

Review

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Review

3D Printing/Bioprinting and Cellular Therapies for Regenerative Medicine: Current Advances

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Abstract: The application of three-dimensional (3D) printing/bioprinting technologies and cell therapies has garnered significant attention due to their potential in the field of regenerative medicine. This paper aims to provide a comprehensive overview of 3D printing/bioprinting technology and cell therapies, highlighting their results in diverse medical applications, while also discussing the capabilities and limitations of their combined use. The synergistic combination of 3D printing and cellular therapies has been recognised as a promising and innovative approach, and it is expected that these technologies will progressively assume a crucial role in the treatment of various diseases and conditions in the foreseeable future. This review concludes with a forward-looking perspective on the future impact of these technologies, highlighting their potential to revolutionize regenerative medicine through enhanced tissue repair and organ replacement strategies.

Keywords: 3D printing; additive manufacturing; biomaterials; biomedical applications; bioprinting; cellular therapies; tissue regeneration

1. Introduction

Nowadays, regenerative medicine is considered an emerging field of research worldwide with the potential to revolutionise healthcare (improving patient outcomes and quality of life) in the 21st century [1]. The field of regenerative medicine is focused on the replacement, engineering and regeneration of human cells, tissues and organs. Its aim is to repair, restore, supplement or replace the normal function of a biological system following treatment with autologous, allogeneic stem and stromal cells [2–4].

In recent years, two promising approaches that have gained attention, namely three-dimensional (3D) printing and cellular therapies. 3D printing has revolutionized tissue engineering through the manufacture of complex, patient-specific, whilst providing precise control over the spatial distribution of cells and biomaterials. On the other hand, cellular therapies encompass the use of living cells to replace, repair, or regenerate injured tissues. The integration of 3D printing with cell therapies provides personalised, cell-laden constructs that mimic the complex architecture and

function of native tissues. This approach stimulates cell-cell interactions, cell-matrix interactions and the creation of functional tissue-like constructs [5,6].

This review presented an overview of current advances in the combination of 3D printing/bioprinting and cell therapies in the field of regenerative medicine. Firstly, the principles and applications of 3D printing/bioprinting and cell therapies were addressed individually. Subsequently, we explored recent advances in the integration of these technologies to create 3D-printed cellular structures. The limitations and challenges associated with these approaches were also mentioned, and the current state was analysed. Finally, we presented a future perspective on the potential impact of these technologies in the field of regenerative medicine and concluded with a summary of the main findings.

By providing a detailed understanding of the current state and future directions, this review seeks to contribute to the growing body of knowledge in this challenging and rapidly evolving field.

2. Three-Dimensional Printing

Three-dimensional (3D) printing, also known as additive manufacturing (AM), or biofabrication, is a burgeoning technique for rapid prototyping of structures in diverse fields, encompassing regenerative medicine [7–11]. Over the last two decades, 3D printing has been widely researched due to its simplicity and its highly flexible manufacture provides unlimited possibilities for creating complex structures [12,13]. Several examples of 3D printed medical devices include: instrumentation, such as guides to facilitate the correct surgical placement of devices; implants, such as hip joints; and external prostheses, such as bionic hands [14].

This advanced manufacturing technology was initially developed by Charles Hull in the early 1980s and can be used to create customized and complex structures with high precision and accuracy [15]. The technology operates by using computer-aided design (CAD) software to create a virtual model of the structure, which is subsequently converted into a physical object through the use of various 3D printing techniques [16]. The International Standard Organization (ISO) and the American Society for Testing and Materials (ASTM) [17] have established a classification system comprising seven overarching categories to categorize 3D printing methods based on part manufacturing approaches, as outlined in **Figure 1**. The subsequent sections provide a succinct overview of 3D printing processes.

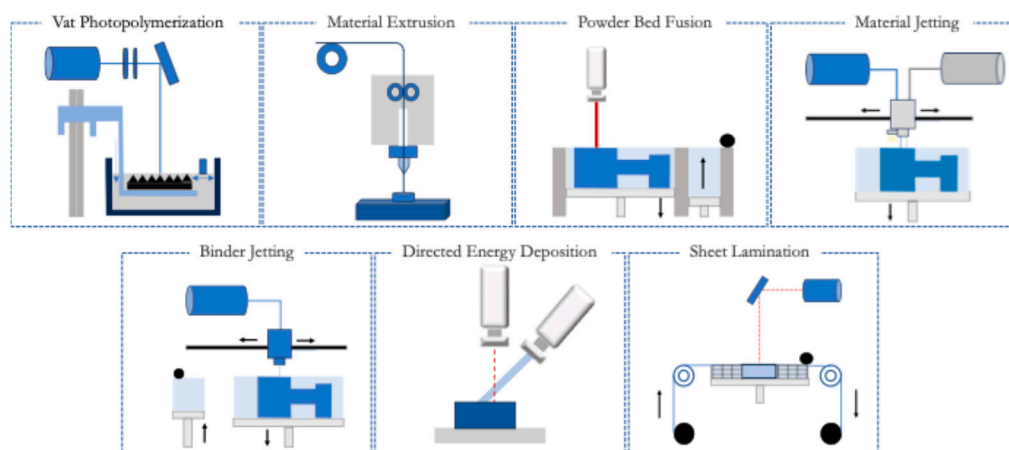


Figure 1. Diagram of the different AM processes.

2.1. Vat Photopolymerization

This method is characterized by principle of selectively solidifying a liquid photopolymer resin by exposing it to specific light sources or patterns. In the procedure, a vat contains the liquid resin, which is selectively cured layer by layer in the desired regions, gradually constructing the intended object. The laser, guided by predetermined digital coordinates, provides the necessary information to cure the resin at precise locations within each layer. To facilitate this curing process, a

photoinitiator is introduced into the resin material. The photoinitiator absorbs the incident ultraviolet (UV) radiation and generates active species that initiate the photopolymerization reaction [18,19].

The vat photopolymerization technique encompasses several commonly utilized processes, including stereolithography (SLA), digital light processing (DLP), continuous light interface production (CLIP), and two-photon polymerization (2PP) [20]. These methods employ the SLA utilizes a laser or digital light projector to cure the resin layer by layer, while DLP utilizes a digital light projector to project an entire layer simultaneously. CLIP employs a continuous liquid interface production approach, where a continuous liquid interface is established between the resin and a transparent window, enabling continuous printing. Lastly, 2PP utilizes a focused laser beam and a photosensitive resin to achieve high-resolution printing via a nonlinear optical process [21]. These vat photopolymerization techniques offer versatile and AM capabilities for fabricating complex and detailed structures.

2.2. Material Extrusion

Materials extrusion is a widely adopted AM technique due to its fast manufacturing, cost efficiency, simplicity, user-friendly nature and the potential to produce complex components [22–24]. This technique involves extruding the material through an orifice and depositing it on a construction platform [25–27].

The most common technique is the fused filament fabrication (FFF), also known as fused deposition modeling (FDM) [28]. In this process the filament is melted (usually in the range of 150–250°C), and the molten material is squeezed through a nozzle [25]. The extruded material is then placed onto a build plate, which can be adjusted in the z direction. This technique usually uses more polymers, although it is also used to print metal and ceramic components. FDM uses a support to build over-hang features, but this material can be easily removed, mechanically by removing it from the printed part, or chemically by dissolving it in a solvent solution [29].

2.3. Powder Bed Fusion

The powder bed fusion (PBF) process uses a laser or an electron beam to melt and fuse the material into powder [30]. The principle used in this technique is to produce the product layer by layer and melt it. A heat source concentrates its heat on a powdered base material and heats the cross-sectional area [31].

PBD is mainly used due to the low cost of producing the object and the powder can be recycled to produce another piece from it [32]. This method encompasses several commonly utilized processes, including direct metal laser sintering (DMLS), selective laser melting (SLM), selective laser sintering (SLS), and electron beam melting (EBM). PBF uses a laser source (DMLS, SLM and SLS) or an electron beam (EBM) to directly and selectively melt or sinter layers of materials to produce a solid component. This technique can be used to process various powder-based materials, although the most frequently used are metals and polymers [29].

2.4. Material Jetting

The material jetting (MJ) deposits the liquid in droplets to bind powder material [33]. In the MJ, the material is injected into the surface/building platform, where it solidifies, and the model is assembled layer by layer. The layers are subsequently cured or toughened with ultraviolet light. In this method, the material must be deposited in droplets, and therefore the materials used are limited. Generally, polymers and waxes are used, considering their viscous nature and ability to produce droplets [34]. This is a fast and proficient method and offers greater freedom when designing and printing complex models [24].

2.5. Binder Jetting

In the binder jetting (BJ), a liquid bonding compound is applied selectively to bind powder materials. A relevant feature of this technique today is the possibility of using color inkjet technology to create colored objects in the binders [35].

Binders are used to ensure adhesion between the powdered material particles. These binders contribute to obtaining the strength of the part and the desired form of the final product [31,36]. The materials commonly used in this method are metals such as stainless steel, ceramics such as glass and polymers such as acrylonitrile butadiene styrene, polyamide and polycarbonate [32].

2.6. Directed Energy Deposition

The directed energy deposition (DED) is a method of using focused thermal energy to melt materials, fusing them as they are deposited. In this AM technique, an energy source (such as an electron beam, a laser and a plasma) is used to melt the materials which are then deposited [35].

The DED consists of a nozzle assembled on a multi-hub arm that deposits the dissolved material at a surface layer where it solidifies. A significant advantage of this method is its ability to precisely control the grain structure of the deposited material [32,37,38].

This process is analogous to material extrusion; however, the nozzle in DED can move in multiple directions rather than being fixed to a specific axis. While the process can be employed with polymers and ceramics, it is predominantly used with metals, supplied in the form of either powder or wire [39].

2.7. Sheet Lamination

Sheet lamination consists of the layer-by-layer bonding of thin sheets of material, normally fed through a system of feed rollers [40]. This technique can use a variety of materials, including paper, polymer and metal [28]. Although this is a less precise method, it has the advantage of being quick and low cost [40].

The most widely used sheet lamination methods are laminated object manufacturing (LOM) and ultrasonic additive manufacturing (UAM). LOM employs a layer-by-layer approach similar to other AM techniques, but it uses paper as the primary material and adhesive for bonding instead of welding. UAM, on the other hand, uses metals such as aluminum, stainless steel, and titanium. This process operates at low temperatures, allowing for the creation of complex internal geometries [41].

3. Bioprinting

Considering the incorporation of active substances such as biomaterials, living cells and active biomolecules, three-dimensional printing can be progressed to bioprinting, thus providing the manufactured structures with biological functions [12].

Bioprinting technology allows for the manufacture of complex, functional structures that promote cell growth and tissue formation. The prospect of manufacturing complete tissues or organs using 3D printing is very promising and has the potential to revolutionise regenerative medicine [42].

In the past decades, there has been a significant progress in 3D bioprinting (**Figure 2**) [43–46]. The evolution of this technology began with the invention of SLA by Charles Hull in 1984, which marked the beginning of 3D bioprinting [44]. Subsequently, in 2002, Landers *et al.* introduced extrusion-based bioprinting technology, which was later commercialized under the name “3D-Bioplotter” [47,48]. In 2003, Thomas and Boland's research team adapted a conventional inkjet printer to develop the first inkjet bioprinter capable of printing living cells [47,48]. Later, the engineering of scaffold-free vascular tissue via bioprinting was achieved by Norotte *et al.* [49]. The subsequent years witnessed the development and introduction of various bioprinted constructs, including an artificial liver in 2012, full human skin in 2014, a heart valve in 2016, and a lung-mimicking air sac with surrounding blood vessels in 2019, among other innovations [43–46].

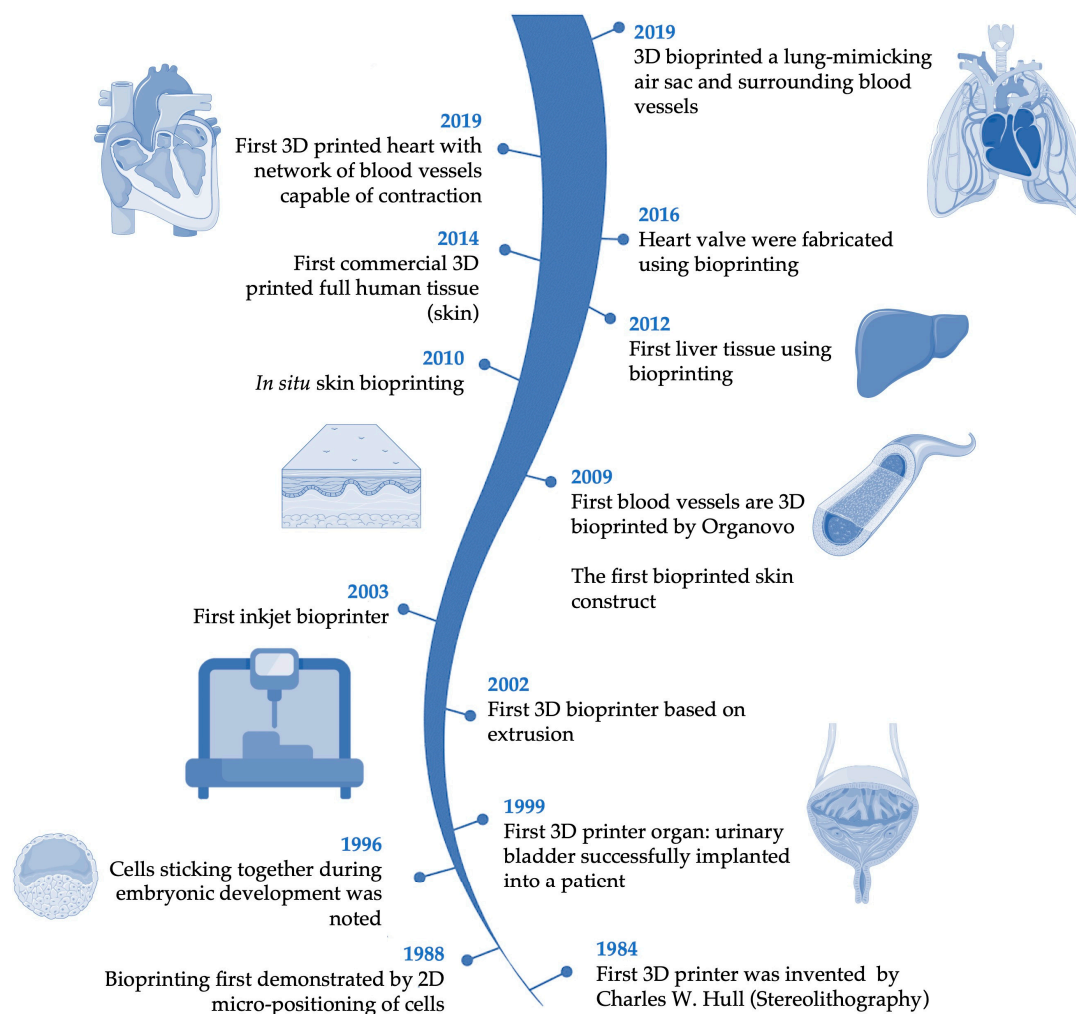


Figure 2. Major milestones in the history of 3D bioprinting.

3.1. Techniques

Among the diverse AM techniques, the most widely used in bioprinting are laser-based printing (SLA and SLS), extrusion printing and inkjet printing [50–52].

SLA is a vat photopolymerization process and consists of photocurable bioinks that are subjected to UV, infrared or visible light to produce 3D pieces using the layer-by-layer procedure [53,54]. Among the advantages of SLA is its capacity to cure quickly at physiological temperatures, allowing the manufacture of constructions for applications in regenerative medicine. This technique has been widely used to fabricate hearing aids, micro-needles for transdermal drug delivery, surgical guides for placing dental implants, temporary crowns and bridges, and supports for tissue engineering with/without encapsulated cells [53,55].

During SLS printing, fine particles of the entire substance are fused by the heat of a high-powered laser to produce a 3D structure [56,57]. In this technique, several categories of powders, including polymers, metals and ceramics, must be processed into powder. SLS is a PBF process and is used in numerous applications in the medical field, namely the fabrication of physical models used in surgery, prototypes for medical devices and scaffolds for tissue engineering. The popularity of SLS printers is attributed to their affordability, high productivity and material versatility [57,58].

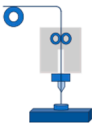
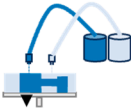
Extrusion printing is considered the most popular bioprinting technology [59]. In this technique, material is melted and extruded, through a nozzle, orifice or needle, using a screw, piston, or high-pressure pneumatic force, to form successive layers of the part [60–64]. This technique has been used

extensively in the medical field, enabling the biofabrication of tissues, organs, implants and personalised drug delivery systems. It is also highly applicable in the field of disease modelling. Models made by extrusion can provide a baseline for the comprehension of the underlying biological mechanisms behind disease progression, thus contributing to the identification of effective treatments [65].

Inkjet bioprinting is considered as the pioneering technology in the field of bioprinting. The printing process utilizing this technique comprises two phases: the production of discrete droplets that are directed to a specified location on the substrate, and the subsequent interaction between the droplets and the substrate [44,66]. This technique has significant potential in non-contact bioprinting for hard and soft tissue regeneration. It is used in a wide range of medical applications, such as the manufacture of patient- or project-specific implants, including those for static load-bearing applications (such as dental crowns and prosthetic structures), joint applications (such as osteochondral cartilage implants), as well as for *in vivo* blood vessel formation and tissue regeneration [67].

Table 1 presents the aforementioned techniques, as well as the advantages and drawbacks of each.

Table 1. Comparison of the bioprinting techniques [56,68–71].

Printing Technology	Laser-based Printing		Extrusion Printing	Inkjet Printing
	Stereolithography	Selective Laser Sintering		
Advantages	✓ High accuracy/resolution ✓ Printing time independent of complex geometries ✓ Fast fabrication speed ✓ Good vertical printability ✓ Smooth surface finish	✓ No need for support structures ✓ Suitable for a wide range of materials ✓ High mechanical strength of printed constructs ✓ High productivity ✓ Ability to print complex structures	✓ Relatively simple and cost-effective setup ✓ Versatile material choices ✓ Ability to print with high cell densities and high viscosity biomaterials ✓ No need for support materials	✓ Low cost and scalable ✓ High printable resolution/precision ✓ Fast fabrication speed ✓ Wide availability ✓ Ability to print low viscosity biomaterials ✓ Low material wastage ✓ Capable of depositing multiple cell types simultaneously
Drawbacks	▪ Limited to photocurable biomaterials ▪ Potential cytotoxicity of photoinitiators ▪ Lack of printing multi-cells ▪ Need for support materials ▪ Expensive equipment and materials	▪ Rough surface ▪ High equipment cost ▪ High energy consumption ▪ Difficulty in printing cell-laden constructs ▪ Slow fabrication speed ▪ Post-processing required to remove excess powder	▪ Slow fabrication speed ▪ Low printable resolution/precision ▪ Possible nozzle clogging ▪ Potential shear stress-induced cell damage during extrusion	▪ Lower mechanical strength of printed constructs ▪ Limited printable materials/requires low viscosity materials and low cell densities ▪ Need for support materials ▪ Potential clogging of print nozzles

3.2. Materials Used in 3D Bioprinting

Nowadays, a wide variety of biomaterials are being used in 3D bioprinting [72]. The interaction between biomaterials and cells is fundamental for cell viability, proliferation, and differentiation. Therefore, it is fundamental to consider the characteristics of biomaterials, such as non-toxicity, biocompatibility, and the absence of immune reactions and foreign body responses [73].

The materials used for 3D bioprinting can be classified into two categories: natural and synthetic biomaterials. Natural biomaterials are particularly attractive due to their bioactivity, being similar to the extracellular matrix (ECM) and biocompatible. The following are some examples of natural materials used in this field: collagen, xanthan gum (XG), silk fibroin (SF), gelatine, pectin, gellan gum, albumin, chitosan, sodium alginate, agarose, fibrin, keratin and hyaluronic acid (HA). However, these types of materials generally exhibit poor mechanical properties [74–95]. Conversely, synthetic

materials offer advantages over natural materials, as they can be tailored to possess specific physical properties and present greater uniformity. The following are some examples of synthetic materials used in this field: poly (ϵ -caprolactone) (PCL), poly(lactic-co-glycolic) acid (PLGA), poly(l-lactic) acid (PLA), poly (glycolic acid) (PGA), polyurethane (PU), polyethylene glycol (PEG), polyether ether ketone (PEEK), polyvinylpyrrolidone (PVP) and pluronic. Nevertheless, synthetic materials for 3D bioprinting have some disadvantages, such as poor biocompatibility, the release of toxic degradation products and the lack of bioactive ligands [96–109].

To address the drawbacks, a more comprehensive understanding of the physiological properties of the ECM and a higher ability to replicate the complex 3D structures mimetic of the ECM would represent a significant advance in 3D bioprinting. Furthermore, the development of composite or hybrid bioprinting materials and multimaterial bioprinting technologies has emerged in the area of tissue regeneration [110,111]. However, the living tissues and organs of the human body operate in a dynamic biochemical environment. Artificial constructions bioprinted with traditional materials often do not adapt to the spatial and temporal development of tissues/organs. Consequently, interest in the development of advanced stimuli-sensitive biomaterials for 3D bioprinting is growing. These materials would allow artificial biological constructions to adapt to the complex and dynamic physiological environment [111–113].

4. Cellular Therapies

Cellular therapies, also known as cell therapy, cell transplantation, or cytotераpy involve the injection, grafting or implantation of cells into a patient autologous or allogeneic to achieve a medicinal effect [114,115]. Cell therapies are frequently applied in combination with biomaterial supports, which are designed to support and guide the cells both during and after transplantation [116].

Advances in 3D printing technology allow for the precise spatial distribution of multiple cells and biomaterials, thereby enhancing the efficiency of cell delivery and the loading of cells onto scaffolds. There are two approaches to delivering cells via 3D printed scaffolds: seeding cells after scaffold printing and embedding cells in bioinks via 3D bioprinting [117–120]. The approach of seeding the cells after printing the scaffold offers user-friendly requirements for the conditions and 3D printing, although is limited by a low cell adhesion rate on the scaffolds. To address this issue, a strategy consists of using hydrogels to encapsulate cells and then integrating them with 3D printed scaffolds. The bioprinting approach, on the other hand, can enhance cell loading efficiency and precisely control the spatial distribution of several cells. Nevertheless, this approach has stringent requisites for the printing environment and parameters, as the cells are sensitive to inappropriate conditions. This necessitates requires detailed knowledge of the parameters of the bioinks and printing conditions beforehand [70,121–124].

The selection of a specific type of cell therapy is highly important, since it affects the function and design of the tissue engineering model [125,126]. There are three categories of cell therapies that can be applied to printed scaffolds, namely stem cell-based, non-stem cell-based and multicellular therapies [114].

4.1. Stem Cell-Based Therapies

One of the most used cells are stem cells, which are unspecialized with the capacity to self-renew and differentiate into multiple cell lines [11,125,127,128]. Their capacity to differentiate into specific cell types while continuously dividing and self-renewing makes them interesting prospects for medicine regenerative. They have successfully been used to create functional tissues that replicate the properties of natural organs. These stem cells can be sourced from several origins [129]. In the field of bioengineering, the three most commonly used types of stem cells are mesenchymal stem/stromal cells (MSCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [130].

Also known as multipotent cells, MSCs can be isolated from different tissues, including bone marrow, muscle, lung, teeth, adipose tissue, liver, and perinatal/extra-embryonic associated tissue.

Furthermore, they have the ability to proliferate and differentiate into a wide variety of cell lines such as osteoblasts, chondrocytes, and adipocytes [131]. Nevertheless, they can also be differentiated into other types of mesenchymal and non-mesenchymal cells, such as myocytes, tendocytes, neural cells, ligament cells, smooth muscle cells, endothelial cells, cardiomyocytes, and hepatocytes [131,132]. In regenerative medicine, these cells offer numerous advantages, including ease of expansion in culture and the capacity to differentiate into desired cell lines. They also possess specific immunological properties, such as being immunoprivileged and immunomodulatory, and have tropisms for injury sites. Additionally, they can stimulate trophic responses and modulate tissue functions and inflammation through the secretion of essential bioactive molecules [133]. However, MSCs present some possible challenges, such as poor-quality control and inconsistency regarding heterogeneity, stability, differentiation, immunocompatibility, and migratory capacity [134,135].

In turn, ESCs are pluripotent cells originating from the inner cell mass of blastocytes (an early embryo). They can differentiate into almost any type of cell derived from the germ layers, except the trophoblastic cell line [136]. However, these present some ethical concerns, their differentiation is not easily controlled, and the cells can form teratomas and be immunogenic [130,137–140].

Finally, iPSCs are pluripotent stem cells derived from adult somatic cells that have been genetically reprogrammed (by inducing genes and factors) to a state similar to ESCs, presenting a high level of multipotency [141]. Although they are similar to ESCs, they do not present the ethical and immunogenic concerns of ESCs. Nevertheless, as with ESCs, iPSCs present a risk of teratoma formation *in vivo* [130].

4.2. Non-Stem Cell-Based Therapies

Non-stem cell therapies commonly use somatic cells isolated from humans, subsequently cultured and expanded *in vitro*, and then applied to patients for therapeutic, preventive, or diagnostic treatment [142]. These can be categorized into immune cells and non-immune cells. Immune cells, such as, natural killer cells, dendritic cells and macrophages can be engineered to target specific antigens. This approach is used in therapeutic strategies, including cancer, infections, autoimmune diseases, and allogeneic transplantation. On the other hand, non-immune cells, such as chondrocytes, fibroblasts, hepatocytes, keratinocytes, and pancreatic islet cells, normally are involved in the host's defense response, as structural architectures, regulators and effectors of its protective immune reaction [114,142–146].

These somatic cell-based therapies are usually used as an *in vivo* resource of cytokines, enzymes, and growth factors. Additionally, they are frequently used in adoptive cell therapy for cancer treatment and as transplanted cells (such as hepatocytes or pancreatic islet cells) to correct inborn metabolic errors. They also find applications as cell-based or scaffold-free systems in the treatment of burns, ulcers, and cartilage injuries [114,143,147].

4.3. Multicellular Therapies

Multicellular therapies comprise at least two types of cultured stem and/or non-stem cells. This emerging approach considers that using a combination of cell types is more effective for promoting long-term tissue repair compared to single-cell therapy. This effectiveness is attributed to cell-cell interactions that extend beyond embryogenesis and play a crucial role in regenerative procedures [114,145,148].

Examples of multicellular therapies include scaffold-based or scaffold-free cell products, bone marrow aspirate-derived therapies, adoptive cell therapy products, stem cell transplantation, and stromal vascular fraction [114,143,149–151].

5. Advancements in 3D Printed/ Bioprinting and Cellular Therapies for Regenerative Medicine

The advancements in combining 3D printing/bioprinting and cellular therapies for regenerative medicine have the potential to enhance tissue engineering and provide innovative treatments for

various diseases and conditions. These developments could significantly improve patient quality of life and potentially save many lives [13,152,153].

This approach has been successful in printing the structures of different tissues, including cardiovascular, bone, liver, skin and neural tissues (**Figure 3**). The following sections analyze the advancements in the integration of 3D printing/bioprinting and cellular therapies in several tissue engineering applications.

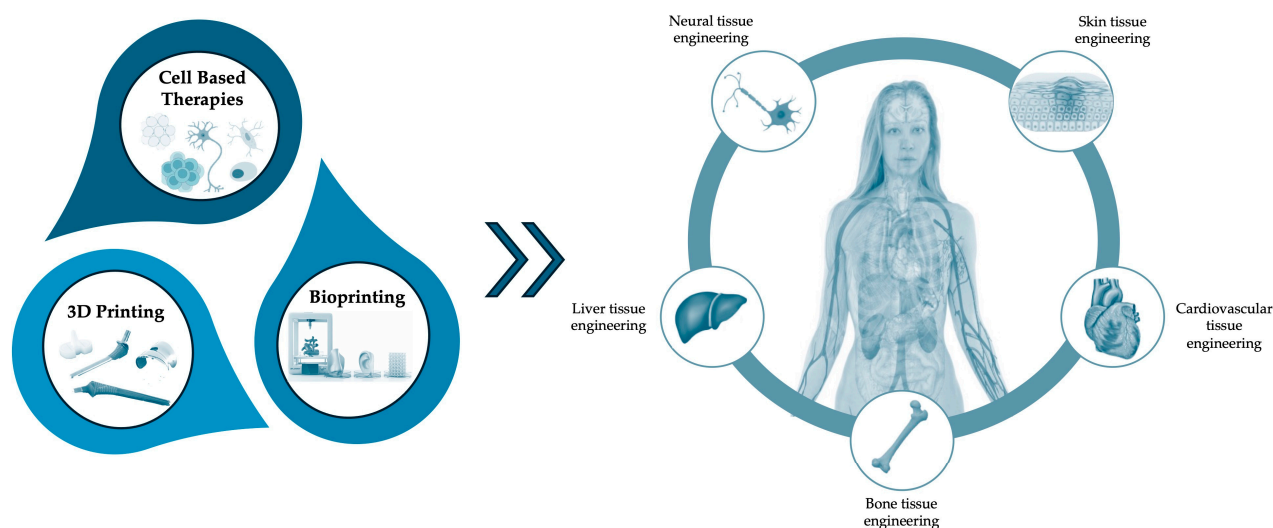


Figure 3. Advances in cell therapies, 3D printing, and bioprinting are addressing the challenges in engineering cardiovascular, bone, liver, skin, and neural tissues.

5.1. Cardiovascular Tissue Engineering

Cardiovascular diseases, which include pathologies affecting the myocardium, heart valves, and body's vasculature, are highly prevalent worldwide. These diseases are a leading cause of morbidity and mortality, especially in developed countries [154–157]. Current therapeutic approaches include cellular therapies, bypass grafting, implantation of medical devices, cardiac tissue patches, and organ transplantation [154,158–161]. Organ transplantation is often not the optimal solution due to the imbalance between the availability of donor organs and the high demand. Additionally, the success of organ transplants is frequently compromised by complications related to immune rejection [155,162]. Among the various therapeutic approaches, cell therapy has demonstrated success in regenerating cardiovascular tissue. Nevertheless, the absence of ECM limits cell survival following injection, resulting in reduced long-term viability [162].

To address these concerns, 3D printing and bioprinting have emerged as effective approaches for developing scaffolds that incorporate ECM components and enhance cell viability [163]. These structures can more accurately the spatial and mechanical properties of native tissues, which is relevant for their functionality and integration *in vivo* [157]. Currently, scaffolds for cardiovascular tissue engineering have been fabricated using a range of 3D printing technologies, including inkjet printing, extrusion-based techniques, and SLA.

The scaffolds commonly incorporate biocompatible materials of natural origin, including fibrin, alginate, gelatin, collagen, HA, and fibrinogen [164]. However, to achieve complex structures with optimal physical, chemical, and mechanical properties, synthetic materials, such as, poly (glycerol sebacate) (PGS), PCL, and poly (ethylene glycol) methacrylate (PEGMA) are also employed. Additionally, some studies utilize a combination of natural and synthetic materials or semi-synthetic derivatives, such as gelatin methacrylate (GelMA) and hyaluronic acid-gelatin methacrylate (HAGM), to optimize scaffold properties while maintaining cell viability [13]. Furthermore, decellularized matrices are extensively utilized due to their provision of a porous, interconnected polymeric network that facilitates cell migration, proliferation, and the delivery of essential nutrients for cell survival [165].

Maiullari *et al.* developed a method to fabricate 3D cardiac tissue models with a vascular network (**Figure 4-A**). They created multi-cellular constructs using human umbilical vein endothelial cells (hUVECs) and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), encapsulated in alginate and PEG-Fibrinogen (PF). These constructs were extruded through a custom microfluidic printing head. Their study demonstrated that bioprinted endothelial cells can form functional vasculature in the transplanted tissues and interact with the host's existing vessels [166].

In order to repair damaged myocardium, Beijleri *et al.*, developed a 3D-printed cardiac patch composed of decellularized cardiac extracellular (cECM) matrix hydrogel and GelMA, for delivery of pediatric human cardiac progenitor cell (hCPC) (**Figure 4-B**). The GelMA-cECM bioinks ensure uniform distribution of cECM and hCPCs, with the hCPCs maintaining over 75% viability. Additionally, conditioned media from GelMA-cECM patches demonstrate more than a 2-fold increase in angiogenic potential. The patches also remain adhered to rat hearts and show vascularization over a 14-day period *in vivo* [167]. Also, in the development of tissue patches, Melhem *et al.* proposed a hydrogel patch embedded with multiple microchannels to enhance cell retention and factor delivery at the target tissue (**Figure 4-C**). They integrated bone marrow-derived MSCs (BMSCs) into a hydrogel by cross-linking a poly (ethylene glycol) dimethacrylate (PEGDMA) solution containing the cells. Microchannels with precise diameters were created within the cell-loaded hydrogel using an SLA unit for *in situ* cross-linking. This 3D-printed, microchanneled hydrogel, was designed as an advanced therapeutic tool for sustained delivery of multiple therapeutics, aiming to improve outcomes in ischemic heart injury [168].

Noor *et al.* developed a method to 3D print thick, vascularized cardiac patches tailored to a patient's specific properties. They used patient-derived cells, reprogrammed into cardiomyocytes and endothelial cells, and a personalized hydrogel made from the patient's extracellular matrix. These components were combined to create bioinks for printing cardiac tissue and blood vessels. The patches, optimized for oxygen transfer, demonstrated proper structure and function *in vitro*, and the approach was further validated by successfully printing cellularized human hearts with natural architecture (**Figure 4-D**) [169].

In turn, to mimic human microvasculature, Cui *et al.* developed a bioink combining human microvascular endothelial cells (hMVECs) and fibrin, used to fabricate micron-sized fibrin channels via drop-on-demand polymerization (**Figure 4-E**). This aqueous printing process minimizes cell damage. hMVECs printed with fibrin aligned within the channels and proliferated to form confluent linings, resulting in a 3D tubular structure. The study concludes that simultaneous cell and scaffold printing promotes hMVEC proliferation and microvasculature formation [170].

Several researchers have employed 3D printing or bioprinting, with or without cell therapies, to advance cardiovascular tissue engineering. A summary of these studies is presented in **Table 2**.

Table 2. Recent advances in 3D printing/bioprinting and cellular therapies for cardiovascular tissue engineering. Abbreviations: human microvascular endothelial cells (hMVECs); mouse embryonic fibroblasts (mEFs); human cardiac-derived cardiomyocyte progenitor cells (hCMPCs); human umbilical vein endothelial cells (hUVECs); cardiomyocytes (H9c2 cells); induced pluripotent stem cell-derived cardiomyocytes (iCMs); human cardiac fibroblasts (CFs); human coronary artery endothelial cells (hCAECs); gelatin methacrylate (GelMA); decellularized cardiac extracellular matrix hydrogel (cECM); human cardiac progenitor cells (hCPCs); heart tissue-derived extracellular matrix (hdECM); neonatal rat cardiomyocytes (NRCMs); poly(ethylene glycol)-fibrinogen (PF); induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs); digital light processing (DLP); poly (glycerol sebacate) (PGS); poly (ε-caprolactone) (PCL); fused deposition modeling (FDM); not applicable (n/a); stereolithographic assembly (SLA); poly(ethylene glycol) dimethacrylate (PEGDMA); bone marrow-derived mesenchymal stem cells (BMSCs); mesenchymal stem/stromal cell (MSC); hyaluronic acid (HA); human embryonic stem cells (hESCs); early vascular cells (EVCs); hyaluronic acid glycidyl methacrylate (HAGM); neonatal rat cardiomyocytes (NRCMs).

3D printing technology	Biomaterials	Cellular Therapies	Application	Outcomes	Reference
Inkjet Printing	Fibrin gel	hMVECs	Microvasculature construction	The construction promoted hMVEC proliferation and microvasculature formation.	[170]
	NovoGel	mEFs	Aortic tissue construct	Support structure and mEF were successfully printed with the self-supporting approach.	[171]
	Alginate	hCMPCs	Construction with cardiogenic potential for use <i>in vitro</i> and <i>in vivo</i>	The printed hCMPCs were able to migrate in the alginate matrix while maintaining their functional properties.	[172]
	Alginate/ Gelatin	hUVECs, H9c2 cells	Cardiac tissue engineering	A novel angular structure mimicking the orientation of the heart fibers presents high cell viability (for both hUVECs and H9c2 cells), high mechanical strength, and suitable dilation and degradation properties.	[173]
Extrusion 3D Bioprinting	Alginate/Gelatin	iCMs, CFs, hCAECs	Cardiac tissue patches	3D bioprinted AlgGel-based patches for epicardial transplantation improved cardiac function.	[174]
	GelMA, Alginate	hUVECs	Endothelialized myocardial tissues	New method for generating endothelialised fabricated organoids.	[175]
	GelMA	cECM, hCPCs	Cardiac tissue patches	Cardiac patches printed with cECM and GelMA demonstrated significantly higher viability of hCPCs and exhibited a 30-fold increase in the expression of cardiogenic genes compared to patches composed solely of GelMA. <i>In vivo</i> studies on rats revealed vascularization over a period of 14 days.	[167]

	Collagen	hdECM, NRCMs	Cardiac tissue model	It promoted early differentiation and improved the maturation of cardiomyocytes in hdECM.	[176]
3D bioprinting with microfluidic printing head	Alginate, PF	iPSC-CMs, hUVECs	Vascularized cardiac tissue	A 3D cardiac tissue comprising iPSC-CMs with a high orientation index caused by the different defined geometries and blood vessel-like shapes produced by hUVECs.	[166]
DLP-based 3D printing	PGS/PCL/Gelatin	hUVECs	Heart valve substitute	A crosslinked 3D valve analog with elastomeric characteristics.	[177]
FDM 3D printing	PGS/PCL	n/a	Myocardial remodeling	It improved and preserved heart function.	[178]
SLA	PEGDMA	BMSCs	Gel patch to damaged cardiac tissue	Placement of the MSC-laden, microchanneled gel patch improved in the ejection fraction, fractional shortening, and stroke volume.	[168]
Pneumatic-extrusion	Fibrinogen, gelatin, aprotinin, HA	hESCs-derived EVCs	Cardiac tissues	Spheroids derived from hESC-derived endothelial and EVCs offer greater potential for engineering complex vascular structures compared to single cells.	[179]
Micro-continuous optical printing	GelMA, HAGM	hiPSC-CMs	Cardiac micro-tissue (for drug testing)	The micro-tissue exhibited a well-organized sarcomere structure and a marked upregulation in the expression of maturity markers.	[180]
Pneumatic 3D printing	Fibrinogen, gelatin, aprotinin, glycerol, HA	NRCMs	Functional and contractile cardiac tissue constructs	They produced an organised structure with physiological and biomechanical characteristics similar to native heart tissue.	[181]

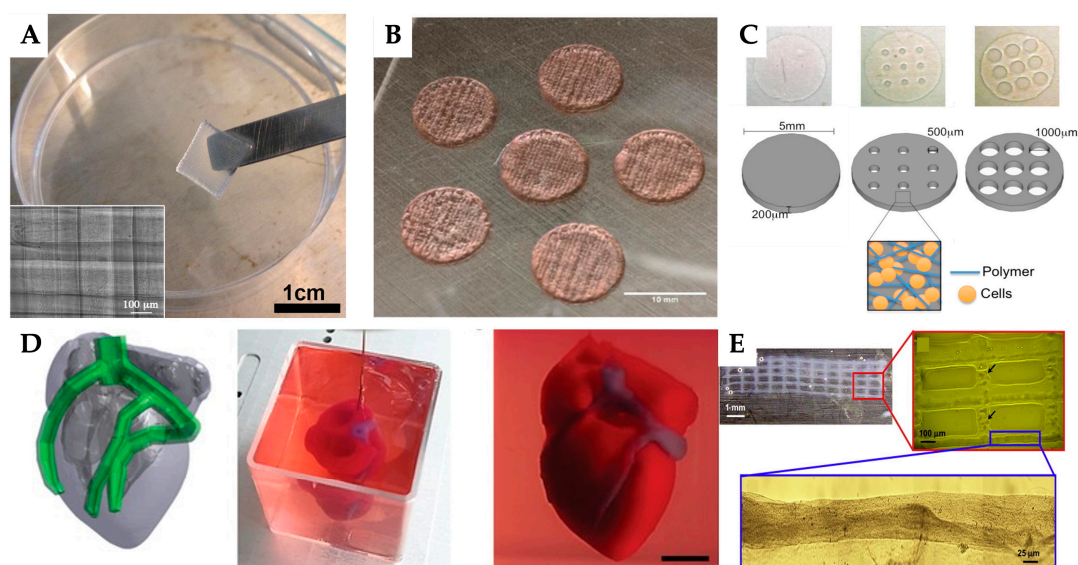


Figure 4. Advances 3D printing/ bioprinting and cell therapies in engineering cardiovascular: **A:** Bioprinted cellularized construct composed of human Umbilical Vein Endothelial Cells (hUVECs) and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), encapsulated in alginate and PEG-Fibrinogen (PF) (adapted from [166]); **B:** Printed patches composed of decellularized cardiac extracellular (cECM) matrix hydrogel and GelMA, for delivery of pediatric human cardiac progenitor cell (hCPC) (adapted from [167]); **C:** MSC-loaded gel patches with microchannels of controlled diameters. Three models tested: one without microchannels and two with nine evenly spaced microchannels, maintained at diameters of 500 μm and 1000 μm (adapted from [168]); **D:** A 3D-printed cellularized human heart is shown. (Left: CAD model of the human heart; Middle and right: The printed heart within a support bath) (adapted from [169]); **E:** Printed fibrin scaffold using modified thermal inkjet printer (adapted from [170], Copyright (5839410409139) with permission from Elsevier).

5.2. Bone Tissue Engineering

Severe bone defects resulting from trauma, aging, osteoporosis, degenerative diseases (such as osteoarthritis), autoimmune diseases (such as rheumatoid arthritis), or tumor removal are a leading cause of disability globally, affecting an estimated 1.71 billion people [182,183]. Current treatment options for severe bone defects include autografts, allografts, xenogeneic grafts, and bone transplantation. However, these methods carry risks such as potential transmission of infectious diseases and immunological rejection. Additionally, graft sources are limited, and xenogeneic grafts lack the ability to participate in metabolic processes [184,185]. To address the limitations of traditional bone defect treatments, 3D printing and bioprinting techniques, with or without cellular therapies, have been developed for bone tissue engineering. These methods enable the large-scale fabrication of custom-tailored bone tissues, meeting the growing demand for functionalized bone implants [186]. The 3D-printed scaffolds serve as a support for cell growth and differentiation, forming a hierarchical bone microvascular structure [187]. An ideal scaffold must exhibit biocompatibility, sterility, osteoconduction, biodegradability, a porous and interconnected structure for cell infiltration and nutrient transport, and the ability to repair bone defects while mimicking native bone tissue [188–190].

The most commonly employed printing techniques in bone tissue engineering are laser powder bed fusion, vat photopolymerization, and extrusion-based methods.

Various biomaterials are utilized for printing scaffolds: ceramics (such as beta-tricalcium phosphate (β -TCP), hydroxyapatite (HAp), and amorphous calcium phosphate (ACP)); natural polymers (such as, matrigel, alginate, HA, and dextran emulsion); synthetic polymers (such as, GelMA, PLGA, PCL, and polyethylene glycol diacrylate (PEGDA)); metals (such as titanium alloy (Ti6Al4V), tantalum (Ta), and titanium (Ti)) and the combinations of these materials.

Lei *et al.* produced high-interface-strength Ti6Al4V-based porous Ta scaffolds using laser powder bed fusion, where porous Ta was directly fabricated on a solid Ti6Al4V substrate (**Figure 5-A**). *In vitro* biocompatibility assays with rat bone marrow mesenchymal stem cells (r-BMSCs) demonstrated that the scaffolds were biocompatible. The results indicated strong mechanical compatibility and osteointegration performance of the Ti6Al4V-based porous Ta scaffold, highlighting its significant potential for orthopedic clinical applications [191].

For mandibular bone defect reconstruction, Yu *et al.* encapsulated BMSCs in matrigel and infiltrated this mixture into porous Ti6Al4V scaffolds. The results indicated that rats with full-thickness critical mandibular defects treated with matrigel-infiltrated Ti6Al4V scaffolds showed superior new bone formation compared to those receiving local BMSC injection or matrigel treatment alone (**Figure 5-B**). These findings suggest that matrigel creates a more conducive 3D microenvironment for BMSCs, making matrigel-infiltrated scaffolds a promising approach for enhancing bone formation in 3D-printed Ti6Al4V scaffolds [192].

Wu *et al.* developed a 3D-bioprintable scaffold combining alginate and β -TCP, for the treatment of bone defects (**Figure 5-C**). MG-63 cells were seeded onto these scaffolds. The 3D-printed scaffolds using a 10% alginate/ β -TCP bioink demonstrated superior physical properties and significantly enhanced cell viability and alkaline phosphatase activity, indicating strong potential for personalized bone regeneration therapies [193].

To produce a scaffold that replicates bone microstructure, Ressler *et al.* developed trabecular-like porous scaffolds using ceramic vat photopolymerization with HAp powders doped with magnesium (Mg^{2+}), strontium (Sr^{2+}) and zinc (Zn^{2+}). Scaffolds sintered at 1100-1300°C exhibited mechanical properties similar to trabecular bone, with optimal performance at 1300 °C. The microstructure resembled cancellous bone, and the incorporation of trace elements resulted in a biphasic calcium phosphate system (HAp/ β -TCP), potentially enhancing bioactivity.

Numerous studies have utilized 3D printing or bioprinting, either with or without cellular therapies, to advance bone tissue engineering. An overview of these studies is provided in **Table 3**.

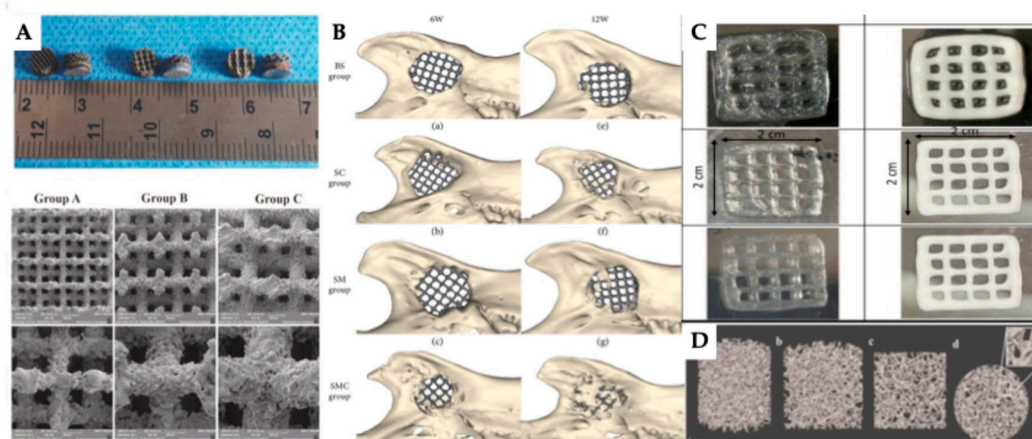


Figure 5. Advances 3D printing/ bioprinting and cell therapies in bone tissue engineering: **A:** Ti6Al4V-based porous tantalum (Ta) scaffolds and scanning electron microscopy images (adapted from [191]); **B:** Newly formed bone within the scaffold based on *ex vivo* micro-CT scans at 6 and 12 weeks (adapted from [192]); **C:** Images of 3D-printed at different alginate concentrations, alginate alone or alginate with beta-tricalcium phosphate (β -TCP) (adapted from [193], Copyright (5841970729622) with permission from Elsevier); **D:** Bone scaffolds based on magnesium (Mg^{2+}), strontium (Sr^{2+}) and zinc (Zn^{2+})-substituted hydroxyapatite, as represented in the CAD model [194].

Table 3. Recent advances in 3D printing/bioprinting and cellular therapies for bone tissue engineering. Abbreviations: selective laser melting (SLM); bone marrow-derived mesenchymal stem cells (BMSCs); beta-tricalcium phosphate (β -TCP); gelatin methacrylate (GelMA); Xonotlite (Sr-CSH); poly(lactic-co-glycolic acid) (PLGA); bone morphogenetic protein 2 (BMP-2); human umbilical cord mesenchymal stem cells (hUCMSCs); alkaline phosphatase (ALP); hydroxyapatite (HAp); Magnesium (Mg^{2+}); Strontium (Sr^{2+}); Zinc (Zn^{2+}); not applicable (n/a); polycaprolactone (PLC); amorphous calcium phosphate (ACP); dental pulp stem cells (DPSCs); diacrylate poly(ethylene glycol) (PEGDA); digital light processing (DLP); tantalum (Ta); rat bone marrow mesenchymal stem cells (r-BMSCs); diamond (DO); rhombic dodecahedron (RD); iron (Fe); manganese (Mn); calcium (Ca); magnesium (Mg); titanium (Ti); niobium (Nb); zirconium (Zr); stromal cells from apical papilla (SCAPs); human umbilical vein endothelial cells (hUVECs).

3D printing technology	Biomaterials	Cellular Therapies	Application	Outcomes	Reference
SLM	Ti6Al4V, Matrigel	BMSCs	Mandibular bone defect reconstruction	Scaffold-Matrigel-BMSCs with enhanced bioactivity and mechanical properties for bone repair.	[192]
3D bioprinting	Alginate, β -TCP	MG-63 fibroblasts	Bone defects	10 % alginate/ β -TCP improved cells proliferation and alkaline phosphatase activity	[193]
	GelMA, Sr-CSH	BMSCs	Critical-size bone defects	GelMA-Sr-CSH scaffolds indicated complete regeneration of critical-size bone defects.	[195]
	Alginate, PLGA	BMP-2, hUCMSCs	Bone tissue engineering	hUCMSCs printing with Alginate/ BMP-2 loaded into PLGA improved osteogenesis of the printed cells as demonstrated by higher rates of ALP activity, calcium deposition, expression of genes associated with osteogenesis, and mineralization when compared with controls.	[196]
Vat photopolymerization	HA multi-substituted with Mg^{2+} , Sr^{2+} , and Zn^{2+} ions	n/a	Cancellous bone defects	It is a suitable technique for manufacturing highly complex trabecular structures for bone regeneration.	[194]
Extrusion 3D Bioprinting	PCL, ACP	n/a	Bone defects	It presented suitable properties, such as compressive strength, pore size, rigidity and repeatability (related to the interconnectivity between the pores).	[50]
	GelMA	DPSCs	Bone regeneration	DPSCs in GelMA bioprinted presented better osteogenic differentiation potential	[197]
	PCL, HAp, PEGDA	DPSCs	Bone regeneration	PCL/HANp/PEGDA revealed hydrophilic properties, suitable mechanical performance and	[188]

				significantly higher cell viability than the other groups.	
DLP-based bioprinting	GelMA, dextran emulsion	BMSCs	Bone regeneration	It promoted the proliferation, migration, dissemination and differentiation of encapsulated BMSCs.	[198]
Laser Powder Bed Fusion	Ti6Al4V, Ta	r-BMSCs	Orthopedic clinical applications	It is mechanically compatible, favourable to the adhesion, proliferation and differentiation of r-BMSC in osteoblasts.	[191]
	PCL, HAp	BMSCs	Human mandibular trabecular bone regeneration	The biodegradable structures (with DO and RD elementary unit cell geometry) demonstrated their suitability as supports for bone regeneration.	[199]
Binder-jetting	Fe-Mn-Ca, Mg	n/a	Cranio-maxillofacial bone defects	The Fe-Mn and Fe-Mn-1Ca constructs confirmed higher degradation from the addition of Ca to the 3D printed constructs and good cytocompatibility.	[200]
Laser Directed Energy Deposition	Commercially pure Ti	n/a	Dental restoration	Commercially pure grade 4 titanium produced by Laser Directed Energy Deposition has a higher mechanical response than other techniques, which can be attributed to the modification of the microstructure inherent in the process.	[201]
	Ti-Nb, Ti-Zr-Nb	n/a	Orthopedic and dental applications	Ti-35Zr-25Nb presented a lower modulus of elasticity, higher hardness, good corrosion resistance and <i>in vitro</i> biocompatibility.	[202]
Sheet lamination	AISI 302	n/a	Bone regeneration	The scaffold prototype was designed and fabricated with the parameters selected through experimental tests and using the mathematical model.	[203]
Laser-assisted bioprinting	BioRoot RCS®	SCAPs, hUVECs	Bone regeneration	The application of laser-assisted bioprinting techniques with this ink failed to provide complete bone repair, whether the SCAPs were printed in direct proximity or not.	[204]

5.3. Liver Tissue Engineering

The liver plays a vital role in blood protein synthesis, glucose metabolism, and the detoxification of metabolites [205]. It is also the only organ in the human body capable of efficient regeneration. However, this regenerative capacity can be compromised by excessive drug use or viral infections, which can cause irreversible damage to hepatocytes and lead to liver failure [206,207]. Chronic liver diseases, including fibrosis, cirrhosis, chronic viral hepatitis, and fatty liver disease, significantly contribute to global morbidity and mortality. Unfortunately, advancements in treatment options for these conditions remain limited [208,209].

The primary medical intervention for liver failure is partial or total liver transplantation. Nevertheless, this approach faces challenges, including limited donor availability, immune rejection, and variable graft success rates. Alternative tissue engineering treatments include bioartificial liver systems, which involve creating tissue *in vitro* to repair or replace damaged liver parts, as well as hepatocyte transplantation and cellular therapy approaches [206]. There has been a continuous search for a reliable and reproducible source of hepatocytes, whether for liver regeneration therapy, seeding liver support devices, or *in vitro* screening applications [207].

Numerous studies have investigated 3D bioprinting of liver tissue using stem cells or immortalized hepatic cell lines [210,211]. Stem cells are particularly promising due to their ability to express hepatocyte-like phenotypes. In contrast, adult hepatocytes are limited in availability, challenging to isolate, exhibit poor propagation, and experience rapid functional deterioration *in vitro* [212].

Various biomaterials are used for liver tissue engineering, and they can be divided into natural and synthetic polymers [213]. Natural polymers, such as, alginate, HA, collagen, cellulose nanocrystal (CNC), and gelatin, present the advantages of enhanced cell compatibility and ease of manipulation. Nevertheless, they are limited by weaker mechanical properties, limited availability, and variable degradation rates. Consequently, synthetic polymers, such as PCL, have been developed, which are characterized by high mechanical strength, flexibility, processability, and tunable degradability. Despite these advantages, they lack cell recognition and adhesion sites, leading to lower biocompatibility compared to natural polymers [214]. To address these concerns, combining natural and synthetic polymers is a strategy for developing suitable bioinks for 3D bioprinting. A common biomaterial for creating microenvironments for liver cells is liver decellularized extracellular matrix (dECM), derived from animal sources. This material is favored due to its cross-species tolerance, which reduces the risk of immune rejection [215–217].

Yang *et al.* developed a liver tissue model using 3D bioprinting with HepaRG cells, a commonly used hepatic progenitor cell line. This model, known as 3D bioprinted hepatorganoids, demonstrated functional liver activities including albumin secretion, drug metabolism, and glycogen storage after 7 days of differentiation (**Figure 6-A**). *In vivo* studies showed that the 3D bioprinted hepatorganoids further matured, exhibiting enhanced synthesis of liver-specific proteins and more human-like drug metabolism. Notably, transplantation of 3D bioprinted hepatorganoids significantly improved the survival rates of the recipient mice. These results suggest that 3D bioprinted hepatorganoids can effectively undergo hepatic differentiation and ameliorate liver failure *in vivo* [218].

In the study conducted by Xie *et al.*, a patient-derived 3D bioprinted hepatocellular carcinoma model was developed using isolated primary hepatocellular carcinoma cells combined with gelatin and sodium alginate to create a bioink for printing (**Figure 6-B**). These models were successfully established and demonstrated robust growth during extended culture periods. They preserved key characteristics of the original hepatocellular carcinoma tumors, including consistent biomarker expression, stable genetic alterations, and expression profiles. Thus, 3D bioprinted hepatocellular carcinoma models prove to be reliable *in vitro* systems, suitable for long-term culture, and capable of predicting patient-specific drug responses for personalized therapy [219].

Lewis *et al.* studied a technique for 3D printing gelatin into precisely defined geometries, which exhibit distinct biological effects on seeded hepatocytes (**Figure 6-C**). Their study demonstrates that gelatin, can significantly influence biological processes when formed into specific 3D structures. An undifferentiated hepatocyte cell line showed high viability and proliferation on 3D-printed scaffolds

with two different geometries. However, hepatocyte-specific functions—such as albumin secretion, cytochrome P450 activity, and bile transport—increased in more interconnected 3D-printed gelatin cultures compared to less interconnected geometries and two-dimensional (2D) controls. The study also highlights the disparity between gene expression and protein function in simple 2D cultures, emphasizing the necessity of a physiologically relevant 3D environment to fully induce hepatocyte expression and function [220].

Jeon *et al.* employed 3D bioprinting technology to reconstruct liver tissues and organs using human hepatocellular carcinoma (HepG2) cells, a liver cancer-derived cell line. They created multi-layered 3D structures by mixing alginate with HepG2 cells (**Figure 6-D**). The study demonstrated that replicating the 3D hepatic architecture using this technology enhances the stability and gene expression profiles of HepG2 cells. Cells cultured on these 3D alginate scaffolds for three weeks were analyzed via fluorescence microscopy, histology, and immunohistochemistry. Results indicated that HepG2 cells exhibited improved growth and liver-specific gene expression in 3D cultures compared to 2D cultures, highlighting the effectiveness of 3D bioprinting in mimicking liver architecture and enhancing cellular function [221].

Using the same 3D bioprinting technique, Wu *et al.* developed a novel bioink containing alginate, CNC and GelMA (namely 135ACG hybrid ink), aimed at fabricating both cell-laden and acellular structures (**Figure 6-E**). The bioink presented excellent shear-thinning behavior and solid-like properties, ensuring high printability and minimal cell damage. After crosslinking, it formed a rigid ECM conducive to stromal cell growth. The team engineered a GelMA bioink with suitable mechanical properties to mimic human liver tissue, enabling the printing of liver lobule-mimetic constructs with precise cell placement (fibroblasts and hepG2) in different ECMs (135ACG and GelMA). These constructs were used to study the impact of mechanical cues and cell interactions on behavior, revealing that fibroblasts thrived in the stiff 135ACG matrix while hepG2 cells formed spheroids in the softer GelMA. Co-cultures of hepG2 and fibroblasts cells showed increased albumin production, highlighting the role of soluble factors in enhancing hepatic function. The study demonstrated that the developed bioinks and printing methods are effective for creating complex, multi-cellular constructs with varied ECMs, advancing both fundamental research and tissue engineering applications [222].

Several studies have employed 3D printing/bioprinting techniques, with or without the incorporation of cellular therapies, to further the field of liver tissue engineering. A summary of these studies is presented in **Table 4**.

Table 4. Recent advances in 3D printing/bioprinting and cellular therapies for liver tissue engineering. Abbreviations: digital light processing (DLP); methacrylated gelatin (GelMA); decellularized extracellular matrix (dECM); human-induced hepatocyte (hiHep cells); hyaluronic acid (HA); primary mouse hepatocytes (PMHs); human induced pluripotent stem cells (hiPSCs); human embryonic stem cells (hESCs); human pluripotent stem cells (hPSCs); induced pluripotent stem cells (iPSC); 3D bioprinted hepatorganoids (3DP-HOs); hepatocellular carcinoma (HCC); poly (ϵ -caprolactone) (PCL); human bone marrow-derived mesenchymal stem cells (BMMSCs); human hepatocellular carcinoma (hepG2); cellulose nanocrystals (CNCs); bioink with alginate (1%), cellulose nanocrystal (3%), and gelatin methacryloyl (5%) (135ACG); undifferentiated hepatocyte cell line (HUH7); primary rat hepatocytes cells (PrHCs); human umbilical vein endothelial cells (hUVECs); human lung fibroblasts (hLFs); human extracellular matrix (hECM); human hepatic stellate cell line ($L \times 2$); primary fetal activated hepatic stellate cells (aHSC).

3D printing technology	Biomaterials	Cellular Therapies	Application	Outcomes	Reference
DLP bioprinting	GelMA	dECM, hiHep cells	Hepatic functional restoration	It was found that the addition of hepatic dECM to the biotints improved printing capacity and cell viability. Moreover, hiHep cells spread more and showed better functions in the liver microtissue.	[223]
	HA, alginate, gelatin	dECM, PMHs	Functional <i>in vitro</i> liver tissue models	The inclusion of dECM enhanced the hepatic function of hepatocyte spheroids.	[224]
	Alginate	hiPSCs, hESCs	Mini-liver tissue structures	Using this approach, researchers have been successfully bioprinting hPSCs whilst preserving their pluripotency or directing their differentiation towards specific cell types.	[225]
3D bioprinting	NovoGel 2.0	iPSC-derived hepatocyte	Functional <i>in vitro</i> liver tissue models	It demonstrated a method for rapidly fabricating multicellular 3D liver constructs in a multi-well format, displaying critical liver functions such as albumin production, cholesterol biosynthesis, fibrinogen and transferrin production and inducible CYP 1A2 and CYP 3A4 activities.	[210]
	Alginate	HepaRG cells	Hepatorganoids: Liver tissue model	The transplantation of 3DP-HOs significantly improved the survival of mice (with liver failure)	[218]
	Gelatin, Alginate	Primary HCC cell lines	<i>In vitro</i> models for patient-specific drug screening for HCC	The generated models preserved the key characteristics of the original HCCs, including consistent biomarker expression, stable genetic alterations, and maintained expression profiles.	[219]

	PCL	dECM, BMMSCs, HepG2	3D cell printing-based liver tissue engineering	The liver dECM bioink proved to have excellent printing capacity without significant cell death during the process. It also improved stem cell differentiation and HepG2 cell function.	[226]
	Alginate	HepG2	Reconstruction of liver tissues or organs	The cells proliferated well in the scaffold and the expression of liver-specific genes increased.	[221]
	GelMA, alginate, CNC	NIH/3T3 fibroblasts, hepG2	Bicellular liver lobule-mimetic structures	NIH/3T3 cells proliferated within the rigid 135ACG matrix and aligned along the 135ACG/GelMA interface due to durotaxis, whereas HepG2 cells exclusively formed spheroids within the GelMA matrix. High albumin production was noted in the 3D two-cell co-culture of hepG2 and NIH/3T3, indicating that the improvement in liver cell function can be contributed to soluble chemical factors.	[222]
Pneumatic extrusion	Gelatin	HUH7	Model hepatocyte system	It has been proved that the scaffold geometry, using well-defined gelatine constructs, modulates hepatocyte function.	[220]
	PCL, collagen	PrHCs, hUVECs, hLFs	Liver tissue engineering	The 3D cell printed construct comprising a capillary-like network enhanced the protein secretion and metabolism of PrHCs. It demonstrated a great potential for functional liver tissue regeneration.	[227]
	Alginate, gelatin	hECM, human HepaRG liver cells	3D liver model for infection and transduction studies	It was demonstrated that supplementing an alginate/gelatin bioink with hECM enhanced cell viability and hepatic metabolic activity in a 3D-printed humanized liver model.	[228]
Extrusion bioprinting	Alginate	HepG2/C3A, EA.hy926	Hepatic lobules within a highly vascularized construct	The bioprinting of multiscaled hepatic lobules within a highly vascularized construct was successfully produced, presenting higher albumin secretion, urea production, albumin, MRP2, and CD31 protein levels, when	[229]

			compared to other groups, and cytochrome P450 enzyme activity	
HA, Collagen I	L × 2, aHSC	3D bioprinted liver model	The formulations seemed to facilitate cell viability, in line with the biomatrices used. The printed bioinks containing primary human hepatocytes were monitored over 2 weeks, showing that they maintain urea and albumin production and responded adequately to acetaminophen.	[230]
Alginate, CNCs	Fibroblasts, Human hepatoma cells	Liver-mimetic structures	This bioink showed excellent shear-thinning property, extrudability, and shape fidelity after the deposition. The bioprinting caused minimal cell damage.	[231]

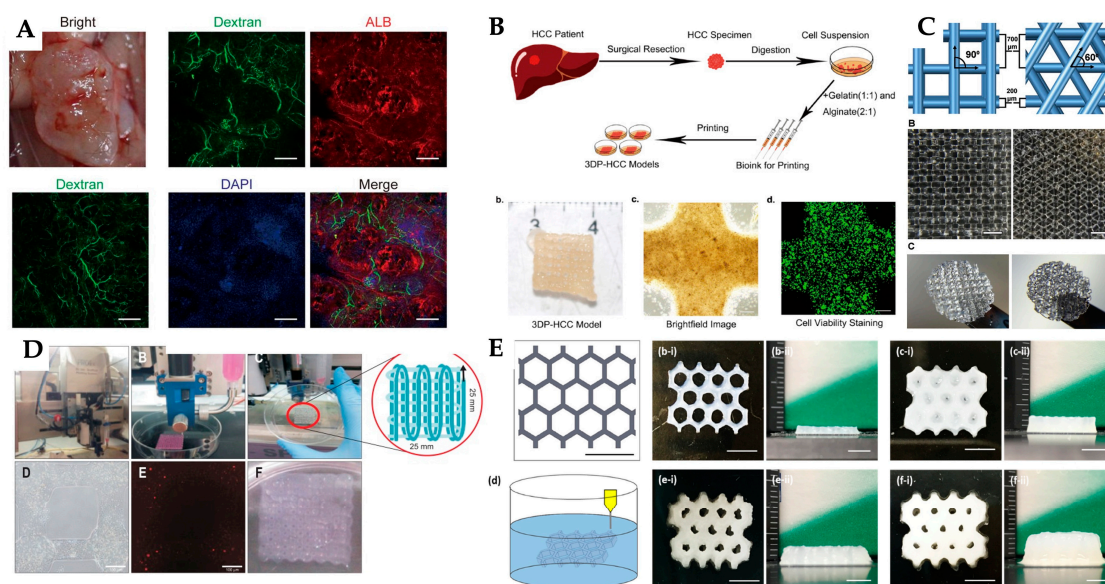


Figure 6. Advances 3D printing/ bioprinting and cell therapies in liver tissue engineering: **A:** Human liver functions of 3D bioprinted hepatorganoids in mice. First column: Macroscopic observation of transplanted 3D bioprinted hepatorganoids. The vascular system is shown after dextran infusion. Second and third column: The figure shows the dextran infusion with the formation of functional vessels and the red fluorescence showing the expression of human ALB at 4 weeks (adapted from [218]); **B:** The study details the production of patient-derived 3D bioprinted hepatocellular carcinoma models. Initially, HCC samples were collected post-surgical resection and processed into cell suspensions. These suspensions were then mixed with sodium alginate and gelatin to create the bioink used for bioprinting. The images provided in the second line include: (1) the general appearance of the 3D bioprinted hepatocellular carcinoma model, (2) a light microscopy image showing the distribution of cells within the model, and (3) an *in vitro* view of the model, where live cells are stained green and dead cells are stained red (adapted from [219], Copyright (5842091348696) with permission from Elsevier); **C:** First line: schematic representation of the 3D printed structure, including strut spacing, nozzle diameter and angle between adjacent layers (90 or 60 degrees). Second line: large-scale 3D printed gelatin structure. Third line: cross-linked scaffolds and biopsy-punched samples (adapted from [220], Copyright (5842111318276) with permission from Elsevier); **D:** Construction of 3D-printed mCherry-HepG2 hepatic structures: (A) 3D bioprinting machine; (B) extrusion of alginate mixed with mCherry-HepG2 cells through nozzle pressure; (C) layer-by-layer deposition of cross-linked structures in square arrays; (D) phase contrast microscopy showing a confluent monolayer of HepG2 cells; (E) fluorescence microscopy image of mCherry-HepG2 cells within the alginate scaffold; (F) multilayered mCherry-HepG2 cells solidified and stacked repetitively (adapted from [221]); **E:** Schematic representation of the liver lobule-mimetic honeycomb structure and top views and side views of the embedded-printed structures (adapted from [222]).

5.4. Skin Tissue Engineering

The skin is considered the largest human organ and performs important functions, such as, providing a protective barrier, regulating temperature, and preventing water loss [232]. Cutaneous wounds, particularly extensive full-thickness wounds that compromise damage blood vessels, pose significant risks to human life due to the resulting cellular hypoxia and nutrient deficiency [233]. Although autografts are regarded as the "gold standard" for treating severe skin injuries, their use is limited by challenges such as donor site availability and associated morbidity [234]. Furthermore, existing commercial skin substitutes lack sufficient vascular networks necessary for effective nutrient delivery in full-thickness wounds. Consequently, artificial skin substitutes are seen as promising alternatives, offering the potential for vascularized skin reconstruction with tailored cell composition and controlled geometrical morphology [233,235].

Over the past decade, 3D printing and bioprinting have arisen as an innovative technological approach, in skin tissue engineering, for engineering structures by depositing cell-laden bioinks in a layer-by-layer fashion [236,237]. This technique includes several methods, such as, DLP, extrusion bioprinting, electrospinning, and inkjet printing. The materials commonly used in this field are natural polymers (such as collagen, alginate, HA, gelatin, and fibroin) and synthetic polymers (such as PCL, PLGA, polyglycolic acid, polyurethanes, polycarbonates, and PEGDA). These materials, often referred to as biopolymers, are biocompatible and biodegradable. In wound healing applications, bioinks may be combined with antibiotic substances or antimicrobial peptides, along with growth factors (such as epidermal, fibroblast, or vascular endothelial growth factors) to promote cell stimulation, growth, proliferation, and migration during the healing process [236,238]. Recent advancements in wound healing and skin regeneration involve incorporating cells, such as fibroblasts, hUVECs, keratinocytes, and human umbilical cord mesenchymal stem cells (hUCMSCs) directly into bioinks. Liu *et al.* fabricated vascularized full-thickness skin substitute by printing an alginate-gelatin hydrogel to simulate the epidermis, and a phosphosilicate calcium bioglass (PSC)-alginate-GelMA hydrogel containing hUVECs and hUCMSCs to replicate the dermis (**Figure 7-A**). They showed a marked enhancement in blood vessel formation and collagen deposition, demonstrating the effectiveness of these skin substitutes in reconstructing full-thickness skin injuries in rat models [233]. Also, with a view to developing a full-thickness skin substitute, Admane *et al.* employed extrusion-based 3D bioprinting to create a silk fibroin-gelatin construct containing fibroblasts to mimic the dermis, and a silk fibroin-gelatin layer with keratinocytes to replicate the epidermis (**Figure 7-B**). The 3D bioprinted full-thickness skin model showed extensive keratinocyte migration and differentiation, mimicking reepithelialization. Analysis revealed similarities to native human skin, involving pathways related to skin development, extracellular matrix organization, and keratinization [239]. Also, Jin *et al.* developed an advanced 3D bioprinted structure designed to mimic natural full-thickness skin, incorporating the epidermis, dermis, and a vascular network. This model utilized GelMA with HaCaTs for the epidermal layer, an acellular dermal matrix (ADM) with fibroblasts for the dermis, and a GelMA mesh with hUVECs for the vascular network (**Figure 7-C**). They demonstrated that this functional skin model not only enhanced cell viability and proliferation but also supported epidermal reconstruction *in vitro*. *In vivo*, the functional skin model maintained cell viability for at least one week and promoted wound healing, re-epithelization, dermal ECM secretion, and angiogenesis, thereby improving wound healing quality [240].

Song *et al.* developed a drug-loaded bilayer skin scaffold for repairing full-thickness skin defects. Briefly, amoxicillin (AMX) was loaded on PCL nanofiber via electrospinning to form the antibacterial nanofiber membrane as the outer layer of scaffold to mimic epidermis (**Figure 7-D**). In order to maintain wound moisture and facilitate healing, external human epidermal growth factor (rhEGF) was incorporated into sodium alginate-gelatin to form a hydrogel (SG-rhEGF) as the inner layer of the scaffold to mimic the dermis. AMX and rhEGF were successfully loaded into the scaffold. The scaffold presented excellent physicochemical and drug release/ antibacterial properties. Both *in vitro* and *in vivo* studies demonstrated that the fabricated scaffold enhanced cell adhesion and proliferation, facilitated skin wound healing, and exhibited favorable biocompatibility. These findings suggest that the scaffold holds significant promise for applications in skin regeneration [241].

Numerous studies have utilized 3D printing and bioprinting techniques, both with and without the integration of cellular therapies, to advance skin tissue engineering. A summary of these studies is provided in **Table 5**.

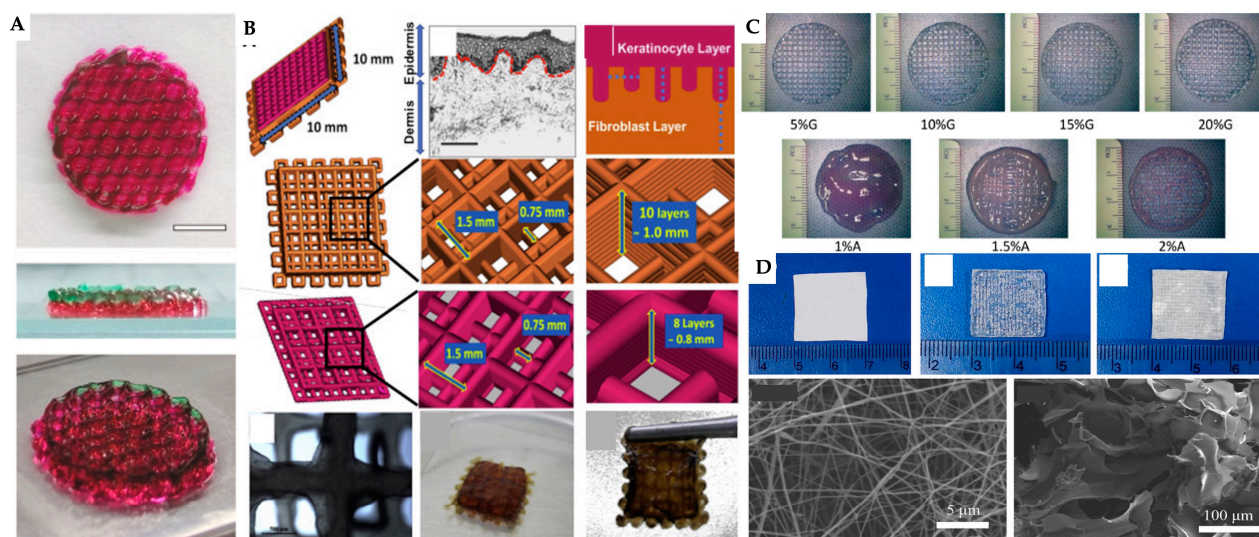


Figure 7. Advances 3D printing/ bioprinting and cell therapies in skin tissue engineering: **A:** The image shows the printed epidermal layer (red) composed of alginate-gelatin hydrogel, and the dermal layer (green) made of phosphosilicate calcium bioglass (PSC)-alginate-GelMA hydrogel embedded with hUVECs and hUCMSCs (adapted from [233], Copyright (5842131198782) with permission from Elsevier); **B:** schematic overview of the bioinspired 3D bioprinted construct design: diagramme illustration of the CAD model; illustration of human skin anatomy, with the dermis and epidermis; design strategy for the dual-layered 3D printed construct; detailed dimensions of the dermal and epidermal layers: the dermal layer consists of 10 layers, while the epidermal layer has 8 filaments, oriented perpendicularly with an interfilament spacing of 0.75 mm and a Z-axis increment of 0.08 mm per layer; microscopic image of the bioprinted construct; dual-layered 10 × 10 mm 3D printed construct in culture; and the mechanically stable 3D printed construct provides suitable handling properties for effective characterization (adapted from [239], Copyright (5842131492259) with permission from Elsevier); **C:** Printability test of acellular dermal matrix and GelMA hydrogels through printing the same structure (adapted from [240], Copyright (5842140280407) with permission from Elsevier); **D:** Macrographs of PCL-AMX, SG-rhEGF, and PCL-AMX-SG-rhEGF. SEM images of the fabricated PCL-amoxicillin (AMX) (D1), SG-rhEGF (E), and PCL-AMX-SG-rhEGF (adapted from [241], Copyright (5842140587126) with permission from Elsevier).

Table 5. Recent advances in 3D printing/bioprinting and cellular therapies for skin tissue engineering. Abbreviations: strontium silicate (SS); matrix hydrogel with 2.8% of gellan gum, 1.6% of alginate, and 2.8% of methyl cellulose (GAM); human dermal fibroblasts (hDFs); human umbilical vascular endothelial cells (hUVECs); murine umbilical vein endothelial cells (MUVECs); dextran (Dex); vascular endothelial (VEGF); gelatin (Gel); keratin (Kr); not applicable (n/a); bi-layer (BL); poly (ε-caprolactone) (PCL); amoxicillin (AMX); external human epidermal growth factor (rhEGF); Digital light processing (DLP); gelatin methacrylate (GelMA), hyaluronic acid (HA); N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide (NB); lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP); human skin fibroblasts (hSFs); silk fibroin (SF); 4-arm polyethylene glycol acrylate (PEG4A); platelet-rich plasma (PRP); dermal fibroblasts (DFs); epidermal stem cells (ESCs); acellular dermal matrix (ADM); polyurethane (PU); endothelial progenitor cells (EPCs); human keratinocytes (hKCs); pectin methacrylate (PecMA); human neonatal dermal fibroblasts (hNDFs); extracellular matrix (ECM); human endothelial cells (hECs); human placental pericytes (hPCs); phosphosilicate calcium bioglasses (PSCs); human umbilical cord mesenchymal stem cells (hUCMSCs); poly-(lactic-co-glycolic acid) (PLGA).

3D printing technology	Biomaterials	Cellular Therapies	Application	Outcomes	Reference
3D bioprinting	Collagen, SS, GAM	hDFs, hU-VECs, MUVECs, BALB/3T3 fibroblast	Vascularized Skin substitute	Collagen-2SS-GAM presented a high capacity of blood vessel formation, graft-host integration, and wound repair <i>in vivo</i> .	[242]
Extrusion-based 3D printer and electrospinning	Dex-VEGF, Gel-Kr	n/a	Construct to accelerate wound healing	The BL-VEGF structure is presented as an ideal sample for accelerating the healing of full thickness skin wounds.	[243]
	PCL, AMX, alginate, gelatin	rhEGF	Repair defective skin tissue and wound healing	The PCL-AMX®SG-rhEGF scaffold presented great drug release and antibacterial characteristics. <i>In vitro</i> and <i>in vivo</i> studies suggested that the structure could promote cell adhesion and proliferation and the healing of skin wounds, presenting biocompatibility.	[241]
DLP-based 3D printing	GelMA/HA, NB/LAP	hSFs, hUVECs	Functional living skin	Bioink demonstrated rapid gelation, adjustable mechanical properties, good biocompatibility and adhesion to tissues. The <i>in vivo</i> study demonstrated that the living skin had an immediate defence response and was more effective in promoting dermal regeneration, including the formation of skin appendages, in large animals models.	[244]
	SF, PEG4A	NIH 3T3 fibroblas, keratinocytes	Artificial skin model	SF-polyethylene glycol hydrogels presented higher cell proliferation, and the thickest keratin layer was produced with SF-PEG4A hydrogels when compared to PEG4A alone.	[245]

Extrusion-based bioprinting (<i>in situ</i>)	Alginate, gelatin, PRP	DFs, ESCs	Wound repair	The addition of PRP enhanced the cellular behaviour of the cells, regulated vascular endothelial cell tube formation and macrophage polarisation in a paracrine manner. In in-situ bioprinting, the addition of PRP accelerated high-quality wound closure, modulated inflammation and started angiogenesis when compared to alginate-gelatine bioink.	[246]
Extrusion-based bioprinting	GelMA	ADM, HaCaTs, hUVECs, fibroblasts	Functional skin model	The <i>in vivo</i> results showed that the functional skin model stimulated wound healing and re-epithelialisation, promoted the secretion of dermal extracellular matrix and angiogenesis, and enhanced the quality of wound healing.	[240]
	PU, gelatin	Fibroblasts, keratinocytes, EPCs	Skin tissue engineering.	Curvilinear-bioprinted hydrogel showed superior structural integrity over planar-bioprinted hydrogel. In the treatment of large and irregular rat skin wounds, the curvilinear-bioprinted tri-cell-laden hydrogel achieved complete repair within 28 days.	[247]
	Alginate, honey	3T3 fibroblast	Skin tissue engineering	The incorporation of honey resulted in bioprinted scaffolds with enhanced cell proliferation, without any visible decrease in printability.	[248]
	SF, gelatin	hDFs, hKCs	Full-thickness skin constructs	A silk and gelatine bioink have been printed that can be used to recapitulate a series of biological and design parameters comparable to human skin.	[239]
3D Bioprinting	PecMA	hNDFs	Biomimetic skin constructs	The hydrogels produced showed to be highly versatile, allowing fine-tuning of the rheological and viscoelastic properties. Furthermore, they provided a suitable microenvironment that supports the deposition of endogenous ECM, rich in collagen and fibronectin, by entrapped hNDFs.	[249]
	Rat tail type I collagen	hDFs, hKCs, hECs, hPCs,	Multilayered vascularized human skin grafts	<i>In vitro</i> , hKCs replicate and mature to become a multi-layered barrier, whilst hECs and hPCs self-assemble into interconnected microvascular networks. <i>In vivo</i> , the presence of hPCs in the printed dermis increased	[250]

				the invasion of the graft by the host's microvessels and the creation of an epidermal network.	
	PSCs, alginate, gelMA, gelatin	hUVECS, hUCMSCs	Skin tissue grafts	The incorporation of PSCs revealed enhanced cell proliferation and higher expression of genes related to angiogenesis <i>in vitro</i> . <i>In vivo</i> experiments showed an improved wound healing effect, characterized by an increase in angiogenesis and collagen deposition.	[233]
Pneumatic-assisted extrusion freeforming	Alginate, gelatin	hSFs	Bioactive dermal substitute scaffold	The three-phase crosslinking technique can produce dermal substitute supports with physicochemical and biological properties suitable to be used in skin tissue engineering.	[251]
Inkjet printing	PLGA, prednisolone	n/a	Personalized dermal patches for treatment of skin diseases	The first steps have been made towards the concept of manufacturing personalized patches by inkjet printing that can be made for poorly soluble pharmaceuticals. The model drug prednisolone was successfully processed and printed in the format of a nanosuspension. The PLGA nanoparticles and the patches produced demonstrated a prolonged release of the pharmaceutical.	[252]

5.5. Neural Tissue Engineering

The human nervous system represents one of the most complex and intricate biological systems formed during development [253]. The nervous system is divided into two primary components: the central nervous system, consisting of the brain and spinal cord, and the peripheral nervous system, which includes cranial and spinal nerves along with associated ganglia [254]. Traumatic injuries, including traumatic brain injury and spinal cord injury, as well as neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and multiple sclerosis, pose major public health challenges, with limited treatment options that mainly offer symptomatic relief. Autologous nerve graft transplantation is considered the gold standard for treating severe nerve injuries. However, its application is hindered by significant challenges, including limited donor availability and potential mismatches between donor and recipient nerves [255–257]. Despite ongoing clinical advancements, fully effective therapies for neural regeneration are still in early stages, driving interest in neural tissue engineering [258]. Neural tissue engineering aims to create biological substitutes that combine biomimetic 3D scaffolds with cells to enhance neural tissue function. 3D printing technology has been increasingly utilized in neural autograft engineering, ushering in a new era of innovation in the fabrication of tissue-engineered neural grafts. A range of 3D printing technologies has been employed to create precisely structured implants for *in vivo* nerve injury repair, as well as models and devices for *in vitro* nervous tissue engineering. These technologies include extrusion-based printing, laser assisted bioprinting, SLA, and 4D printing.

The biomaterials chosen for 3D printing of neural constructs encompass biocompatible polymers (e.g. PCL and PU), composites (e.g. reduced graphene oxide (rGO)), and hydrogels (e.g. GelMa, HA, collagen and fibrin). These materials must meet specific requirements for printability and biocompatibility, as well as possess suitable physicochemical properties and mechanical strength [257].

Researchers have combined 3D printing/bioprinting with cellular therapies for neural tissue engineering applications. Lee *et al.* developed a photocrosslinkable methacrylated silk fibroin-pectin bioinks (**Figure 8-A**) and they demonstrated adjustable mechanical properties, favourable biocompatibility, and an environment highly conducive to neural induction on the neural stem/progenitor cells spheroid laden 3D bioprinting [259].

For spinal cord injury treatment, Song *et al.* developed PCL microfiber-reinforced spinal cord ECM hydrogel-based scaffolds loaded with oxymatrine (OMT) through electrospinning (**Figure 8-B**). These scaffolds promoted neuronal differentiation of neural stem cells (NSCs) and inhibited astrocyte formation *in vitro*. *In vivo*, they recruited NSCs, enhanced neuron growth, reduced glial scar formation, and improved motor function recovery in rats with spinal cord injury [260].

In turn, Lin *et al.* developed a model designed to forecast cell growth and distribution, aiming to minimize trial-and-error experimentation. They established a multiphysics model that integrates oxygen diffusion and substrate consumption kinetics within a rat adrenal medullary pheochromocytoma (pc-12) cell-laden nerve scaffold (**Figure 8-C**). This model was utilized to simulate and predict oxygen concentration and cell growth patterns. The scaffold was fabricated using SLA, and the distribution of cells was assessed through fluorescence staining to validate the model. The results demonstrated that the model effectively predicted cellular growth patterns [261].

Numerous studies have utilized 3D printing and bioprinting technologies, both with and without the integration of cellular therapies, to advance the field of neural tissue engineering. **Table 6** provides a comprehensive overview of these investigations.

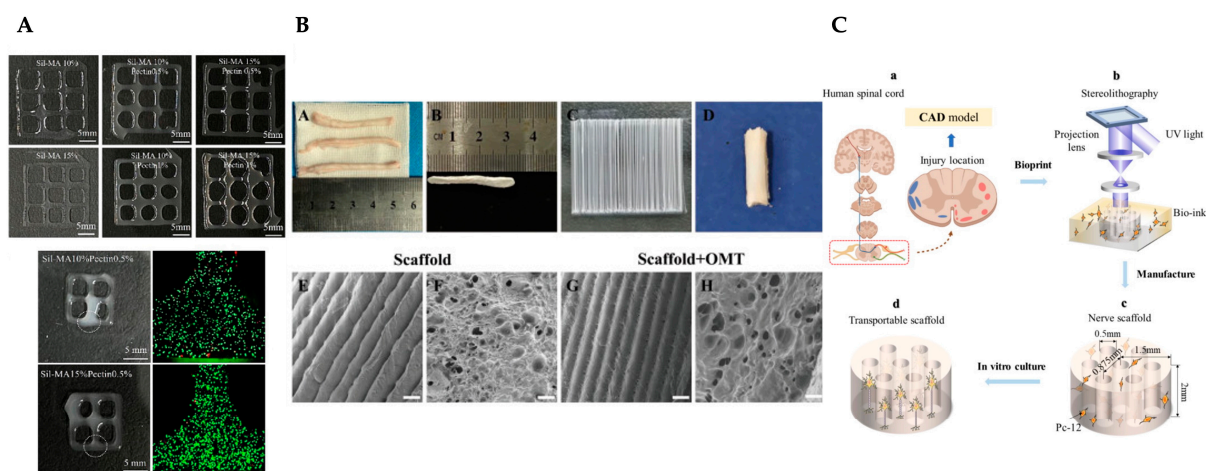


Figure 8. Advances 3D printing/ bioprinting and cell therapies in neural tissue engineering: **A:** Evaluation of bioink printability was conducted for various compositions of methacrylated silk fibroin and methacrylated silk fibroin/pectin hydrogels. Optical microscopy images were captured to examine the morphology, while a live/dead assay was performed on L929 cells embedded within 3D bioprinted constructs using bioinks composed of 10% methacrylated silk fibroin/0.5% pectin and 15% methacrylated silk fibroin/0.5% pectin (adapted from [259], Copyright (5842151229827) with permission from Elsevier); **B:** Characterization of the scaffolds was conducted as follows: (A) Normal spinal cord tissue; (B) Decellularized spinal cord scaffold; (C) Polycaprolactone (PCL) microfiber structure; (D) Overall morphology of the 3D-bioprinted composite scaffold; (E–H) Microstructure of scaffolds visualized through scanning electron microscopy (SEM). The SEM images of both the scaffold alone and the scaffold combined with OMT reveal parallel microfibers of consistent thickness, with hydrogels adhering to the fibers. The interior of the scaffold exhibited uniform and densely packed pores, which are conducive to neural stem cell (NSC) growth and efficient nutrient exchange (adapted from [260]); **C:** Schematic representation of the experimental procedure for spinal cord injury repair utilizing stereolithography apparatus (SLA) to create neural scaffolds. (a) Computer-aided design (CAD) to develop the scaffold model corresponding to the injured region for bioprinting; (b) Construction of neural scaffolds employing the SLA technique; (c) Utilization of the fabricated scaffolds for *in vitro* cell culture; (d) Assessment of the functionality of scaffolds following a period of *in vitro* cultivation (adapted from [261], Copyright (5842160814781) with permission from Elsevier).

Table 6. Recent advances in 3D printing/bioprinting and cellular therapies for neural tissue engineering. Abbreviations: methacrylated silk fibroin (SilMA); pectin methacryloyl (PecMA); silk fibroin (SF); neural stem/progenitor cells (NSPCs); polyurethane (PU); neural stem cells (NSCs); hyaluronic acid (HA); neural progenitor cells (NPCs); poly(lactic-co-glycolic acid) (PLGA); primary human dermal fibroblasts (phDFs); vascular endothelial growth factor (VEGF); murine neural stem cells (C17.2); interferon-gamma (IFN- γ); poly (ϵ -caprolactone) (PCL); oxymatrine (OMT); polyethylene glycol diacrylate (PEGDA); digital light processing (DLP); gelatin methacrylate (GelMA); Poly(3,4-ethylenedioxythiophene) (PEDOT); not applicable (n/a); chondroitin sulfate methacrylate (CSMA); bone marrow-derived mesenchymal stem cells (BMSCs); stereolithography (SLA); rat adrenal medullary pheochromocytoma (pc-12); reduced graphene oxide (rGO).

3D printing technology	Biomaterials	Cellular Therapies	Application	Outcomes	Reference
Extrusion-based 3D Bioprinting	SilMA, Pectin, PecMA, SF	NSPCs	Neural tissue engineering applications or <i>in vitro</i> brain models	The SilMA/pectin biotints showed adjustable mechanical properties, biocompatibility and a highly propitious environment for neural induction.	[259]
	PU	NSCs	Neural tissue engineering	NSCs proliferated and differentiated favourably in PU2 bioprinted hydrogels. In the <i>in vivo</i> model of neural injury in the zebrafish embryo, the injection of hydrogels loaded with NSCs promoted the repair of the damaged central nervous system. Additionally, the function of adult zebrafish with traumatic brain injury was recovered after the injection of constructs with NSCs.	[262]
	Gelatin, alginate, fibrinogen, nanofibrillate cellulose, matrigel, HA	NPCs, astrocytes	Study the connection between human neuronal networks, model pathological processes and provide a platform for drug testing.	Imprinted neuronal progenitors differentiate into neurons and form functional neuronal circuits within and among tissue layers with specificity within weeks.	[263]
Laser Assisted Bioprinting	PLGA	NE-4C	Artificial neural tissue constructs	The comparative study demonstrated that the topological clue plays a relevant role in the development of clusters on the support, but the bioprinted laminin dots appeared to regulate the strength of the bond between them, paving the way for controlling the functional morphology of artificial neural tissue constructions.	[264]

3D Bioprinting	PU	FoxD3 plasmids, phDFs	Personalized drug screening or neuroregeneration.	The phDFs printed with FoxD3 in the thermo-responsive PU hydrogel demonstrated that they could be reprogrammed and differentiated into a neural tissue-like structure at 14 days after induction.	[265]
	Collagen, fibrin gel	VEGF, C17.2	Neural tissue regeneration	The bioprinting of fibrin gel incorporating VEGF supported the sustained release of growth factors on the collagen support.	[266]
3D printing technology based on low-temperature extrusion	Collagen, chitosan	IFN- γ , NSC-derived exosomes	Neurological recovery after traumatic brain injury	The 3D printed collagen/chitosan structure showed good biological and mechanical properties, providing a suitable microenvironment for the differentiation of NSCs and the secretion of exosomes. Furthermore, this scaffold was involved in multiple pathological processes after traumatic brain injury in rats, significantly enhancing neurodeficiency.	[267]
Electrospinning	PCL, OMT, gelatin, PEGDA, β -cyclodextrin	Spinal cord extracellular matrix	Treatment of spinal cord injuries	The manufactured scaffolds led to the differentiation of NSCs into neurons and inhibited their differentiation into astrocytes. These scaffolds created a suitable microenvironment for spinal cord tissue regeneration <i>in vivo</i> and guided the directional growth of axons. Furthermore, transplantation of the scaffolds with OMT enhanced the motor function of rats with spinal cord injuries.	[260]
DLP printing	GelMA, chitosan, PEDOT	n/a	Neural tissue repair	The GelMA/Chitosan-PEDOT hydrogel showed good and stable electrical conductivity, enhanced mechanical strength and noticeable biocompatibility. <i>In vivo</i> tests showed that the hydrogels promoted nerve regeneration and helped muscle recovery in the repair of sciatic nerve defects in rats.	[268]
Microextrusion-based 3D bioprinting	GelMA, PEGDA, PEDOT, CSMA	NSCs	Nerve injury repair	The 3D bioprinted electroconductive hydrogel showed biocompatibility, suitable mechanical	[269]

				strength and good conductivity, thus promoting the adhesion, growth and proliferation of NSCs.	
Additive-lathe 3D bioprinting	GelMA, PEGDA	BMSCs	Peripheral nerve injury repair	An integrated two-layer nerve conduit was obtained, in which the BMSCs inside the printed nerve conduits demonstrated very good cell viability and extended morphology. <i>In vitro</i> culture of PC12 cells revealed that the growth of neurons is significantly enhanced in nerve conduits embedded with BMSCs.	[270]
SLA	GelMA, PEGDA	Pc-12	Multi-physical model for cell-laden nerve scaffolds	This model can predict in advance, in <i>in vitro</i> culture experiments, which are the main areas of cell growth in the neural scaffold and can thus increase the oxygen concentration in these areas in order to further increase the concentration of cells.	[261]
Electrohydrodynamic jet	PCL, rGO	PC-12	Peripheral nerve injury repair	The addition of rGO to scaffolds results in softer materials that enhance neural differentiation. <i>In vitro</i> studies with PC12 cells show increased cell proliferation and improved support for neural differentiation in PCL/rGO scaffolds compared to PCL-only scaffolds, as confirmed by RT-PCR and immunocytochemistry analyses.	[271]

6. Limitations and Challenges

Despite the potential of 3D printing/bioprinting and cellular therapies for regenerative medicine, there are several limitations and challenges that need to be addressed. Although extensive research trials have been conducted in recent years, the clinical translation of these technologies has been limited. The lack of sufficient animal studies and the absence of viable 3D models in clinical trials underscore the need for further focus and development in these critical areas [272–274].

One of the current challenges is the development of functional vascular networks within bioprinted tissues and organs. Specifically, the formation of a vascular network that can integrate with the host's native blood vessels is hindered by structural complexity and the heterogeneity of tissue components [275–277]. Vascularization is crucial for providing nutrients and oxygen, which are vital for maintaining cell viability and ensuring tissue functionality. A robust, multi-level vascular network is necessary to support the long-term survival and growth of bioprinted organs, incorporating smooth muscle cells and hUVECs into the blood vessels [278]. To address transport limitations, researchers have employed proangiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (BFGF) to stimulate the formation of blood microvessels. Additionally, incorporating endothelial cells into the culture medium has been shown to promote micro vessel formation and the development of angiogenic sprouts in engineered constructs [279]. However, endothelial cells and angiogenic factors generally do not produce perfusable constructs in a fast way [280]. Bioreactors offer a solution by continuously supplying media to porous constructs, reducing the reliance on arterial scaffolds and large tissue samples. Despite this, these constructs often lack micro vessels and are stored outside of bioreactors, which can compromise cell survival. Microfluidic systems represent a potential alternative for vascular network fabrication, though scaling these systems to larger physiological sizes remains a significant challenge [275].

The selection and sourcing of cells is another challenge in regenerative medicine. Cells used in bioinks must possess some key characteristic: high proliferative capacity, printability, functionality, safety, and economic viability [278]. The choice of cells encapsulated in bioinks critically influences their differentiation potential and ability to develop into various lineages. Although live cells, including primary and stem cells, are very promising, their practical application is limited by issues related to availability and ethical concerns. Researchers have reported successful integration of stem cells with bioprinting technologies [272]. An additional challenge is the mass production of cells, which needs substantial quantities of cells and increases the demand on *in vitro* expansion cultures. Addressing the cost-effectiveness of large-scale cell production is a crucial challenge [278]. Extended processing times and mechanical forces experienced during 3D printing can adversely affect cell viability by altering cell geometry and disrupting signaling pathways [281]. To address these issues, it is essential to enhance existing bioprinting techniques to minimize processing duration and to develop specialized buffers that can protect cells throughout the printing process.

In 3D bioprinting of tissues and organs, the selection of optimal biomaterials is crucial for the successful production of clinically relevant tissues [281,282]. Biomaterials are essential for providing structural support, maintaining cellular viability, and ensuring long-term tissue integration. Although many polymers used in conventional 3D printing and traditional tissue engineering have been investigated for bioprinting due to their availability and prior usage, they are not always the most biologically suitable regarding bioprinting applications [283]. These materials may exhibit excessive biological reactivity, leading to unwanted cellular interactions and premature or undesired stem cell differentiation. Bioinks must possess specific properties to be suitable for clinical use, including structural stability, capacity of promoting cell growth, and an appropriate degradation rate that aligns with tissue regeneration requirements. Additionally, bioinks must be compatible with bioprinting technologies to facilitate rapid prototyping [13]. A major challenge is ensuring that printed structures are biocompatible and provide an appropriate environment for cell growth. Current research is focusing on novel biopolymers and hydrogels that better mimic the nanoscale features and responsiveness of the ECM and the native tissue microenvironment [284]. However, these advanced materials often face compatibility issues with conventional bioprinting methods.

Many of these materials lack the structural integrity needed for effective bioprinting and may collapse if they are too soft [285]. One promising strategy involves combining different substances to leverage the strengths of each: integrating the mechanical properties of firmer materials with the cell-proliferative and cytocompatible attributes of softer materials [130,286].

Furthermore, the development and implementation of 3D printing and cell therapies in regenerative medicine pose significant financial challenges, which could prevent the widespread adoption of these technologies. The costs associated with research, development, and clinical trials are substantial, and securing funding for these endeavors can be difficult. Additionally, the cost-efficiency of 3D bioprinting needs careful consideration, particularly in relation to the high expenses of 3D printers, cellular materials, and associated computer software [287]. Generally, the costs of maintaining and scaling bioprinting technologies limit the rapid integration of 3D printing capabilities into clinical settings [284]. Another challenge is the size of bioprinted tissues. Currently, bioprinted constructs are typically small and consist of a limited number of cell types, which restricts their functionality and scalability [284,287–289]. Moreover, 3D printers are often constrained by their build volume, which limits the maximum size of bioprinted tissues and complicates the creation of entire 3D-printed organs [284].

Lastly, the use of 3D printing/bioprinting and cellular therapies in regenerative medicine is subject to regulatory approval processes. Although these processes are often time-consuming and complex, important guidelines can be derived from regulatory bodies such as the Food and Drug Administration (FDA) in the United States of America and the European Medicines Agency (EMA) in the European Union, particularly concerning 3D-printed medical devices. For tissue bioprinting to achieve clinical translation, it is essential to establish a clear and defined regulatory pathway [290]. Additionally, ethical challenges and concerns related to biosafety and liability arise when fabricating internal tissues and organs. The clinical translation of bioprinting techniques will depend on regulatory bodies' thorough evaluation of safety, efficacy, and risk. Globally, regulatory authorities face challenges in addressing the potential and uncertain risks associated with 3D bioprinting, such as immune responses to bioinks or materials [284]. In the absence of specific regulations, the FDA is currently relying on the Center for Biologics Evaluation and Research (CBER) guidelines for 3D bioprinting products. These products require FDA approval, and adherence to regulatory guidelines is mandatory from the initial stages of product development. As the field advances, more 3D bioprinting products are likely to emerge, highlighting the need for more specific regulatory guidelines. Currently, only South Korea's Ministry of Food and Drug Safety (MFDS) and Japan's Pharmaceuticals and Medical Devices Agency (PMDA) have developed specific guidelines for 3D bioprinting. Thus, the global development of comprehensive regulations for 3D bioprinting techniques, bioinks, and printers is of increasing importance [13].

These limitations and challenges highlight the complexity of integrating 3D printing with cellular therapies in regenerative medicine, emphasizing the need for continuous research and development in this field. Overcoming these obstacles requires transdisciplinary collaboration among cell biologists, engineers, physiologists and pharmaceutical industry partners, to advance and expand the potential of this technology [291]. Despite the challenges, progress continues, and it is likely that these technologies will play an increasingly significant role in treating a wide range of diseases and conditions in the future. With ongoing research and innovation, the goal of creating viable, safe, and fully functional 3D-bioprinted organs is becoming increasingly attainable [278].

7. Current State and Future Outlook

The current state of clinical trials combining 3D printing/bioprinting and cellular therapies in regenerative medicine is promising, though still in its early stages. Numerous research groups are actively developing and testing new treatments utilizing these technologies, and several clinical trials have been initiated in recent years to assess their safety and efficacy.

Tissue engineering currently has broad applications, including the development of various tissues such as cartilage, skin, cardiac, vascular, bone, neural, and retinal tissues. Traditionally, this approach involves seeding cells onto a porous and mechanically stable scaffold to promote cell

proliferation and tissue formation [292]. This method presents several advantages, including optimal structural support with appropriate degradation kinetics, modulation of the cellular microenvironment, and facilitating the exchange of nutrients and waste between cells and the scaffold [293,294].

3D bioprinting has opened a new era in bioengineering and the biomedical field allowing the production of patient-specific autologous organs and tissues [18,295–298]. This rapid prototyping technique allows for the development of complex organ and tissue structures by directly depositing living cells and biomaterials in a layer-by-layer process, guided by a CAD model. Through this method, 3D products can be manufactured with precise control over positioning and architecture, including features such as shape, pore geometry, and interconnectivity, thereby creating tissue and organ models that closely mimic the human body with high reproducibility and repeatability [299–301]. The structural and biochemical complexity of living tissues and organs can be achieved by co-printing multiple cell types alongside various materials, creating a hetero-cellular microenvironment at targeted locations [214,302,303].

Although *in vitro* models have advanced significantly for developing new therapies, their application in surgical settings is still not fully realized. However, significant progress is being made in hydrogel design and the development of advanced technological tools. These advancements are bringing us closer to meeting the fidelity and safety standards required for bioprinted constructs to be consistently and personalized used in patients in the near future [304].

The future of tissue-engineered materials is expected to replicate not only the structural design and characteristics of organs and tissues but also their dynamic, functional behaviors [159,160]. The concept of time as the fourth dimension (4D) has emerged in the context of biofabrication and bioprinting, introducing two key aspects: materials capable of deformation and structures that mature after printing [305,306]. This new approach to bioprinting addresses the complexity of the system, which is very important for fully understanding the behaviour of functional living materials during the post-processing stage [304].

8. Conclusion

The combination of 3D printing/bioprinting technologies with cellular therapies represents a significant advancement in regenerative medicine. Advances in this field are leading to the creation of functional tissues that closely resemble native tissues. This review has detailed the fundamental principles and applications of each technology individually and has elucidated how their convergence is pushing the boundaries of tissue engineering.

The review highlights that recent developments have markedly enhanced the ability to fabricate complex, 3D cellular structures with high precision. Innovations in bioprinting techniques, coupled with improvements in biomaterials and cellular engineering, have enabled more sophisticated control over tissue architecture and cellular organization. These advancements facilitate the creation of more accurate and functional tissue models, which hold promise for personalized regenerative therapies.

Nevertheless, there are still several critical barriers that need to be addressed, such as the development of functional vascular networks, regulatory and ethical issues, and the development of suitable biomaterials.

In the future, the potential impact of these integrated technologies on regenerative medicine is expected to be high. As the field progresses, solving these challenges will be essential to realizing the full potential of 3D printing/bioprinting and cell therapies. Ongoing research and innovation in these areas is therefore expected to produce transformative advances in personalized medicine and tissue regeneration, ultimately improving outcomes for patients and advancing therapeutic options.

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Abbreviations

2D	Two-dimensional
132ACG	Bioink with alginate (1%), cellulose nanocrystal (3%), and gelatin methacryloyl (5%)
2PP	Two-photon polymerization
3D	Three-dimensional
3DP-HOs	Three-dimensional bioprinted hepatorganoids
4D	Four-dimensional
ACP	Amorphous calcium phosphate
AD	Additive manufacturing
ADM	Acellular dermal matrix
aHSC	Primary fetal activated hepatic stellate cells
ALP	Alkaline phosphatase
AMX	Amoxicillin
ASTM	American Society for Testing and Materials
BFGF	Basic fibroblast growth factor
BJ	Binder jetting
BL	Bi-layer
BMP-2	Bone morphogenetic protein 2
BMSCs	bone marrow-derived mesenchymal stem/stromal cells
C17.2	Murine neural stem cells
Ca	Calcium
CAD	Computer-aided design
CBER	Center for Biologics Evaluation and Research
cECM	decellularized cardiac extracellular
CFs	Human cardiac fibroblasts
CLIP	Continuous light interface production
CNC	Cellulose nanocrystal
CSMA	Chondroitin sulfate methacrylate
dECM	Decellularized extracellular matrix
DEP	Directed energy deposition
Dex	Dextran
DFs	Dermal fibroblasts
DLP	Digital light processing
DMLS	Direct metal laser sintering
DO	Diamond
DPSCs	Dental Pulp stem/stromal cells

EBM	Electron beam melting
ECM	Extracellular matrix
EMA	European medicines agency
EPCs	Endothelial progenitor cells
ESCs	Embryonic stem cells
ESCs	Epidermal stem cells
EVCs	Early vascular cells
FDA	Food and Drug Administration
FDM	Fused deposition modeling
Fe	Iron
FFF	Fused filament fabrication
GAM	Matrix hydrogel with 2.8% of gellan gum, 1.6% of alginate, and 2.8% of methyl cellulose
Gel	Gelatin
GelMA	Gelatin methacrylate
H9c2	Cardiomyocytes
HA	Hyaluronic acid
HAGM	Hyaluronic acid-gelatin methacrylate
HAp	Hydroxyapatite
Hap/ β -TCP	Biphasic calcium phosphate system
hCAECs	Human coronary artery endothelial cells
HCC	Hepatocellular carcinoma
hCMPCs	Human cardiac-derived cardiomyocyte progenitor cells
hCPCs	Human cardiac progenitor cells
hdECM	Heart tissue-derived extracellular matrix
hDFs	Human dermal fibroblasts
hECM	Human extracellular matrix
hECs	Human endothelial cells
hepG2	Human hepatocellular carcinoma
hESCs	Human embryonic stem cells
hiHep	Human-induced hepatocyte
hiPSCs	Human induced pluripotent stem cells
hKCs	Human keratinocytes
hLFs	Human lung fibroblasts
hMVECs	Human microvascular endothelial cells
hnDFs	Human neonatal dermal fibroblasts
hPCs	Human placental pericytes
hPSCs	Human pluripotent stem cells
hSFs	Human skin fibroblasts
hUCMSCs	Human umbilical cord mesenchymal stem cells
HUH7	Undifferentiated hepatocyte cell line
hUVECs	Human umbilical vein/vascular endothelial cells
iCMs	Induced pluripotent stem cell-derived cardiomyocytes
IFN- γ	Interferon-gamma
iPSC-CMs	Induced pluripotent stem cell-derived cardiomyocytes
iPSCs	Induced pluripotent stem cells
ISO	International Standard Organization
Kr	Keratin
L x 2	Human hepatic stellate cell line
LAP	Lithium phenyl-2,4,6-trimethylbenzoylphosphinate
LOM	Laminated object manufacturing
mEFs	Mouse embryonic fibroblasts
MFDS	Ministry of Food and Drug Safety
Mg	Magnesium
MJ	Material jetting
Mn	Manganese
MSCs	Mesenchymal stem/stromal cells
MUVECs	Murine umbilical vein endothelial cells
n/a	Not applicable

Nb	Niobium
NB	N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide
NPCs	Neural progenitor cells
NRCMs	Neonatal rat cardiomyocytes
NSCs	Neural stem cells
NSPCs	Neural stem/progenitor cells
OMT	Oxymatrine
PBF	Powder bed fusion
pc-12	Rat adrenal medullary pheochromocytoma
PCL	Poly (ε-caprolactone)
PecMA	Pectin methacrylate
PEDOT	Poly(3,4-ethylenedioxythiophene)
PEEK	Polyether ether ketone
PEG	Polyethylene glycol
PEG4A	4-arm polyethylene glycol acrylate
PEGDA	Diacrylate poly (ethylene glycol)
PEGDMA	Poly (ethylene glycol) dimethacrylate
PEGMA	Poly (ethylene glycol) methacrylate
PF	PEG-Fibrinogen
PF	Poly (ethylene glycol)-fibrinogen
PGA	Poly (glycolic acid)
PGS	Poly (glycerol sebacate)
phDFs	Primary human dermal fibroblasts
PLA	Poly(l-lactic) acid
PLGA	Poly (lactic-co-glycolic) acid
PMDA	Pharmaceuticals and Medical Devices Agency
PMHs	Primary mouse hepatocytes
PrHCs	Primary rat hepatocytes cells
PRP	Platelet-rich plasma
PSC	Phosphosilicate calcium bioglass
PSCs	Phosphosilicate calcium bioglasses
PU	Polyurethane
PVP	Polyvinylpyrrolidone
r-BMSCs	Rat bone marrow mesenchymal stem cells
RD	Rhombic dodecahedron
rGO	Reduced graphene oxide
rhEGF	External human epidermal growth factor
SCAPs	Stromal cells from apical papilla
SF	Silk fibroin
SilMA	Methacrylated silk fibroin
SLA	Stereolithography
SLM	Selective laser melting
SLS	Selective laser sintering
Sr-CSH	Xonotlite
SR ²⁺	Strontium
SS	Strontium silicate
Ta	Tantalum
Ti	Titanium
Ti6Al4V	Titanium alloy
UAM	Ultrasonic additive manufacturing
UV	Ultraviolet
VEGF	Vascular endothelial
XG	Xanthan gum
Zn ²⁺	Zinc
Zr	Zirconium
β-TCP	Beta-tricalcium phosphate

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