Chapter

3D Printing for Tissue Regeneration

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Abstract

Tissue engineering is an interdisciplinary field and 3D bioprinting has emerged to be the holy grail to fabricate artificial organs. This chapter gives an overview of the latest advances in 3D bioprinting technology in the commercial space and academic research sector. It explores the commercially available 3D bioprinters and commercially printed products that are currently available in the market. It provides a brief introduction to bioinks and the latest developments in 3D bioprinting various organs. The chapter also discusses the advancements in tissue regeneration from 3D printing to 4D printing.

Keywords: 3D bioprinting, bioink, tissue engineering, regenerative medicine, 4D bioprinting, scaffold

1. Introduction

Tissue engineering is a branch of biomedical engineering that focuses on repairing and/or replacing diseased and damaged organs. This is done primarily via developing artificial organs using natural or synthetic materials. Organ shortage is a severe problem worldwide due to the non-availability of donors and tissue engineering strategies enable to produce a scaffold that mimics the organ of interest [1]. Three-dimensional (3D) bioprinting has great potential in this field and was developed in the early 1990s, and has evolved ever since. It is an additive manufacturing (AM) technique that uses computer-aided design (CAD) models to deposit biomaterials on the substrate along with living cells, extracellular matrix (ECM) components, biochemical cues, and drugs. The three basic steps in the 3D bioprinting process are: (i) preprocessing - includes developing CAD models to develop in-vitro scaffolds or to develop organ blueprints from imaging modalities such as computer tomography (CT) and magnetic resonance imaging (ii) processing - produces a physical structure that mimics the organ/tissue of interest from the designed model (iii) postprocessing - improves the bioprinted organ model and scope for transplantation if required. Over recent years, there has been a huge demand and interest in 3D bioprinting due to its potential to produce high-throughput biomimetic organ scaffolds. Several technological advancements have come up in 3D bioprinting which are mentioned in **Figure 1.** The goal of 3D bioprinting is to provide alternative approaches to autologous and allogeneic implant treatments and avoid animal testing in drug studies and disease models. 3D bioprinting has several biological applications in the fields of

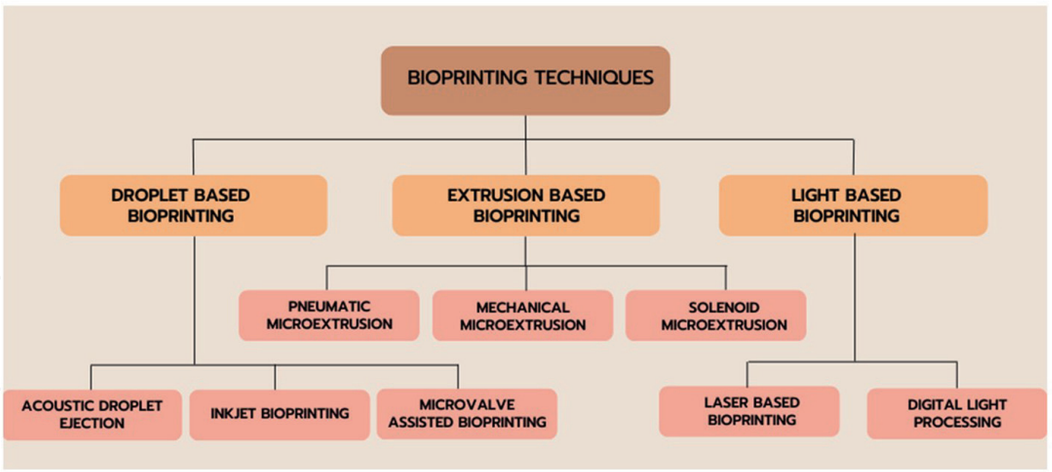

Figure 1.
Various classifications of 3D bioprinting.

tissue engineering, materials science, pharmaceutical drug development and validation, cosmetics testing, personalized medicine, regenerative medicine, cell-based biosensors, and bionics.

2. Commercial 3D bioprinters

A 3D bioprinter is an automated device that enables the development of functional tissue and organ models. The 3D bioprinting technology is generally classified into three types – (i) droplet-based bioprinters (ii) extrusion-based bioprinter (iii) light-based bioprinter (**Figure 1**). Extrusion-based bioprinters are widely used and are based on the principle of depositing the material layer by layer. Laser-based 3D bioprinters deposit the bioink drop by drop, the principle is like an inkjet 3D bioprinter. Some companies and universities have developed 3D bioprinting technologies that cannot be easily classified into widely known technologies. For example, Cyfuse Biomedics has developed a technique where cells are 3D printed on a needle array. A scaffold is not required in this method instead only a cluster of cells (not mixed with other biomaterials) are skewered onto vertical needles to fabricate 3D tissue structures. Companies like rainbow biosciences have developed a bioprinter called BiOassay where biocompatible magnetic nanoparticles are used to print the 3D structures and use the working principle of magnetic levitation [2]. There are many emerging bioprinting technologies being developed by researchers across the world to make the process more efficient and cost-effective. Currently, the 3D-printed organs can be used for research only; however, in future, they can be transplanted into human patients.

The wide range of applications has driven many companies/universities to develop bioprinting technology. The following are the types of business models utilized by these companies that exploit bioprinting technology – (i) Manufacturing bioprinters (ii) Providing bioprinting services (iii) providing cell therapies that utilize bioprinting technology. Commercially available 3D bioprinters have increased in the market over the past decade and have rapidly advanced the tissue engineering field. The 3D Bioprinting Market is expected to reach USD 3261.31 Million by 2027, from USD 796.9 Million in 2020 growing at a compound annual growth rate of 22.3% during

2021–2027 [3]. The growth of this market is due to a limited number of organ donors, and an increase in the aging population with chronic diseases. The rise in R&D investment in this sector, advancement in commercially available products, and increment in the incidence of chronic diseases are other vital factors that are likely to boost market growth during the coming years [4]. **Table 1** provides a list of commercially available bioprinters in the market.

3. Formulation of bioinks

In bioprinting, cells are placed at user-defined coordinates, along with biomaterials that are either (i) mixed with cells before printing, or (ii) printed simultaneously with one print head while the cells are deposited via the other print head (**Figure 2A** and **B**). Materials used in bioprinting that contain cells in the mixture are termed as bioink. The biomaterial is usually a polymer (natural or synthetic) that has biocompatible components and provides favorable rheological properties for the desired organ of interest. Hydrogels are the most used bioinks. However, hydrogel precursors are widely in use these days as they can be cross-linked into hydrogels post-biofabrication. Another method is to crosslink the precursor solution to obtain a viscous ink, followed by crosslinking the scaffold post-printing [5].

An ideal bioink should have the desired physicochemical properties to print mechanically stable scaffolds which mimic the organ of interest (**Figure 2C**). These properties are determined by the mechanical strength of the scaffold, viscosity of the ink, chemical structure of the polymer, and biological characteristics of the desired tissue. These properties should lead to: (i) mechanically stable scaffolds, that have the mechanical strength similar to the native tissue (ii) adjustable rheological properties (gelation, viscosity) to help in ease of bioprinting the constructs while retaining the desired structural shape (**Figure 3**); (iii) biocompatibility, biodegradability if required; (iv) not be cytotoxic to be suitable for in vivo studies and possible transplantation in future; and (v) large scale reproducibility of the ink [6]. Optimizing the bioink formulation is a vital step toward successful bioprinting and this is represented in **Figure 4** in the form of a flowchart. **Table 2** provides a few examples of different polymers that have been used in the formulation of bioinks.

4. 3D bioprinting for hard tissues

4.1 Bone

Bone is a complex tissue that has mechanical, metabolic, and hemopoietic functions. It provides structure and function to the surrounding tissues. Currently, bone defect repairs are treated by grafts: Autologous grafts, allografts, and synthetic grafts. Alternative methods like cadaver allografts and xenografts are available but they have poor biological properties like lower biocompatibility and risk of infection. Osteoconductive properties are seen in synthetic grafts but are degraded by osteoclasts and hence are suitable for small defect repairs only. Bone tissue engineering offers solutions to treat bone defects and one effective way is via 3D bioprinting. However, a major concern is to provide a solution that overcomes the challenges faced in conventional treatments by improving osteoinduction and osteoconduction. Studies have shown that 3D bioprinted bone constructs avoid the possibility of immune rejection which was

Sl. No	Company name	Model	Technology	Country	Website
1	Cellink	INKREDIBLE™ Series, BIO CELLX, BIO X™, BIO X6™	Extrusion based	Sweden	https://www.cellink.com/
		BIONOVA X, LumenX+™, Quantum X bio	Light-based		
2	Allevi	Allevi (1,2,3)	Extrusion-based	United States	https://www.allevi3d.com/
3	Advanced Solutions Life Sciences	BioBotBasic, BioAssemblyBot (200,400,500)	Extrusion-based	United States	https://www.advancedsolutions.com/
4	RegenHu	R-GEN (100,200)	Extrusion-based	Switzerland	https://www.regenhu.com/
5	Rokit Healthcare	Dr.INVIVO (4D2, 4D6)	Extrusion-based	South Korea	https://rokithealthcare.com/?ckattempt=1
6	Fluicell	Biopixlar, Biopixlar AER	Extrusion-based	Sweden	https://fluicell.com/
7	Envision Tec	3DBioplotter (started, developer, manufacturer) series	Extrusion-based	United States	https://etec.desktopmetal.com/
8	Inventia Life Science Operations	RASTRUM™	Digital bioprinting	Australia	https://inventia.life/
9	3D bioprinting Solutions	Fabion, Fabion 2	Extrusion-based	Russia	https://bioprinting.ru/
10	Poietis	NGB-R Bioprinter	Extrusion-based, laser-assisted, micro-valve bioprinting	France	https://poietis.com/
11	Organovo	NovoGen Bioprinter®	Extrusion-based	United States	https://organovo.com/
12	nScript	The BAT Series	Extrusion-based	United States	https://www.nscrypt.com/
13	Cyfuse Biomedics	Regenova	Extrusion-based	Japan	https://en.cyfusebio.com/
14	SunP biotech International	BIOMAKER, ALPHA-CPT1, ALPHA-BP11	Extrusion-based	United States	http://sunpbiotech.com/

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Sl. No	Company name	Model	Technology	Country	Website
15	Next Big Innovation Labs	TRIVIMA	Extrusion-based	India	https://nextbiglab.com/
16	Axolotl Biosystems	Axo A3, Axo A6	Extrusion-based	Turkey	https://www.axolotlbio.com/
17	Brinter	Brinter®One	Extrusion-based	Finland	https://www.brinter.com/
18	GeSiM	BS5.3/E, BS5.3, BS3.3, BS3.3 Prime	Extrusion-based	Germany	https://gesim-bioinstruments-microfluidics.com/
19	Regemat 3D	BIOV1, REG4LIFE	Extrusion-based	Spain	https://www.regemat3d.com/
20	CLECELL	U-BIOLET™, U-BIOXT	Extrusion-based; laser-assisted	South Korea	https://www.clecell.co.kr/
21	UpNano	NanoOne Bio	multiphoton lithography	Austria	https://www.upnano.at/

Table 1.
List of commercially available 3D bioprinters.

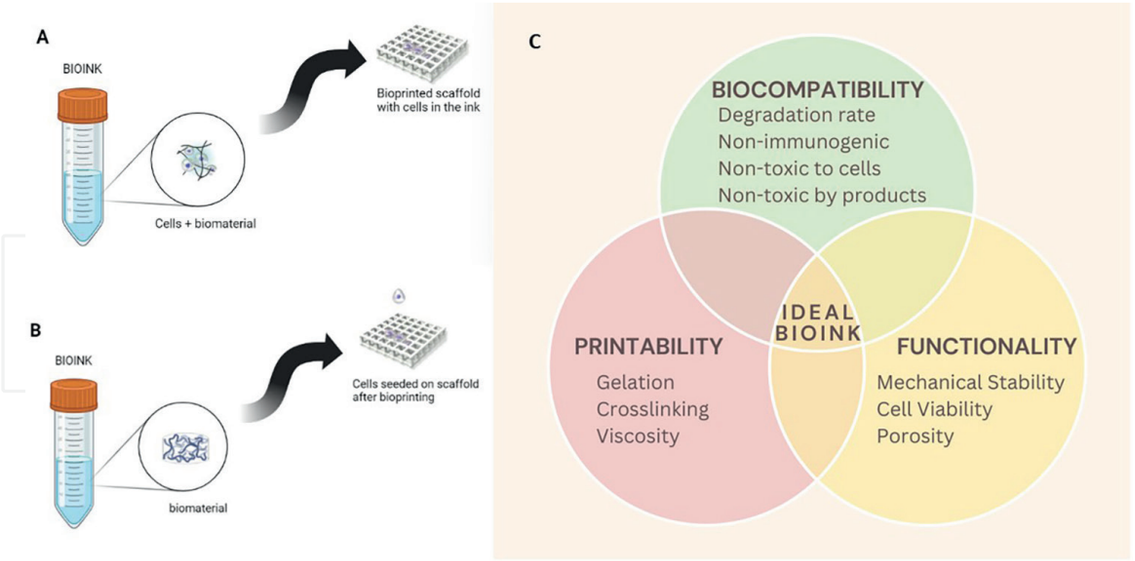

Figure 2.
(A) Bioprinted scaffolds with cells in ink (B) cells seeded on scaffolds after bioprinting (C) properties to consider for the formulation of an ideal bioink.

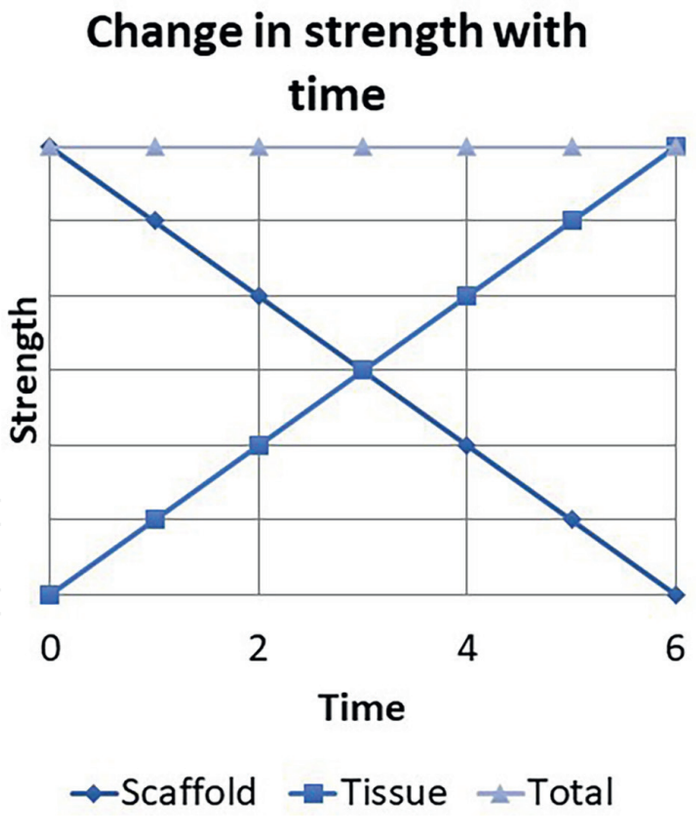

Figure 3.
Ideal strength and degradation profile of a bioprinted scaffold.

observed earlier in the use of grafts which may have otherwise led to inflammation, fibrosis, scarring, and transplant failure. The advantage of 3D bioprinting over the current grafting technique is that the cells are spatially distributed within the construct, thus optimizing tissue regeneration. 3D-bioprinted bone tissues have a huge impact

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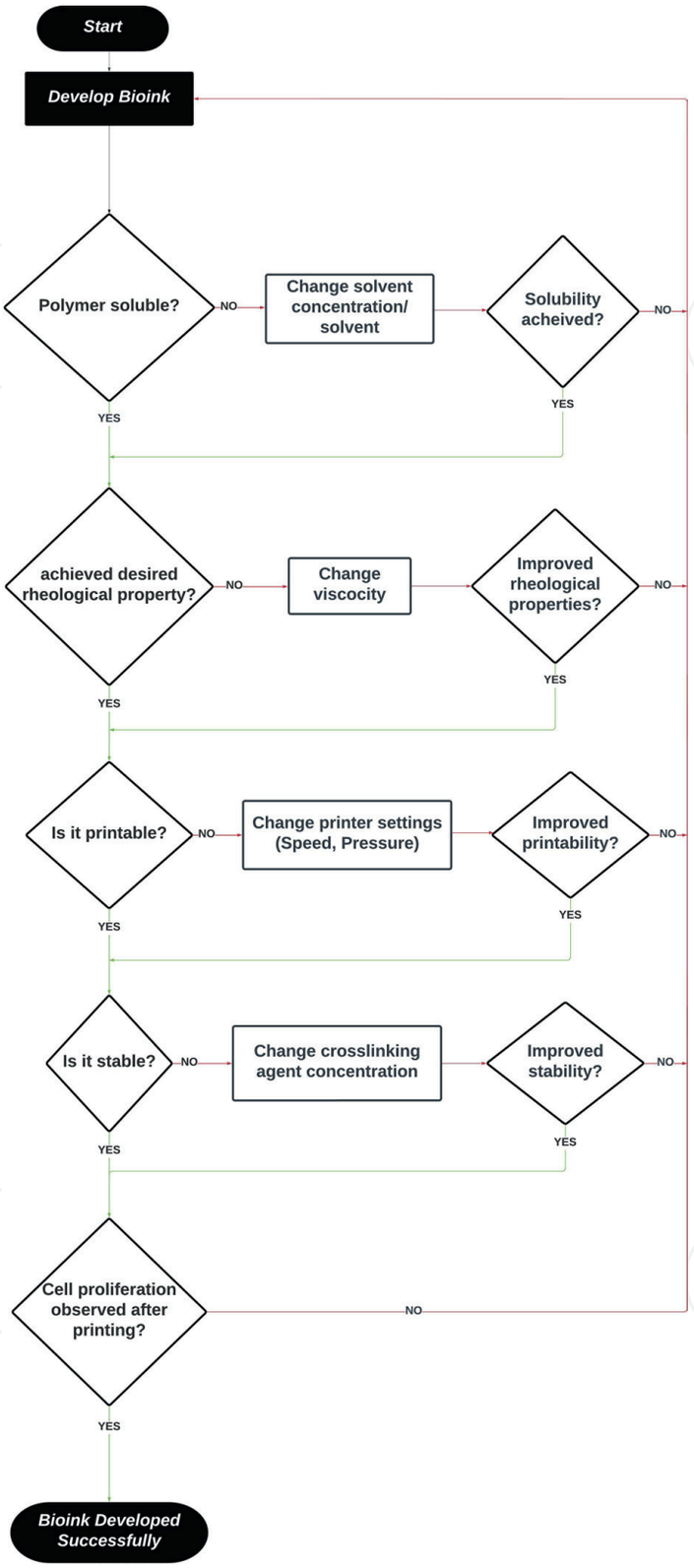

Figure 4.
Flowchart depicts the process of bioink development.

on clinical practice as it makes reconstruction of bone defects with complex shapes efficient and less time-consuming, by translating defect data from image modalities like CT to CAD designs which make it possible for patient-specific bioprinting. The ideal

Polymer origin	Constituent materials	Constituents of polymers
Natural Polymers	Polysaccharides	HA[1], dextran, chitosan, agarose, Alginate
	Decellularized Extra Cellular Matrix	dECM[2]
	Proteins	Collagen, lysozyme, silk, Matrigel™, gelatin, fibrin
Synthetic polymers	Biodegradable synthetic polymers	PCL[3], PLA[4], PLGA[5], methacrylated HA, GelMA[6]
	Non-biodegradable synthetic polymers	HEMA[7]
	Bioactive synthetic polymers	Proteins, peptides, carbohydrates, PLA
Hybrid polymers	Synthetic polymer, modified natural polymers	Pluronic® 127/carboxymethyl hexanoyl chitosan
	Synthetic peptide-modified proteins or polysaccharides	Glucose, Gluconic acid
	[9]PNIPAAm modified polymers	PNIPAAm/Collagen, PNIPAAm/Chitosan, and PNIPAAm/Alginate
	[10]PEG-modified natural polymers	Heparin, dextran, HA, fibrinogen, and albumin, HAP[8]

[1]*HA- Hyaluronic acid.*
[2]*dECM - decellularized extra cellular matrix.*
[3]*PCL-Polycaprolactone.*
[4]*PLA -poly lactic acid.*
[5]*PLGA- poly(lactide-co-glycolide).*
[6]*GelMA-methacrylated gelatin.*
[7]*HEMA- 2-Hydroxyethyl methacrylate.*
[8]*HAP- Hydroxyapatite.*
[9]*PNIPAAm- Poly(N-isopropylacrylamide).*
[10]*PEG- Polyethylene glycol.*

Table 2.
Polymers used in bioinks.

scaffold should mimic the bone structure and composition, have a good resorption rate, allow for vascularization, and have a higher bone healing/formation ability compared to ceramics and metals. Fabrication of bone constructs with various geometries, porosity, and sizes, which are specific to each patient's features is possible via 3D bioprinting. It also helps to fabricate osteoinductive scaffolds [7].

Bioink is necessary to bioprint bone and it should have good mechanical strength without losing cell viability and bioactivity. Bioinks can be classified in three categories- (i) first generation – materials that are bioinert and biocompatible. Chances of rejection are minimized in this case. The scaffold remains in vivo to provide mechanical support and does not degrade, e.g., metals (stainless steel and titanium) and polymers (ii) second generation – materials that are biocompatible and bioactive simultaneously. They allow mineralization and biodegradation over time so that the cells can replace the scaffold. (iii) third generation – bioresponsive materials. They contain growth factors and stimulatory molecules that trigger osteoblast differentiation including bone morphogenetic proteins (BMP) and fibroblast growth factors (FGF). A composite bioink is most beneficial for use since it combines the best of all three generation of bioinks i.e., a balance between mechanical and functional properties is obtained to meet the needs of the desired tissue [8].

Bone regeneration requires osteoinductive cues which include growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), bone

morphogenetic proteins (BMPs), parathyroid hormone, and platelet-derived growth factor (PDGF). Growth factors are delivered in 3D-printed bone scaffolds during or after the printing process. The biochemical factors can be delivered on already printed scaffolds by adding them on the top surface after printing or within the micropores during printing. These osteogenic factors help in enhanced cell proliferation, differentiation, and angiogenesis. Bioinks were formulated using BMP-2 loaded PLGA nanoparticles, alginate, and mesenchymal stem cell (MSC). Composite bioink showed enhanced printability and yielded stable constructs post-printing. Sustained in vitro release for up to two weeks was observed in BMP2-loaded PLGA nanoparticles and was also noted to induce osteogenesis of the MSCs [9]. Bone healing is linked to the relationship between blood vessels and bone cells. It is known that VEGF is released during fracture healing. VEGF inhibition has been shown to interfere with fracture repairs and bone defects. However, it was not sufficient to heal large defects. Furthermore, it was observed that VEGF did not drive progenitor cells toward the chondrogenic or osteogenic lineage. Hence, combination therapies with BMPs are being developed to advance the regeneration of large bone defects. VEGF and BMP-2 were delivered to enhance the regeneration of large bone defects. The release of these growth factors was studied by 3D bioprinting alginate-based bioinks with nanoparticles. The bone formation was enhanced in vivo by slowing the release of BMP-2. Enhanced vascularization was found in vivo when VEGF was introduced in the study. Accelerated large bone defect healing was observed in this case using 3D-printed implants containing VEGF and BMP-2 [10].

Osteoinductive materials are preferred for studies in bone regeneration as they enhance regenerative properties. A study has shown that the NICE bioink (7.5% methacrylated gelatin, 1% kappa carrageenan, 2% nano silicates) showed both high print performance and enzymatic degradability. This bioink provided cell friendly microenvironment for bone bioprinting [11]. Jakus et al. developed a bioink composed of 90% HAP and 10% PCL or PLGA. It was found that the bioprinted scaffolds promoted osteogenic differentiation without additional biochemical factors. Higher biocompatibility, tissue integration, vascularization, and mineralization were observed in animal studies with no immune rejection [12].

Cell adhesion, viability, and metabolism are influenced by a scaffold's internal architecture like pore size and shape which in turn affect the bone regeneration capacity. A major challenge in developing a scaffold is to obtain a balance between the mechanical strength of the scaffold and mimicking the native strength of the tissue. A comparative study was done in the internal architecture of the scaffold- (i) continuous pattern (ii) zigzag-Spiral pattern, in treating bone defects treatments. It was found that the printed scaffolds showed characteristics like that of a native bone – permeability, porosity, and mechanical properties which are owed to the microarchitecture of the scaffold design. Human mesenchymal stem cells seeded scaffolds determined the effects of geometrical microstructure on cell attachment and morphology. The cells in the scaffold with a zigzag pattern infilled pores quickly in comparison with the other pattern [13]. **Tables 3** and **4** outline the latest developments in 3D bioprinting of bone in-vivo and in-vitro studies respectively.

4.2 Teeth

Teeth are the hardest part of the human body and have limited capacity for repair and regeneration. The gold standard treatment for permanent tooth loss by disease, defect, or injury has been dental implants. Titanium alloys are widely used to

Sl. No	Animal model	Material	Printing method	Cells	Growth factors	Time (weeks)	References
1	Rat	Fibrin, GelMA, Y-irradiated RGD modified Alginate	Extrusion based	Human umbilical vein endothelial cells, human bone marrow stem cells	VEGF	2	[14]
2	Rat	HAP, PLGA	Extrusion based	[1]C3H/10 T1/2 cells, human patient-derived osteoblasts	—	12	[15]
3	Rat	GelMA, Calcium silicate nanowires	Extrusion based	Bone mesenchymal stem cells, Schwann cells	—	8	[16]
4	Rabbit	Silk fibroin	Extrusion based	Bone marrow-derived mesenchymal stem cells	BMP 2, [2]TGF-β	12	[17]
5	Rat	Calcium phosphate-based materials	Extrusion based	Rat mesenchymal stromal cells	—	12	[18]
6	Rat	β-tricalcium phosphate and osteogenic peptide (OP) containing water/PLGA/dichlo romethane emulsion inks	Extrusion based	Rat endothelial cells and rat bone marrow-derived mesenchymal stem cells	—	12	[19]
7	Rabbit	Sodium alginate, Gelatin, HAP	Extrusion based	Bone marrow mesenchymal stem cells	—	24	[20]
8	Rabbit	GelMA	Extrusion based	Bone marrow mesenchymal stem cells	—	12	[21]
9	Rabbit	Bioactive glass	Extrusion based	Bone marrow mesenchymal stem cells	—	12	[22]
10	Rat	GelMA/Gelatin/PEG/[3]MSN	Extrusion based	Bone marrow mesenchymal stem cells	BMP 4	3	[23]

[1]C3H/10 T1/2 cells – Mouse embryo fibroblast cells.
[2]TGF- transforming growth factor.
[3]MSN- mesoporous.

Table 3.
3D bioprinting for bone: in vivo studies.

Sl. No	Model	Material	Printing method	Cells	Growth factors	Time (weeks)	References
1	Square scaffold	Gelatin-nano HAP	Extrusion-based	Human mesenchymal stem cells, human umbilical vein endothelial cells	—	2	[24]
2	Square scaffold	Graphene oxide/alginate/gelatin	Extrusion-based	Human mesenchymal stem cells	—	6	[25]
3	Square scaffold	Y-irradiated RGD modified Alginate, GelMA, [1]PEGMA	Extrusion-based	Mesenchymal stem cells	—	4	[26]
4	Round scaffold	Alginate-[2]PVA-HAP hydrogel	Extrusion-based	[3]MC3T3-E1	—	2	[27]
5	Square scaffold	[4]PCL/bioactive borate glass composite	Extrusion-based	Human Adipose stem cells	—	1	[28]
6	Square scaffold	Wood-based nanocellulose and bioactive glass modified Gelatin/ Alginate	Extrusion based	Human bone marrow-derived mesenchymal stem cells	—	2	[29]
7	Round scaffold	Polypropylene fumarate-PEG-PCL	Extrusion based	—	—	—	[30]
8	Round scaffold	Octacalcium phosphate, GelMA	Digital Light Processing	C3H/10 T1/2cells, Human Umbilical vein endothelial cell spheroids	—	2	[31]
9	Square scaffold	PCL/polydopamine/HA	Extrusion based	MC3T3-E1	BMP 2	3	[32]
10	Square scaffold	Silk Fibroin-MA	Digital Light Processing	MC3T3-E1	—	2	[33]

[1]*PEGMA-Poly (ethylene Glycol) Methyl Ether Methacrylate.*
[2]*PVA- Poly Vinyl Alcohol.*
[3]*MC3T3- mouse preosteoblasts.*
[4]*PCL- polycaprolactone.*

Table 4.
3D bioprinting for bone: in-vitro studies.

manufacture dental implants. The tooth has multiple internal organs and its connection to surrounding tissues (periodontal ligament and alveolar bone) is important for its function. Hence, the entire tooth unit i.e., tooth root and the adjacent connecting tissues should be considered for tooth regeneration. The 3D printing technique is beneficial for tooth regeneration and building patient-specific supporting structures for teeth (e.g., dentures, dental implants, aligners etc). Dentistry applications of scaffolds are in the limelight of research with the aim of enhancing the regeneration of dental tissues. Bioprinting dental and periodontal tissues is a primary focus of research in dental regeneration [34].

In the native tooth, two mineralized tissues are present – dentin and enamel. Dentin provides strength and toughness while enamel is hard and resistant to both fracture and wear. Titanium alloys are commonly used implants, and they exceed the required strength and stiffness that is normally found in native teeth. This leads to alveolar bone resorption post-implantation. A study used collagen, agarose, and fibrin-based bioink to bioprint dental pulp. The study was successful in vascular tube formation at the root. Human dental pulp cells and Human primary umbilical vein endothelial cells were used in the bioink. Injecting the prepared bioink by the hand-held bioprinter in-vitro showed vascularization and proved to be effective in comparison to filling up the canals with inert materials and sacrificing the tooth [35]. Yi-Ting Lin et al., developed calcium silicate-reinforced gelatin methacrylate bioink and bioprinted dental pulp stem cells along the scaffolds for dental regeneration. The release of silicon ions from the scaffolds contributed to enhanced regenerative properties by upregulating the expression of various odontogenic-related biomarkers. It was also found that these increased calcium mineralization. The developed bioink enhanced the mechanical properties of the scaffold and contributed to increased regenerative properties [36]. Jonghyeuk Han et al., developed a new Demineralized Dentin Matrix particle (DDMp) bio-ink. This bioink is composed of human tooth-derived DDMp, fibrinogen–gelatin mixture, and dental cells. It was found that the DDMp bio-ink improved odontogenic differentiation [37]. **Tables 5** and **6** outline the latest developments in 3D bioprinting of teeth in-vivo and in-vitro studies, respectively.

5. 3D bioprinting for liver

Liver disorders like acute liver failure, chronic liver disease, liver fibrosis, viral hepatitis, and carcinoma have led to high mortality which requires liver transplantation [58]. 3D printing has been used as an alternative strategy to generate organs in vitro as being a shortage of organ donors [59]. 3D printed patient-specific liver models are being used and are showing great potential in disease treatment while the constructs having scaffolds and cells (bioprinted) are being used for the fabrication of liver tissue-like constructs and whole artificial livers [60].

It is very important to select the appropriate kind of cells and scaffold when considering 3D printing of liver tissues [61]. The viability of the hepatocytes reduces in vitro and there is a loss of hepatic phenotype [62]. Many studies have focused on liver regeneration using patient-specific functional cells and pluripotent stem cells. Valve-based inkjet bioprinting has been used by Faulker-jones et al. to print human induced pluripotent stem cells and human embryonic stem cells. The cells were able to differentiate into hepatocyte-like cells post-printing, and there were positive results for nuclear factor 4-alpha and albumin secretion. The cells were compatible to fabricate mini livers as drug testing models [63]. Primary

Sl. No	Animal model	Material	Printing method	Cells	Growth factors	Time (weeks)	References
1	Rat	PCL/HA	Extrusion-based	—	[1]SDF-1, BMP-7	9	[38]
2	Rat	[2]PU, [3]POSS	Extrusion-based	MC3T3-E1	—	6	[39]
3	Rat	GelMA	Extrusion-based	Dental papilla cells, Hertwig's epithelial root sheath	—	8	[40]
4	Mouse	[4]PEGDA and sodium alginate composite	Stereolithography	Human dental pulp stem cells	FGF	4	[41]
5	Dog	HAP/PLA	Extrusion-based	Human dental pulp stem cells		40	[42]
6	Mouse	Alginate, Gelatin	Extrusion-based	Gingival fibroblasts	platelet-rich fibrin	8	[43]
7	Mouse	PCL/PGA	3D wax printing	Human primary gingival fibroblast cells	BMP-7	6	[44]
8	Rat	PCL	Inkjet-based	Human Periodontal Ligament Cells	—	6	[45]
9	Mouse	PCL/HA	Extrusion-based	Human dental pulp stem cells	—	6	[46]
10	Rat	PCL	Solid-free form fabrication method	Primary human periodontal ligament cells	BMP-7	3	[47]

[1]PEGMA- Poly (ethylene Glycol) Methyl Ether Methacrylate
[2]PVA- Poly Vinyl Alcohol
[3]MC3T3- mouse preosteoblasts
[4]PCL- polycaprolactone.

Table 5.
3D bioprinting for teeth: in vivo studies.

Sl. No	Model	Material	Printing method	Cells	Growth factors	Time (weeks)	References
1	Patient-specific	Bioglass, HAP, porcelain	Digital Light Processing	—	—	—	[48]
2	Patient-specific	PCL, Fibrin	Extrusion-based	Human dental pulp stem cells	—	2	[49]
3	Round scaffold	PCL/45S5 bioglass composite and PCL/hyaluronic acid	Extrusion-based	Human gingival fibroblast cells	—	3	[50]
4	Square scaffold	GelMA	Extrusion-based	Human dental pulp stem cells	BMP	3	[51]
5	Square scaffold	GelMA and HAP-magnetic iron oxide nanoparticles	Extrusion-based	Human osteoblasts and human periodontal ligament fibroblasts	—	1	[52]
6	Square scaffold	Alginate, dentin matrix	Extrusion-based	[1]OD 21 cells	—	1	[53]
7	Round scaffold	Poloxamer-407	Extrusion-based	Apical papilla stem cells	—	2	[54]
8	Round scaffold	PLGA, HAP and β-tricalcium phosphate	Extrusion-based	—	—	—	[55]
9	Square scaffold	Alginate, gelatin, nano-HAP	Extrusion-based	Bone marrow-derived mesenchymal stem cells, gingival fibroblast cells	—	1	[56]
10	Square scaffold	GelMA	Extrusion-based	Primary human periodontal ligament cells	—	2	[57]

[1]*OD 21 cells – undifferentiated dental pulp cells.*

Table 6.
3D bioprinting for teeth: in-vitro studies.

rat hepatocytes, HUVECs, and human lung fibroblasts were bioprinted by Lee et al. using multiple nozzle-based extrusion printing. Collagen-based bioink was mixed with the cells and a 3D construct was fabricated by infusing the bioink into PCL framework. There was enhanced survival and functionality of the HCs in the printed liver construct due to the 3D environment-induced interaction among cells. This study showed potential in the liver tissue regeneration field for the capillary-like networked 3D constructs [64].

Robbins et al., used iPSC-derived HLCs, and endothelial and hepatic stellate cells to fabricate highly reproducible 3D liver constructs. These constructs had high viability, multi-layered architecture, tissue-like cell density. There was improved reproducibility, durability, and biological complexity in a study conducted by Nguyen et al. the liver constructs were able to show more biological functions including storing lipids and glycogens and retaining their viability, and compartmentalized structure. Kim et al., used an alginate scaffold and primary mouse hepatocytes to fabricate liver constructs [65]. The cells were viable for 14 days and there was an increase in albumin, HNF-α. Zhong et al., fabricated 3D-printed hydrogel and they were implanted in mice in different groups acting as a control, hydrogel, hydrogel with cells, and hydrogel with hepatocyte growth factor. The viability of the cells was not affected by the hydrogel. The group implanted with cells showed significant improvement in levels of albumin, bilirubin, and the group with HGF, had the longest survival time [66].

6. 3D bioprinting for tubular organs

Tubular organs like Esophagus, blood vessels, urethra, etc. are very prone to infection and can be treated via surgery, stent insertion, and organ transplant that is dependent on suitable donors and autologous organs [67]. Tissue engineering has emerged as an alternative approach for developing grafts and scaffolds.

6.1 Blood vessels

Vascular systems are the most common tissue-engineered structures in the body. Development and discoveries have happened in the past years toward the fabrication of vascular networks in all organ systems. An arterial scaffold consisting of three layers of polydioxanone, fibrin, and gelatin was fabricated by Thomas et al. [68]. The Polydioxanone (PDS) layer provided mechanical integrity and the protein layers had a similar functional extracellular matrix as blood vessels. Nguyen et al., fabricated a tubular scaffold made up of PCL/PU using electrospinning for artificial blood vessels, which demonstrated improved cell attachment and proliferation [69].

6.2 Trachea

Tracheal disorders are rare but still life-threatening like tracheal stenosis and narrowing, and such disorders require immediate treatment. 3D printing of trachea constructs is gaining popularity in the field of regenerative medicine [70]. 3D bioprint tracheal constructs were fabricated by Taniguhi et al. using chondrocytes and mesenchymal cells [71]. Spheroids were fabricated and matured in a bioreactor; then as tracheal grafts transplanted in rats. Silicone stents were used as a framework to provide support and prevent collapsing of the stent. Vascular and epithelium networks

were observed over the grafts thus successfully making a way in tracheal engineering. Goa et al., fabricated a porous PCL construct that would mimic the native trachea of rabbits [72]. The graft was cytocompatible as it was observed when seeded with chondrocytes. There was successful formation of cartilage tissue in the subcutaneous spaces of the mice. Later, it was transplanted into the rabbit, and the survival time was observed as 10 weeks. The fabricated scaffolds can be used for tracheal replacement therapies and for repairing whole-segment tracheal defects.

6.3 Excretory organs

Many organs in the excretory systems are hollow and tubular in morphology including Bowman's capsule, tubules in renal nephron urethra, etc. 3D printing has been utilized and applied in the fabrication of tissues and organs in this organ system. Zhang et al., fabricated cell-laden urethra using PCL and poly (lactide-co-ε-caprolactone) (PLCL) polymers having spiral scaffold design that could mimic the native properties of the urethra of rabbits [73]. Urothelial cells and smooth muscle cells of the bladder were added to the hydrogel comprising gelatin, Dulbecco's Modified Eagle Medium (DMEM) and hyaluronic acid, and the cell-laden hydrogel was fabricated. The urethra was 3D printed by adding PCL/PLCL polymers blend in one syringe and cell-laden hydrogels in another. The polymers provided with the structural framework and the cell-laden hydrogels contributed to mimic the microenvironment. It was observed that the scaffold had the mechanical properties equivalent to native rabbit urethra and the hydrogel was able to maintain a suitable microenvironment and the results set up a strong foundation for future studies on 3d bioprinting of urethra. Pi et al., used a coaxial extrusion-based printing technique to fabricate complex tubular hollow fibers which were made up of blend bioink consisting of PEG, and GelMA/alginate hydrogel [74]. The main objective of this study was to avoid the pre/post-processing step as the coaxial nozzle allows the printing of multiple layers in one step. The team was successfully able to print cannular urothelial tissue constructs using human urothelial cells and human bladder smooth muscle cells. This kind of fabrication is a fundamental step toward creating human cannular tissues.

6.4 Gastrointestinal tract

The esophagus is the tubular tube connecting throat to stomach. Many congenital and acquired disorders of GI tract have only esophageal replacement as the treatment option. 3D printed scaffolds are being considered to repair damaged esophagus. Esophageal reconstruction has been done using resorbable materials, acellular matrices, decellularized patches, and implants of synthetic polymers [70]. Pisani et al., fabricated a biodegradable patch using PLA-PCL polymer. Two different techniques- electrospinning and temperature-induced precipitation were used to develop the cellularized patch. The protocol was repeatable, reproducible, and simple [75]. Haghdel et al., fabricated a flexible esophageal stent to treat esophageal strictures using PLA, polyurethane, and Polyvinyl alcohol (PVA) [76]. The stent was assessed in vitro and in vivo canine esophagus. The stent was implanted in a 16-year-old boy who had esophageal stricture, and it was observed for 2 months. No major inflammatory effects and cytotoxicity were observed, and the mechanical tests revealed that the nature and behavior did not change significantly. This biocompatible polymeric stent can be used as an individualized treatment for treating esophageal structures.

7. Commercial 3D bioprinted products

Manufacturing companies have been using 3D printing for years, mostly to create product prototypes. Models and molds are produced by several manufacturers using huge and quick 3D printers referred to as "rapid prototyping machines" [77] There are many.stl files that may be used for business. Many of these printed goods are comparable to those that are made traditionally [78]. There are now businesses that employ 3D printing for industrial medical purposes [79]. These include Organovo, Helisys, and Ultimateker, a business that creates living human tissue through 3D printing. The use of 3D printing in medicine, however, is still relatively new. The market value of 3D printing is $700 million out of which only 1.6% of it is devoted to medical uses. If we look at the numbers, it is anticipated that the market value of 3D printing will expand to be a sector of $8.9 billion in the next 10 years out of which 21% of it is estimated to go toward its usage in medical applications [78].

The democratization of product design and production is another advantageous aspect of 3D printing [80].

A significant shift has been made in the manner hearing aids are made, currently 99% of hearing aids that fit in the human ear are fabricated via 3D printers. Every individual has a unique ear canal shape, and 3D printers make it possible to build custom-shaped devices quickly and affordably [81]. Another productive commercial use of 3D printing is the production of 50,000 Invisalign braces per day. Each user's set of these transparent, removable, 3D-printed orthodontic braces is unique and is created to order. This item serves as an excellent illustration of how 3D printing can be utilized effectively and commercially to create unique, personalized, complex items [80].

In 2010, Organovo made its first noteworthy business using just primary human cells to successfully bioprint entirely functional blood arteries. The year 2014 saw the introduction of Organovo's ExVive™ 3D bioprinted human liver tissue models. There were histological and functional resemblances to the natural liver, and albumin, ATP, and CYP3A4 activity are consistently expressed for up to 28 days. Drugs like Valproic acid and Monocrotaline have their therapeutic effects demonstrated using tissue models [79].

Organovo released ExVive™ Human Kidney Tissue in 2016, a complete three-dimensional bioprinted human tissue made of primary renal fibroblasts and endothelial cells at the tubule-interstitial interface, which is rich in collagen IV, and polarized primary renal proximal tubule epithelial cells (RPTECs) in the apical layer [77]. ExVive™ Human Kidney Tissue displays in vivo-like renal transporter expression, barrier function, and the production of the crucial enzyme gamma glutamyl transferase (GGT). When subjected to the chemical Cisplatin, this bioprinted kidney tissue produces kidney damage indicators and shows transporter-dependent (OCT2) drug uptake [82]. The world's first animal thyroid gland was successfully 3D printed by 3D Bioprinting Solutions (3dbio) in March 2015 and then implanted into the mouse when it was alive. In addition, to create artificial tissues in the International Space Station using a magnetic 3D bioprinter, 3dbio has been collaborating with Russia's national space agency, United Rocket and Space Corporation (URSC). The company hopes to fabricate synthetic thyroid and kidney tissue using this technology [83].

The most recent RX1TM bioprinting from Aspect Biosystems makes use of their exclusive Lab-on-a-Printer™ microfluidic technology. Contains a coaxial flow-focusing system that guarantees the direct extrusion of biological fibers in a range

of diameters. The device was utilized to show how to fabricate the 3DBioRingTM artificial airway. Primary human airway smooth muscle cells make up contractile smooth muscle tissue that lines the airway. When histamine is present, the airway tissue responds with proper and repeatable contractions, and when pharmacological stimuli are present, it dilates (B2-agonist) [84].

A new biotech company called BIOLIFE4D was established in 2015. The business hopes to 3D bioprint patient-specific, perfectly operational hearts for secure and reasonably priced organ transplantation. They are a strong group of biomedical researchers and businesspeople that are now supporting their research through equity crowdfunding. The goal of the BIOLIFE4D technique is to 3D bioprint a human heart using adult induced pluripotent stem cells (iPSCs), following a complete MRI (Magnetic Resonance Imaging) scan to determine the precise dimensions needed for its production [85].

Poietis makes use of INSERM and the University of Bordeaux technology. The business focuses on D laser-assisted bioprinting technology and collaborates with BASF and L'Oréal to develop bioprinted skin models and hair follicles, respectively [86]. Their NGB 17.03 bioprinting machine, which has an eight-axis motion, can print 3D models down to the level of a single cell. Early in 2018, Poietis introduced the first bioprinted human full-skin model made with their NGB bioprinter, called Poieskin® [87].

In collaboration with scientists at Sichuan University's West China Hospital, Revotek has had success implanting 3D-printed arteries within simian test subjects. In 30 rhesus monkeys, a replacement of a 2-centimeter portion of the abdominal artery was done with a 3D-printed blood conduit, and the stem cell bioink was created using the monkeys' own autologous adipose mesenchymal stem cells (ADSCs) [88]. Using a print head with two nozzles, the printer can presently manufacture 10-centimeter blood arteries in about two minutes [89].

TeVido biodevices make use of patented Clemson University technology. TeVido's initial product is a bioprinted nippular-areola implant for breast reconstructive surgery. In two to three years, clinical trials are expected to begin. The second product from TeVido is intended for Vitiligo sufferers who desire to print skin tissues to lessen the contrast in colors [90].

8. Advancements in 4D bioprinting

The fourth dimension (4D) which is 4D printing incorporates time, and it is an improved production method based on 3D printing. With this technique, external stimulation can cause the printed constructions to alter form over time. 4D bioprinting refers to the recent expansion of 4D printing to include the printing of complex constructions from biocompatible materials or even live cells. If one of the following criteria is met, 4D printing can be referred to as 4D bioprinting. 1) Biomedical engineering may make use of printed products, such as biomedical gadgets. 2) The printed materials are transplantable into the human body and are biocompatible. 3) The printed materials are loaded with living cells. When using 4D bioprinting, the bio constructs size, form, and/or functionality might vary over time [91].

The benefits that 4D printing has over 3D printing might prove to be the necessary proof of concept and accelerate wider adoption. More precisely, 4D printing enables the implementation of micro or nano actuators by providing sensation, knowledge of the movement, and programmability embedded into the material

without any requirement for an external source or system like the batteries, wires, engines etc. Additional advantages of these systems include decreased installation time, expense, human effort, mistakes, storage, and the number of components in a prototype or system.

There have been reports of 4D printing applications in several industries, including medicinal devices, security, the creation of precisely patterned surfaces for optics, electrical devices, constructions with multidirectional capabilities, and soft actuators. Recent years have seen a huge increase in the popularity of soft robotics, which attempts to emulate biology by building flexible and rigid controlled objects, notably for the medical industry. Researchers have recently been more interested in the usage of Shape memory alloys and electroactive polymers which are the materials that change their shape and size according to the temperature and electric field respectively [92], pressurized fluid or gas-operable elastomers, chemical stimuli, and light-sensitive materials with a focus on soft robotics and the biomedical area. As a result, many new opportunities and chances are anticipated to materialize soon as the development of 4D printing technology would open several new possibilities [93].

Zhang et al. modified cellulose with stearoyl moieties to create a material that responds to moisture. They created a film out of this material that, when exposed to an environment with a moisture gradient, would bend because of the non-uniform absorption of moisture [94]. To expand its biological uses or improve the control of printing accuracy, certain novel techniques are emerging. In certain ways, recent advances in 4D bioprinting have resolved issues that were once seen as obstacles, such as the development of microscale vascular models and medication delivery systems for the stomach and muscular actuators. Now that 4D bioprinting is more understood, it has drawn a lot of attention to the research of tissue regeneration and biomedical devices [95]. The fact that 4D bioprinting can better suit the physiological aspects of the body is now widely acknowledged by experts. Instead of being in a static environment like 3D printing, the 4D bioprinted devices may integrate dynamic modification. It has been demonstrated that 4D bioprinting has enormous potential to change tissue engineering, medication delivery, and other sectors. It offers up a new path for bio fabrication. We have a thorough grasp of the biomedical area thanks to the innovative features of 4D bioprinting, not only in terms of tissue and organ regeneration but also in terms of illness therapy. It totally advances the idea of biomedicine while innovating traditional industrial techniques. The tissues in the human body are exceedingly malleable, non-static, and have special roles that are ideal for dynamic alterations. Conventional 3D-printed objects may have certain forms, topologies, or cells, but they are unable to demonstrate dynamic processes. Given this, 4D bioprinting effectively satisfies the need for biomedicine. To the greatest degree possible, 4D bioprinting aims to emulate biological functions in vivo. The bodily reaction cues that trigger the shift should be secure and simple to manage [96].

9. Conclusion

In this chapter, we have reviewed the basics of 3D printing, the various types of bioprinters available like droplet-based, extrusion-based, light-based bioprinters. There are many commercially available bioprinters discussed, developed by various companies fulfilling the requisites of bioprinting. Bioinks are the core part of bioprinting, and it is important to formulate them properly to get the constructs that can be stable, biodegradable, biocompatible, and able to mimic the native

microenvironment of the tissue. Numerous studies have been mentioned where 3D printing has been used to fabricate bone grafts, dental implants, liver disease models, liver tissue constructs, vascular structures, tracheal constructs, cell-laden urethra, and esophageal stent. Some of the mentionable commercially available 3D printed products available include ExVive™ 3D bioprinted human liver tissue model, ExVive™ human kidney tissue, animal thyroid gland by 3dbio, etc. The future of 3D printing is 4D printing which utilizes time as a fourth dimension. The smart materials used for 4D printing change the shape and size under the influence of an external stimulus. 4D printing will open several new possibilities in the field of biomedicine.

10. Future scope

3D printing is the latest technology creating a buzz in all fields including artificial intelligence, advanced simulations, biomedicine, and engineering. The scope embraces objects like human organs, aircraft components, and much more. The technique is being widely accepted due to the several advantages its offers including patient-specific design, high complexity, cost-effective fabrication, and high productivity. The possible uses of 3D printing are endless now, from decreasing the cost of health care to the construction of houses. The cost of production of the prosthetic limb has been reduced to 75% by using 3D printing by the company Mercuris. Rice university has developed a 3D bioprinter that can print narrow blood vessels and which led to developing lung model. 3D printing is being explored by various researchers but now many are working around 4D printing as it is the upcoming technique that is beginning to establish. There are still many challenges and hurdles that must be addressed including the lack of multi-material printers, lack of low-cost printers, and smart materials. The area is still new and unexplored.

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Conflict of interest

The authors declare no conflict of interest.

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