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Review

The Art of Building Living Tissues: Exploring the Frontiers of Biofabrication with 3D Bioprinting

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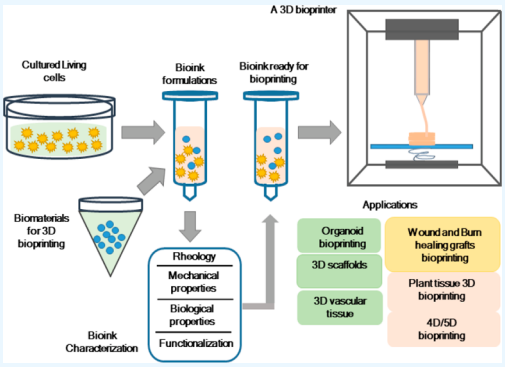
ABSTRACT: The scope of three-dimensional printing is expanding rapidly, with innovative approaches resulting in the evolution of state-of-the-art 3D bioprinting (3DbioP) techniques for solving issues in bioengineering and biopharmaceutical research. The methods and tools in 3DbioP emphasize the extrusion process, bioink formulation, and stability of the bioprinted scaffold. Thus, 3DbioP technology augments 3DP in the biological world by providing technical support to regenerative therapy, drug delivery, bioengineering of prosthetics, and drug kinetics research. Besides the above, drug delivery and dosage control have been achieved using 3D bioprinted microcarriers and capsules. Developing a stable, biocompatible, and versatile bioink is a primary requisite in biofabrication. The 3DbioP research is breaking the technical barriers at a breakneck speed. Numerous techniques and biomaterial advancements have helped to overcome current 3DbioP issues related to printability, stability, and bioink formulation. Therefore, this Review aims to provide an insight into the technical challenges of bioprinting, novel biomaterials for bioink formulation, and recently developed 3D bioprinting methods driving future applications in biofabrication research.



1. INTRODUCTION

The worldwide requirement for grafts and transplants has significantly increased in past years, as indicated by the number of transplants performed in the United States during January − April 2022, i.e. 13 567. Moreover, the problem does not end there, as 106 132 patients are waiting for their donors for either single or multiple organs. In this regard, three-dimensional bioprinting (3DbioP) technology is ready to deliver the global demand for bioengineered tissue grafts, prosthetics, and ready-to-print (RTP) bioink formulations for cardiac, liver, hepatic, and corneal tissue regenerative therapy. Furthermore, the above number emphasizes the importance of developing artificially prepared natural biomimics of human organs, which is possible through 3DbioP technology.[9]

3DbioP is a sophisticated, sensitive, and labile technique relying heavily upon the availability and compatibility of live cells derived from humans and animals.[1,2] The stem cells show pluripotency, the ability to differentiate into various types of live cells that attain a structure in a specific pattern to form a functional unit of live cells. Thus, the primary challenge in tissue engineering is to replicate tissue complexity in arrangement and networking to create a functional live tissue, i.e., ready-to-be-transplanted or grafted.[3,4] Initially, the stem cells are cultured in vitro and later added to a matrix, forming a biomaterial such as a hydrogel to form a biologically active ink, also known as a bioink. This bioink is the key to developing and augmenting 3D bioprinted products from laboratories to medical facilities worldwide. The developments in the technique of 3D printing, the easy availability of raw material, and the ever-broadening application spectrum have allowed a conglomeration of researchers and clinicians to work together and develop 3D bioprinted products.[5−7]

The advancements in techniques, tools, and biomaterials forming the bioprinted products have taken a giant leap in past years. Research in this area provides a unique opportunity to improve tissue and regenerative medicine procedures.[8] The cutting-edge bioink formulations, biomaterials, and scaffolds have accelerated the growth of tissue engineering-based services and research. Thus, the entire 3D bioprinting industry now thrives upon improvements in bioink formulation to recreate organs and prosthetics to replace them in patients whenever and wherever required by the clinicians.

3DbioP has vast applications in every sphere of life and is evolving at an exponential pace. The current Review, in principle, summarizes the requirements and advances in bioink formulations and recent advances in the techniques associated with 3DbioP and artificially prepared natural biomimics of human organs. A brief introduction to the utility of 3DbioP in

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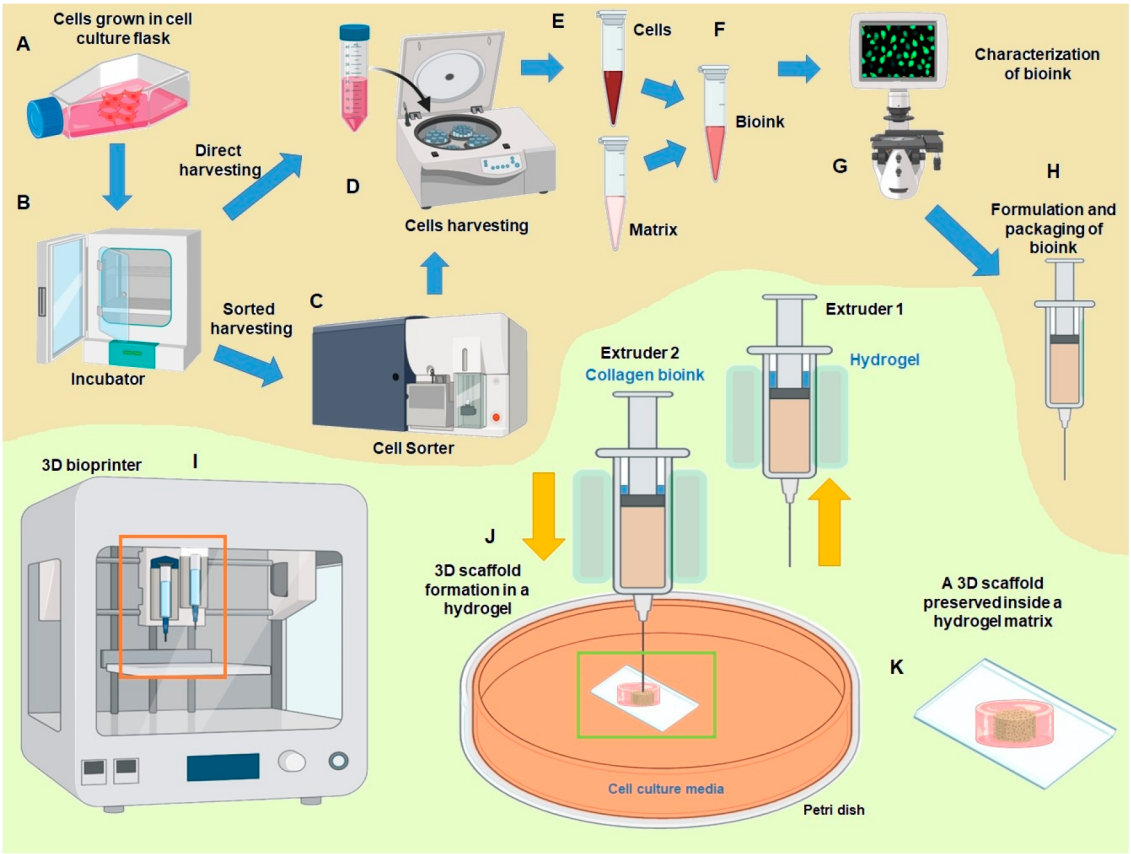

Figure 1. Illustration of various stages in 3D bioprinting. Phase 1 starts with (A) a cell culture of required cell lines in (B) flasks incubated in a CO_2 incubator. The cells are then sorted and collected using (C) a cell sorter and (D) centrifugation. The cells are mixed with (E) a suitable matrix and (F) formulated as a bioink. The bioink is then (G) characterized using microscopy and other suitable techniques. (H) The validated bioink is then appropriately packaged into a ready-to-use bioink. Phase II starts with (I) the preparation of a 3D design and a bioprinting environment, followed by (J) optimization of bioprinting in a 3D scaffold (hydrogel) and (K) obtaining a finished product stabilized inside a matrix/hydrogel. This illustration has been partially adapted from "Bioengineering and Biomaterials" by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

plant biology is also incorporated in the manuscript to widen the horizons of the researchers and clinicians. Lastly, we have discussed the key challenges and Authors' outlook on the current concepts and understanding. We have also attempted to concise the existing literature on 3DbioP in tabular form to comprehend recent advances quickly.

1.1. Biofabrication. 3DbioP technology is an additive manufacturing (AM) technique used to assemble live-cell-laden printing materials to produce a functional structure.[10,11] This process involves the generation of a biologically and functionally active structural organization, containing a mixture of living cells, bioactive molecules, biomaterials, and growth factors, to produce cellular aggregates, microtissues, or hybrid assemblies, which can be collectively termed "biofabrication".[12] The living cells, bioactive molecules, biomaterials, and cell aggregates form a biologically functional unit through assembly and subsequent tissue maturation processes. When mixed as a suspension, biomaterials and living cells are often referred to as a "bioink" in 3DbioP.[13,14] The "bioink" differs from "biomaterial inks" due to the presence of living cells and requires more stringency in its physicochemical parameters, thus not allowing complete experimental flexibility. This means that the biomaterial inks can be used with more flexibility related to temperature and pressure and provide a suitable organic/inorganic environment for stability and maintaining structural integrity.[15−17]

The biofabrication process involves the *in silico* design of a biological scaffold, followed by selecting a suitable biomaterial to prepare a supporting matrix/scaffold for the live cells to adhere to and, subsequently, for their growth, regeneration, and proliferation. The formulation is then tested for compatibility with the bioprinting technique employed for deposition and the biological and physicochemical properties. After that, the live cells are cultured, mixed with the prepared bioprinting formulation, and then tested for cell viability, proliferation and growth kinetics.[8,17] Once the formulation passes all tests, the bioink is subjected to final bioprinting and later examined for the functionality and stability of the bioscaffold (Figure 1).

Diverse 3D printing technologies have evolved in past years, with many variations in the working principle and the materials employed.[16,18] However, due to extreme temperature and energy radiation, conventional 3D printing (3DP) cannot be implemented in bioprinting cell-laden constructs. Hence, many live-cell-friendly versions of 3DbioP have been developed to overcome the past limitations and efficiently print the cell-laden constructs under physiological conditions. Extrusion-based bioprinting technology has emerged as the approach-to-go in 3DbioP, followed by stereolithography (SLA)-based technologies. Inkjet-based bioprinting is the earliest technique used for the 3D deposition of biomaterials as a continuous

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droplet or on a droplet-on-demand basis using thermal and piezoelectric actuators.[5,6]

Further, the droplet-based technique can be classified into acoustic and microvalve-based bioprinting, where the bioink droplets are forced onto the surface using acoustic waves or a pneumatic pressure over the electromechanical valve, respectively.[7,16] Recently, microfluidic devices have been introduced for the bioprinting of low-viscosity, finely organized 3D tubular and vascularized structures.[19] The in-depth description of each technique is beyond the scope of this Review; hence, the readers are referred to other commanding contributions to the literature in this field.

1.2. Bioinks. Bioinks are essential modules of the 3DbioP technique that offer growth and function support to the live cells for proper association and function. Besides, bioinks help minimize the influence of printing on the viability of cells without conceding the resolution shape and firmness of the construct.[20] Moreover, they allow the distribution of different types of cells and biomaterials at other locations within the bioprinted scaffolds.[15,21] The significant challenges in developing a successful bioink are to enhance the physicochemical properties, i.e., the printability,[20] biocompatibility,[18] biodegradability, and sterilization stability[22] of the biomaterials. The ability to print accurately into a defined design and structure within a specified time requires rapid cross-linking for precision layering.[16,23] Additionally, the coexistence of the live cell and biomaterials is essential in providing solutions for biological problems such as regenerative therapy, where stem cells are supplied at the site of interest in the case of implantable prostheses, scaffolds, and organ and tissue reconstruction.[8,13] In such cases, the properties such as chemical composition, structural morphology, mechanical strength, surface charge, and surface characteristics become imperative in the determination of biocompatibility.[16,20,24] The biocompatibility of a biologically functional scaffold thus refers to a substrate capable of supporting the critical cellular activity, i.e., regulation of mechanomolecular signaling pathways for optimal tissue regeneration, without eliciting adverse effects over the cellular machinery structure and functioning. In this regard, surface modification, compositing of natural and synthetic biomaterials, and biomimicry approaches have successfully developed biotechnologically and pharmaceutically compatible bioink.[13]

The mechanical strength conferred by the bioink is necessary for various biofabrication applications.[21] It could be derived from the cross-linking ability[25,26] or by adding sacrificial materials.[27−29] The sacrificial material provides additional support to the scaffold, as it can be deposited along with the bioink during printing and later could be removed through temperature or UV treatment. Biodegradability becomes an important parameter when designing implants, as the nontoxic degradation process should maintain an equilibrium between the rate of production of cells and the rate of degradation of the biomaterial used for providing the substrate/matrix.[7,24] Thus, selecting an appropriate bioink/biomaterial requires careful experimental and physical examination of the contents and their cumulative response during deposition using various techniques. In particular, the following parameters need to be considered for any type of bioink formulation, with special emphasis on extrusion bioprinting.

1.2.1. Rheology. Among all of the parameters, the rheological characteristics control the printability of the biomaterials. The rheology describes the flow perturbations due to the resistance offered by the force applied for extrusion/printing, e.g., shear stress, viscosity, viscoelastic shear moduli, and elastic recovery post-printing.[30] The shear thinning is the most commonly observed non-Newtonian fluid property, which is inversely proportional to the viscosity.[14] Many partially cross-linked hydrogels, colloidal suspensions, polymer melts, and polymeric solutions used in extrusion bioprinting undergo shear-thinning above their critical concentrations.[31] These biomaterials can exhibit a time-dependent viscosity, i.e., for thixotropic materials.[32] The viscosity decreases at a constant shear rate, whereas the opposite is demonstrated by the rheopectic materials.[33] Thus, the time dependency of the shear-stress profile could increase the complexity of extrusion-based bioprinting techniques.[34] Therefore, the strategy for designing such bioinks should be carefully monitored. The biomaterials such as carrageenan,[35] gellan, hyaluronan and gums typically increase the yield stress when added into a bioink, resulting in increased stiffness and improved filament formation.[36,37] Additionally, rheology might inhibit cell encapsulation during bioprinting. The higher yield stress results in the bioprinting of filaments that can only be deformed if the acting force is above a yield threshold value such as gravity, the weight of the filaments, capillary forces, surface tension, and the weight of all the layers above them.[30]

1.2.2. Cell Density. Cell density is another parameter that significantly affects the bioprintability of the 3D tissue construct.[24,38] It has been observed that the increase in cell density results in a higher-viscosity bioink, whereas in some other biomaterials it may decrease viscosity.[16] Living cells have their volume, size, and density inside bioinks, potentially modifying the interspatial interactions in the biomaterial. Hence they might impact cross-linking and the overall rheological behavior of the bioinks. The extent of this impact is greatly influenced by the metabolic state, encapsulation density, aggregate formation, and subtype of cells employed in bioprinting.[21,38,39]

1.2.3. Printability. Printability can be referred to as the degree of dimensionally faithful extrudability of the filaments, such as the printed structure mimicking the designed system exhibiting high shape fidelity or printing accuracy.[20,30] The shape fidelity is an important parameter governed by the biomaterials' rheological properties, i.e., the shear-thinning kinetics of the elastic recovery and yield stress.[40,41] Various techniques have evolved to assess shape fidelity; in particular, the damping factor (δ) provides information on the balance between the viscous and elastic deformation properties.[41] Further, the angle of deflection (θ), a measure of the filament deformation due to its weight, gravitational forces and inertia, is measured by yield stress and storage modulus of the ink.[40] Another value, i.e., the integrity index, could be defined as the calculated ratio of the postprinted structure compared to the designed one, which could be used to assess the shape fidelity through 3D computed tomography (CT scan) and optical coherence tomography (OCT).[42−45] The feed rate/print speed/flow rate is detrimental in determining the successful deposition and the time required to print a bioink. Flow rate is an essential parameter in extrusion bioprinting and microextrusion bioprinting. In contrast, the print speed and droplet deposition rate govern the quality of 3D bioprinted constructs in stereolithography and inkjet-based bioprinting.[30]

The above-mentioned parameters can substantially alleviate the 3D bioprinting enigma and help refine the present bioinks.

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Further, biomaterial design and engineering could be automated based upon expanding our knowledge and understanding of the "good", the "bad", and the "ugly" bioink formulations.

2. PROMISING STRATEGIES FOR 3D BIOFABRICATION

2.1. Microfluidic Extruder-Assisted Bioprinting: A Remedy for Efficient Control over the Printed Object'S Morphology, Direction and Dimension. Extrusion-based bioprinting is by far the most popular and promising approach for 3DbioP technology due to its simplicity, cost-effectiveness, flexibility, robustness, and durability.[46] The extruder-dependent technique employs layer-by-layer deposition of the bionks extruded through a nozzle/needle having an optimal geometry using either a pneumatic, mechanical piston, or screw-driven displacement technique. Extrusion-based bioprinting is further divided into direct ink writing (DIW), coaxial printing, coagulation bath printing, and free-form reversible embedding.[16] DIW is suitable for bioprinting highly viscous ink with shear thinning upon extrusion, forming struts upon recovering the initial viscosity. Besides, due to the simplicity of the extrusion system employed in DIW, the rheological behavior can be tuned by adjusting the concentration or increasing the complexity of the bioink/biomaterial components.[20,30,47]

Contrary to the simple nozzle-based extrusion technique, the coaxial nozzles can be used for bioprinting perfusable hollow vascular structures where the wall thickness, diameter, and geometry can be controlled using tunable bioinks.[48−50] The mechanical strength in this type of hollow microfibers can be provided by increasing the cross-linker flow rate and adding vasculature-related cultured cells, i.e., smooth muscle cells (SMCs) and endothelial cells (ECs), and GelMA (∼7%), Alginate (∼3%), and PEGDA (∼2%) as wall biomaterials.[51,52] The vascular structures with hollow microchannels were bioprinted with the help of EC, and fibroblasts embedded in fibrin gel were reported to have ∼67% cell viability after 7 days (cell viability using solid fibers is ∼50% after 7 days).[49,53,54] Therefore, the coaxial extrusion strategy could be employed in bioprinting blood vessels and capillaries requiring an enhanced oxygen and nutrient supply through perfusion for their cellular viability.

Recently, the amalgamation of extrusion bioprinting with microfluidics-based bioprinting technology allowed control, switching, and mixing of bionks/biomaterials within microchannels in a precise manner.[19,24] Microfluidic mixing allows efficient control over the printed object's morphology, direction, and dimension, thus resulting in better stability and resolution of the 3D bioprinted product. Additionally, implementing microfluidic channels for extrusion reduces material wastage, manufacturing cost, and printing and analysis time and allows biologically safe disposal of waste biomaterials,[55,56] thus allowing mixing,[57] on-the-fly cross-linking,[58−60] coaxial filament formation,[56] tunable multilayer hollow fiber formation,[61,62] and cell-laden microsphere generation for the bioprinting of biological constructs. Constantini et al. fabricated a microfluidic chip head coupled to a coaxial syringe for the biofabrication of muscle precursor cells, i.e., C3Cl2 cells, encapsulated in alginate hydrogel fibers, and PEG/Fibrinogen, a photocurable semisynthetic biopolymer, was used as a bioink.[63] They showed successful migration and fusion, forming multinucleated myotubes offering a high degree of alignment along the direction of hydrogel deposition. Besides, the in vivo grafting of this multicellular construct allowed the generation of organized artificial muscle tissue. Therefore, it can be used to fabricate macroscopic artificial muscle for human clinical applications, such as drug testing and burn therapy. A similar type of system was used to generate a 3 mm thick synchronously beating cardiac tissue formed by stacking porous alginate/GelMA fibers laden with ECs that were cross-linked using $CaCl_2$ under UV light exposure.[64] Zhang et al. used a similar system and bioink formulation, except that they used induced pluripotent stem cells−cardiomyocytes (iPSC-CMs) to generate uniformly beating endothelialized myocardium and a two-step cross-linking using Irgacure 2959 and $CaCl_2$.[65] The alginate dissolved in 5 days leaving a porous membrane behind that facilitated cell growth and proliferation. This 3D bioprinted construct was purposed to find widespread application in regenerative medicine, drug screening, and disease modeling studies.

2.2. Biofabrication of Multiscale Heterogeneous Bioscaffolds. *2.2.1. Preset Extrusion Technique.* The multicellular, multiscale, and heterogeneous nature of human organs require a set of complex bioprinting system that should involve the incorporation of extrusion, inkjet, DLS, and SLS technologies and the usage of multiple bioink formulations with various properties and printing requirements.[29] This makes whole-organ printing tricky, complex, expensive, and time-consuming. One possible solution to the above problem could be the preset extrusion bioprinting of the heterogeneous tissue constructs using preset precursor cartridges designed separately depending on the application of biofabrication.[66] The miniaturized parallel printing design allows multimaterial bioprinting to be achieved by extrustion through the preset nozzles without substantial deformation.

The authors could bioprint an artificial heterogeneous spinal cord, capillaries, blood vessels, hepatic lobule, and an S-shaped object. The bioprinting of NIH/3T3 cell-laden bioink with precursor nozzles was performed to compare cell viability with the conventional extrusion-based bioprinting. The cell viability was indeed high in preset extrusion, i.e. ∼90% compared to ∼70% in conventional bioprinting. Additionally, the time taken in conventional bioprinting was far more than that in preset bioprinting. The hepatic lobules bioprinted using precursor cartridges also showed ∼90% cell viability until the fifth day, with a similar cell viability and proliferation rate compared to the conventional 3DbioP construct. However, the cellular connectivity formation was better in the case of preset 3DbioP due to ECs covering the collagen bioink compared to the mixing of ECs in the case of conventional 3DbioP. Therefore, the preset 3DbioP technique holds promise in future bioprinting of functionally better multiscale heterogeneous tissue constructs with high cell viability and cell connections and requiring lesser time and labor.

2.2.2. Fast Hydrogel Stereolighography (FLOAT). The FLOAT[67] technique can print multiscale solid hydrogel-based centimeter-sized call-laden structures within a few minutes. It is based on the phenomenon of photopolymerization-based curing, occurring in the presence of a low-suction-driven high-velocity flow of hydrogel. This enables a constant supply of fresh hydrogel prepolymer during the ongoing curing process to allow scaling of the 3D printed structures. This technique allows rapid, multiscale 3DbioP without structure deformation, strain, or depletion in the physical design of the engineered tissue model. Using the FLOAT method, a 2.6 × 1.7 × 5.6 cm

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hydrogel-hand model was bioprinted in ∼20 min compared to the SLA method that took ∼2 and 6.5 h for the 150 and 50 μm layer thick models of the same dimension, respectively.[67] This method has been adopted for application in the meat industry for bioprinting multiscale cultured meat in the hydrogel using muscle and fat cells cultured using differentiating fibroblasts.[68] Here, the mechanical strength of the bioprinted meat was ∼6× less than that of the actual raw beef steak; however, there was no significant difference between the stiffness in the pan-fried meat samples, with a marked decline in texture and rigidity of the cultured hydrogel meat. Thus, the lab-grown and bioprinted engineered meat may be a big boon for regions experiencing subzero and extremely high temperatures where food cultivation is nearly impossible.

2.2.3. Intravital 3D Bioprinting (i3D). The photo-cross-linking techniques in 3DbioP have helped transition bioprinting applications from in vitro to in vivo environments.[25] Intravital three-dimensional bioprinting (i3D) is capable of performing biofabrication both in in vitro environments (Matrigel) and across various organ tissues in live organisms with augmented support through live-cell monitoring.[69] Urciuolo et al.[69] demonstrated the above concept by fabricating a spatially controlled 3D construct in live mice using photo-cross-linking polymers and donor-muscle-derived stem cells. This technique made in vivo 3DbioP possible by overcoming hurdles related to biofabrication across tissues, i.e., without causing physical damage to the surrounding tissues and without hindering the physiological functions and their efficiency, achieving the accurate orientation and positioning required for precision 3DbioP. They exploited the near-infrared laser light for photo-cross-linking of the modified hydrophilic biopolymer with a hydrophobic photoactive cross-linking functional group, i.e., a coumarin derivative. This photoactive biopolymer undergoes cycloaddition when the two photons get excited at wavelengths greater than 850 nm. This wavelength range allows deeper tissue penetration of the laser light to allow efficient photo-cross-linking. The best coumarin derivative, i.e., 7-hydroxycoumarin-3-hydrocarboxylic acid (HCCA), results in the formation of photo-cross-linking products with linear and branched (4- and 8-arm) PEG (HCC-PEG polymer) and gelatin formulations that avoid possible cellular toxicity and cellular damage. The comparison between single- and two-photon-irradiated cells resulted in 90−99% cell viability when compared to unirradiated controls (at 1 mW laser power). The stiffness of the bioprinted scaffolds was thus fine-tuned in the biologically viable range, i.e., 1−20 kPa, by modulating the laser power. Using the i3D printing, the bioink containing HCC-PEG polymer and fibroblasts, MuSCs, and NPS (neural progenitor cells), was introduced into the mice skin, muscle, and brain through injection, respectively, followed by two-photon irradiation.[69] Thus, direct bioprinting of cells with regenerative properties shall provide us with more insights into the regulation of self-organization, morphogenesis, maintenance of cell functionality, mechanotransduction, and any mechanism underpinning the specific responses in a three-dimensional environment.

2.3. Nanoparticle-Based "Smart" Bioink Formulation Techniques for Various Biological Applications. The bioink/biomaterial formulation techniques used for 3DbioP are essential for a 3D organization with biological function. In this regard, live cells such as stem cells, primary cells, and organoids require growth factors, adhesion signaling molecules, and other additives to maintain average cellular growth and metabolism.[70−72] Recently, "smart" bioinks have been referred to as biologically active formulations that contain multiple materials and respond to environmental stimuli by releasing the appropriate growth factors and adhesion signaling molecules.[73] Nanomaterials (NMs) have emerged as a popular and biologically compatible additive for "smart" bioinks.[74] NMs can be one-, two-, three-, or four-dimensional and possess different physicochemical properties as their dimensionality changes.[75−77] NMs can be synthesized into various 3D forms, i.e., rods, cages, cubes, and stars. These 3D NMs have been used to fabricate biosensors and drug delivery carriers.[74] Besides, they can also be functionalized through various surface treatments and chemical modifications.[15] Therefore, NMs have been used as bioreactive inorganic fillers (BIFs) to form hybrid/composite bioinks/hydrogels for 3DbioP live constructs.[78,79] Recently, bioink formulations based on nano-engineered ionic−covalent entanglement (NICE) have been used in 3DbioP of advanced cell-laden 3D structures with superior printability, high elasticity, and mechanical strength. In brief, the NICE bioink formulation uses nanosilicates to form an ionic−covalent entanglement (ICE) hydrogel containing GelMA and κ-carrageenan (κ-CA).[80] The resulting 3D bioprinted constructs showed high structural fidelity and mechanical stiffness when printed as a ten-layered 3 cm tall elastic structure, which maintains its shape and geometry for more than 120 days while efficiently proliferating and forming interconnections. In another study, an anticancer, 3DbioP-compatible, thermosensitive injectable bioink formulation was prepared with a β-glycerophosphate-bound chitosan (CGP) and dopamine-modified alginate containing polydopamine gold nanorods (AuNRs) (AuNR-PDA).[81] This bioink supported high biocompatibility to normal cells and mouse fibroblast cells but inhibited HepG2 (hepatocellular carcinoma) cells during photothermal therapy (PTT). The property of electrical conductivity in GNRs was exploited in a GelMA-based bioink used for 3DbioP of a cardiac tissue construct.[82] Additionally, the GNRs promote the high-cell-density organization of the cardiac construct apart from bridging the electrically neutral polymer surface, improving cell adhesion, intercellular coupling, and last but not least, they promote synchronous cardiac impulse in the bioprinted constructs.[77]

Boularaoui et al. added AuNPs and MXene nanosheets (2D transitional metal carbides) to the GelMA to enhance human skeletal muscle cells, i.e. C2Cl2 cross-linking at low temperature, printability, and electrical conductance, leading to a better differentiation of encapsulated myoblasts with high viability, i.e., > 90%.[83] The MXene nanosheets help improve electrical conductivity, increase the hydrophilicity, and are generally stable at varying temperatures, thus finding application in water desalination, photocatalysis, and biosensors.[83] Adding MXene and AuNPs enhanced the sheer thinning property, thereby enhancing the extrudability, shape recovery, and printability of the GelMA bioink formulation without affecting its mechanical stiffness. In a study on hepatocellular proliferation for liver regeneration and drug-induced hepatotoxicity, the addition of cerium oxide NPs has been shown to enhance the proliferative nature of the cells.[84] Bai et al. developed a single-walled carbon nanotube (SWCNT) containing dECM for 3DbioP of the conductive neural cells that promoted differentiation, thus allowing its application in neurodegenerative disease modeling.[85] Apart from the above, the nanoparticles derived from hyaluronic acid

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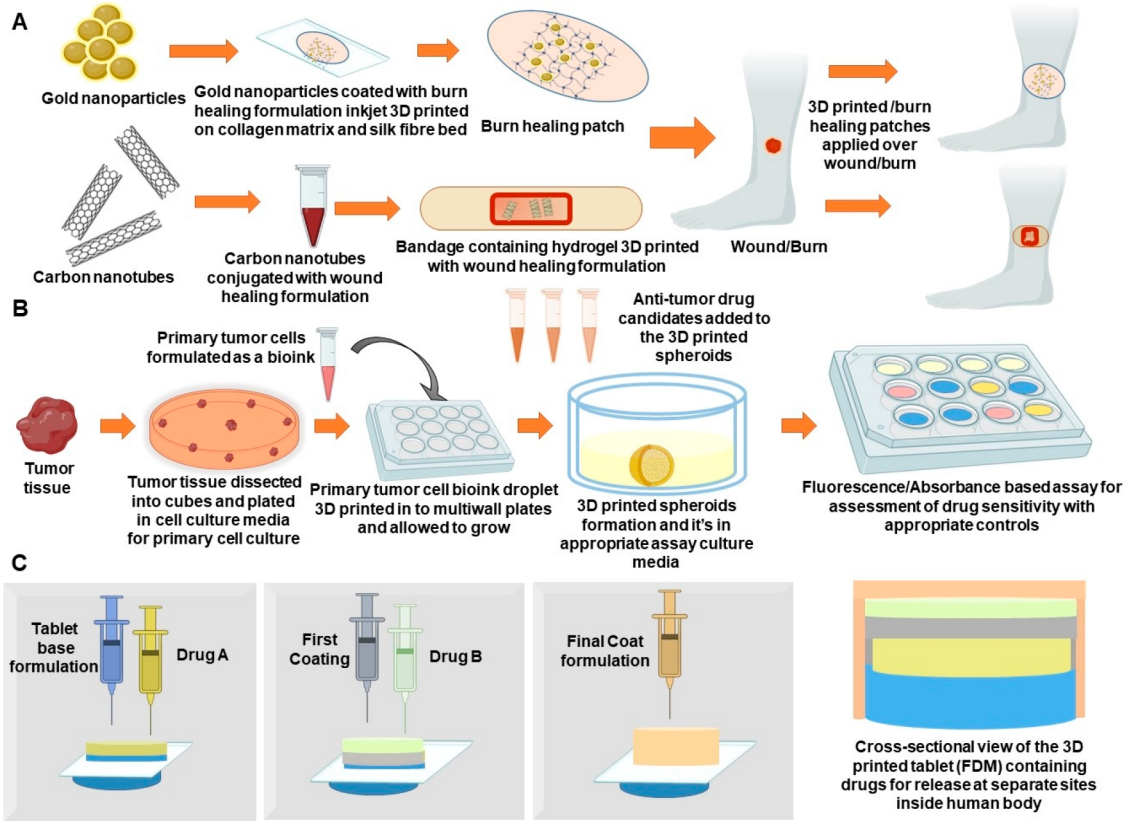

Figure 2. Illustration of bioengineering and biopharmaceutical applications of 3D bioprinting. (A) Illustration of the oxygen-generating substances (OGSs) containing bioinks and nanoparticle (NP)-based bioink and the fabrication of wound healing bandages. The OGSs/NP containing hydrogels are printed as sheets and applied directly on the wounded/burnt areas. (B) 3D spheroid/organoid-based bioprinting of primary tissue and induced pluripotent stem cells (iPSCs) as an organ-on-a-chip (OOC) for diagnostics and drug screening. (C) "Multipill" formulation containing multiple compartments for the sustained and immediate release of drugs for a personalized drug formulation and drug release profile in a biopharmaceutical. This illustration has been partially adapted from "Bioengineering and Biomaterials", "Nanotechnology", "Cancer Cell" and "Anatomy and Clinical" by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

(HA),[86] bioceramics,[87] nanoclays,[88,89] bioactive glasses,[90] and silica[91] nanoparticles have been extensively used in bioink formulations for 3DbioP of osteogenic cells, chondrogenic cells, adipogenic cells, and neurogenic cells.[16]

Further, microsphere technology has also emerged as a promising technique that allows the controlled release of encapsulated growth factors when placed appropriately in a bioink formulation along with the live cells. In particular, Banche-Nicolet et al. developed pH-triggered large mesoporous silica (LPMS) using 1,3,5-trimethylbenzene as a swelling agent.[92] LPMS can release large biomolecules in the 3DbioP scaffolds, i.e., mimicking a bone matrix. The LPMS was coated with PEG as a pH-responsive polymer that undergoes protonation in a low-pH environment. The PEG-coated LPMS carrier released horseradish peroxidase, an enzyme that initiates the polymer hydrolysis reaction, in an acidic environment. The same technology can be used in pH-responsive osteogenic and osteoconductive patches, wound healing patches,[93] and drug delivery and dosage control.[94] Therefore, adding NPs and microspheres is a promising avenue in 3DbioP of biomimetic constructs and drug delivery through 3D printed scaffolds.

2.4. Enhance Viability Using Oxygen-Releasing Biomaterials for Vascular Tissue Bioprinting. The purpose of biofabrication of vascular tissue is squandered if it does not harbor the ability to foster gaseous exchange through micro- or macroporous tissue walls.[95] The gaseous exchange plays a crucial role in many diseases, especially the oxygen tension in various pathological diseases, e.g., coronavirus infectious disease (COVID) mediated hypoxia in lungs.[96] We know how the COVID-19 disease damages the alveoli and reduces the lungs' gaseous exchange efficiency. In this regard, oxygen-releasing biomaterials (ORBs) are promising for developing clinically viable trachea, alveoli, and other alveolar origin tissues using 3D bioprinting.[97] Additionally, the biofabrication of large constructs requires efficient oxygen transfer through tissue layers to maintain various metabolic processes and signaling cascades. Oxygen plays a vital role in maintaining physiological function and an adequately oxidizing environment.[98,99] Under the hypoxic condition, the tissue undergoes immense stress due to nutritional deprivation and the accumulation of reactive oxygen species (ROS); it fails to maintain cellular activity and undergoes necrosis.[100,101]

In tissue engineering, various solid and liquid peroxides, fluorides, and percarbonates have been used as popular bioink additives to initiate cellular gaseous exchange through bioscaffold layers.[98,99,102] These oxygen-generating substances (OGSs) produce hydrogen peroxide (H_2O_2) when exposed to water; subsequently, the H_2O_2 dissociates into water and oxygen.[103] Among the solid peroxides, calcium and magnesium peroxide have been used in their encapsulated form, i.e., hydrophobic materials such as polydimethylsiloxane (PDMS)

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and PLGA. The oxygen is then released into the surrounding tissue through diffusion, as the hydrophobic layers act as a barrier in reducing the rate of hydrolysis of water molecules. Therefore, the availability of water, solubility of peroxides, pH, temperature and peroxide-to-water ratio affect the release rate of oxygen.[102] In contrast, the catalase enzyme is chemically bound to the alginate and surrounded by a hydrophobic layer of PDMS or PLGA used for the controlled release of oxygen.[104] The fluorinated compounds such as perfluorodecalin, perfluorooctanoic acid (PFOA), perfluorooctanesulfanoic acid (PFOS), perfluoroalkylated amine oxides, perfluoromethyl-cyclohexylpiperidin ($C_{12}F_{23}N$), and fluorocarbon/hydrocarbon amphiphile FnHm ($C_6F_{13}C_{10}H_{21}$) have been used in emulsion form to treat the hypoxic environment in various tissue engineering applications.[98,105]

PLGA-H_2O_2/poly(vinylpyrrolidone) shell−core oxygen-carrying microparticles were synthesized and coimplanted into pancreatic islets, resulting in the improvement of graft function through the reduction of hypoxia-induced cell dysfunction and inactivation of the HIF-1α pathway.[106] Similarly, CaO_2 (CPO) was incorporated into a collagen-based cryogel and was applied in diabetic mice, resulting in improved glycaemic control with enhanced cellular viability.[107]

The CPO/PLGA matrices were employed in the vascularized bone to stimulate the migration of host cells toward the OGB matrix, resulting in better regeneration and cell survival for more than 8 weeks.[108] The OGBs have been extensively used in wound healing therapy (Figure 2A) in the form of exosome-laden oxygen-releasing cryogels, CPO-containing thiolated gelatin implants, and CPO in combination with sodium peroxide encapsulated in PCL-poly(vinyl alcohol) wound patches that showed better vascular endothelial cell infiltration, forming structures mimicking capillaries and large vessels (in pigs after 8 weeks). Thus, the OGBs, when used in a suitable formulation, can support angiogenesis, an essential requirement in the wound and burn rehabilitation patients requiring tissue therapy. Although the development of OGSs-based hydrogel formulations has succeeded in alleviating hypoxia-induced necrosis and cell damage, long-term controlled oxygen release is the current limitation of this technology. However, this technology holds promise for developing short-term regenerative medicine and 3D bioprinting.

2.5. Organ-on-a-Chip (OOC) Technology and Organ Building Blocks (OBBs): a "New Hope" For Organ Transplant and Therapeutics Research. Another exciting application of microfluidics-based technology is the development of organs-on-a-chip (OOC) systems.[109] The resulting OOCs are promising tools for both biomedical engineering and biopharmaceuticals. They have been used for exploring various mechanisms involved in human diseases,[110,111] the functioning of organs,[112,113] and drug efficacy and toxicity screening[114] procedures. The advantages of using 3D printed microchips and integrated microfluidics-based extruders could be attributed to the ability to the control gas permeability, perfusion properties, precise positioning of cells, pore size, and morphology required for conducting the above experiments and screening procedures. These OOCs have been used to mimic organ systems such as the kidney, liver, heart/vasculature, brain−blood barrier (BBB), gut, cancerous tissue like tumors, bone/cartilage, and placenta.[115−119]

Multiscale, multicellular, and complex 3DbioP requires multidimensional support differentiation and maturation into functional tissue constructs of multicellular spheroids, organoids, embryoid bodies, and scaffold biomaterial.[120,121] The advent of iPSCs and recent progress in developing advanced hydrogels has made the 3DbioP of these complex tissue forms possible within the structured organ building blocks (OBBs).[122] The conventional 3DbioP approach involves directly printing cell-laden bioinks into the required shape and geometry to form a functional tissue construct (Figure 2B). However, bioprinting of functional organoids is somewhat complex and requires a build-up scale that is unachievable with the conventional approach.[17,123,124] The direct printing of multicellular organs in an OBB is, however, possible if the suitable growth environment and signaling molecules are provided to the iPSCs that differentiate and organize themselves into cardiac, hepatic, lung, capillary, and cartilage tissue patterns, achieving macroscale constructs, i.e., "mini organs".[125] Although there are many approaches to 3DbioP of organs in OBBs, the embedded and sacrificial writing into functional tissue (SWIFT) techniques have been popular choices as they support both bulk and vascularized tissue bioprinting.[126] The embedded organ bioprinting involved an "ink-in-matrix" approach where a viscoelastic, self-healing matrix formulation is molded as a block, with the extrusion of viscoelastic bioink directly into it. The extrusion needle/nozzle can freely translate inside building blocks without causing wear and tear to the structure. The bioprinted structures/scaffolds remain suspended in the 3D space with a degree of freedom to migrate and grow without steric inhibition, sagging, or sinking.

Conversely, the sacrificial bioink formulation can be removed after tissue construct/scaffold bioprinting. The remaining construct could be a vascularized network or an organ-building bioink that raises the structured construct from multicellular organoids and spheroids within the sacrificial matrix. The SWIFT approach has been used to bioprint a branched vascularized network within the sacrificial bioink embedded within the iPSC-enriched OBBs; further, the vascularized tissue construct generated synchronously beating cardiac tissue after 1 week of perfusion. Using the FRESH (freeform reversible embedding of suspension hydrogels) approach,[127] a miniaturized human heart containing 0.5 mm thick walled dual chambers and in- and out-vessels was allowed to form for 2 weeks after the fabrication of iPSCs within OBB. Postformation, this miniaturized heart showed synchronous beating with the electrical impulse for up to 6 weeks. Thus, direct embedded writing of vascularized network tissue[128−132] in OBB holds future promise in developing functional, upscaled, and biostable 3Dbioprinted organs for human transplantation.

3. INNOVATIONS IN 3D PRINTING DRIVING THE FUTURE OF BIOENGINEERING RESEARCH

3.1. 3DbioP in Plant Biology. 3DbioP in plants is an upcoming and very promising avenue for exploration. The 3DbioP technology involves plants and associated organisms like algae, and the process is also called "green bioprinting".[133] Plants are an essential source of food, fuel, fiber, and oxygen to human beings. Therefore, plants hold the key to the future sustainability of food and the environment. It has been reported that plants possess totipotent and differentiating cells like animal stem cells, called plant stem cells (PSCs).[134] These PSCs can provide specialized functionally differentiating cells that allow for plant growth and development in four

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dimensions.[135] It is also imperative that these PSCs retain their proliferation, differentiation, and self-renewal functionality for hundreds of years when their surrounding niche is maintained under normal physiological conditions.[136,137] These PSC niches are present in shoot apical meristem (SAM) and root apical meristem (RAM) and are under transcriptional and post-transcriptional control of cell molecular machinery.[136] The stem cells generate differentiated cells that push surrounding cells in different directions according to anatomical programming in plants. Therefore, the bioink formulation containing PSCs with suitable growth factors and supporting matrix has the potential to provide experimental scaffolds and self-assembling scaffolds that could be employed to form root/shoot model systems for a deeper understanding of the molecular mechanisms as well as the fibrous tissues for food and engineering applications.[138,139]

Varma et al. reported 3D bioprinting of transgenic *Oryza sativa* (rice), which produces recombinant butyrylcholinesterase (BChE)[140] as a prophylactic/therapeutic against organophosphate nerve agent poisoning, cocaine toxicity, and neurodegenerative diseases like Alzheimer's, and cells were immobilized in a polyethylene glycol-based hydrogel.[141] The cells maintained viability similar to those in suspension cultures. They displayed similar sugar consumption trends for 14 days in the semisolid matrix, undergoing a growth phase from days 0−6, a BChE production phase in a sugar-free medium from days 6−12, and a growth/recovery phase from days 12−14. The rice cells in the bioprintable hydrogel also produced a significant amount of active BChE, comparable to the levels produced in liquid cultures. A considerable fraction of this BChE was secreted into the culture medium, allowing for easier product separation. Recently, the researchers reported the development of tunable, lab-grown plant materials generated from a *Zinnia elegans* cell culture,[142] which respond to various concentrations of hormones in the growth medium.[143] The 3DbioP and casting of this bioink resulted in net-shaped structures with varying hardnesses at scales and forms that do not occur naturally in plants. The PSCs have found widespread application in the cosmetic industry due to their ability to provide the tissues of plant origin (TPOs) with antioxidant, antibacterial, and antifungal properties.[144] Besides, the application of TPOs has already shown potential in human stem cell regeneration and UV protection, apart from stimulating the antioxidant system in mammalian cell lines. PSCs from apple, argan, samphire, and tomato have significantly reduced transepidermal water loss (TEWL), thereby reducing the wrinkles in the human volunteers in the clinical trial on skin.[145]

Additionally, the 3DbioP technology can potentially provide bioprinted wood, root/shoot architecture models, and artificial plant environments for plant−microbe interaction-based studies, as well as eco-toxicological studies.[146]

3.2. 4D and 5D Biofabrication/Bioprinting. The four-dimensional (4D) printing (4DP) technologies are shifting self-assembling architectures toward the new paradigms of environment-responsive architectures.[147] The 4D biofabrication technology is based upon the principle that exposing the bioinks to a specific external stimulus, i.e., temperature, light, pH, electrical impulse, or any other energetically critical stimuli that could trigger a physio-chemical response, results in the attainment of functional and dynamic 3D structures.[148,149] Most 4D bioprinted products undergo geometrical changes under thermal, electrical, magnetic, or light stimulus by the congruent incorporation of multiple materials. Therefore, the primary application of 4DP is in the fabrication of precise "programmed" geometrical structures that can transform/recover their shape in response to external stimuli.[150−152] Such materials capable of geometrical morphogenesis can be termed "smart" materials. Zhang et al. classified the "smart" materials as thermo-, moisture-, photo-, electro-, and magneto-responsive according to environmental or temporal stimuli.[153−155] Thermoresponsive materials are primarily driven by the shape memory effect (SME) and shape change effect (SCE).[31] Shape memory polymers (SMPs) are the most researched because of their ease of printability. The glass transition temperatures of SMPs are usually higher than their operating temperatures. They are programmed under specific temperatures and mechanical conditions above the transition temperature ,followed by cooling to maintain the temporary shape. They regain their shape upon heating above the transition temperature.[156,157] Moisture-responsive materials primarily include hydrogels, which uniformly swell up in an aqueous solution until their moisture saturation point.[148,158] Biocompatibility and enhanced printability are the two main advantages that make them a first choice for a broad range of applications. Bioengineering of the hydrogels through anisotropic swelling, controlling the temperature of water in which hydrogels are immersed, and unique hinge designs is contemplated by various researchers to program them according to the requirements.[147,149] Both photo- and electroresponsive materials exert their stimuli indirectly through heat. The heat produced following the light and electrical impulse stimulates the deformation of the structures.[31]

The concept of a "multisome",[159] a smart material with pH and temperature-dependent release bilayer structure, has the potential to be applied to drug delivery and studies related to synthetic biology. A similar "programmed deposition" approach resulted in the fabrication of cohesive, communicating synthetic cells by a single lipid bilayer.[160] This approach can potentially fabricate rapid communicating structures with patterns and light-activated gene/protein-controlled switching in synthetic biology research.[161,162] The injectable conductive cryogel fabricated with glycidyl methacrylate and CNTs showed blood-triggered shape recovery and high blood uptake capacity for hemorrhagic wound healing and hemostasis.[163] The electroactive "smart" biopolymers were developed to facilitate the regeneration process and physiological activities through the SME mechanism, i.e., due to high shape recoverability and shape fixation ratio under physiological conditions.[73,164]

Recently, different chromophores were incorporated into various positions in the polymer gel block so that only these parts swell up after getting the suitable light wavelength.[165,166] Okuzaki et al. used polypyrrole films to control water absorption or desorption. The electroresponsive materials may include volatile compounds such as ethanol, whose evaporation upon the application of the electrical current initiates the expansion of the matrix.[167] The Ferric-oxide nanosized particles incorporated into the bioinks respond to the magnetic field changes by modulating the shape of the bioprinted structure.[154] The magnetoresponsive material has the potential for polymer printing and metal printing as well.[168,169]

Adding another dimension to the 3DP structure has enhanced the adaptability and placement of the medical

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devices/stents inside the human body with minimal access. Ge et al. fabricated a 4D stent using high-resolution microstereolithography with SMPs and demonstrated its applicability and efficiency.[170] Similarly, Wei et al. used a magnetoresponsive material to fabricate a 4D stent with the remote-controlled placement using a magnetic field.[171,172] Moreover, targeted drug therapy in the intended body part and at specific times could be achieved more precisely using 4DP. Khaled et al. fabricated a 3D printed "polypill" containing five drugs for cardiac patients.[173] This polypill formulation was designed for three sustained-release drugs, i.e., pravastatin, atenolol, and Ramipril, containing a compartment separated by cellulose acetate shell and covered with an immediate release compartment containing aspirin and hydrochlorothiazide. Such a 3D printed formulation has the potential to provide the convenience of optimized and personalized drug dosage with a controlled and tunable drug release profile (Figure 2C). Malachowski et al. fabricated "theragrippers", multifingered grippers[174] that are activated under thermal conditions and release a controlled drug dosage in the gastrointestinal cavity.[175]

Owing to the wide-spectrum applications of dynamic smart bioinks, the need for the development of 4D-based fabricated structures must promise morphogenic dynamic implants to mimic the natural systems and their environment.[176]

5D printing (5DP) technology involves a fabrication process where the printing head rotates in three planes and the stage rotates in two planes, thus resulting in the fabrication of nonplanar structures, i.e., convex/concave shaped structures. The technology can print curvilinear, complex structures with improved inherent characteristics.[177] The curved structures printed by 5DP eliminate the weak points at the edges of flat surfaces, thus improving the strength of the resultant structure as much as five times.[178,179] Moreover, the technology is efficient in material management, with approximately 25% less material consumption. On the other hand, researchers have added dimensions in the form of "artificial intelligence-based algorithms"[180] that control the robotic extrusion mechanism by continuously monitoring the printing procedure in the active mode.[10,181] Thus, future bioprinting technologies may be a hybrid of five-axis printing along with "smart" bioinks capable of stimulating geometric change with time.

4. KEY CHALLENGES AND OUTLOOK

The 3DbioP technology is revolutionary and evolving with time. It has enormous potential to provide plausible solutions in tissue bioengineering and the pharmaceutical industry. However, the technical hurdles associated with printability, biocompatibility, stability, cost, and ethical considerations have taken it a step back.

Printability is essential, as it controls the morphology of the 3D scaffold and the growth of live cells of a bioengineered structure after printing.[20] Biomaterial compatibility is another issue, as different bioprinting techniques demand materials with different characteristics.[46,73] For example, inkjet technology requires materials capable of rapidly cross-linking to ensure the layered formation of complex structures.[14,182] On the other hand, extrusion bioprinting requires highly viscous bioink where the initial layer maintains the 3D structure, and cross-linking occurs after printing.[16,30] Therefore, the "one-bioink-fits-all" concept is hard to apply to different bioprinting techniques.

Conversely, biocompatibility is necessary to avoid the immune system response or any adverse reaction locally or systemically.[183] The requirement of biocompatibility is low when 3D bioprinted devices are used externally, as in the case of surgical/guiding stents, prostheses, and rehabilitation aids. However, the issue cannot be overlooked when the bioprinted implants are placed intrinsically.[87,184] The biocompatibility studies of such implants are lacking and pose a significant challenge to the success of tissue-engineered organs. The biodegradability rate of the implanted bioprinted organ is an essential aspect in the tissue regenerative process, where the implanted tissue should biodegrade at a similar pace as new tissue formation and promote the proliferation of cells along with the production of the extracellular matrix.[92,185] At the same time, the residue of biodegradation should be nontoxic. This aspect requires further exploration and research. The sterility of the bioprinted model is another critical challenge where the technique should not alter the mechanical and biological properties of the 3DbioP organ.[22]

The tissue engineering industry faces the most challenging task of generating clinical-grade human iPSCs due to the cost of manufacturing the iPSC cell lines and the conditions required to initiate and maintain these cell lines.[186,187] Although the discovery of low-cost precursor molecules like CHIR-99021 has partially reduced the cost,[188,189] the main challenge is adopting good manufacturing practices (GMPs) at a large scale. For this, the suspension culture-based bioreactors with appropriate biosensors and real-time monitoring could be a plausible solution for maintaining optimal GMP standards while culturing organ-grade iPSCs.[190−192] Further, the cost could be reduced by culturing patient-specific derived ECMs instead of relying on iPSCs and other synthetic sources.[193−196] The tumorigenicity is another "shot in the eye" for iPSC-based therapeutics and organ culturing due to the genomic instability introduced in iPSC as a reprogramming mechanism.[122] The possible solution to the above approach could be to employ engineered drug-sensitive kill switches in the iPSCs to eliminate undifferentiated cells during the selection procedure.[197,198] The OOCs were developed as an alternative for animal drug trials; however, the use of iPSCs and their susceptibility to the flow, shear stress, and flow pressure restrict their reproducibility and robustness, requiring higher optimization GMP standards and regulations. The optimization could be achieved by integrating the OOCs with machine learning (ML) and artificial intelligence (AI), giving rise to the next generation of OOC platforms for the acceleration of drug discovery and cost reduction. The ML approach can potentially simplify the task of representing complex biological tissue images in 3D tissue models with optimal cellular resolution, and the biocompatibility of the biomaterial could also be predicted a priori.[180,181,199] The amalgamation of big data with ML/AI could help reduce multiscale and multiparameter complexities that are difficult for manual operators to handle, including postprocessing operations.[200] To date, small-scale organ fabrication involves delineating an appropriate balance between maturation before and after biofabrication. Thus, OBB maturation is a tough challenge in large-scale human tissue bioassembly.[123,187]

The 3DbioP skin models have been attractive alternatives for animal substitution; however, the absence of immunogenic components has been a critical challenge.[73,201] The addition of the immunogenicity aspect to the skin models could help in the treatment of infections, inflammasome research, and the

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Table 1. Some Recent Innovations in 3D Bioprinting Techniques and Bioink Formulations

technique	innovation	property modified	product/application	future prospective	reference
microfluidic assisted digital light processing (DLP) bioprinting	microfluidic platform with four on and off pneumatic valves	can offer fast switching between different hydrogels for heterogeneous hydrogel construct	multimaterial (PEGDA and GelMA with different viscosity) fabrication with high spatial resolution	models for tumor angiogenesis and musculoskeletal systems	Miri et al., 2018[207] and 2019[29]
microfluidic with extrusion-based bioprinting (EBB)	addition of micromixers and microconcentrators in the print head	micromixers can perform the last stage of mixing the bioink components for complex applications and enhanced resolution, and microconcentrators allow an increase in the cell concentration	improves dispensing function with varying ratios, high speed, higher accuracy, and capability of fabricating complex constructs	for the bioengineering of organs	Serex et al., 2018[208] and 2021[209]
microfluidic with EBB	multimaterial multinozzle 3D printing in a voxelated fashion	materials with different viscosities can be printed simultaneously	fabrication of a voxelated soft structure using epoxy and silicone resin	it could be used to fabricate complex structures in a voxelated fashion	Skylar-Scott et al., 2019[126] and 2022[131]
microfluidic with EBB (coaxial nozzle)	passive mixer in a microfluidic chip extruded through a coaxial nozzle	mixing of two inks with different volumetric proportions	an osteochondral analogue was prepared, closely resembling an in vivo environment	better prospects for tissue bioengineering	Idaszek et al., 2019[210]
void free-3D printing (VF-3DP)	deposition of template bioink (containing endothelial cells) alongside extrusion of matrix bioink for cross-linking	prevents structural collapse and deformation and continuous oxygen supply for constructs	fabricates endothelium with more excellent cell uniformity	it can be used to fabricate self-supported hydrogel-based constructs	Ouyang et al., 2020[211]
microfluidic with droplet-based bioprinting	glass capillary-based microfluidic device	encapsulation of mesenchyme cells in alginate hydrogel droplets	developed a device which generated 200 droplets/min	applicability of regenerative medicine strategies	Mesquita et al., 2021[212]
microfluidic with DLP	bioprinted hydrogel-based microfluidic chip for lab on a chip	it uses lesser reagent quantity, involves less specimen handling, and requires lesser cells for detection	measures reactive oxygen species	high-throughput screening device	Bhusal et al., 2022[213]
digital mask projection-stereolithography (MP-SLA)	low suction force, the high-velocity flow of a prepolymer hydrogel	a higher flow of prepolymer enables adequate replenishment of prepolymer, thus aiding in faster growth of the part	it prevents part deformation and environmental stress on the cells and can fabricate more extensive size constructs	can produce large-sized tissue structures	Anandakrishnan et al., 2021[67]
gold nanoparticles (AuNP) in bioink	incorporation of AuNP for the synthesis of semisynthetic ECM	AuNPs-ECM-encapsulated fibroblast used for 3D printing of tubular tissue constructs	the cells secreted ECM for 4 weeks	closely mimics the in vivo cellular environment	Gungor-Ozkerim et al., 2018[214]
AuNP in bioink	AuNP coated with polydopamine (PDA) in a chitosan-based hydrogel	the mixture is more biocompatible to normal cells and mouse fibroblasts and showed suppression of HepG2 cells	showed antitumor activity when used with photothermal tests	it may be used as a treatment for carcinomas using a photothermal technique	Zeng et al., 2019[81]
nanosilica for bioink	Incorporation of silica nanoparticles and ruthenium-loaded PEGylated liposomes (RLS)	RLS has better mechanical properties and is porous enough to release drugs in a consistent manner	RLS malfunctioned mitochondria, leading to apoptosis of human osteosarcoma cells	promising candidate in the treatment of osteosarcoma	Ye et al., 2019[215]
silica nanoparticle containing bioink	SiNPs were added to the oxidized alginate containing chondrocytes	electrostatic interactions between cationic silica and anionic polysaccharides	resulted in enhanced rheologic properties, good mechanical strength, structural fidelity and porous structure	it may be used for the bioengineering of cartilage	Lee et al., 2020[216]
nanoinks	silica nanoparticles	improved mechanical properties, enhanced electroconductivity for neural and cardiac bioengineering, nanospheres prevent biomolecules from degradation/burst release	application in drug delivery, tissue bioengineering, precision medicine	tissue bioengineering, therapeutics, regenerative and precision medicine	Maia et al., 2023[217]
carbon family nanoparticles in bioink	graphene oxides, carbon nanotubes, functionalized carboxyl group	better mechanical properties, good neural and skeletal cell proliferation and viability, increased oxygen metabolism	particularly useful in neural and muscle cell bioengineering	neural tissue and muscle bioengineering	Huang et al., 2017;[218] Ajiteru et al., 2020;[219] Kang et al., 2021[220] and 2022;[68] Mendes et al., 2021[221]
GelMA hydrogel-based bioink	incorporation of nanomaterials with GelMA	enhanced mechanical properties, electro-conductive, better viscoelastic properties, stimuli-responsive drug delivery	extraordinary behavior in bone, skin, cardiac and neural tissues	it could be used in novel drug delivery systems, diagnosing devices and tissue bioengineering	Kurien et al., 2022[222]
oxygen generating biomaterials	calcium peroxide, magnesium peroxide, sodium percarbonate, hydrogen peroxide, and fluorinated compounds	releases oxygen by hydrolytic mechanism or enzymatic decomposition	cardiovascular tissue engineering, pancreatic tissue engineering, muscle bone tissue engineering	bioengineering of various tissues and wound healing	Suvarnapathaki et al., 2019[223]

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Table 1. continued

technique	innovation	property modified	product/application	future prospective	reference
oxygen generating biomaterials	calcium peroxide (CPO) and cardiomyocyte-laden GelMA	CPO has high purity and provides sustained release	3D printing can provide constructs with high-quality products, having moderate O_2 release profile and with a relatively more uncomplicated technique	provided future directions of carrier materials with metered degradation, coupling sensors for measuring oxygen release	Erdem et al., 2022[98]
3DbioP and organ on a chip	various materials are used for the fabrication of different tissues/organs.	for the fabrication of large-scale artificial organoids/tissues, EBB and light-based bioprinting is good; however, for precision printing, droplet/jet generation-like printing is suitable	for fabrication of vascular/endothelial systems, kidney, heart, ovaries, urothelium, lungs	for fabrication of different body organs, precision medicine, and drug trials	Chliara et al., 2022[224]
3DbioP and plant systems	green bioprinting	photosynthetically active scaffolds containing green algae, land plant cells, and callus cells, usually immobilized in Ca-alginate, cellulose-containing bioinks.	living scaffolds designed for energy harvesting, gene delivery, and physiological and developmental studies	microfactories for the production of cellulose, active metabolites, and green constructs.	Landerneau et al., 2022;[146] Günter et al., 2023[225]

development of novel skin therapeutics.[202,203] Another critical challenge in tissue engineering is to manufacture biofabricated constructs with a good shelf life, i.e., the storage life and shelf availability. In this regard, a cryobioprinting technology was developed to allow direct fabrication and in situ freezing of tissue scaffolds to allow their shelf availability.[204] The advent of in situ mobile bioprinting devices is a plausible solution to this problem. However, the sterility and stability of fabricated constructs are daunting challenges in this approach.[205]

The present nozzle technologies have evolved with tremendous modifications and improvements due to the addition of multiaxial tips and microfluidic-based extruders in FDM-based bioprinting technology.[48,60,64] The above advances have resulted in spatial improvements in the fabrication techniques. However, this comes with the loss of temporal advantage, i.e., printing time increases. However, simultaneous printing with multiple extruder units (micromixers and gradient formation devices) and automated valves could allow the formation of variable hollow fibers and the deposition of variable biomaterials to increase the temporal resolution of bioprinting.[58,61,121] Alternatively, computed axial lithography could be employed in the biofabrication photo-cross-linking bioink, which is fast and accurate.[206]

The bioprinting of time-scale-dependent flexible structures and vascular networks with irregular concave/convex geometry is a critical limitation in the success of tissue engineering. However, the advent of SMEs and 5DP technology could be employed with the incorporation of "smart" bioinks that can adapt to their environment and space.[179]

5. CONCLUSION

3DbioP are a novel, versatile, robust, and revolutionary technology that can potentially solve existing problems. This Review envisages the ideal parameters for bioinks and promising technologies to fabricate tissue-engineered structures. Notably, developing novel "smart" bioinks to support high precision, rapid prototyping, and functional compatibility with various bioprinters and bioprinting techniques has become an inevitable trend. Incorporating microfluidic technology with OOC, OBB, organoid, in situ, 4D, and 5D bioprinting techniques can take tissue fabrication to another level. These 4D bioprinting technologies could provide great potential for personalized treatment and precision medicine, regarded as an important trend in tissue engineering. Additionally, the newer role of 3DbioP in plants, 4D and 5D printing, was discussed to appreciate the extent of the range of its applications for humankind. No doubt, the technology has the potential to serve humanity; however, the challenges pertaining to printability, biocompatibility, biodegradability, and sterilization should be adequately addressed through a series of high-quality research studies. Additionally, the ethical and regulatory aspects of bioprinted tissue/organs not discussed in the paper should be considered before their introduction for service.

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Notes

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