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Review article

Bioprinting: revolutionizing biology with 3D innovation

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Abstract

Three-dimensional (3D) bioprinting is an innovative technology that enables the fabrication of bioengineered structures through computer-guided processes. It has been in use for over 35 years, contributing hugely to biomedicine. Utilizing biomaterials with regenerative properties has significantly impacted tissue engineering, regenerative medicine, and pharmaceutical research. Various bioprinting techniques, including inkjet, extrusion, laser-assisted, and stereolithography, offer unique advantages for precise tissue construction. The "bioinks" used for such printing can be derived from natural sources or synthesized; the ink ensures biocompatibility, mechanical integration, and controlled degradation. Advanced bioinks—such as nanoengineered, biomolecular, multimaterial, self-assembling, and stimuli-responsive variants—enhance applicability in tissue regeneration. Bioprinting is widely employed, including for organ fabrication, cancer detection, and in food technology. However, its efficiency is limited by challenges like cell positioning and nozzle clogging. Techniques such as pollen-based bioinks and the Freefrom Reversible Embedding of Suspended Hydrogels help address these issues by improving precision and structural stability. This review summarizes the types of 3D printing, their applications in medicine and industry, as well as their advantages and limitations. Owing to ongoing and achieved advancements, the potential uses of bioprinting continue to expand in personalized medicine, organ transplantation, and sustainable food production.

Keywords: Bioinks, biomedical, personalized medicine, regenerative medicine, three-dimensional bioprinting, tissue engineering.

Tissue engineering is an advanced branch of biomedicine where tissues can be created by using a combination of engineering, biology, and materials science. Damaged tissues and organs can be replaced, repaired, or enhanced with the help of scaffolds, cells, and bioactive molecules. The field of regenerative medicine holds enormous potential, offering plausible therapeutic solutions for conditions such as tissue damage, organ failure, and degenerative diseases.[1, 2] Stem cells from any origin—adult, embryonic, and induced pluripotent stem cells—primary cells harvested from patients or donors, and cells genetically modified for enhanced function are the key components of tissue engineering. Then, these cells are hosted by natural (collagen, fibrin) or artificial (polymers, hydrogels) scaffolds for attachment and tissue development. Finally, based on the requirement, certain growth factors are added to enhance their proliferation and differentiation. Artificial scaffolds play different roles in determining the fate of stem cells. Scaffolds fabricated with various polymers have been reported recently.[3] Remodeling of the extracellular matrix (ECM) plays a pivotal role in the development of cancer.[4] ECM extracted from different types of cancer cells induced varying effects on stem cell growth.[5] Regenerative medicine often involves the creation of skin grafts and/or the replacement of bones. Wound healing can also be enhanced by accelerating the repair of surrounding

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tissues.[6] Immune rejection poses an enormous challenge; so does angiogenesis, necessary for the survival of grafted tissue. With advances in stem cell research, bioprinting, and biomaterials, lab-grown organs and tissues can become a reality, sooner rather than later.

Three-dimensional (3D) bioprinting is gaining recent popularity as a novel biofabrication technology; it uses computerized processes to bioengineer structures comprising biomaterials with renewal properties, presenting significant potential for facilitating advancements in regenerative medicine and the development of personalized body parts.[7] It is widely applied in medicine, in areas ranging from pharmaceutics to cancer research and from tissue engineering to regenerative technology,[8] as well as in drug testing and high-throughput assays. It overcomes the shortcomings of traditional tissue fabrication methods and is mainly used for printing tissues that would be transplanted.[9] Its primary application is the printing of model human organs, to avoid animal testing, while minimizing the wait for organs. It forms a part of an autonomous robotic system and would be helpful for doctors treating patients at risk.[10] Ongoing advancements have ensured steady progress in the field of bioprinting, opening up new possibilities for personalized medicine and organ transplantation.[11] This mini-review discusses different types of bioprinting, the various kinds of bioinks used for it, as well as its applications and challenges.

Types of bioprinting

Extrusion, inkjet-based, laser-assisted, and lithography are the different types of bioprinting available.[12] **Figure 1** presents the various applications of bioprinting and bioinks used.

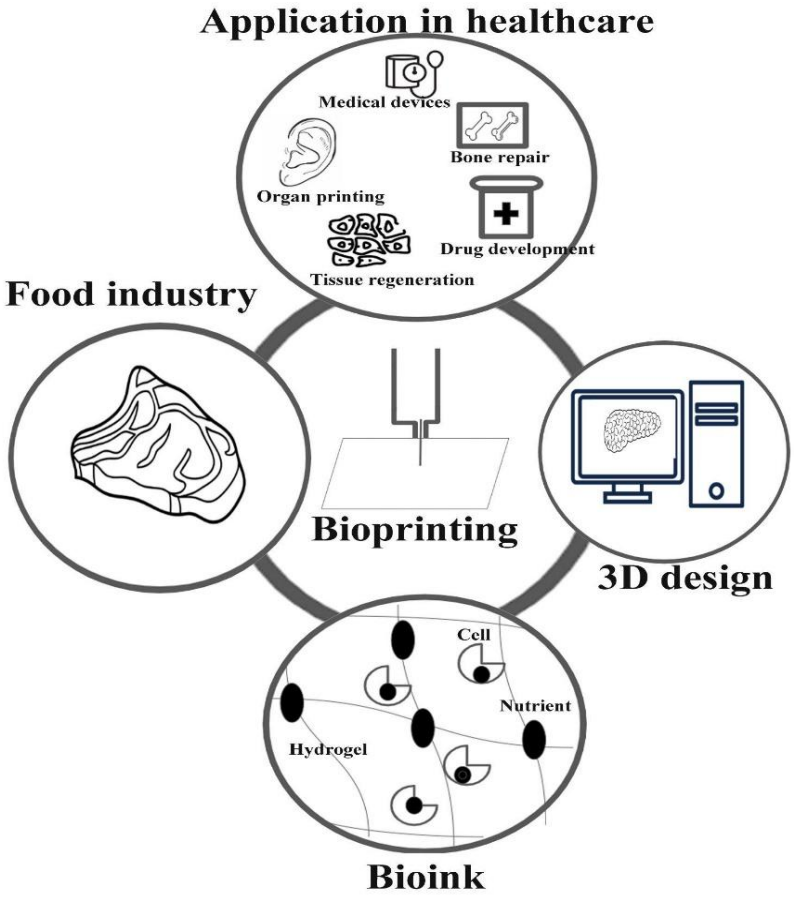

Figure 1. Bioprinting applications and bioink types.

Inkjet-based bioprinting

Inkjet-based bioprinting is based on the principles of conventional inkjet printing and allows for precise, contact-free deposition of bioink droplets onto a substrate. It operates through two primary mechanisms: thermal and piezoelectric actuation. Thermal bioprinting relies on heat to create a pressure pulse that propels ink through the nozzle. While affordable and widely utilized, it is prone to nozzle clogging, inconsistent droplet sizes, and potential induction of thermal stress in cells. In contrast, piezoelectric bioprinting employs an actuator-generated pressure to dispense droplets, improving uniformity and reducing nozzle blockages. However, frequent use may damage cell membranes. Despite challenges, piezoelectric methods maintain a viability of over 90% in various mammalian cells. [13]

Pressure-assisted bioprinting

Pressure-assisted bioprinting constructs 3D structures by extruding biomaterial filaments through a fine nozzle. It uses pneumatic, plunger, or screw-based pressure to control the flow of solutions, pastes, or dispersions, allowing room-temperature operations. It has been successfully applied to print various cell types, including pre-osteoblasts, mesenchymal stem cells, and endothelial progenitors.[14]

Laser-assisted bioprinting

Laser-assisted bioprinting (LAB) uses a pulsed laser to transfer liquid biomaterials onto a receiving surface. The laser heats the ribbon, causing the materials to vaporize and land on the substrate as droplets, where they support cell adhesion and growth. LAB typically employs nanosecond ultraviolet (UV) or near-UV lasers to print hydrogels, cells, proteins, and ceramics. The resolution, ranging from pico- to microscales, is influenced by factors like material thickness, laser pulse energy, rheological properties, substrate wettability, and printing speed. [15]

Stereolithography

Stereolithography (STL) is a nozzle-free, free-form method that uses light to solidify photo-sensitive liquid materials—mainly acrylics and epoxies—and can construct a single structure comprising multiple resins. The light intensity is controlled through digital micromirror arrays. STL offers high precision, layer-by-layer printing, applicable to light-sensitive hydrogels, with a print duration determined by the structure's thickness. However, challenges include the lack of suitable biocompatible polymers, toxic residual curing agents, difficulty in removing the support structures, and limited ability to create horizontal gradients within the printed designs. [16]

Cell-spheroid-based printing

Cell-spheroid-based bioprinting forms tissue-like 3D structures using clusters of cells, or spheroids, which replicate the natural organization observed in tissues. The spheroids are printed using cell aggregate or hydrogel-based bioinks with high precision. They improve cell–cell interactions and tissue functions, and are applied in research areas such as drug testing, disease modeling, and regenerative medicine. Such a technique holds remarkable potential for advancing tissue engineering and therapeutic applications.[17]

Types of bioinks and their uses

Bioinks are materials used to create tissue structures via 3D bioprinting. While many biomaterials aid tissue regeneration, most are incompatible with current bioprinting methods, especially those that require high temperatures or solvents. Thus, to serve as a bioink, a biomaterial must ensure cell protection while offering printability, mechanical strength, stability, biocompatibility, controllable biodegradation, and cell adhesion. Additionally, their production should be easy, facilitating commercial viability. Bioinks can be made from naturally sourced or synthetic biomaterials and are classified into scaffold-free and scaffold-based—the former uses tissue spheroids or cell pellets to fabricate new tissues, and the latter incorporates cells within hydrogels or decellularized matrices.[18, 19] A range of materials, such as ceramics, polymers, elastomers, hydrogels, and lipids, have been utilized as bioinks for the fabrication of 3D structures.[20] **Table 1** summarizes the most significant studies showcasing the diversity of bioinks, presently under investigation.

Table 1. Overview of bioink types and their applications in 3D bioprinting.

Type of bioink	Composition	Key features	Main applications
Hydrogel-based[19]	Natural (alginate, collagen, gelatin) or synthetic (polyethylene glycol, pluronic) hydrogels	High water content, adaptable mechanical properties, biocompatible	Tissue engineering, wound healing, and drug delivery
Cell-embedded bioinks[21]	Living cells suspended in hydrogels or extracellular matrix components	Promotes cell viability, supports growth and differentiation	Organ printing, tissue regeneration, disease research
Decellularized ECM[22]	Matrix materials derived from tissues or organs	Replicates the native cell environment, highly compatible with biological systems	Scaffold development, organ and tissue engineering
Composite bioinks[23]	Blend of natural and synthetic biomaterials	Improved mechanical strength, bioactive properties	Bone and cartilage repair, load-bearing tissues
Sacrificial bioinks[24]	Pluronic, gelatin, or carbohydrate-based materials	Provides temporary structural support, dissolves post-printing	Creating vascular channels, complex tissue formation
Conductive bioinks[25]	Carbon-based materials, conductive polymers, or nanoparticles	Enables electrical conductivity for cell signaling	Neural tissue repair, bioelectronics, and electroactive tissues
Hybrid inks[26, 27]	Nanoclay, epoxy, photopolymer, and glass fiber reinforcement.	Hybrid 3D printing is an advancing technique used to create soft electronic devices by combining direct ink writing of conductive and dielectric elastomers with automated pick-and-place placement of surface-mounted components. This integrated additive manufacturing approach enables precise fabrication of insulating structures and conductive electrodes in tailored configurations, allowing smooth incorporation of mechanical flexibility with electronic performance.	Passive and active electronic components are integrated to form the desired circuitry by employing an empty nozzle operating in vacuum-on mode to pick up each component, accurately position it on the substrate, and then release it in vacuum-off mode at the designated location.

Chemical, physical, and biological properties of bioinks directly impact their performance and influence the printing strategy employed. To overcome these issues, advanced bioinks with improved properties, including viscosity, viscoelasticity, hydration, and/or gelation kinetics, shear thinning, biodegradation, and biocompatibility, have been designed. Such bioinks are classified into five types: 1) nanoengineered; 2) biomolecular; 3) multimaterial; 4) self-assembling; and 5) stimuli-responsive.[28] Nanoengineered bioinks are those incorporated with nanomaterials such as nanoparticles, nanofibers, and carbon-based structures to enhance mechanical strength, cell attachment, and bioactivity, making them ideal for use in tissue engineering and regenerative medicine.[29] Multimaterial bioinks combine various biomaterials, including hydrogels, synthetic polymers, and bioactive compounds, to enhance mechanical strength, cell compatibility, and functional diversity, thereby facilitating the creation of complex, multi-layered tissues for biomedical applications.[30] Biomolecular bioinks contain bioactive compounds, like growth factors, peptides, and proteins, which stimulate cell signaling, differentiation, and aid in tissue regeneration. They play a key role in regenerative medicine, wound healing, and organ development by enhancing cell–cell interactions and tissue maturation.[31] Self-assembling bioinks are composed of biomaterials that naturally organize into structured frameworks, replicating tissue formation. Such an arrangement improves cell–cell interactions, structural integrity, and tissue regeneration, facilitating the creation of biologically complex constructs.[32] Stimuli-responsive bioinks react to external cues, including temperature, pH, light, or magnetic fields, allowing controlled changes in their properties. This feature improves printability, structural integrity, and cell–cell interactions, making it valuable for use in advanced tissue engineering and biomedical applications.[33]

Bioprinting in medicine

Bioprinting has several biomedical applications: tissue regeneration; the treatment of deformed bones and brain injury; and diseases of the heart, eyes, and kidneys; cancer, etc.[34, 35]

1) Artificial human skin

Human skin was the first organ to be bioprinted. Skin tissue can be printed using two methods. The first involves the use of allogenic skin, which consists of a stock of cells, and is performed on a large scale. The second type employs autologous skin made up of the patient's cells and is used to treat burn injuries. These bioprinted skins can serve as a material for testing pharmaceuticals, cosmetics, and other chemicals.[36] Certain polymeric hydrogels can be effective substitutes for tissues because of their biocompatibility and mechanical properties, which closely mimic those of biological structures. These hydrogels create an O_2 and nutrient-rich hydrated environment, supporting healthy cell growth. Biomedical constructs can be fabricated through 3D printing, by combining materials such as polymers, ceramics, liquids, powders, and even living cells to produce anatomically precise, micron-scale structures. Thus, the integrated use of polymer composites, hydrogels, and 3D bioprinting technologies has gained prevalence in applications such as skin bioprinting and, more broadly, tissue engineering.[37] Full-thickness skin constructs replicating the epidermal and dermal layers were produced employing a digital light processing 3D printer and bioinks composed of methacrylated silk fibroin (silk–GMA) and gelatin (gel–GMA), seeded with keratinocytes, fibroblasts, and vascular endothelial cells. Researchers evaluated the printability, mechanical strength, and cell viability of hydrogels containing silk–GMA and gel–GMA at varying ratios to determine the optimal formulation. A combination of 15% sel–GMA and 5% silk–gMA was identified as most suitable for fabricating stable artificial skin. Following printing, culture, and air-lifting for 4 weeks, histological analysis revealed a robust proliferation of keratinocytes and fibroblasts, along with an increased expression of cytokeratin 13, phalloidin, and CD31, indicated by immunofluorescence intensity. Full-thickness wound models have also been printed to assess functionality. Treatment with epidermal growth factor has remarkably improved the repair of wounded epidermal and dermal layers, along with the enhanced proliferation of keratinocytes and fibroblasts. Semi-quantitative reverse transcriptase-polymerase chain reaction demonstrated elevated levels of cytokeratin 13, fibroblast growth factor, and CD31 compared with untreated controls. Overall, this artificial skin model remained biocompatible and mechanically stable for > 4 weeks, highlighting its robust potential for application in future skin tissue engineering endeavors.[38]

2) Artificial bones

3D bioprinting has opened up new avenues in bone tissue engineering by enabling the fabrication of patient-specific grafts. Restoring anatomy, structure, shape, and function in the longterm is critical for treating bone trauma, tumors, infections, fracture non-union, or congenital defects. However, the structural and mechanical complexity of bone, coupled with variations in the type of defect and patient anatomy, makes the application of current treatment methods difficult—especially for large, load-bearing defects—to replicate the architecture of bones. The technology of 3D bioprinting addresses these challenges by allowing precise control over the chemistry, shape, porosity, and surface topography of materials that can substitute bone. It enables the creation of bone grafts tailored to specific patients and clinical conditions, offering a promising strategy for restoring the structural integrity and functionality of complex bone defects.[39] The key requirements for any material to serve as artificial bone fall into two main categories. Structurally, it must be customized to fit a specific defect site and also contain a microporous network that supports bone tissue formation and vascular ingrowth. In terms of physical properties, the scaffold material should possess mechanical strength sufficient to enable stabilization of the defect area, along with strong biofunctionality to stimulate and support bone regeneration.[40]

Researchers have employed 3D bioprinting to design and evaluate artificial bone scaffolds composed of hydroxyapatite (HA), zirconia (ZrO_2), and polyvinyl alcohol (PVA). In this composite system, ZrO_2 functions as a strengthening agent, while PVA acts as a binder to facilitate printing. Scanning electron microscopy images indicated that the resulting scaffold possessed a porosity of ~65%, a level considered favorable for chondrocyte proliferation and neovascularization. Further, c5.18 chondrocytes were cultured on the printed scaffolds to assess their biocompatibility. The composite supported excellent cell growth, with cell proliferation increasing by nearly 100% compared to the control group. Overall, the HA–ZrO_2–PVA composite exhibited robust biocompatibility and is a promising artificial bone candidate.[41]

A biomineralization-inspired synthetic approach, which can replicate the nanostructural features of nacre in chitosan hydrogel 2D films, was recently evaluated for its suitability in producing similar structures within a 3D scaffold. In this study, researchers applied the McGrath method to a porous chitosan hydrogel 3D scaffold to generate chitosan–$CaCO_3$ composites that mimic nacre-like, flat tabular $CaCO_3$ morphologies and associated nanoscale features. The scaffolds were fabricated using 3D bioprinting, employing a custom-built printer, and via the nozzle-based extrusion of a chitosan hydrogel formulated as the printing ink. Ink rheology and printing parameters were optimized to produce cylindrical constructs containing a cuboid lattice-like internal architecture. Additionally, the influence of different dehydration methods—critical point, air, and freeze-drying—on the structural integrity of the scaffolds was assessed from nano- to macroscale. The findings demonstrated that the McGrath method is effective in generating 3D chitosan–calcium carbonate composites in which the mineral phase and the chitosan matrix are closely integrated at the nanoscale, highlighting its potential for use in the development of advanced biomimetic scaffolds.[42] To produce a strong biocomposite, researchers have used the bacterium *Sporosarcina pasteurii* as bioink; it produces $CaCO_3$ when exposed to a solution containing urea. This polymeric scaffold dried within a minute, giving rise to a strong bone-like structure.[43]

3) Artificial brain tissue

The use of 3D models in modern neuroscience to investigate neural circuitry, nerve regeneration, and the mechanisms underlying neurological diseases is increasing. Various biofabrication strategies have been explored to create such structures. Of these, 3D bioprinting emerges as a particularly promising approach due to its high precision, scalability, and an ability to produce complex architectures. As the demand for physiologically relevant 3D neural models grows, 3D bioprinting offers the potential to fabricate reproducible, high-throughput neural tissues. Researchers have outlined key design principles for engineering neural tissues, emphasizing the importance of biomimetic architecture, appropriate mechanical properties, and controlled spatial organization of cells. However, a major challenge in adapting 3D bioprinting technologies for neural model fabrication lies in developing effective neural bioinks—materials that not only exhibit suitable printability and gelation behavior but also provide a microenvironment supportive of neuron survival, differentiation, and functional maturation.[44] Researchers have successfully bioprinted 3D brain-like structures using a bioink

composed of discrete layers of primary cortical neurons encapsulated within hydrogels, formulated from a novel peptide-modified biopolymer, gellan gum–RGD (RGD–GG). The bioink was specifically optimized for a modified reactive printing approach, enabling the fabrication of neural constructs in routine cell-culture conditions without the need for highly specialized bioprinting equipment. Critically, the incorporation of RGD peptides into the gellan gum hydrogel markedly enhanced the proliferation of primary neurons and promoted the formation of a robust neural network, demonstrating its strong potential for advanced neural tissue engineering and *in vitro* brain modeling.[45]

An *in vitro* layered brain-like tissue construct was developed using 3D cell printing. The study systematically evaluated the construct, beginning with the standardization of printing parameters and progressing to bioperformance assessments. The optimal printing conditions were identified as a nozzle with a 0.51 mm diameter and a printing speed of 5 μL/s, producing a construct with an elastic modulus of ~6 kPa, closely resembling the softness of brain tissue. The viability and growth of printed cells were examined using live/dead cell assays and immunostaining; these confirmed the distribution and maturation of healthy neurons. When comparing neuron survival in 2D and 3D cultures, the layered 3D-bioprinted structures demonstrated a significant improvement in culture conditions, enhancing cell survival rates and better mimicking the native architecture and physiology of neural tissues.[46] Researchers have used human induced pluripotent stem cells to print a two-layered brain cortical structure, which may help in treating brain injury.[47]

The treatment of myelin disorders, including multiple sclerosis and leukodystrophies, is difficult largely due to the nonavailability of reliable *in vitro* models that mimic the complex physical and biochemical environment of neuronal axons. To overcome this limitation, scientists have engineered synthetic axon mimics that replicate the geometry, mechanical softness, and surface chemistry of actual axons. Similar to human axons, these artificial axons are extremely flexible and can span distances without any support, enabling glial cells—particularly oligodendrocytes—to engage, wrap, and form myelin. Key features, such as stiffness, diameter, and biochemical ligand-based coatings, can be independently adjusted using 3D bioprinting, allowing the precise modeling of varied developmental, diseased, or injured axonal states. Experiments demonstrated that these parameters can remarkably influence myelin production by oligodendrocytes and axonal wrapping. Such a biofidelic platform enables the direct visualization and quantification of myelination, providing a powerful tool to study the mechanisms by which physical cues and pharmacological agents regulate myelin formation, offering marked potential for advancing remyelination therapies.[48]

4) Wound healing

Wound healing can be achieved by utilizing different types of hydrogels infused with natural products and nanoparticles. [49-51] Using a novel portable 3D-printing pen, a bioactive ink has been successfully employed for healing wounded tissues. It was applied to wound sites in test mice, forming a sturdy gel. The bioink was able to treat wounds pre-exposed to immune system vesicles or extracellular vesicles (EVs), more rapidly than the untreated ones. This ink contained macrophages secreted from EVs combined with sodium alginate[52]. These EVs promoted angiogenesis, reduced the levels of epithelial inflammatory markers, and increased collagen fiber formation. This non-complex process could easily repair a wound.[53] A portable handheld bioprinter, termed "SkinPen," was designed, which utilizes a biomaterial-based ink for onsite tissue printing. This hydrogel ink was composed primarily of gelatin methacrylate combined with copper-containing bioactive glass nanoparticles. This formulation exhibited remarkable biocompatibility along with robust antibacterial and angiogenic properties, making it suitable for applications in skin tissue regeneration.[54]

5) Artificial heart collagen

Based on human heart MRI data, researchers have bioprinted heart and collagen tissues that function like the heart. The aortic valves were also printed, and the pumping action of the heart was mimicked. The heart and aorta were surrounded by sleeves similar to blood pressure cuffs, which, when connected to a pneumatic system, can help control the air outflow and sustain the heartbeat rhythm. The 3D-printed heart can be used to test treatment methods for various heart diseases.[55]

6) Artificial liver cells

Isolated primary hepatocytes closely resemble the native *in vivo* hepatocytes, but their lifespan during conventional 2D culture is limited. Although techniques such as sandwich cultures or 3D organoids incorporating mesenchymal stem cells (MSCs) can extend survival, prolonged 3D culture often results in hypoxic cell death. Additionally, these systems still fall short of replicating the true liver architecture. To address these limitations, researchers have employed a 3D bioprinting strategy using alginate hydrogels containing primary hepatocytes and MSCs. These printed constructs maintained cell viability at >90%, and the morphology of the hepatocytes was preserved for 7 days, supported by MSCs. Compared with traditional 2D cultures, the 3D system simultaneously exhibited a higher expression of critical hepatic genes and proteins. These findings indicated that 3D bioprinting and MSC-derived paracrine signals synergistically enabled sustained hepatocyte culture without structural deterioration. This approach supports large-scale cell expansion, the formation of multi-cellular liver-like aggregates, and holds promise for drug screening applications and the development of artificial liver systems.[56] Scientists bioprinted blood vessels and hepatocytes in separate compartments to cure a mouse suffering from chronic liver injury.[57, 58]

7) Bionic eye

A bionic eye was created by printing an array of light receptors on a hemispherical surface using a 3D bioprinter.[59, 60] Sight can be restored in visually impaired individuals through several advanced techniques, including 3D bioprinting, stem cell and gene therapy, optogenetics, and implantable prosthetic devices.[61] A biomimetic apposition compound eye was created using a microfluidic-assisted 3D printing technique. In this design, each microlens is connected to the basal surface of the eye through internal, refractive-index-matched waveguides that prevent cross-talk and functionally replicate the natural rhabdoms found in compound eyes. The artificial eye enables full-color, wide-angle panoramic imaging and accurate positioning of light sources when placed on an imaging sensor. As a biomimetic of native compound eyes, the ability of this system to map 3D visual information from 2D images presents promising potential for applications such as advanced endoscopic imaging, machine vision, and human–robot interactions.[62]

8) Artificial bones

The technique of 3D imaging has also been used in forensics. The prints and 3D models of burned human bone fragments could effectively fit the physical deformity. 3D CT scans of the knee can aid in planning and executing surgery. Screw threads for use in high tibial osteotomy plates can also be printed, allowing these plates to be placed correctly and secured against the bone. Finger joints can also be printed according to the finger bone size of a patient and the strength required. These bioprinted materials possess antibacterial properties and hence avoid the excessive use of antibiotics.[34] **Figure 2** presents the use of 3D bioprinting in tissue engineering and regenerative medicine.

9) Dental coping

Another medical application of 3D printing is the production of dental copings, which serve as the frameworks for crowns and bridges. These structures can be rapidly fabricated, offer high durability, and can be customized to precisely match the oral dimensions of a patient. This technology is being utilized to improve patient care by enabling the fabrication of personalized dental and orthodontic devices. [63, 64]

10) Vascularization

Vascularization strategies range from direct methods—such as promoting vascular ingrowth from the host through postimplantation scaffold modifications—to more advanced approaches in which these scaffolds are engineered with prefabricated vascular networks that can be directly anastomosed or microsurgically connected to the host's blood vessels, ensuring efficient and stable integration.[65] One study developed a biomimetic, nano-bone tissue construct featuring a perfusable, endothelialized vascular channel obtained by combining the 3D bioprinting techniques of SLA and fused deposition modeling (FDM). The vascular channel was formed within the SLA-printed bone scaffold using a sacrificial PVA template created by employing FDM. Within these constructs, bone tissues developed through the osteogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs), while capillary networks were synthesized via angiogenesis using human umbilical vein endothelial cells (HUVECs) perfused through the channel. The constructs were cultured under physiologically relevant conditions to simulate *in vivo* tissue development.

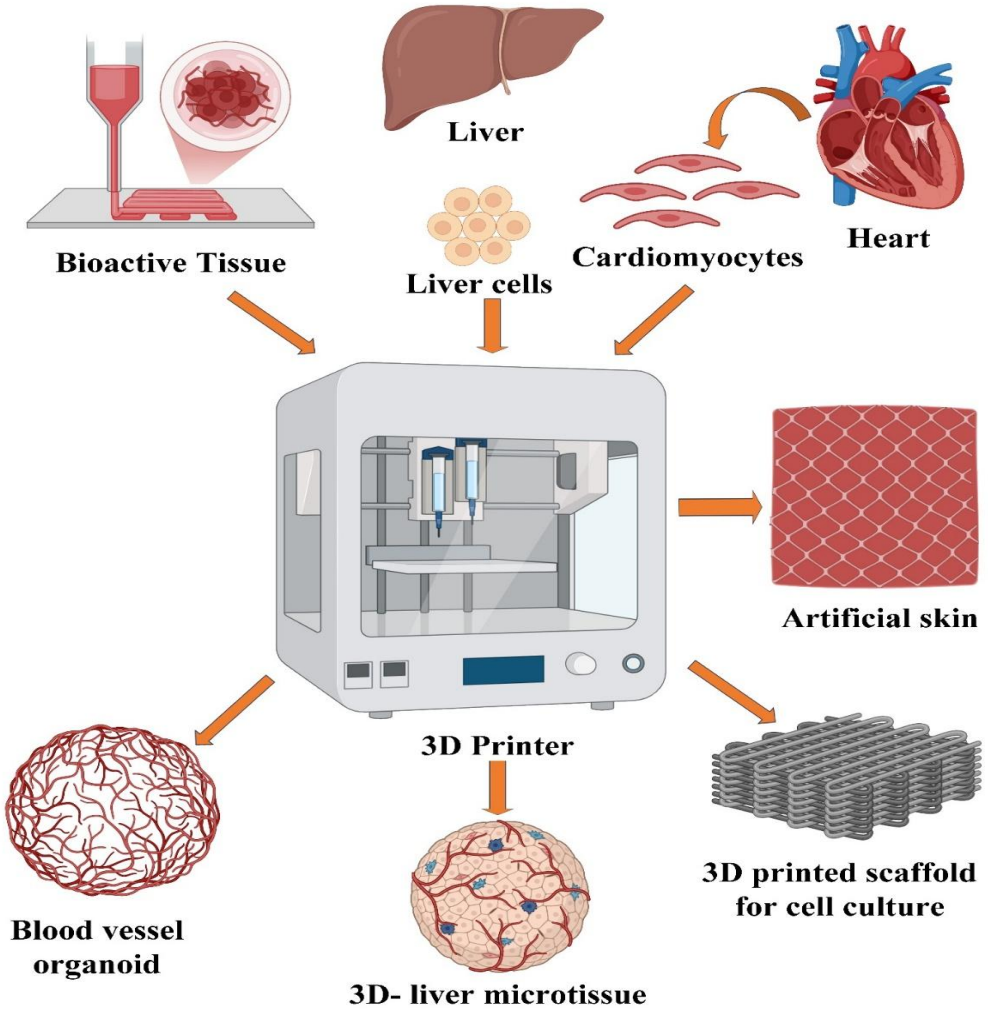

Figure 2. The application of a 3D bioprinter in tissue engineering and regenerative medicine. The bioinks are provided in the 3D printer to obtain artificial scaffolds suitable for growing different cells, liver, skin, blood vessels, and heart. The image was drawn by using different images from BioRender and assembling them in Photoshop.

The findings demonstrated that such a custom-designed bioreactor and an hMSC–HUVEC coculture system markedly promoted vascularization and enhanced osteogenic maturation for over 20 days.[66] Another study developed a 3D-printed biodegradable scaffold designed for the controlled release of deferoxamine via surface aminolysis and applying a layer-by-layer assembly approach. It aimed to promote both angiogenesis and osteogenesis, aligning with the requirements of bone repair and regeneration. It effectively enhanced vascular network formation by HUVECs, the deposition of mineralized matrix, and the expression of osteogenesis-related genes during the osteogenic differentiation of MSCs. *In vivo* experiments in a rat large bone defect model demonstrated that deferoxamine significantly promoted vascular infiltration and bone regeneration at the defect site. Additionally, the scaffold was excellently biocompatible, supporting the growth and differentiation of MSCs, while maintaining mechanical properties comparable to those of the natural cancellous bone.[67]

11) Organ-on-a-chip and microfluidic devices

Organ-on-a-chip platforms represent a rapidly advancing technology that can mimic the crucial functions of human tissues and organs, helping bridge the gap between traditional 2D cell culture or animal models and the complexities of the human body.[68] As a result, their applications in drug discovery, toxicology, disease modeling, and personalized medicine are increasing. A powerful approach for fabricating these systems is 3D printing, which enables the precise, layer-by-layer construction of the intricate microarchitectures needed for constructing organ-mimicking devices. In parallel, 3D bioprinting—a specialized branch of 3D printing—focuses on the controlled deposition of cell-laden hydrogel-based bioinks to build living, tissue-engineered structures. Together, these technologies offer complementary

strengths: 3D printing provides structural complexity and microfluidic features, while 3D bioprinting introduces biofunctionality through a spatial organization of living cells.[69] To achieve these effects, artificial organs must be microfabricated with an appropriate ECM and multiple cell types, enabling them to recapitulate the native organ morphogenesis, cell differentiation, and physiological functions. Fully 3D-printed organs-on-chips can seamlessly integrate mechanical, electrical, and microfluidic components during fabrication. These offer distinct advantages by not only enhancing device functionality but also supporting automated and large-scale manufacturing, thereby making commercial production more feasible.[70]

Microfluidic organs-on-chips have been used to investigate the physiology and pathology of major organs, including the liver, gut, kidney, and heart. The capacity to recreate vital aspects of the tissue microenvironment, combined with the use of human cells, allows these systems to overcome many limitations associated with traditional animal models. As a result, organ-on-chips provide platforms that can more accurately replicate human tissues for studying disease mechanisms and drug responses, as well as in personalized medicine.[71] Researchers have demonstrated an easily accessible approach to microfluidic and organ-on-a-chip fabrication by combining low-cost liquid crystal display (LCD) photopolymerization-based 3D printing (costing 150–600 USD) using a customized PEG diacrylate-based resin (PLInk), which is optimized for resolution, speed, and biocompatibility. Despite the relatively low illumination power and nonuniformity, typical of LCD printers, PLInk enabled the fabrication of lateral features as minute as 75 μL, 22 μL-thick embedded membranes, and circular channels with a radius of 110 μL. Using this method, 3,420 organ-on-a-chip devices were produced during a single 8-hour-long print run in a 384-well plate format. These chips supported the co-culture of two spheroids separated by a vascular barrier for 5 days, during which endothelial sprouting, cell reorganization, and migration were observed. This work demonstrates that LCD 3D printing, combined with tailored biocompatible inks, can democratize the global manufacturing of high-resolution, ready-to-use microfluidics and organ-on-a-chip devices.[72]

Artificial intelligence-assisted bioprinting

Artificial intelligence (AI) has been applied to nanomedicine for superior outcomes.[73] The incorporation of AI into 3D printing systems enables the real-time optimization of printing parameters, precise prediction of material performance, and early identification of defects through computerized vision and sensor feedback. Machine learning further enhances this process by automating design-related tasks, generating complex geometries, optimizing slicing, and enabling adaptive, self-correcting printing control. These capabilities align with the Industry 4.0 and 5.0 principles, which emphasize cyber–physical integration, autonomous decision-making, and human–machine collaboration for smarter manufacturing. The synergy between AI and 3D bioprinting not only boosts operational efficiency and product consistency but also fosters the evolution of self-learning, intelligent industrial fabrication systems. As this convergence advances, it can gain transformative potential across multiple sectors, including consumer goods, healthcare, construction, and aerospace. The authors also highlighted the role of AI in augmenting 3D bioprinting through process optimization, defect detection, and intelligence-based control strategies.[74]

Researchers optimized the 3D-bioprinting process to obtain a component of the hand exoskeleton via the computational analysis of design features and biomaterial selection to achieve maximum tensile strength. This technique integrated an artificial neural network (ANN) with genetic algorithms (GA). The selected component was modeled in Cura 0.1.5 and fabricated using fused filament fabrication technology. To refine the material and process-associated parameters, two printing materials were evaluated based on ten variables —polylactic acid (PLA) and PLA+—employing the ANN–GA model developed and trained in MATLAB. Mechanical testing was used to measure the tensile strength with a 5966 universal testing machine (INSTRON, MA, USA). The optimized parameters favored PLA+, which demonstrated superior performance. Although further assessments are needed to balance technical performance with user safety, the study highlights that additive manufacturing can effectively produce functional exoskeleton parts. Moreover, AI-driven optimization holds marked potential for improving the performance and safety of patient-specific medical devices.[75]

Bioprinting in biomedical engineering

Three key components for bioprinting in biomedical engineering include ultrasound lens, particle sorter, and capture filters. (i) ultrasound lens: the lens used in an ultrasound machine is cylindrical or spherical, but 3D bioprinting can fabricate a complex lens, which produces sharper images. Such a lens focuses the ultrasound waves at multiple sites or at a specific target, per requirements. This imaging technique can differentiate between benign and malignant tissues.[76, 77]; (ii) particle sorter: 3D bioprinting platforms can be added with a particle sorter, which can sort out cancer and normal cells, making their detection easier.[78] ; (iii) capture filters: CO_2 capture filters can also be printed using hydrogels; these can hold carbonic anhydrase, which catalyzes bicarbonate formation.[79]

Advantages, limitations, and future perspectives

Advantages: 3D printing, also known as additive manufacturing (AM) or rapid prototyping, has existed for several decades. Similar to an inkjet printer depositing ink on paper, a 3D printer builds an object layer by layer using various materials. AM serves as an effective tool for streamlining supply chains through multiple means. Compared to conventional manufacturing, it offers several key advantages—cost efficiency, faster production, enhanced quality, innovation potential, and a broader impact. With this technology, consumers can produce replacement parts for their home appliances by downloading a digital 3D printing file, effectively becoming small-scale manufacturers. It also enables the printing of components for customers residing in distant regions, eliminating geographical barriers to physical delivery. As a result, the supply chain shortens and becomes more efficient, reducing the need for shipping and inventory storage. In healthcare, 3D printing has significantly improved the quality of life through customized implants and prosthetics that enhance patient care. Additionally, AM is also environmentally friendly since it minimizes residual waste by using only the exact amount of material required for production.[80]

The demand for enhanced visualization and better precision during surgery has led to the development of 3D-printed anatomical models, customized surgical guides, and patient-specific prosthetics. The ever-expanding surgical use of 3D printing across fields—orthopedics, maxillofacial, cranial, and spinal—has sparked interest in evaluating its current applications. The use of 3D printed surgical models has reduced the surgery duration and exposure to ionizing radiation due to decreased fluoroscopy time. Tactile anatomical models have also helped medical students to learn the subject better. However, the expenses related to printing and additional imaging procedures can contribute to the higher overall procedural costs.[81, 82] With surgical implants, 3D printing can be used as a tool for personalized medicine by designing patient-specific scaffolds for better adaptability. [83]

Limitations: The use of 3D printing in surgery is not often considered cost-effective. However, many researchers suggest that in extremely complex cases, the benefits of patient-specific surgical guides may justify the added expenses. With increasing financial constraints in the healthcare sector, evaluating the economic implications of emerging medical technologies and procedures has become essential.[81, 84] The major disadvantage lies in the limited ability to control cell position in 3D, as cells often move and can be penetrated by the scaffolding support material. The second drawback concerns bioinks, such as hydrogel-based inks, which clog the nozzle.[85] To overcome these challenges, pollen grains are used as bioinks. Such an ink maintains shape when deposited on the surface, and the hollow-structured pollen grains can carry drugs, cells, and molecules; they also respond to pH changes. This ability enables the use of pollens for the controlled release of drugs. The FRESH technique also helps to overcome the problems associated with 3D printing, as a support gel is used while printing, which can then be easily melted by heating from room to body temperature after printing is completed.[86]

Limited resolution, bioink batch variability, and printer calibration are the major challenges to the use of 3D printing technology. [87- 89] Within the fields of tissue engineering and biofabrication, high-definition bioprinting refers to the ability to reliably create 3D structures with feature sizes < 50 μL using bioinks that contain living cells. Among the existing HD bioprinting methods, multiphoton lithography currently offers the maximal resolution—below 1μm—enabling precise crosslinking or structural modifications within transparent, cell-laden materials. The resolution to be achieved can be adjusted through repeated processing. Since the focal spot size determines the number of lines needed to construct each layer, the ability to modulate it according to the desired resolution can substantially reduce fabrication time. Although increasing laser power elongates voxels along the vertical axis, this limitation can be overcome through spatiotemporal focusing, which enables the formation of nearly spherical voxels with adjustable dimensions.[90, 91]

The primary components of bioprinting are hydrogel-based bioinks, which are printed into structures that accurately replicate the computer-aided design. For optimal cell activity, soft and easily printable inks are generally preferred; however, their low mechanical strength can lead to deformations during printing, compromising structural fidelity. Although the notion of printability is conceptually straightforward, its quantitative assessment remains undefined and depends on several rheological and chemical characteristics of the bioink. A key rheological property relevant to bioprinting is shear thinning, a type of time-independent non-Newtonian behavior in which viscosity decreases as the shear rate increases. This property is typical of materials used in extrusion-based printing, including polymer melts, polymer solutions above their critical concentration, partially cross-linked hydrogels, and colloidal suspensions.[92, 93]

Future perspectives: A regulatory authority should be constituted to properly monitor the use of 3D bioprinting in the medical and manufacturing sectors. The robust use of varied types of 3D bioprinters can be standardized to reduce product cost. This effect will facilitate patients as well as customers to implement the technology more easily and effectively. Moreover, the bio-scaffolds manufactured for the 3D printing of tissues and organoids should be tested for long-term safety using artificial biofluids.[94, 95]

Conclusion

Three-dimensional bioprinting is transforming tissue engineering, regenerative medicine, and pharmaceutical research by enabling the fabrication of functional tissues and organs. Despite challenges like cell positioning and nozzle clogging, advancements such as pollen-based bioinks and the FRESH technique are enhancing precision and biocompatibility. The integration and performance of 3D-printed scaffolds *in vivo* have been significantly hindered by the absence of effective methods to induce vascularization. Over the past decade, researchers have investigated various strategies to enhance vascular growth within these scaffolds, aiming to improve their functionality and compatibility with host tissues. Such strategies range from simple modifications that promote host vessel infiltration after implantation to advanced designs incorporating pre-formed vascular networks that can be surgically connected to the host's blood supply for better integration. Optimizing pore size and porosity of the scaffold is a critical factor influencing vascularization. While growth factors can effectively stimulate angiogenesis, their concentrations must be carefully controlled to avoid the formation of irregular, leaky, or hemorrhagic vessels. Currently, no single technique has been proven fully effective in ensuring that the implanted grafts, several mm thick, develop a stable and functional vascular network. Therefore, for greater clinical success, a multifaceted approach combining several complementary strategies is recommended to achieve efficient vascularization in 3D-printed scaffolds.[65, 96]

A wide range of applications have been proposed for bioprinted constructs, particularly for creating artificial tissues and organs for transplantation. Currently, the most successful ones have been in the fields of drug discovery, toxicology, and cancer research. The development of bioprinted artificial skin and partial liver tissues has also progressed. However, it is essential to carefully evaluate long-term biocompatibility and potential individual variations in biological response before large-scale clinical use.[97]

Future developments in bioink formulations, vascularization, and AI-driven optimization could accelerate the fabrication of transplantable organs and expand applications in food and biofabrication. Continued innovation in this field holds great promise for revolutionizing healthcare and biotechnology.

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Conflict of interest

The authors declare no conflict of interest.

Data sharing statement

The present review is based on the references cited. All data generated or analyzed during the present study are included in this published article and the citations herein. Further details, opinions, and interpretation are available from the corresponding author on reasonable request.

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