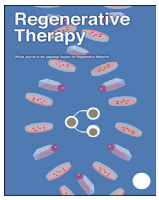
Regenerative Therapy 18 (2021) 133–145

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth



Review

The promising rise of bioprinting in revolutionalizing medical science: Advances and possibilities

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ARTICLE INFO

Article history:
Received 16 November 2020
Received in revised form
19 February 2021
Accepted 26 May 2021

Keywords:
Bioprinting
Bioinks
Tissue engineering

ABSTRACT

Bioprinting is a relatively new yet evolving technique predominantly used in regenerative medicine and tissue engineering. 3D bioprinting techniques combine the advantages of creating Extracellular Matrix (ECM)like environments for cells and computer-aided tailoring of predetermined tissue shapes and structures. The essential application of bioprinting is for the regeneration or restoration of damaged and injured tissues by producing implantable tissues and organs. The capability of bioprinting is yet to be fully scrutinized in sectors like the patient-specific spatial distribution of cells, bio-robotics, etc. In this review, currently developed experimental systems and strategies for the bioprinting of different types of tissues as well as for drug delivery and cancer research are explored for potential applications. This review also digs into the most recent opportunities and future possibilities for the efficient implementation of bioprinting to restructure medical and technological practices.
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Contents

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Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2021.05.006
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1. Introduction

1.1. Bioprinting

In the present age of technology and automation, tissue engineering has blurred the lines between reality and science fiction through the advancement of bioprinting technologies [1]. Bioprinting is an adaptation of additive (3D) printing, incorporating layer-by-layer deposition of biomaterials and bioactive molecules e.g. cells, growth factors, etc. This technology enables the designing and manufacturing of 3D constructs of tissues and organs mimicking the structure of their human counterparts [2]. The architecture is achieved precisely by the formulation of predefined locations and structures in computer-aided design software (CAD) [1]. The success of utilizing the bioprinting method depends on the functionality of the resulting structures. Design considerations have to be made based on three fundamental variables-bioactive components, cellular interactions, and the bioprinting device [3]. The core biomaterial for 3D printing called bioink comprises cells to be deposited, often enveloped in a carrier matrix that aims to imitate the physical and biochemical environment of native tissues and support adhesion, cell proliferation, differentiation [4]. Can bioprinting revolutionize biomedicine and biomedical engineering with the proper utilization of bioinks and different techniques, or is it limited to experimental use?This thorough review primarily centers around the potential implementation of 3D bioprinting in regenerative medicine and other medical sciences while also encompassing current tactics and future prospectives in bioprinting. (see Table 1)

1.2. Bioinks for tissue engineering

Bioinks used in bioprinters consists of an extensive range of materials that is continuously expanding [5]. Bioinks can be cell-laden, cell-only (scaffold-free), or even cell-free (cell-adhesive channel) depending on tissue requirements [6]. Water-soluble polymers known as hydrogels are one of the predominant biomaterial components of 3D bioprinters [7]. Permeability to nutrients, oxygen, and other water-soluble molecules makes cell-laden hydrogels convenient [8]. Hydrogels can be natural, synthetic, or a blend of both. Naturally derived polymers vastly used for tissue engineering include collagen, fibrin, gelatin, alginate, hyaluronic acid, decellularized extracellular matrix, chitosan, etc. [9]. The purpose of the carrier material of bioink is to provide structural and biochemical support similar to the native Extracellular Matrix (ECM). Collagen is favorable for being a major component of native ECM. However, compensations have to be made as it has low mechanical properties [10]. Both collagen and gelatin are naturally cell-adherent [5]. Fibrin and hyaluronic acid have excellent biodegradability. Fibrin aids in angiogenesis and promotes cell growth whereas hyaluronic acid has flexible usage [11]. Alginate is a biocompatible, low-cost natural polysaccharide commonly used in a blend with gelatin or fibrinogen [8]. Synthetic and biosynthetic bioinks serve additional benefits such as improved mechanical stability, photo cross-linking ability, controlled biodegradability, etc. Examples of synthetic bioinks include PEG-based bioinks, polyester-based bioinks, pluronic-based bioinks, polyurethane-based bioinks [12]. More advanced bioinks developed or developing include multi-material bioinks [13], functionalization/dual cross-linking, supramolecular hydrogels, interpenetrating network, nanocomposites, thermoplastic reinforcement [14], self-assembling bioinks, stimuli-responsive bioinks [8], etc.

Bioink design is pre-eminent for an efficient bioprinting process. Thus, it involves considerations like mechanical properties such as permeability, viscosity, and tensile strength [15], biochemical properties, cytotoxicity of components, printability, degradability, and biocompatibility [11]. To maintain high cell viability after printing the materials used must not be susceptible to shear stress. In addition, the bioinks used must be biocompatible and biodegradable to successfully generate bioprinted tissue [16]. The choice of cells and hydrogel in a cell-laden bioink principally depends on the target tissue to be reconstructed.

1.3. Fundamental steps in bioprinting

Generally, the bioprinting process consists of three major steps [17]. For the **pre-bioprinting stage**, medical images are used as a guide to design the blueprint of the tissue using computer-aided design software (CAD) [18]. The **physical bioprinting step** comprises bioink composition, compact and good resolution bioprinter selection, and lastly, repeatable and biocompatible bioprinting [17]. This step is crucial for achieving affordable and functional bioprinting. Finally, the **post-bioprinting stage** refers to the mechanical and chemical conditioning of the constructs, usually performed in a bioreactor. To ensure the functionality of the bioprinted construct optical image analysis, mechanical stability assessment, swelling and degradation analysis, etc. are performed [19].

2. Techniques for 3D bioprinting

Bioprinting of desired tissues may utilize different techniques according to their individual principles, material demands, and considering their advantages, disadvantages. Based on the principle of operation, 3D bioprinting can be categorized into three types: droplet-based, extrusion-based, and photocuring-based [20].

2.1. Droplet-based bioprinting

Droplet-based bioprinting uses droplets of controlled volumes of bioink todeposit at predetermined locations. Due to its precise

Table 1
Summary of other applications of bioprinting in cancer tissue engineering.

Cancer Type	Bioink Composition	Bioprinting Method	Observations	Ref
Ovarian cancer	Ovarian cancer cells + Matrigel	Pneumatic cell droplet	Micropatterning ovarian cancer cells (OVCAR-5) and fibroblasts (MRC-5) with spatial control, characterization of acini growth kinetics	[174]
Liver cancer	Hepatic carcinoma cells + Matrigel	Pneumatic Extrusion	Radiation shielding capabilities of the prodrug amifostine, benefits in dual-cell model	[175]
Brain cancer	Endothelial cells + Glioma stem cells + Collagen/Laminin	Extrusion	Tumor microenvironment of glioma/vascular system with dynamic flow to model cell–cell interaction of neoplastic glioma cells and ECs	[176]
Brain cancer	U118 glioma + Pluripotent Stem Cell-Derived Neural Organoid	Extrusion	Invasion of human tumour cells using different neural progenitor cell lines, cell-tracking dyes and 3D laser scanning confocal microscopy	[177]
Cervical cancer	HeLa/10T1/2 + Poly (ethylene glycol) diacrylate	Projection stereolithography	Comparison between cancerous and non-cancerous cell lines (HeLa vs 10T1/2)	[178]
Oral cancer	β-Tricalciumphosphate	Extrusion	Incorporating oral squamous cell carcinoma (OSCC) cell line spheroids to a 3Dbioprinted model to depict the stages of oral cancer	[179]
Cervical cancer	HeLa + Gelatin/Alginate/Fibrinogen	Extrusion	Viability, proliferation, Matrix Metalloproteinase (MMP) expression, and chemoresistance	[180]
Breast cancer	Breast Adenocarcinoma + Mouse Macrophage + Sodium alginate	Coaxial extrusion	Tumor microenvironment to explore migration of segregrated tumor cells and macrophages (>90% viability)	[181]
Breast cancer	Breast cancer cells + (Fetal osteroblasts/ Mesenchymal stem cells) + Gelatin methacrylate	Stereolithography	Observation of interactions between BrCa and MSC/ osteoblasts, and VEGF secretion in artificial bone microenvironment	[172]
Breast cancer	Breast cancer cells + Poly (ethylene glycol)	Continuous 3D projection	Breast cancer spheroids showed hypoxic coresand signs of necrosis, key features of tumor environment	[182]
Breast cancer	MCF-7 + Poly (ethylene glycol)	Inkjet	*In situ* cell seeding for the formation of breast cancer cellular spheroids and analysis as potential microenvironment (>90% viability)	[183]
Brain cancer	Human glioma stem cells + Gelatin/ Alginate/Fibrinogen	Extrusion	Tumor microenvironment with over 87% cell viability; potential for vascularization, tumor angiogenesis, and VEGF secretion	[184]
Brain cancer	Glioblastoma-associated macrophages + glioblastoma multiforme + Gelatin methacryloyl/ Gelatin	Extrusion	In the printed mini-brains, glioblastoma cells actively recruited macrophages and polarized them into a GAM-specific phenotype. Also, macrophages induced glioblastoma cell progression and invasiveness	[173]
Brain cancer	GSC23 + U118 + Sodium alginate	Coaxial extrusion	Glioma microenvironment evaluation for invasion and drug screening	[185]
Brain cancer	GSC23 + HMSCs + Sodium alginate/ Gelatin	Coaxial extrusion	Tumor-stroma cells interaction, transcription of RFP	[186]
Breast cancer	Breast cancer cells + Gelatin	Laser-assisted	Laser direct-writing on rat mesentery tissues, quantitative study of cancer cell activity, angiogenesis, lymphangiogenesis	[187]
Cervical cancer	HeLa + Collagen (printed on a nanofibrous membrane in co-culture with fibroblasts)	Inkjet	Matrix Metalloproteinase 2 (MMP2) and Matrix Metalloproteinase 9 (MMP9), drug screening	[188]

control of deposition, simplicity, and versatility, it has a large area of application including regenerative medicine, transplantation, high-throughput screening, oncology, etc. [21].

2.1.1. Inkjet bioprinting

Inkjet bioprinting is a non-contact technique of bioprinting in which droplets of bioinks are ejected under pressure [6]. Inkjet techniques make use of the physical properties of bioinks, such as viscosity, surface tension, density, etc. [20]. Inkjet bioprinting can be continuous or drop on demand (DOD). Whereas continuous inkjet bioprinters continuously release bioinks, DOD inkjet bioprinters make use of pressure pulses to eject droplets when required. The pressure pulses are usually generated by thermal, piezoelectric, or electrostatic actuators [22]. The thermal actuator in a thermal inkjet bioprinter is an electric heating unit that vaporizes the bioink solution to form a vapor bubble. Eventually, the vapor bubble expands due to pressure and rapidly explodes, generating a pulse pressure that ejects a bioink droplet [3]. Such temperature changes do not significantly affect cell viability as the processes only take a few microseconds. The bioprinted cells have been assessed to maintain their proliferation capacity, genotype, phenotype, and function [23]. On the other hand, in a piezoelectric inkjet bioprinter, a voltage pulse causes the piezoelectric actuator to change its shape. Sudden change in the volume of the fluid chamber containing bioink subsequently causes the release of a droplet [24]. There was no issue in sustaining the cell viability even after the process [25]. Electrostatic actuators work quite similarly. When a voltage pulse is applied between a pressure plate and an electrode, the pressure plate deflects. There was about an 80–95% yield in the cells printed from thermal, piezoelectric and electrostatic inkjet bioprinting method, confirming a high cell viability [26]. Removal of the voltage pulse brings the pressure plate back to its original shape ejecting a bioink droplet [21].

Inkjet bioprinting techniques show promise as they provide high-resolution printing due to its fine control over the ejection of droplets and pico-liter sized ink droplets [27].

2.1.2. Electrohydrodynamic jet (EHDJ) bioprinting

EHDJ bioprinting technique uses an electrical field to drive the bioink droplets. The bioink solution is pumped through a needle

connected to a high voltage generator [28]. High-resolution printed tissue can be achieved, firstly because the nozzles are much smaller in diameter than inkjet printers. This allows the droplets to be much more focused and precise. Secondly, electrohydrodynamics generates droplets that can be significantly smaller than this diameter. The size of the droplet is also influenced by the voltage applied-high voltage bringing smaller droplets. Lastly, lateral variations are minimal in droplet placement due to the focused distribution of electric field lines [29]. EHDJ printing is a comparatively complex process. Careful selection, control, and optimization of bioink are essential for this technique as it is not only dependent on the viscosity, surface tension, and density, but also the electrical conductivity and evaporation rate of the bioink [30]. Cell viability depends on the applied voltage, bioink flow rate, and bioink properties.

2.1.3. Acoustic bioprinting

The acoustic bioprinting technique keeps the biomaterials free from detrimental stress like heat, high voltage, high pressure, and any form of shear stress. Droplets are ejected using a gentle acoustic field through a nozzle [31]. However, gentle acoustic fields are not capable of ejecting droplets of bioinks that are viscous or have high cell concentration. Studies on this technique are quite limited.

2.1.4. Micro-valve bioprinting

Micro-valve bioprinting employs an electromechanical valve to control the ejection of droplets. The nozzle opening of the device is gated by the valve which is unlatched by a magnetic field created by a voltage pulse. The pressure in the fluid chamber containing bioinks overcomes the surface tension resulting in the generation of a droplet [32]. Cells are less prone to damage through this process than piezoelectric bioprinting due to the low range of pneumatic pressure used.

Droplet-based bioprinting offers excellent spatial resolution which makes it desirable for application in tissue engineering and regenerative medicine. These techniques also provide a good resolution and relatively high cell viability at a lower expense [22]. However, droplet-based methods also have their drawbacks. The most prominent problem is the clogging of the nozzle when the bioink is too viscous [33].

2.1.5. Laser-assisted bioprinting

With similar mechanisms as inkjet printers, laser-assisted bioprinting uses laser pulses to induce microbubbles. Support comprising bioink in the form of a thin sheet or film Is attached to laser-absorbing metal or metal oxides, commonly gold or titanium [34]. A laser beam is pulsed at the interface of the target substrate and the absorptive layer, causing thermal volatilization and subsequently formation of a microbubble [35]. Bioink droplet is ejected through the expansion of the microbubble.

Overcoming the limitations of other droplet-based methods, laser-assisted bioprinting supports bioinks with higher viscosities [8]. The clogging problem is absent in the case of laser-assisted technique as it is nozzle-free. The non-contact, nozzle-free process also protects cellular components from shear stress resulting in higher cell viability [36]. However, the cell viability also gets slightly reduced due to the pulsed laser technology involved in this mechanism [37]. The laser-assisted bioprinting also provides high-resolution printing. Yet, it is highly expensive and complex, leading to several operational issues [4]. Even though the process is fast, droplet size limits the overall volume deposition over time.

2.2. Extrusion-based bioprinting

In extrusion-based bioprinting, ongoing filaments are produced through continuous extrusion force instead of single droplets [38]. This technology is suitable for printing highly concentrated cells which means high viscosity bioinks. The ink used in extrusion bioprinting is distributed by mechanical force like screw or piston, or via a gas or pressurized air [39].

2.2.1. Pneumatic-driven extrusion

The extrusion technique involving pneumatic force utilizes compressed air with valve-free or valve-based configuration [9]. The air pump with sterilized air is connected to a bioink-filled syringe [40]. Pneumatical extrusion of the bioink causes shear stress, which means only the type of bioinks that have shear-thinning properties can maintain filamentous shape after extrusion. Valve-free extrusion is relatively simple. For high-precision performance, however, valve-based extrusion is preferred [41]. This is one of the most convenient techniques for printing cell-laden bioink [42].

2.2.2. Mechanical micro-extrusion

Micro-extrusion refers to when the nozzle orifice has a diameter of less than 1 mm [43]. Mechanical driven extrusion is suitable for highly viscous bioinks, such as synthetic and natural polymers. One commonly used mechanical micro-extrusion technique is the piston-based extrusion, which employs a piston connected to an electric motor. Rotation in the motor through an electrical pulse drives the piston forward thereby pushing bioink through the nozzle [40].

The screw-driven extrusion technique provides more volumetric control and is useful for higher viscosity biomaterials [44]. In this technique,a screw connected to the motor instead of a piston drives the release of the bioink. This process can accommodate larger pressure drops through the nozzle. Mechanical methods provide higher resolution and better printability for a larger range of biomaterials, though it requires a tighter tolerance selection of the ram and nozzle [9].

Extrusion-based bioprinting is preferable for high cell densities and is relatively fast-paced [45]. As native tissues contain densely packed cells, printing cells at high density is significant for use in regenerative medicine [22]. A variety of bioinks can be used in this technique, which is an advantage. One of the most commonly used bioinks for extrusion is alginate [46]. Bioinks used for extrusion-based bioprinting has to be fairly viscous in order to enhance resolution and remain stable in the mechanically stressful process. However, the resulting high shear forces in pneumatic driven extrusion lead to low cell viability [47]. Nevertheless, the cell viability is still about 90% in cells manufactured from extrusion-based bioprinting [48]. The resolution is relatively low [4].

2.3. Photocuring-based bioprinting

2.3.1. Stereolithography (SLA)

Stereolithography utilizes a photocuring-based technique solidifying photosensitive polymers to form tissue constructs under precisely controlled lighting [20]. The ultraviolet ray is directed at a reservoir of photosensitive polymers. For each layer deposition, laser scans a 2D pattern by passing through its path point-by-point and the controlled light interacts with the bioink material to polymerize it according to a specific design [49]. After one layer is cured, the printing platform moves upwards or downwards, away from the laser source, so that new unpolymerized ink material can flow into position for the next layer [50].

2.3.2. Digital light processing (DLP)

The technique is quite similar to SLA, except for the different light scanning mode. Instead of point-by-point, in DLP the light is projected onto the surface of the layer at once [18]. This has a significant advantage in processing time.

Photocuring-based bioprinting approaches have a rapid fabrication time [51]. Less dependency on mechanical forces generates higher cell viability. In addition, complex architectures of tissues can be constructed using these techniques with high resolution [52]. However, the processes require a very careful selection of biomaterials, and photoinitiators are often introduced to the bioink to improve photosensitivity which can affect cell viability [53]. Also, the cumulative UV exposure is a drawback [45].

3. Applications of 3D bioprinting in tissue engineering and biomedicine

3.1. Bioprinting for tissue and organ regeneration

Severe damage in human tissues caused by trauma or diseases requires medical attention for regeneration or transplantation. Previously existing tissue engineering techniques alone are not sufficient to produce tissues and organs for medical use except drug testing. The success of organ transplantation is also limited due to donor shortage and immune reactions [54]. The heterogeneous structures of natural tissue, ECM organization, and gradients play a significant role in cell migration, proliferation, and differentiation [55]. The aim of using bioprinting in tissue engineering is to achieve well-vascularized, functional, and reproducible complex tissue structures of heterogeneous compositions, suitable for future clinical use. The architecture of the target tissue to be printed is constructed by computer-aided design/computer-aided manufacturing tools based on medical images obtained from patients [56]. As discussed before, printed tissues have the potential to provide the necessary behavioral cues to cells as well as facilitate vascular network generation [6]. The choice of bioinks and the techniques of printing are influenced by the structure of the target tissue. Recent advances in regenerative medicine using bioprintingexamine restoration, replacement, and regeneration of damaged or injured skin, bone, cartilage, neural tissue, heart, blood vessels, and so on. Bioprinted tissues can be achieved using tissue-specific bioinks or from stem cells [57]. This section emphasizes the established studies and applications of 3D bioprinting for the regeneration of hard and soft tissues.

3.1.1. Hard tissue

3.1.1.1. Bone and cartilage. Bone is a complex composite of minerals and organic matrix with a distinct structural organization [58]. Bone is a self-healing tissue, yet, the self-regeneration is typically limited. Cartilage is a connective tissue primarily comprising collagen and proteoglycans [59]. Hyaline cartilage that is found between joints is an important factor when reconstructing bone tissue [60]. Avascular cartilage does not regenerate spontaneously. Thermoplastics such as polycaprolactone (PCL) and PLA have been utilized for 3D bioprinting of cartilage tissue. Bioprinting can precisely mimic the complex architecture of bone tissue and provide fast and often inexpensive mandibular, skull bolt, and maxillar bone reconstruction and regeneration.

Endothelial progenitor cells and multipotent stromal cells encapsulated in Matrigel or Matrigel and alginate have been studied [61] for the development of bone-like tissue by implantation into subcutaneous dorsal pockets of mice. A fibrin-collagen hydrogel with rabbit articular chondrocytes has been printed using an inkjet-based technique onto anelectrospun PCL matrix [62]. The inclusion of the PCL enhanced the mechanical properties of the construct. The cartilage tissue formed within the bioprinted scaffolds over a period of 8 weekswas similar to natural elastic cartilage. Human chondrocytes have also been utilized, suspended in poly (ethylene glycol) dimethacrylate (PEGDMA) solution [63]. The scaffolds were supplemented with either fibroblast growth factor-2 (FGF-2) or transforming growth factor-β1 (TGF-β1) to benefit cartilage development. An *in situ* printing approach by Cui et al. utilized the same printing method wherein the scaffolds were printed either separately or directly into osteochondral plugs that had been harvested from bovine femoral condyles [64]. Even though the cell viability was promising, low compressive modulus compared to the human articular cartilage of the lower limbs indicates the need for further structural development of the scaffold before human implantation. Decellularized bone matrix loaded with human adipose-derived stem cells (hASCs) in a PCL matrix was studied for craniofacial regeneration in mice [65]. Due to the tissue's complex structure, chondral and osteochondral injuries are difficult to heal. An approach called “in-situ biopen” was developed by O'Connell et al. to reconstruct chondral, maxilla, and mandible defects. A human infrapatellar fat pad (IPFP) adipose stem cell-ladenGelMA or hyaluronic acid-methacrylate hydrogel construct was fabricated utilizing the biopen [66]. Dhariwala et al. used poly (ethylene oxide) (PEO) and CHO cells to achieve a high resolution bioprinted construct using the SLA technique, however, lacking structural strength [67]. Lu et al. also used the SLA technique with stem cells derived from mouse bone marrow stromal cells in PEDGA hydrogel [68]. Cartilage is avascular and has low cell densities, which makes regeneration of cartilage tissue difficult. To address this issue, trophic factors have been added into cell-laden cartilage scaffolds [69]. For the rapid development of adequate vasculature, a pre-vascularization approach was developed through laser-assisted positioning of human umbilical vein endothelial cells (HUVECs) [70]. More recently, Sun et al. [71] developed a dual-factor (BMP4 and TGFβ3) releasing gradient structured human and rabbit cartilage construct using mesenchymal stem cells (MSC) and poly (lactic-co-glycolic acid) (PLGA) resulting in good interconnectivity. Overall, bioprinting of bone and cartilage tissue can provide a potential alternative to xenogeneic or allogeneic bone grafts.

3.1.1.2. Dental applications. Bioprinting offers a wide array of applications in dentistry, including dental aligners, dental and orthodontic models, direct crowns and bridges, surgical drill guides, flexible gingiva masks, night guards, and denture bases [72]. 3D bioprinting enables the use of digital models to fabricate orthodontic models with accuracy and reproducibility. In orthodontics, high-resolution printing in resin is already utilized practically and commercially. Resins named E-Model and E-Denstone which can be photopolymerised for use in DLP printers have been developed by EnvisionTEC [73]. Similarly, VisiJet M3 Dentcast and Visijet M3 Pearlstone-two UV curable plastic polymers have been produced by 3D Systems [73]. Restorative dentistry is also utilizing similar processes. In maxillofacial and implant surgery, the use of anatomical models produced by bioprinting is on the rise. Bioprinting technology has been applied to develop periodontal regeneration by constructing hierarchical scaffolds, mimicking the physiological properties and architecture of the periodontium, consisting of both soft (gingiva, periodontal ligament) and hard (bone, cementum) tissues [74]. Different biomaterials used for dental applications include ceramics, composite materials, cell aggregates and spheroids, etc. Three-dimensional dentin–pulp complex with patient-specific shapes was developed by Han et al. inducing localized differentiation of human dental pulp stem cells in a fibrin-based hydrogel [75]. The future of implants and teeth will be heavily influenced by bioprinting. Periodontal ligament

stem cells (PDLSCs) in GelMA and poly (ethylene glycol) (PEG) dimethacrylate have been shown to have the potential for periodontal tissue regeneration [76]. Reconstruction of maxillary bone in a canine using a printed PCL/βTCP scaffold was achieved by Kim et al. [77].

3.1.2. Soft tissue

3.1.2.1. Skin. Human skin serves as a barrier to protect against potential physical damage, radiation, water loss, pathogens, toxic chemicals, etc [78]. The skin is composed of three layers, epidermis, dermis, and hypodermis. Epidermis primarily consists of keratinocytes and melanocytes, the dermis is rich in collagen and other ECM proteins excreted by fibroblasts, and hypodermis is predominantly composed of fat tissues [79]. The dermis is the layer containing additional functional structures such as sweat glands and hair shafts [80]. Treatment techniques like split-thickness skin grafts for skin injuries fail to provide complete restoration of sensory and motors neurons as well as structures like hair shafts and sweat glands [81]. Bioprinting also gets an additional advantage for its precision in cell deposition, automation, and standardization [82].

Skin bioprinting can be *in situ*, where the pre-cultured cells are directly printed at the wound site, allowing maturation in the natural environment [83]. The research on *in situ* skin bioprinting in limited. On the other hand, *in vitro* skin bioprinting has been vastly experimented, which achieves maturation of the tissue construct in a bioreactor before transplantation into the wound site [84]. Several bioinks and cell types have been tested by researchers to generate viable, biocompatible, and functional skin substitutes using bioprinting technology.

Lee et al. produced a skin graft utilizing microvalve droplet-based techniques, printing a layered structure using adult human dermal fibroblasts and adult human epidermal keratinocytes in between layers of collagen [85]. The number of cells per droplet was 68 ± 13 for keratinocytes and 93 ± 13 for fibroblasts. The technique was tested on a polydimethylsiloxane (PDMS) mold with 3D surface contours as a target substrate to demonstrate the ability to print and culture multi-layered cell—hydrogel composites on a non-planar surface. The epidermis was dense and formed tight junctions with neighboring cells composing an effective barrier. Further studies confirmed the advantages of bioprinting such as retention of tissue shape, flexibility, and reproducibility [86]. Quílez et al. produced skin tissue using human plasma-derived fibrin matrix populated with human fibroblasts and human keratinocytes [87]. The structure and function were analyzed in immunodeficient athymic mice. Another study by Rimann et al. involved a PEG-based approach using the microvalve droplet system [88]. Alternating layers of PEG-based bioink and fibroblasts were deposited to print a scaffold at 20 °C. Each layer of the bioink was immediately polymerized upon exposure to ultraviolet light. DNA damaged due to UV exposure was quantified by the formation of Cyclobutane Pyrimidine Dimer. However, cell viability before and after printing was similar, indicating insignificant damage to DNA. Over a period of 3 weeks, the cells were fully capable of proliferation and production of ECM. The absence of keratinocytes in the scaffold affected cell distribution and positioning. A comparative study in mice by Yanez et al. showed bioprinted skin tissue to successfully adhere to the wounded site in 14 days whereas a commercial graft dried out and detached from the site [89]. Other bioinks such as alginate, gelatin, fibrinogen, decellularized ECM, or mixtures of multiple biomaterials have also been studied for viability in skin tissue printing [4]. A stem cell-based *in situ* approach has been tested [90] using amniotic fluid-derived stem cells and bone marrow-derived mesenchymal stem cells suspended in fibrinogen-collagen solutions, separately. Even though the procedures showed great promise in coverage and secretion of trophic factors, the printed cells did not integrate with the tissue. Another study demonstrated a 3D bioprinting strategy using an extrusion-based, integrated composite tissue/organ building system (ICBS) [91]. Bioprinting was achieved using fibroblasts and keratinocytes in PCL and collagen-based bioink. Laser-assisted bioprinting techniques have also been successfully tested using alginate bioink, keratinocytes, fibroblasts, and human mesenchymal stem cells [92] and a simple structure using collagen bioink, keratinocytes, and fibroblasts [93].

3.1.2.2. Cardiovascular and cardiac tissue. To promote heart function as well as facilitate cardiac tissue regeneration, the bioprinted cardiac tissue constructs should be mechanically robust yet flexible, contractile, electrophysiologically stable, and most importantly, vascularized [94]. Large tissues and organs incorporate complex vascular networks for optimal oxygen and nutrition supplies, removal of metabolic waste, and therefore sustaining functionality [95]. Researchers have combined general tissue engineering approaches with 3D bioprinting techniques to demonstrate*in vitro* vasculature constructs. The methods include-cell self-assembly to generate vascular constructs, inkjet-based bioprinting of endothelial cells, angiogenic growth factor delivery in bioprinted constructs, coaxial nozzle-assisted extrusion bioprinting of vasculature, and generation of channel-based vascular tissue [96]. The distinct layers of vascular tissue are tunica intima formed with endothelial cells, sub-endothelial fibro-elastic connective tissue, and internal elastic lamina; tunica media primarily consisting of smooth muscle cells and elastin fibers; tunica externa predominantly made of fibro-elastic connective tissue [97]. A scaffold-free, cell self-assembly approach was developed by Norotte et al. using human umbilical vein smooth muscle cells (HUVSMCs), human skin fibroblasts (HSFs), and porcine aortic smooth muscle cells (PASMCs) for small diameter vascular reconstruction. The study was limited by spatial resolution and the thickness of the vascular wall [98]. Another method [99] involved the printing of $CaCl_2$ as a cross-linking agent into a solution of alginate/gelatin to produce different structures like tubes, branched tubes, hollow cones, and capillaries. Inkjet-based techniques utilizing encapsulation of human microvascular endothelial cells (hMVEC) in fibrin hydrogel [100], Sodium alginate ink with and without the inclusion of NIH-3T3 mouse fibroblasts printed into $CaCl_2$ solution [101], and other bioinks and methods have been tested. In 2014, Kolesky et al. developed a multi-head extrusion-based bioprinting technique to print 4 different inks with good cell viability [102]. Several other approaches have been utilized to produce printed vascular tissue [103–108]. Bioprinting of vasculature still remains the most critical part of developing organs for transplantation-ensuring cell alignment, maturation, delivery of oxygen and nutrients, and diffusion of growth factors [109].

The innermost layer of the heart chamber and heart valves is the endocardium, primarily consisting of endothelial cells. The outer sac protecting the heart is the double-layered pericardium. Myocardium or cardiac muscle tissue has cardiomyocytes as a major cellular component [110]. These three major cardiac tissues along with the ECM control the functionality of the heart. Human-induced pluripotent stem cell (hiPSC) technology has popularly been utilized for personalized heart tissue engineering [111,112]. Cells deriving from mesenchymal stem cells [113] and decellularized structures [114] have also been tested for the production of implantable cardiac patches. Gaetani et al. demonstrated high cell viability using human fetal cardiomyocyte progenitor cells (hCMPC) in EBB, bioprinted alginate, and RGD-modified alginate scaffolds [115]. Another study [116] involved a laser-assisted bioprinting technique to pattern human umbilical vein endothelial cell

(HUVEC) and human mesenchymal stem cell (hiMSC) to fabricate a cardiac patch made from polyester urethane urea (PEUU). Xu et al. printed a half heart shape using cardiomyocytes encapsulated in alginate hydrogel bioink preserving cell viability and contraction in relatively thick constructs [117]. In 2016, Zhang et al. developed an endothelialized-myocardium-on-a-chip method for improved vascularization [118]. Various models for heart valves have also been developed and tested [96]. Even though the current studies are only proof-of-concept, the success indicates an encouraging future for heart transplantation and disease modeling.

3.1.2.3. Neural tissue. Small injuries in the neural tissue of the peripheral nervous system (PNS) may heal and regenerate naturally, whereas larger injuries require surgical care. However, the central nervous system (CNS) is much more complex and barely subject to natural recovery [119]. Due to the substantial chemical and physical inhibitors, autologous grafting for tissue regeneration in the CNS has been particularly hard [120]. Inclusion of supportive ECM components, neurotrophic factors, and bioinks for cell adhesion can make 3D bioprinting a suitable technique for neural tissue regeneration [121].

In 2005, Xu et al. utilized thermal inkjet printing for depositing rat embryonic motoneuron cells suspended in phosphate-buffered saline (PBS) achieving moderately high cell viability [122]. The following year further studies were published, conducted with rat primary embryonic hippocampal and cortical neurons as well as an alternate inkjet printing approach of NT2 cells and fibrin gel [123]. The results were mostly positive as the cells maintained their basic function, phenotype, and electrophysiological properties. Cells of the adult rat central nervous system were the first mature neural tissue to be bioprinted with success, using a piezoelectric inkjet printer [124]. A study in an adult zebrafish traumatic brain injury model, Hsieh et al. achieved successful repair of CNS damage using neural stem cells (NSCs) embedded with polyurethane (PU) nanoparticles [125]. A scaffold self-assembly approach with mouse bone marrow stem cells and Schwann cells was developed using an extrusion-based technique by Owens et al. The cells had produced a substantial amount of ECM and over a period of 40 weeks from surgery, the rats recovered both motor and sensory functions [126]. A study of the differentiation potential of human neural stem cells (hNSCs) into neurons was conducted by Zhu et al. after printing the cells using a stereolithography technique with 10% gelatin methacrylamidehydrogel combined with bioactive graphene nanoplatelets [127]. Cell viability, cell distribution, neurites elongation, and neuron differentiation results from this study have indicated an outstanding potential in neural tissue regeneration for clinical purposes.

3.1.2.4. Liver. The liver is involved in essential metabolic functions such as drug metabolism and detoxification [128]. The liver is primarily composed of hepatic lobules and vascular networks. As the function of the liver largely relies on the vascularization, it becomes a major consideration for building transplantable constructs. The liver is predominantly composed of parenchymal hepatocytes. Other cells in the liver include portal fibroblasts, sinusoidal endothelial cells, biliary epithelial cells, hepatic stellate cells, stromal cells, and Kupffer cells [129]. The ECM largely consists of collagen and glycosaminoglycan [130]. In general, the liver has extensive capacity for self-regeneration. However, severe damage caused by specific diseases or injuries requires liver transplantation.

In 2009, Li et al. demonstrated a study of liver tissue regeneration using hepatocytes encapsulated in gelatin, alginate, and chitosan [131]. The same study used adipose-derived stromal tissue in gelatin, alginate, and fibrinogen hydrogel for vasculature construct. Human liver tissue that remained fully functional for up to 28 days was developed using a syringe-based extrusion printer by Robbins et al. [132]. The study had shown great potential for transplantation of bioprinted liver as functions like albumin production, cholesterol biosynthesis, fibrinogen & transferrin production, and inducible cytochrome (CYP) 1A2 and CYP 3A4 activities, were preserved. In 2013, A complete liver model along with complex networks of vascular and biliary structures was successfully bioprinted [133]. Another study employing a laser-assisted LIFT technique for precise deposition of hepatocytes onto a porous collagen-glycosaminoglycan scaffold showed significant potential for clinical use [134]. A comparative study between single-cell dispersion of iPS-derived parenchymal cells and using iPS-derived hepatocyte-like cells spheroids, both in combination with non-parenchymal cells [135]. The study demonstrated the advantages of spheroid-based bioprinting for liver tissue construction. Ahn et al. had utilized preosteoblast cells, human adipose-derived stem cells, and alginate, cultured using a hepatogenic medium for successful expression of liver-specific genes ALB and TDO2 over a period of 27 days. Ma et al. have produced a hexagonal 3D liver model using a mixture of GelMA and hyaluronic acid with human-induced pluripotent stem cell-derived hepatic progenitor cells (hiPSC-HPCs). Human umbilical vein endothelial cells and adipose-derived stem cells were used as support at spaces in-between hexagons [136]. Several other efforts have been taken to regenerate liver tissues using bioprinting techniques [137].

3.1.2.5. Endocrine glands. Whereas the nervous system deals with quick, short term responses to control physiological activities, the endocrine system regulates long-term metabolic activities. Endocrine cells of the endocrine glands release hormones to transfer information between cells and control metabolic activity [138].

The thyroid gland secretes thyroid hormone which controls the basal metabolic rate, controls the growth and development of tissues, and regulates blood pressure [139]. Bulanova et al. bioprinted a functional vascularized mouse thyroid gland construct from embryonic thyroid spheroids and allantoic spheroids in collagen hydrogel using a self-assembly approach [140]. Right behind the thyroid gland, four small glands collectively called parathyroid glands monitor and regulate blood calcium levels [141]. Tonsil-derived mesenchymal stem cells (TMSC) were demonstrated by Park et al. to have the capability of differentiating into functional cells and tissues of the parathyroid glands [142]. Further work has been carried out for the development of parathyroid tissue [143,144].

The endocrine component of the pancreas consists of islet cells. Two major types of cells, alpha & beta, produce the hormones glucagon and insulin, respectively [145]. Both of these regulate blood glucose levels. Hormones secreted by other hormone-producing cells of the pancreas include somatostatin, vasoactive intestinal peptide, substance P, gastrin, pancreatic polypeptide, ghrelin, etc. [139]. The use of 3D bioprinting for the encapsulation of pancreatic islets has the potential for therapeutic use in a transplantable level [146].

Other endocrine glands have also been tested for regeneration using bioprinting techniques. For example, Leydig cells of gonad or the reproductive organ have been studied for encapsulation and transplantation using biocompatible polylactic acid and polyvinylpyrrolidone-based plasma for increased vascularization [147]. More studies for the bioprinting of gonad have utilized theca and granulosa cells in micro-molded agarose gel [148], ovarian follicles [149], etc. Among others, adrenal glands have also studied for tissue engineering using bioprinting [150,151].

3.1.2.6. Other tissues and organs. Bioprinting technology has been evaluated for implementation in the regeneration of many other

types of soft tissues and organs. One such example is **skeletal muscles and tendons** which provide structural support and contribute in motion. Skeletal muscles are essentially made of myoblasts whereas tendons mainly consist of collagen ECM and a few embedded tenocytes [152]. Various biomaterials have been utilized by different research groups for the development of such tissue [97,153]. Renal tubular tissue of the **kidney** has also been studied for construction using bioprinting [4,154]. Studies for the development of **ear, nose, and throat** tissues have also been carried out [73]. Currently available procedures for the transplantation of human corneal tissue are limited by the quality of visual recovery. Several studies have been conducted for the development of **corneal tissue** [155]. Other types of tissues under research for bioprinting include **airway** [156–158], **lungs** [159], **adipose tissue** [57,160], etc.

3.2. Drug delivery and screening

In regenerative medicine, bioprinting technology has been employed to develop tissues and organs as well as to deliver growth factors and other essentials for vasculature formation. A similar technique can be utilized for the delivery of drugs as an alternative to the oral delivery of conventional medication. The bioinks or hydrogels used for bioprinting have the potential to hold great amounts of drugs and growth factors while releasing them at a relatively slow rate at the target site. It also holds the ability to produce personalized drugs. Crosslinking of the biomaterials plays a significant role in drug release [161]. Tappa et al. applied an extrusion bioprinting technology to print patient-specific intrauterine devices loaded with estrogen and progesterone in different sizes and designs [162]. For this study, PCL pellets were loaded with the hormones enveloped in silicone oil. Martinez et al. fabricated ibuprofen-loaded hydrogel-polyethylene glycol diacrylate (PEGDA) and polyethylene glycol (PEG300) [163]. A printing process was developed by Genina et al. for the combination therapy of two drugs-rifampicin and isoniazid. The results have shown improved treatment efficacy [164]. In addition to drugs and growth factor delivery, bioprinting can potentially provide an efficient and viable medium for gene therapy [165].

3D bioprinting allows the construction of reliable models of human tissues that can mimic natural conditions, which makes it ideal for the development of drugs through drug screening and toxicology analysis in bioprinted tissue models. In order to obtain an accurate architecture and environment in the tissue model, cell types & origin, biomaterials & hydrogel, and printing techniques have to be carefully selected according to the original site of drug delivery [166]. To test drug efficacy and toxicity, several experimental tissue models with different cells, ECMs, and architectures have been developed. Some of these have also been commercially implemented. The influence of differentiation factors on chondrogenicity in cartilage tissue development has been studied using inkjet printing with primary human articular chondrocytes poly (ethylene glycol) dimethacrylate (PEGDMA) hydrogel [167]. In another study, cell-laden hyaluronic acid-PEG microfibers were printed onto a Matrigel matrix for the analysis of cellular behavior and interactions in different cell types upon treatment with a Rho-associated protein kinase (ROCK) inhibitor Y27632 and cadherin antibody [168]. Angiogenic-specific gene CD105 activity was found to be downregulated when exposed to Y27632. Additionally, hMSCs showed higher expression of angiogenic markers such as CD31 or CD105. Further studies have been conducted by the same research group on ROCK inhibition [169]. A scaffold-free liver tissue model was constructed using primary hepatocytes, stellate cells, and endothelial cells for experimental assessment of the toxicity of two drugs-levofloxacin and trovafloxacin [170]. The printed tissue maintained cell viability for up to 42 days while developing microcapillaries, liver proteins like albumin, fibrinogen.

3.3. Tumor modeling

Creating a tumor microenvironment through 3D bioprinting in which tumor cells interact with adjacent stromal cells such as endothelial cells and fibroblasts and an extracellular matrix, serves as a precise model for studying tumor progression, monitoring, and drug response. The conventional approach for tumor or cancer modeling is through either monolayer cell cultures or in animals. However, monolayer cultures lack the complex interactions occurring in natural tissue. Animal models on the other hand may differ from the behavior and response of human tissue. Intracellular molecular interactions, intercellular interactions, enzyme kinetics, changes in protein expression, metastatic progression, etc. [171] can be interpreted using a bioprinted tumor models for specific tissues. Zhou et al. studied the metastasis of breast cancer in bone tissue by developing biomimetic bone constructs through a table-top commercial stereolithographic printer. The study aimed to produce a cell-laden 3D structure of bone tissue and observe the interactions after breast cancer cells (BrCas) were seeded, *in vitro* [172]. Another study bioprinted a mini-brain to demonstrate the interactions between Glioblastoma-associated macrophages and glioblastoma cells during the progression of glioblastoma multiforme [173]. The results of this study indicated that therapeutics can be developed to inhibit the interactions between these cells in order to reduce tumor growth.

One of the major complexities associated with cancer modeling is recreating the angiogenesis of tumor cells due to the lack of vascular networks. Angiogenesis is a key step in cancer progression as the transportation of nutrients and oxygen to the cells is essential [189]. As discussed before, vasculature can be developed using bioprinting techniques with the right bioinks& growth factors can be introduced for further maturation. Potentially, To understand cell–cell interactions and the influence microenvironment factors in glioblastoma multiforme, Lee et al. printed a physiological glioma-vascular niche model using human endothelial cells and patient-derived GBM cells in a collagen matrix [176]. They developed fluidic vascular channels with collagen and gelatin. A recent work by Suarez-Martinez et al. observed cancer cell migration, proliferation, and function during microvascular network growth in a bioprinted tumor microenvironment model of mouse breast cancer cells [190]. Another recent work by Hynes et al. [191] provided a new approach to comprehend cancer metastasis through the development of a bioprinted hydrogel-based vascular flow device.

Commonly used drugs for the treatment of cancer often come with side effects such as bone marrow suppression, gastrointestinal reaction, digestive system toxicity, liver toxicity, urinary system toxicity, renal toxicity, cardiotoxicity, and neurotoxicity [166]. Bioprinted breast cancer cells encapsulated in the peptide-conjugated alginate fibers and macrophages within the channels were subject to heterotypic interactions drugs to evaluate drug efficacy and toxicityb [181]. Another bioprinted model, *HER2*-positive breast cancer cells encapsulated in adipose-derived mesenchymal stem cells (ADMSCs) were used to assess doxorubicin and study drug resistance [192]. A biomimetic ECM consisting of MSC-derived mammary fibroblasts, endothelial cells, and adipose cells was used to evaluate the chemotherapeutic effects of tamoxifen in breast cancer [193]. The drug response was assessed byadenosine triphosphate (ATP) luciferase assay. Anti-cancer drug temozolomide (TMZ) and angiogenic inhibitor sunitinib were assessed in a tumor microenvironment printed by Han et al. The printed tissue contained multicellular tumor spheroids of glioblastoma cells on a

blood vessel layer consisting of fibroblasts and endothelial cells [194]. In another recent study, a Hepatocellular Carcinoma Cell model was constructed by Sun et al. using HepG2 cells with the aim of drug pharmacodynamics research [195].

As the bioprinting techniques develop further, producing more complex biological systems and studying disease interactions not only on a cellular level but also on the surrounding tissue will soon be possible [196].

4. Bioprinting: present and future

4.1. Recent advances and challenges

One of the most promising advances in 3D bioprinting has to be the several approaches for the development of microvascular networks in printed tissues and organs. Human tissue and organs are part of a complex system that requires the flow of blood and lymph, nutrients and oxygen, and the removal of metabolic waste. The experimental small scale studies can only be useful in practical human transplantation through the maturation of proper vascular networks. The established and ongoing studies on vasculature development pave the way for surgical transplantation of bioprinted tissues and organs. One prominent example for that would be the "CT-bone" which is a 3D printed bone made out of calcium phosphate which is the natural makeup of bones. The CT bone can be specifically made for people with facial deformities due to trauma, diseases or cancer. The artificially created CT-bone can be transplanted to human to augment their deformities without any lose of function or other issues and this process is termed the maxillofacial reconstruction [197]. Moreover, important factors in cancer progression such as angiogenesis are also being analyzed using tissue models with microvasculature. Printing in high resolution is being focused to achieve improved vascularization [198]. In addition, Bioink compositions, cross-linking, bioprinting processes, and design specifications are being investigated by researchers for better control of shape and retention of function [199]. In a recent study, to improve cell survival for transplantation, researchers have developed a GelMAbioink utilizing calcium peroxide for controlled oxygen release [200]. This shows great promise in enhancing cell viabilities and aiding in vascularization. Bioprinting is taking another step forward as studies progress towards the popularization of *in situ*/*in vivo* bioprinting. *In vivo* printing is the minimally invasive direct bioprinting at injured or affected sites in living tissues and organs [201]. *In vivo* bioprinting is still in an emerging state, however, studies have been conducted for the regeneration of skin, bone, and cartilage *in vivo*. Chen et al. developed a non-invasive technique for *in vivo* bioprinting of skin tissue [202]. An *in situ in vivo* bioprinting concept was proposed by a research team, using the microbots approach [203]. In the study,a subminiature bioprinter was developed by constructing a Delta robot, which can be endoscopically installed to repair gastric wounds. This innovative and futuristic study truly paves the way for future research in the field. Organ-on-a-chip systems utilizing 3D bioprinting are gaining popularity for the construction of physiological models [204,205]. These are small-scale models mimicking tissue and microvascular networks. Bioprinting-based organ-on-a-chip can be a viable means for drug testing. More recently, techniques for 4D bioprinting is freshly gaining attention. 4D bioprinting method utilizes 3D printing techniques but expands the biomedical applications as the constructs can change with time through external stimuli [206]. Another emerging field in the applications of bioprinting is soft robotics [55].

However, advances in technology often come with more challenges. One of the major challenges is the discrepencies that exist in the application of bioprinting in small animals and large animals. Firstly, one of the limitations that the transplantation of the tissues fabricated from bioprinting are limited to small animals. The possibility of the bioink's molecular properties contradicting with that of the large animal morphology is completely plausible. For example, currently, translation of the bioprinted products and their transplantation is restricted to murine models which are physiologically differentfrom humans. Therefore, the transplantations that successful in the murine models may not be scalable and hence successful in the human trials. This is seen where transplantation of pancreatic tissues containing bioprinted islets are successful in regulating insulin secretion in diabetic murins which have an approximate amount of 10,000 times smaller pancreas than that of a human pancreas. Therefore, the main challenge lies in the scalability and the concurrent changes that may occure due to upscaling [207]. On the other hand, another challenge remains in the development of appropriate **bioinks** to coordinate cell–material interactions. Balancing the printability of bioink with cell viability and function for precise and accurate model construction is crucial. Mechanical, structural, and geometrical properties of the tissue as well as biological activities of the tissue need to be preserved in the bioink [40]. For that purpose, a combination of natural and synthetic polymers is often tested whereby synthetic polymers can provide structural integrity and natural components mimic the biochemical environment. Shear stress can affect the stability of the bioink thus the selection of techniques requires sensitive protocols. Alongside all of these considerations, bioinks must be biocompatible and biodegradable to avoid long term reactions to residual components in tissue engineering applications [19]. Processes like SLA and laser-assisted bioprinting provide high resolution, however, the biomaterial options are limited. The development of next-generation bioinks addresses some of the common concerns associated with biomaterial selection [208,209]. Another challenge is **cell sourcing** for the construction of bioprinted tissue. To achieve the heterogeneous tissue construct through bioprinting, primary cells are often pre-harvested *in vitro* [210]. Applications concerning transplantation seek the primary cells from the patient to prevent an immune response. For the practical implementation of *in vivo*tissue bioprinting, integration between native and engineered tissue is imperative [211]. That can be achieved by the introduction of **growth factors** and **vascular network** development. Replicating the complex hierarchical structure of vascular networks is still under development. Following the current progress, it may be possible to generate capillary beds or large vessels for integration during tissue implantation. **Processing time, scaling up, automation,** and **cost-effectiveness** (e.g. laser-assisted bioprinting), etc. still need improvement [212,213]. Apart from these technical challenges, 3D bioprinting also faces several **ethical concerns** relating to its possible impacts on humans [214].

4.2. Future opportunities

The future of regenerative medicine and healthcare will be heavily influenced by 3D and possibly 4D bioprinting. In the near future, human-scale &stable tissue and organ transplantation may be clinically applied with the incorporation of proper vascular networks, tissue environment, cell viability, biocompatibility, and functionality. Moreover, through the implementation of 3D bioprinting, there can be a paradigm shift in conventional pre-clinical and clinical drug trial procedures. Furthermore, based on the organ-on-chip models, human-on-a-chip models may be developed to evaluate drug reactions in more than one organ at a time [215]. This could also provide a better understanding of interconnected vascular networks and system functions. For stronger interpretations of the tests using bioprinting, advanced 3D

microscopy, genetic and protein assays, culture systems such as bioreactors, and noninvasive monitoring systems need to be improved. In the future bioprinting may also aid diabetic patients through printing pancreas islet tissues overcoming the barriers of immune responses [216]. Besides, pharmacodynamics and drug toxicology studies may set the scene for personalized medicines and therapies. By means of the construction of human-on-a-chip, a superior understanding of the motor functions of the nervous system could be possible in time. Neurodegenerative diseases like Alzheimer's disease, Amyotrophic Lateral Sclerosis, and Parkinson's disease could be studied more thoroughly to develop treatment options in the fullness of time. Further advancement of electrical signal transformation in hydrogels may aid in composing a more comprehensive study of the diseases of electrically active organs. Overall, the possibilities are endless with using bioprinting for developing human tissue and organs, understanding disease mechanisms, evaluating drugs and treatments, etc. Non-clinical applications of bioprinting may also be significantly rising, for example, in the development of soft robotics.

5. Conclusion

Bioprinting is a rapidly expanding area of tissue engineering and regenerative medicine that targets to mimic the intricacies of natural tissues and networks. Significant development of groundwork has laid the foundation for the employment of 3D bioprinting in healthcare and therapeutic use. Starting from the regeneration of skin and bone to the successful experimental reconstruction of organized contractile tissues like skeletal muscle tissue, cardiac tissue, complex organs like liver, kidney, etc. establishes the potential of bioprinting. More research is under progression to overcome challenges such as biomaterial selection, bioink design, printing method optimization, enhanced vascularization, and scaling up. With further improvement of the controlled spatial distribution of cells, mechanical properties, and stability, this technology will create greater opportunities for understanding organ systems, producing transplantable tissues and organs, drug development & screening, and cancer research. Subsequently, biomedical science as we know it may be restructured.

Declaration of competing interest

The authors have declared no conflict of interest.

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