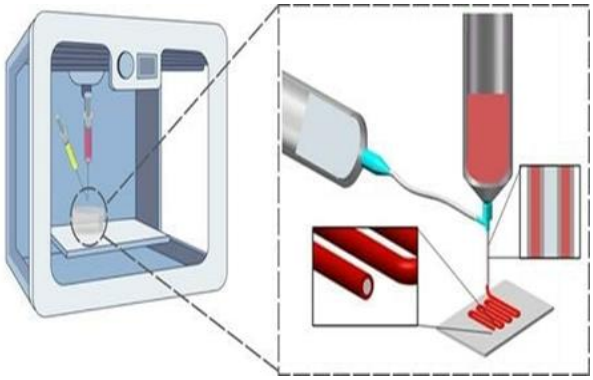

Figure 4. Schematic diagram of coaxial nozzle-assisted 3D bioprinting system (*reproduced from Ref. 62 under CC BY 4.0.).*

5.3. Microfluidic Assisted Bioprinting

Since last few years, 3D-bioprinting has become essential and useful in research on tissue engineering, promoting the simple and tailor-made fabrication of viable tissue structure. This method creates whole 3D tissue structures by layer-by-layer depositing bioinks loaded with cells. This is accomplished via a number of techniques, including as inkjet, laser-assisted bioprinting, and micro extrusion. Among them, microfluidics-driven bioprinting is considered as a unique extrusion-based technique that compiled the interfaces of microfluidic systems with bioprinting, substantially enhanced the structural and material compositional varieties of printed tissue models.[64] Microfluidic devices can handle volumes as small as 10^{-9} to 10^{-18}L, allowing for fine fluid control on a microscale. By enabling precise spatial and temporal control, this lowers expenses, waste, reagent consumption, and analysis time. The use of microfluidic chips, which feature reservoirs and channels, in drug development, diagnostics, and molecular research is growing rapidly. In 3D bioprinting, they are especially useful because of their capacity to regulate fluid flow and bioink mixing.

For microfluidics-assisted bioprinting, the flow of bioink through microchannels, allows precise flow control, modulating, and mixing of components with minimal shear stress. The laminar core's sheath flow improves cell survival by minimizing mechanical damage. Furthermore, printed structures can have their shape, size, and orientation precisely controlled via microfluidics. Microfluidic systems enhance resolution above the conventional micro extrusion limit (~50 µm) when used in conjunction with extrusion bioprinting. The

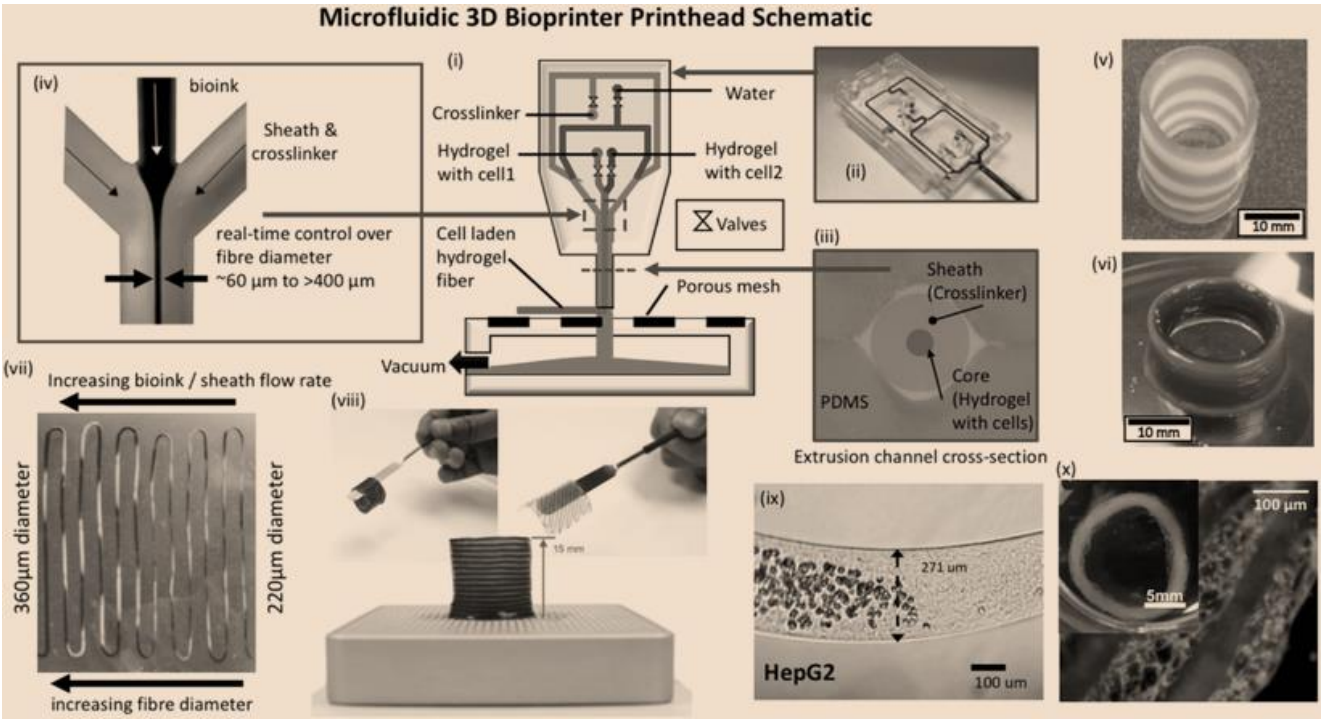

Figure 5. ***Microfluidic bioprinting principles.*** *i) Schematic of a microfluidic 3D bioprinting system depicting a ii) two material PDMS microfluidic printhead with integrated pneumatic valves and iii,iv) coaxial flow focusing extruder capable of generating hydrogel fibers with diameters ≈60 to > 400 µm. Integration with a 3-axis positioning system and custom software enables a variety of multimaterial structures to be fabricated including. v) Tubular structures with inter-layer switching and vi) concentric tubular structures with in-plane intralayer material switching. Flow control over the ratio of hydrogel and crosslinker flow rate enables vii) sequenced 2-material fibers with on-the-fly control over fiber diameter. viii) Printed alginate structures are robust and can be manual manipulated directly postprinting. ix) Abrupt switching between regions containing cells and those without cells is possible. A variety of different cells have been validated in the hydrogel fiber platform including x) human airway primary smooth muscle cells in an alginate collagen fiber and cultured to produce a functional airway contraction model (reproduced from Ref. 64 under CC BY 4.0.).*

combination of microfluidics and bioprinting makes it possible to create functional and incredibly intricate tissue structures, which makes it a proliferating area of regenerative medicine. Such process offers enhanced control and reduced mechanical stress.

In the past few years, the bioprinting process has become more and more efficient. In microfluidic systems (**Figure 5**), T/Y shaped chips inturn facilitate the feeding of multiple materials into the printing head. In order to facilitate abrupt material transitions, bioinks can also be alternately pushed through two channels into a single nozzle using opposed syringe pumps. These developments advance tissue engineering and multimaterial manufacturing by improving the accuracy and adaptability of microfluidic bioprinting.

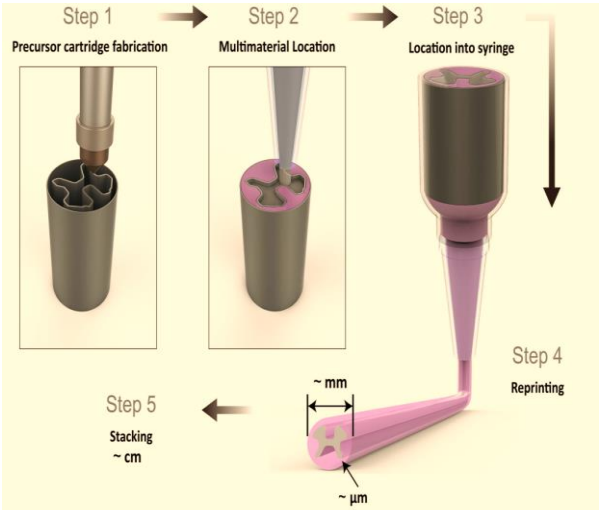

Figure 6. *A schematic illustration of pre-set extrusion bioprinting technique (reproduced from Ref. 66 under CC BY 4.0.).*

By using these methods complex and vascular networks of the organ can be developed for tissue engineering. For mass production of drug testing, personalized medicine and disease modelling it can play a big role.[65]

5.4. Preset Nozzle Bioprinting

Current bioprinting technologies face several limitations in replicating natural tissue-like structures. In order to overcome these difficulties, preset bioprinting has been developed, which makes it possible to create intricate, diverse, multicellular, and multi material structures.[66] In extrusion-based bioprinting, cells can experience damage due to shear stress when passing through small-diameter nozzles. The use of larger-diameter nozzles helps to alleviate this problem by decreasing shear stress and improving cell viability throughout the printing process.[67]

Preset nozzle bioprinting process involves (As illustrated in **Figure 6**)
Step - 1: A compartmentalized precursor cartridge with many segments is present. Its freely adjustable cross-sectional shape enables a variety of adaptable structures

Step - 2: Various types of bioink are added to cartridges as needed.

Step - 3: Cartridges loaded with bioink are inserted into a syringe.

Step - 4: Several bioinks are deposited from the printing nozzle and appear three-dimensionally, matching the cross-sectional geometry of the precursor cartridge.

Step – 5: Create a tissue structure on a large scale by stacking single multi material struts.

The main advantages of preset nozzle bioprinting are its ease of use in printing heterogeneous structures and its ability to reduce structural distortion.[66]

5.5. Sacrificial Bioprinting

Sacrificial bioprinting is a cutting-edge bioprinting method that builds intricate tissue and organ architectures using sacrificial biomaterials. This technique improves the structural integrity and usefulness of printed structures by making it possible to fabricate complex vascular networks. Sacrificial bioinks, sometimes referred to as flexible bioinks, have the special capacity to crosslink gently and reversibly using physicochemical principles. As these bioinks may be removed selectively without compromising the target cells or biomimetic structures, they are essential for producing hollow channels in created tissues.[68]

Using the sacrificial bioprinting method, hollow microchannels can be precisely fabricated inside a hydrogel matrix. This technique proficiently replicates the natural tissue architecture by improving the capacity to produce mammary duct-like structures with high fidelity within an ECM-like microenvironment.

Steps of Sacrificial Bioprinting Procedure:

The crosslinking principle of sacrificial biomaterial in 3D bioprinting process is described in **Figure 7**. To achieve the gelation, a layer of GelMA is poured into a PDMS mold and allowed to cool to ambient temperature. A bioprinter is used to extrude an agarose microfiber onto this GelMA layer. It is necessary to cast and crosslink an additional layer of GelMA to the entire hydrogel. Finally, an external force should be used to extract the agarose microfiber from the GelMA construct. The microchannel will be created here. To give the hydrogel its biological properties, cells can be further seeded into this microchannel.[69]

6. Crosslinking of Hydrogel in Bioprinting Process

Crosslinking of polymer is needed to stabilize one polymer chain with another and form solid or gel like solution from liquid solution. Polymers can be crosslinked by covalent bond formation, condensation of multifunctional groups, irradiation like UV crosslinking or electron beam etc. Hydrogels are water insoluble physical or chemical crosslinked polymers that are used in the bioprinting process as bioink.[70] To obtain proper printability and functionality and good mechanical properties, hydrogels are crosslinked. However, several cross-linking techniques have been developed. Their effectiveness is restricted by the biomaterial nature and concentration that should be balanced carefully to reduce the risk of cell viability and functionality compromising. Now the challenges are to choose crosslinking methods to upgrade efficiency, stability, or medical application of bioprinting strategy.[71] Physical crosslinking of hydrogels involves electrostatic interaction, ionic interaction, hydrogen bond forming, metal coordination, and π–π stacking etc. This is more biocompatible than the chemical crosslinking strategy. These hydrogels display external stimuli responses, self-healing capability, and injectability. It indicates that the hydrogels are appropriate for cell encapsulation and drug delivery. The alginate hydrogels which are mainly used in wound healing and tissue engineering are involved in ionic crosslinking which form gels upon interaction with divalent cations like Ca^{2+}, Ba^{2+}, and Mg^{2+}. On the other hand, cationic biopolymer chitosan forms polyelectrolyte complexes (PECs) via electrostatic interactions with polyanion like pectin, alginate, or polyacrylic acid. Chemical crosslinked hydrogels are respectively strong, stable and mechanically stronger than the physical crosslinked hydrogels. The common chemical crosslinking techniques are free radical crosslinked, enzymatic crosslinking, Diels–Alder click reactions, Schiff base formation, oxime formation, and Michael addition. These hydrogels have adjustable degradation rate and favourable performance in physiological conditions, making them highly suitable for biomedical applications.[72]

7. Applications of Bioprinting

While traditional tissue engineering technologies have showed promising effectiveness in the past, it is vital to understand the limitations of these methods. The major limitations include the inability to create scaffolds that accurately mimic the anatomy of natural tissue, the limitations on the biomaterials that can be transported using traditional engineering techniques, the unreliability of cell delivery, and the inappropriate interactions between various cell lines during in vivo implantation. Moreover, when applied to various in vivo

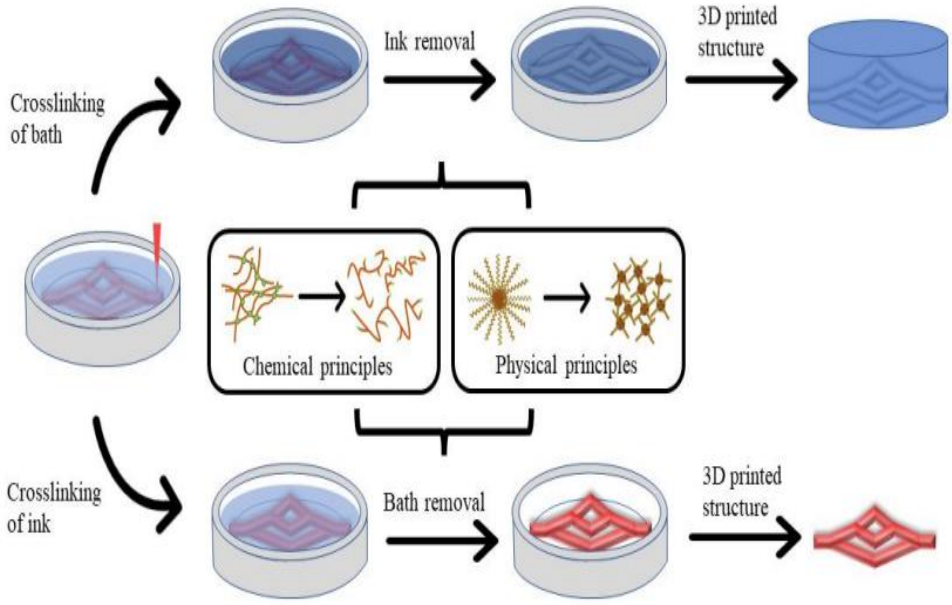

Figure 7. *Overview of the main application scenarios of sacrificial biomaterials based on physical and chemical polymer crosslinking principles in 3D bioprinting (reproduced from Ref. 68 under CC BY 4.0.).*

conditions, certain artificial in vitro structures may not work well. This type of interactions increases the chance of cell damage at the target location. Compared to traditional tissue engineering techniques, 3D bioprinting has numerous benefits. Three-dimensional bioprinting enables a more automated procedure while maintaining high precision and adaptability for each application. Additionally, the scarcity of available donors has led to a major focus on tissue regeneration and organ replacement. Over the past few years, animal models have been utilized to accurately simulate human diseases and improve therapeutic efficacy. However, in this process, the research expenses are likewise high, and therefore, research on human cell lines becomes the focus and 3D bioprinting has offered a way to use tissue engineering to manufacture tissue or functionalized organs.

Nowadays, sophisticated 3D bioprinting techniques can be used to manufacture complex, vascularized, freestanding, and cellular structures. It plays a significant role in personalized organ printing. In this regard, Tal Dvir's team in Israel used the extracellular matrix and the patient's own cells to print a vascularized human heart.[73] Furthermore, to study the onset and progression of cancer, this technique has been utilized to generate cancer models using cells and spheroids. Using bioinks containing cancer cells, extracellular matrix, and signaling chemicals, 3D printed models may accurately depict the intricacy of a tumor. By employing adipose-derived mesenchymal stem cells (ADMSCs) at the periphery and cancer cells at the center, Wang *et al*. have created a bioprinted breast cancer construct and the real tumor behavior in the 3D printed model aids in tumor medication testing.[74]

This method of additive manufacturing emerges as a means of tackling the problems brought forth by novel infections and complicated illnesses. It is highly beneficial for cancer, nerve tissue disorders, skin tissue, and diabetes due to the increasing need in therapeutics and medication discovery. With the use of this technology, 3D models can be created to investigate drug resistance, tumor growth, and cancer metastasis. Additionally, it is utilized to create lung models for viral research, such as those of influenza and SARS-CoV2, cardiovascular models, and brain tissue for CNS repair. Skin bioprinters and cardiac patches are two examples of promising innovations that are still in the preclinical stage. Moreover, there are numerous current clinical trials utilizing 3D organoids, especially in the fields of orthopedics, dentistry, and oncology.[75]

7.1. Drug Screening and Drug Research

In this century, extensive research on drug design and screening is being conducted to find new and effective treatments for different illnesses. However, the process of developing and approving drugs involves a long series of steps and ethical reviews, which can impede achieving successful outcomes.[76] Traditional animal models and in vitro cell culture setups do not fully replicate the conditions of the human body, leading to variations in drug performance and effectiveness.[77]

Additionally, the costs associated with drug production have been rising significantly over time, which can slow down the process. The advent of 3D bioprinting has addressed some of these challenges to a certain degree. This technology has made it possible to create tissue models for drug testing and research. These tissue models can closely resemble the natural human tissue environment and can provide results that closely reflect the effects of drugs.[78] Moreover, the tissue models can be produced in high quantities to enhance the number of trials and expedite the collection of results. The variations in human physiology among individuals can result in unpredictable responses to drug treatments. This gives rise to the concept of personalized drug screening, which aims to mimic the individual's tissue environment and examine the reaction to each drug being evaluated.[79]

7.2. Tissue Engineering

3D bioprinting has attracted significant attention to tissue engineering because of its variety of fabrication techniques, enhanced spatial resolution, and potential for customization.[80] This technology enables researchers to select the fabrication method based on the required resolution and porosity. The selection of materials and types of cells can be customized based on the organ or tissue sections being addressed. Using substances that mimic the extracellular matrix can help improve the incorporation of the seeded cells into the tissue and prevent the immune system from rejecting the scaffolds.[81] The development of 4D printing and multimaterial bioprinting techniques has made it feasible to produce vascularized tissues, patches, grafts, and intelligent scaffolds for use in microdevice and sensing applications. Bioprinting allows tissues to become vascularized, which is not possible with conventional cell culture techniques. It can also provide a sufficient supply of nutrients and oxygen, which promotes rapid cell growth.[82]

7.3. Organ and Disease Models

3D bioprinting is extensively utilized by researchers to develop miniature organs that facilitate in-depth investigations of disease mechanisms and the actions and responses to drugs. Additionally, prosthetic limbs, dental implants, and even miniature human heart replicas can be made with this technology. Therefore, before doing surgery, medical professionals can test these models, improving their comprehension of intricate anatomy and structures to reduce the risk of complications.[83] By utilizing X-rays and scans for proper alignment and comfort, physicians can insert 3D printed dental implants and braces crafted from biopolymer materials. Another significant development in 3D bioprinting is in cancer therapy. Tumour models can be accurately created through printing and utilized for testing chemotherapy drugs and their application, which can reduce the time required and address the challenges encountered by patients.[84]

7.4. Personalised Medical Device

Techniques such as additive manufacturing, including 3D printing, can alleviate the lengthy and expensive processes associated with creating a medical device. Printing using biomaterials can reduce production costs and make property adjustments easier. This method enables the device to be tailored to the individual requirements of the patient, which could increase its efficacy.[85] Furthermore, the use of mechanically stable and biocompatible materials reduces the possibility of rejection, and its price makes it available to the general public. Because of their consistent size and shape, the devices that are currently on the market might not be able to adequately fit all patients. Devices can be precisely tailored to match the target tissue by using 3D printing and sophisticated imaging techniques. These customized medical devices can provide better resolution and more specificity at a reduced cost.[86]

8. Limitations

When it comes to clinical translation, 3D bioprinting has a number of constraints. When the bioprinted tissue organ meets clinical translation, it presents a significant obstacle. Even though employing patient-derived cells reduces immunological rejection, extracting and growing cells and tissues still present difficulties. It is very time taking to expand the cell in clinical laboratory. Pluripotent stem cells (iPSCs) provide an alternate method by allowing fibroblast-like cells to enter cell types that are difficult to reach. This procedure is a quick fix. The creation of genetically modified or neutrally immune iPSCs would be an appropriate remedy. It can overcome the lack of cell source and immune compatibility.[87] Another challenge in the clinical translation of bioprinted tissues is their long-term storage and

transportation compatibility. For newly printed tissue or organ, it takes a long period to print and mature.[88]

9. Future Perspectives

3D bioprinting has made significant progress in improving treatment standards in the healthcare and medical sectors. However, it still encounters various challenges and limitations since we are working with live tissues and organs that possess very intricate structures. In the future, researchers are expecting to bridge the gap between laboratory success of bioprinting and real-life applications. Through the use of smart polymers, 3D bioprinting provides the capacity to customize tissue and organ structures in three dimensions, improving the interaction between cells and the printed scaffolds. The use of smart polymers in multifunctional stimuli-responsive tissue constructions can improve their overall performance and regenerative efficiency.[89,90] Organ and disease models can be created using 3D bioprinting using polymers that mimic the extracellular matrix (ECM). Drug testing, surgical testing, and cancer therapy trials could all be greatly aided by a 3D-printed model of tumors. Additionally, organ models that closely resemble actual human tissue can be produced with high precision for educational purposes and to aid medical students in their training and preparation. The development of bioprinting is concentrated on producing completely vascularized, functional organs that function similarly to those seen in the human body. This could help address the current issues around organ transplantation.[91] Significant obstacles to a successful organ transplantation are the prolonged waiting period for a suitable organ donor, which endangers the patient's health, and the elevated risk of organ rejection and other health issues. Utilizing The use of biocompatible materials in 3D bioprinting that mimic the natural cellular environment may open the door to a future with a higher organ transplant success rate and fewer organ failure deaths. Researchers are working on using 3D printed tissue scaffolds, which can carry out almost all of the functions of real tissue, to replace the damaged tissue in our bodies. In this golden era of stem cell research, the use of patient stem cells to create a whole, healthy organ through 3D bioprinting is anticipated in the upcoming years.[92] Drug screening and the creation of new medications provide additional challenges in the healthcare field. The development of bioprinting, which is characterized by the availability of organ and disease models for drug trial testing, has significantly shortened the time needed to introduce a new medication, which could raise the bar for medical care in near future.[93]

10. Summary

In summary, this technical review demonstrates the possibilities of successful implementation of the 3D-printing strategy in engineering functional translational models,

Rapid advances in additive manufacturing techniques including 3D bioprinting, in medical imaging research, biomaterials and cellular functionalities to ensure future developments in the innovation of patient-specific customized tissue models. Practical challenges however, remain to be addressed, such as cell and material modifications and applications, tissue maturation and responses and appropriate vascularization. Future interdisciplinary research and development are expected to further transform the fields of healthcare with special reference to tissue engineering and biomedical engineering using additive manufacturing.

Supporting Information

The article contains all the information have been appropriately cited in this article.

Biographical Information

Shrabani Bhunia completed her M.Sc. in Polymer Science and Technology from JIS University in 2022. Soon after, she joined as a Project Associate in a DST/AM-funded project and is presently pursuing her Ph.D. under the supervision of Dr. Prosenjit Saha. Her research is focused on the surface modification of natural fibers for applications in small- to medium-scale industries.

Gouripriya D A is graduated from Kerala University with a master's degree in Chemistry in 2021. She qualified GATE (2022) in Chemical Sciences and worked as a JRF in a SERB funded project for 1 year. Currently she is doing PhD under the supervision of Dr. Prosenjit Saha and focusses on developing biopolymer-based 3D printed scaffolds for tissue engineering and wound healing applications.

Poonam Debnath is graduated from JIS University in 2023 in polymer science and technology. She has worked as JRF in a SERB funded project. Her research area is Preparation of scaffold using combined 3D printing and electrospinning procedure for accelerated wound healing application.

Anish Deb is currently pursuing his PhD at JISIASR, JIS University on physico-mechanical characterizations of printable ink prepared from bio-based raw materials. His research focus consists of 3D Printing of polymer and composite, fluid mechanics, non-conventional energy sources, measurement systems.

Sneha Banerjee is a perspective PhD candidate at JISIASR, JIS University. She is presently working as a project assistant after completing her Master's degree in Biotechnology. Her research interests include molecular biology, natural product characterizations, and therapeutic applications of bio-resources.

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Dr. Pooja Ghosh is currently working as an Assistant Professor in the Centre for Interdisciplinary Sciences at JISIASR. She completed her PhD from Indian Institute of Technology (IIT) Kharagpur, West Bengal, India in 2018. In her Post-Ph.D. session, she was involved with the research and academic activities at Indian Institute of Science Education and Research (IISER) Kolkata, West Bengal, India. She has also been awarded International Excellence Fellowship and got the opportunity to visit Germany and work as a guest scientist in Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany in 2022. Dr. Ghosh has published ~35 well renowned peer reviewed journal papers. Her research interest includes designing nanoparticulate systems for drug delivery, interaction of nanomaterials/ligands with biological systems, design of novel therapeutic agents for modulating protein aggregation process.

Dr. Prosenjit Saha has completed his PhD from IIT Kharagpur, India in 2013. Following his PhD work, Dr. Saha joined textile manufacturing industry as Head, R&D at Kharagpur, India. After that he went to South Korea for his postdoctoral study. Dr. Saha has been selected for several prestigious fellowship such as Inspire Faculty Fellowship, DS Kothari PDF fellowship under the schemes of Govt. Of India Dr. Saha has started his independent research group almost one decade back in 2015. Dr. Saha is currently working as an Associate Professor in the Centre for Interdisciplinary Sciences at JISIASR. His research interest includes development of artificial skin scaffold for Bone/Tissue Engineering, 3D printed biomaterials and Bioink, development of eco-friendly fire retardant, hydrophobic, and light-weight structural composites and design of simple low-cost water purifier using natural materials applying basic nanotechnology. Dr. Saha and his group have published more than 40 international research articles, two patents, more than 15 book chapters. Three reference books edited by Dr. Saha were also published by several international publishing houses.

Author Contribution Declaration

Ms. Shrabani Bhunia, Ms. Gouripriya D. A., Ms. Poonam Debnath, Dr. Pooja Ghosh, and Dr. Prosenjit Saha conceptualized the idea of the study, data analysis, manuscript writing and reviewing. Mr. Anish Deb, Ms. Sneha Banerjee, Dr. Subhajit Ghosh helped in data collection and manuscript draft writing.

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Data Availability Declaration

No new data were generated for this study. All referenced data have been properly cited, with proper permissions where required.

Declaration of Competing Interest

The author declares that they have no known competing financial interests.

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