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RESEARCH ARTICLE

Applications of 3D printing in aging

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Abstract

Aging is inevitable, and how to age healthily is a key concern. Additive manufacturing offers many solutions to this problem. In this paper, we first briefly introduce various 3D printing technologies commonly used in the biomedical field, particularly in aging research and aging care. Next, we closely examine aging-related health conditions of nervous system, musculoskeletal system, cardiovascular system, and digestive system with a focus on the application of 3D printing in these fields, including the creation of *in vitro* models and implants, production of drugs and drug delivery systems, and fabrication of rehabilitation and assistive medical devices. Finally, the opportunities, challenges, and prospects of 3D printing in the field of aging are discussed.

Keywords: Aging; 3D-printing; Biomaterials; Disease model; Regenerative medicine, Aging care

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1. Introduction

On November 15, 2022, the United Nations announced that the world's population had reached 8 billion and was expected to exceed 10 billion by the 2080s[1]. The population growth is partly due to improved living standards and medical conditions, which leads to lower mortality rates and increased life expectancy. These huge numbers point to a problem that needs to be taken seriously by all countries: superimposed on declining fertility rates, the global population is rapidly aging as life expectancy increases. In the next 30 years, the global elderly population is expected to more than double. According to the estimates by China's National Health Commission, there will be more than 400 million individuals aged over 60 around 2035, making up more than 30% of the population and entering a stage of significant aging[2]. Aging is a process that human beings must undergo, and the consequences of aging are a gradual and irreversible decline in the physiological functions of all organs, which is caused by the long-term accumulation of various damages[3,4]. Many problems brought about by aging have become the focus of current and future research. First of all, the increase of aging will inevitably lead to an increase in aging-related diseases. To date, biologists have agreed that potentially, there is an unrecognized but important link between aging and many chronic diseases

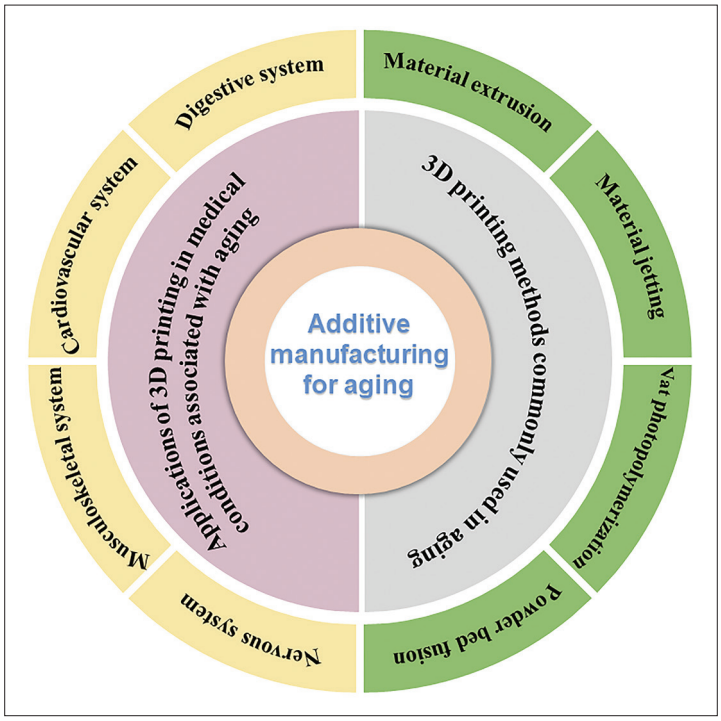

Figure 1. Overview of additive manufacturing for aging from both technology and application perspectives.

in humans, including neurodegenerative diseases (NDD), bone degenerative diseases, cardiovascular diseases, and digestive diseases among others. Aging is one of the major risk factors for severe debilitating as well as life-threatening diseases; as a result, the incidence of these diseases increases with age[4,5]. The treatment of these diseases is an important issue. So far, the pathogenesis of these diseases has not been fully understood owing to the complexity of human body and difficulty in obtaining human tissues for studies. While animal models and *in vitro* cellular models are often used for disease studies, there are many limitations. Furthermore, many severe conditions caused by aging, such as osteoporosis and heart failure, can only be treated by transplantation. However, autologous or allogeneic transplantation have many drawbacks, such as limited source and secondary infection. Therefore, it is important to find a new way to address organ or tissue shortage[6-9]. Finally, elderly people often experience difficulty in eating and drinking due to swallowing disorders as well as tremors and mobility problems caused by degenerative diseases, which adversely affects the normal life of the elderly and entails intensive geriatric care. The development of targeted diets and assistive medical devices tailored to these conditions is one of the keys to improving life quality of elderly people. In short, there are many challenges associated with aging, and we need new solutions[10].

The emergence of additive manufacturing (AM) holds a great promise to address these challenges. AM, frequently referred to as 3D printing, is a process for creating parts with intricate 3D structures by depositing materials layer by layer. 3D printing has been widely incorporated in a diverse range of biomedical applications because of its unique advantages in manufacturing complex structures that were once considered impossible or difficult to fabricate and in producing parts with higher degree of customization. For instance, 3D printing has been extensively used to create anatomical models according to computed tomography (CT) or magnetic resonance imaging (MRI) images for presurgical visualization and practice[11]. 3D printing also enables the precise construction of 3D tissue structures with biologically active materials, biochemical substances, and cells[12]. In regenerative medicine, 3D bioprinting is capable of creating scaffold-based or scaffold-free tissues and organs such as brain, liver, kidney, cartilage tissue, blood vessels, and heart[13-19]. In drug delivery, 3D-printed microneedles are used to control the delivery volume and drug release rate[20,21]. In the area of geriatric care, 3D printing has been used to create dysphagia diet for the elderly with swallowing disorders[22,23]. 3D printing is also widely used to create customized hearing aids, tremor aids, and other rehabilitation and assistive medical devices.

Aging is inevitable, but there are many ways of promoting healthy and active aging with the help of AM technology. This review article first briefly introduces various 3D printing technologies commonly used in biomedical field, either as tools for fundamental research

or healthcare applications. Next, health conditions that are associated with aging are closely examined according to human body systems with a strong focus on the applications of 3D printing in these areas, including the creation of *in vitro* models, synthesis of therapeutics, and fabrication of rehabilitation and assistive devices (Figure 1). Although 3D printing may not be directly used for addressing the aging issues of these human systems, it is used to improve conditions in these systems that are associated with aging. This review also includes some applications for general tissue engineering, but applicable for addressing human aging problems. Some of the 3D printing applications are not exclusively for aging-related conditions, but also used for other purposes. In the end, the opportunities, challenges, and prospects of 3D printing in the field of aging are discussed.

2. 3D printing methods commonly used in aging

In 2009, ASTM International Technical Committee F42 on AM classified AM (which is used interchangeably with 3D printing in this article) into seven processes, including material extrusion, material jetting, binder jetting, vat photopolymerization, sheet lamination, powder bed fusion, and directed energy deposition[24]. Material extrusion is a method in which the ink is extruded from a nozzle and selectively deposited according to the desired pattern. Compared with other 3D printing methods, it has the advantages of low printing cost, broad applicability to many material systems, simple and convenient operation, and fast printing speed. One of the more precise 3D printing methods is material jetting, which prints by ejecting liquid droplets into desired patterns that are subsequently cured by UV or other means. Vat photopolymerization is an AM method that uses photo-activated polymerization to selectively cure liquid photopolymer in a vat layer by layer into 3D structures. It has the benefits of high processing accuracy and smooth surface. Binder jetting creates 3D structures by spraying adhesive onto a powder bed to selectively bind powder materials into desired 3D structures. Postprocessing is often required to permanently set the shape of printed parts. The principle of sheet lamination is that the material sheets are cut by laser, and then bonded or welded together to form a solid block. Sheet lamination printing technology is available in materials other than sheet metal and even in almost any other material that can be curled, such as paper. Powder bed fusion does not use adhesives to bind the powders together. Instead, a high-energy beam, such as a laser beam or electron beam, is directly imparted on the power bed to selectively sinter or melt powder primers into solid parts layer by layer. In the directed energy deposition, the material melts during deposition with a focused thermal energy source (*e.g.*, laser or plasma arc). This process is now only used for metals. Currently, the 3D printing methods that are commonly used in the biomedical field include material extrusion, vat photopolymerization, and material jetting, which are described in detail in Table 1.

3. Applications of 3D printing in medical conditions associated with aging

3.1. Nervous system

By 2040, the World Health Organization (WHO) forecast that due to the aging of the population, neurodevelopmental disorder (NDD) will overtake cancer as the second most deadly human disease[53]. Aging is an important risk factor for NDD, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Frustratingly, almost all these diseases have no effective cures available today. Studies show that the incidence of AD, PD, and ALS is significantly positively correlated with age[54-57]. Aging causes multiple unfavorable changes in the organism, which lead to a progressive loss of synapses and inflammatory response in the nervous system, and finally to neuronal death[3]. These phenomena are observed in cells of neural tissues where degenerative lesions occur, but the sequence and intensity of their occurrence vary.

3.1.1. Alzheimer's disease

According to the latest statistics released by the Alzheimer's Association International, by 2030, 75 million people are anticipated to have dementia globally[58]. The clinical manifestations of AD are mainly cognitive dysfunction and memory impairment, and the main pathological features are the formation of senile plaques by extracellular β-like amyloid deposition in the brain and intracellular tau protein hyperphosphorylation, leading to the formation of intracellular fiber. There are many hypotheses about the pathogenesis of AD, such as amyloid β protein (Aβ) toxicity, tau protein hyperphosphorylation, cholinergic nervous system damage, gene mutation, vascular factors, oxidative stress, neuroinflammatory response, mitochondrial dysfunction, autophagy disorder, insulin signaling abnormalities, and intestinal flora imbalance[59-64].

3.1.2. Parkinson's disease

After AD, PD is the second most prevalent neurodegenerative disease[65]. As a result of the inability to modulate motor activities, PD is characterized by resting tremor, motor bradykinesia, stiffness, and other symptoms that compromise the quality of life and finally result in severe disability. PD is primarily due to the loss of nigrostriatal dopaminergic neuron, resulting in a significant decline in the dopamine levels in the striatum. Studies

Table 1. Summary of 3D printing methods commonly used for biomedical applications

Technology	Category	Introduction	Materials used for biomedical applications	Advantages	Ref
Material extrusion	FDM	FDM uses a heated print head to melt various thermoplastic filaments and extrude the molten material into the desired shape.	Thermoplastic filaments, *e.g.*, wax, ABS, PCL, and PLA.	Low cost; wide range of material selection; environmental protection.	[25-28]
	PEM	PEM is a variation of FDM. Instead of filaments, granular pellets were used as the material feedstock.	Thermoplastic granules, *e.g.*, ABS, PCL, and PLA.	Compared with conventional FDM, the granular raw material is easy to manufacture and costs less. At the same time, the stability of extrusion is greatly improved, and clogging is reduced.	[29,30]
	MEW	MEW deposits molten polymer on the spinning nozzle under a high voltage electric field, forming a jet on the collection plate.	Thermoplastic and functional polymer materials, *e.g.*, PCL and PLA.	The prepared fibers reach the nano- or micron-scale and are similar in structure to the natural extracellular matrix, making them the most promising material for tissue engineering scaffolds.	[31-34]
	DIW	Bioink is extruded directly from the nozzle of the 3D printer, and the ink is deposited onto the printing platform to create the 3D structure.	Hydrogels, *e.g.*, alginate, GelMA, collagen, and CS.	High precision; many hydrogels to choose from; high cell viability.	[24,35,36]
Material jetting	CMJ, NPJ, DOD	Materials are jettisoned continuously or on-demand onto a build platform where they are cured to form 3D structures layer by layer. The machine's complexity and the technique used to manage the ink deposition can differ.	Polymers and plastics, *e.g.*, HDPE, HIPS, and EDP.	High printing accuracy and smooth surface; multi-material integration; fast printing speed.	[37-41]
Vat photopolymerization	SLA	SLA uses a point UV light source to scan the resin from point to line and from line to surface in each layer to form a 3D solid model layer by layer.	Photopolymer, *e.g.*, GelMA, HAMA, and GelMA-PEGDA hybrid hydrogel.	High printing accuracy; high printing resolution; large printing area.	[42,43]
	DLP	DLP uses DMD to project the digital image of the entire slice onto the bottom of the resin tank to cure a thin layer of resin and form 3D structures layer by layer.	Photopolymer, *e.g.*, GelMA, HAMA, and GelMA-PEGDA hybrid hydrogel.	Printing speed generally faster than that of SLA; high printing accuracy.	[44]
	2PP	2PP is one of the vat photopolymerization 3D printing methods based on two-photon optics. Using the near-infrared femtosecond pulsed laser as the light source, the effective focal volume is significantly reduced in 2PP, thus triggering the polymerization of liquid resin in a submicron region.	Photopolymer, *e.g.*, GelMA, HAMA, and GelMA-PEGDA hybrid hydrogel.	It is possible to produce arbitrary 3D structures with high resolution at nanometer scale.	[45-48]
Powder bed fusion	SLS, SLM, EBM	Powder bed fusion applies energy to specific areas of the powder bed to selectively bind the particles into a solid layer and a solid block layer by layer.	Metal and powder-based materials, *e.g.*, titanium and hydroxyapatite.	Relatively cheap; small footprint; multiple material options.	[49-52]

Abbreviations: ABS, acrylonitrile butadiene styrene; CMJ, color material jetting; CS, chitosan; DIW, direct ink writing; DLP, digital light processing; DMD, digital micromirror device; DOD, drop-on-demand printing; EBM, electron beam melting; EDP, environmentally degradable plastic; FDM, fused deposition modeling; GelMA, gelatin methacryloyl; HAMA, hyaluronic acid methacrylate; HDPE, high-density polyethylene; HIPS, high-impact polystyrene; MEW, melt electrowriting; NPJ, nanoparticle jetting; PCL, polycaprolactone; PEGDA, poly(ethylene glycol) diacrylate; PEM, polymer electrolyte membrane; PLA, polylactic acid; SLA, stereolithography; SLM, selective laser melting; SLS, selective laser sintering; 2PP, two-photon polymerization; UV, ultraviolet

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have shown that oxidative stress, defective mitochondrial function, protein misfolding and aggregation, and glial cell proliferation play a significant part in the degenerative death of dopaminergic neurons[66,67].

3.1.3. Amyotrophic lateral sclerosis

A defining feature of ALS is the progressive degeneration of motor neurons in the brain and spinal cord, a rare neurological illness that affects both upper and lower motor neurons. The clinical manifestations are progressive muscle weakness and atrophy throughout the body, and patients eventually die due to swallowing and breathing difficulties. The imbalance of neural protein homeostasis, abnormal proteins proliferation and spreading in a prion-like manner, mitochondria malfunction, glutamate-mediated excitatory neurotoxicity, impaired intraneuronal substance transport, and RNA metabolism disorders, are all currently recognized mechanisms of ALS pathogenesis[68-70].

3.1.4. The role of 3D printing in the treatment and research of neurological diseases

Although great efforts have been made over the years to study NDD, only limited progress is made because the human nervous system is one of the most hierarchically and functionally complicated biological systems. The inherent difficulty in obtaining human tissue samples presents the biggest hurdle to the understanding of human central nervous system development; therefore, research in this area traditionally rely on studies conducted in animal models[71]. Animal models are the gold standard because they have the highest level of physiological relevance. Nevertheless, owing to significant genetic, biochemical, and metabolic differences between species, animal models frequently do not accurately reflect the reality of human patients[72]. Furthermore, animal tests are time-consuming and expensive. Meanwhile, *ex vivo* models cultured with neural sectioning and cell-based 2D *in vitro* culture models have been widely used. The former has the advantage of easy experimental manipulation and easy correction for image analysis. However, once the section is separated from the body, significant functionality is rapidly lost[73]. The latter is also widely used today due to its ease of manipulation and low cost, but 2D cultures are usually insufficient to reproduce specific physiological features due to many limitations such as insufficient intercellular interactions with the extracellular matrix[74]. A more complex environment and longer lifespan are provided by 3D cell cultures which also tend to be more instructive and prescriptive[75]. A superior *in vitro* complement to animal models is thought to be 3D neuronal models because of their closer physiological relevance. As a result of their capability of producing more accurate neural tissue-like structures that combine various cell types and materials to simulate physical and biochemical signaling, various 3D culture systems, such as cell biology-based models (spheres and organs) and engineered models (scaffolds and microfluidic platforms), are now becoming more and more popular[76,77]. For example, human pluripotent stem cells were differentiated by Jo *et al.*[77] into massive multicellular organoid structures with unique neuronal cell layers that expressed markers specific to the human midbrain. More importantly, dopamine synthesis, electrically active, and functionally developed midbrain dopaminergic (mDA) neurons were found in the 3D midbrain-like organoid. The 3D midbrain-like organoids (MLO) were found to be structurally similar to neuromelanin-like particles from human substantia nigra tissue. Unlike human mDA neurons produced using a 2D technique, MLOs are produced from mouse embryonic stem cells. The emergence of pluripotent stem cell-based neural-like organs more realistically simulates the developmental progress of the nervous system. It offers a non-ethically constrained platform for studying human neurodevelopment, a new platform for drug screening, and a highly informative complement to existing 2D culture methods and animal model systems[78]. In addition, organoids have made it possible to obtain cells closer to natural human development for cell therapy. However, the organoid-based 3D models are limited by the relatively simple structure as well as the absence of vascular nerve distribution and extracellular microenvironment.

The emergence of 3D bioprinting has provided a new tool for 3D culture, and the combination of 3D printing and organoid can effectively address the aforementioned problems. 3D printing can automatically reproduce predesigned models using cells and biological materials to simulate the complex tissue structure and natural physiological environment. Lozano *et al.*[13] proposed a new approach for bioprinting 3D brain-like structures consisting of discrete layers of primary neuronal cells encapsulated in hydrogels (Figure 2A). The brain-like structure was 3D-printed by using a bioink composed of peptide-modified gellan gum RGD (RGD-GG; RGD stands for arginine-glycine-aspartic acid) and primary cortical neurons. The bioinks had the ability to accommodate and support the cell growth and network formation in specific hierarchical structures, and can be 3D-printed into multilayer brain-like structures by direct ink writing (DIW). More precise 3D *in vitro* microstructures could be duplicated using these 3D-printed brain-like structures, which would help us comprehend the mechanisms of brain damage and neurodegenerative disorders. NDD usually lead to irreversible neuronal damage and death. Promoting neuronal targeting and regeneration is one solution to treating NDD, and directly 3D printing neural stem cells (NSCs) to create novel scaffolds that promote

neural differentiation and neuronal regeneration is an effective approach. Gu *et al.*[79] constructed a 3D neural mini-tissue construct (nMTC) by 3D-printing human neural stem cells (hNSCs) using DIW. hNSCs differentiated into functional neurons *in situ* and supported glial cells (Figure 2B). Zhou *et al.*[80] fabricated gelatin methacryloyl (GelMA)-dopamine(DA) hierarchical neural scaffolds with NSCs using a customized stereolithography (SLA) 3D printer. The GelMA-DA scaffold had a 3D environment that was extremely porous and connected, which boosted the gene expression of the neuronal markers, namely *TUJ1* and *MAP2*, supported NSC proliferation, and promoted neural differentiation, which all point to the potential of the scaffold for brain repair and regeneration.

At the same time, 3D printing plays a huge role in disease treatment due to its unique and personalized structural customization. For instance, Dong *et al.*[81] prepared helical microcones of photocurable GelMA-based hydrogels by two-photon polymerization (2PP). Following that, composite multiferroic nanoparticles were added to the helices. The microcones therefore displayed magnetoelectric characteristics (MENP) and became multifunctional soft helical microcones with high level of functional integration, capable of targeted delivery of nerve cells, on-demand local wireless neuronal electrical stimulation, and enzymatic digestion after delivery (Figure 2C). MENPs were incorporated into the microcones as part of a magnetic manipulation strategy, acting as magnetically actuated components in low-amplitude rotating magnetic fields. The magnetic input, which was also transformed to electrical output by these MENPs, were used to induce neuronal cell differentiation under electrical stimulation. The microstructure provided a biocompatible matrix that supported the growth of cells and would degrade upon targeted neuronal cell delivery, which opens new avenue for targeted cell therapy for trauma and disease of central nervous system[81].

3D printing also offers significant advantages in drug delivery and release for NDD. Pramipexole is used to treat symptoms and signs of idiopathic PD in adults. The best working dose of pramipexole is patient-dependent. However, only a limited selection of standard doses is available in the market. Gultekin *et al.*[82] used fused deposition modeling (FDM) 3D printing to create dosage forms for pramipexole with varied release characteristics. The results showed that 3D-printed tablets could be successfully manufactured into personalized doses and that the desired drug release profile is achievable by adjusting the formulation (Figure 2D). Saylam *et al.*[83] proposed the use of 3D-printed polylactic acid (PLA) and chitosan (CS) neural tissue scaffolds loaded with levodopa for the treatment of PD and showed that levodopa was released from the 3D-printed scaffolds in a controlled fashion for 2 weeks with good biocompatibility. Systems for implanted drugs provide an alternative to treating chronic illnesses like neurodegenerative disorders. Renishaw used selective laser melting (SLM) to assemble a 3D-printed titanium catheter into an internal drug delivery device that delivered cerebral dopamine neurotrophic factor (CDNF) for the treatment of PD[84]. Preliminary results were encouraging, showing that the internal drug delivery device could be placed accurately with predictable efficacy. This 3D-printed drug delivery device contributed to improved efficacy and safety of CDNF, suggesting that future development of this technology would help alleviate the distress of PD patients suffering from progressive neurological disease.

3D printing also demonstrates important applications in assistive devices for aging care with neurological disorders. In response to the tremor or trembling symptoms of PD, Western University in Canada developed a 3D-printed wearable glove that suppressed tremors or other muscle contractions caused by PD, allowing PD patients to exhibit better motor control[85]. Parizi *et al.*[86] 3D printed a smart ring called AuraRing, which tested the onset of PD by tracking silent hand tremors. Diseases such as ALS cause respiratory muscle weakness and require a noninvasive ventilator with bi-level positive airway pressure/continuous positive airway pressure (BiPAP/CPAP). Wu *et al.*[87] used SLA technology combined with MRI data to customize patient-specific masks for BiPAP/CPAP machines for muscle weakness caused by ALS, increasing patient comfort and reducing air leakage.

In general, the impact of aging on the nervous system increases with age, and the onset of NDD greatly affects the health and life of patients. 3D printing, with its unique advantages, plays an irreplaceable role in the fundamental study and treatment of aging-related NDD, from establishing more intuitive brain-like models for in-depth investigation of the pathogenesis of diseases, to targeted delivery and release of drugs through implantable biological scaffolds, as well as to the manufacturing of rehabilitation and assistive devices. Compared to traditional cell models, tissue sections, and organoids, 3D printing is able to print specific subtypes of neural cells and growth factors in region-specific arrangements to mimic natural tissue structures, which provides precisely choreographed reconstruction of microscale neural networks and connections with internal structures and physiological features of the nervous system in a more 3D and more life-like manner, and hence a complete developmental process of the nervous system. It also provides a method for healing complicated nerve injuries, opening the door to the specialized care of several nerve injuries. In addition,

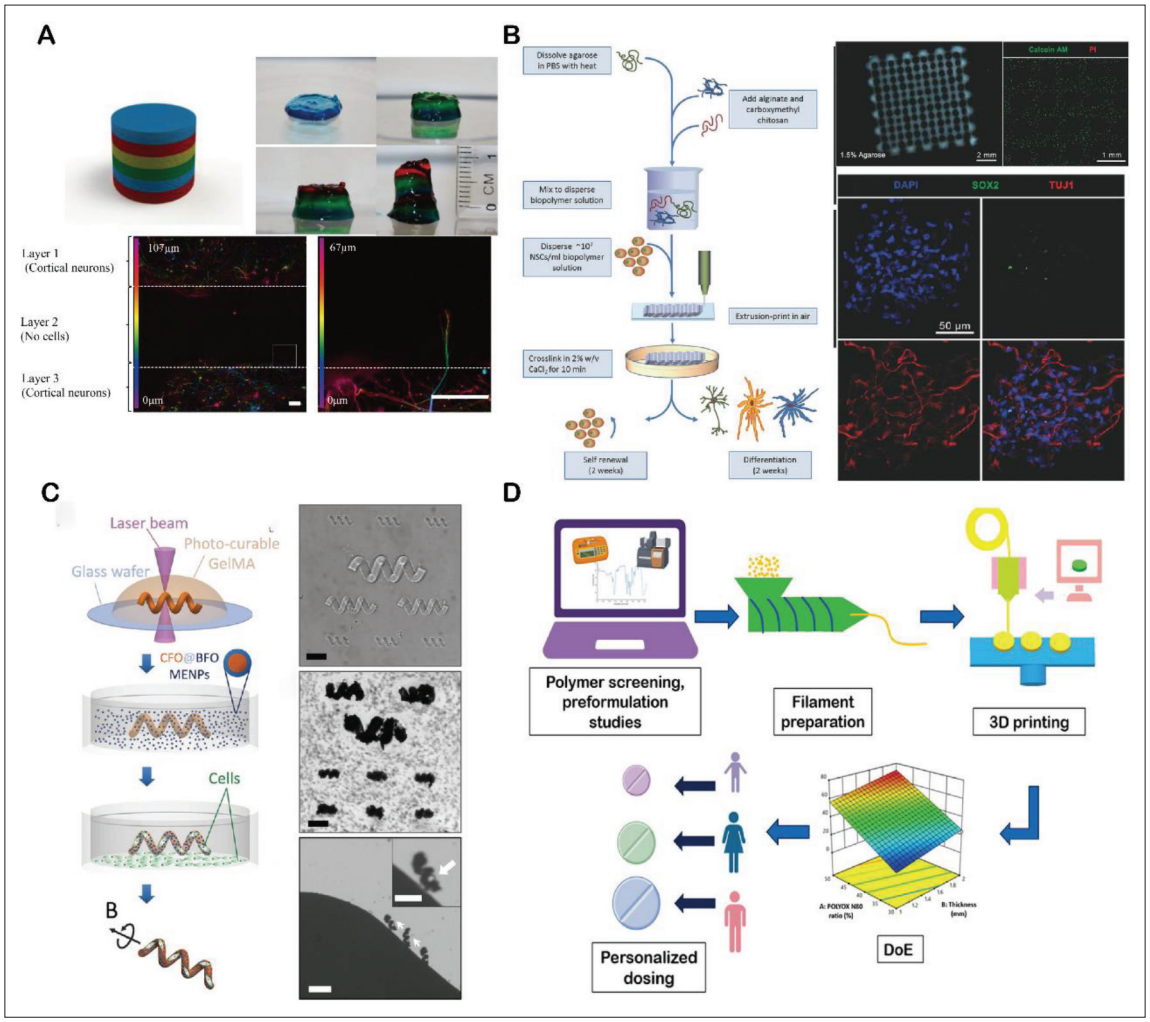

Figure 2. (A) A brain-like structure made of 3D printing; the cells develop and differentiate in a certain layer, where each hue indicates a layer[13]. Reproduced with permission from Elsevier, Copyright © 2015, Elsevier. (B) 3D nMTC produced by printing with hNSC-loaded Al-CMC-Ag bioink using DIW. High expression of *TUJ1* and low expression of *SOX2* followed the induction of differentiation[79]. Reproduced with permission from John Wiley and Sons, Copyright © 2016, John Wiley and Sons. (C) Fabrication process and structural construction of 2PP-printed biodegradable soft spiral microswimmers[81]. Reproduced with permission from John Wiley and Sons, Copyright © 2020, John Wiley and Sons. (D) 3D printing-based personalized drug customization strategies[82]. Reproduced with permission from Elsevier, Copyright ©2022, Elsevier.

3D printing enhances the reproducibility of experiments and facilitates high-throughput screening of drugs.

3.2. Musculoskeletal system

3.2.1. Osteoporosis

Osteoporosis is a common degenerative bone disease in the middle-aged and elderly population and is ranked as one of the top three geriatric diseases in the world by WHO[88]. Patients with osteoporosis often experience back pain as the main symptom and are clinically characterized by impaired bone microstructure[6,89]. Aging is one of the most critical factors closely associated with osteoporosis. With the gradual development of an aging society, the number of patients with osteoporosis has increased dramatically[90]. The condition is linked to the physiological decline of the organism and the imbalance of bone metabolism with age[91]. During bone metabolism in the body, changes in bone resorption occur, leading to a slow decrease in bone density, resulting in osteoporosis. Osteoporosis is most frequently observed in people of advanced age, which results in increased incidence of fractures in patients and has a significant impact on their physical and active functions[92].

3.2.2. Osteoarthritis

The advent of the aging population comes with many problems. Osteoarthritis (OA), which is closely related to age, is one of the most serious conditions. As a complicated chronic disease, OA is a leading contributor to mobility impairment in aged people. OA has long plagued a diverse

group of people, and its incidence is closely related to aging and obesity, with the WHO reporting that about 1 in 10 middle-aged and elderly people over the age of 60 suffer from OA. One of the most significant risk factors for OA is aging[92]. Studies have shown that the incidence of OA with imaging changes and painful symptoms increases with age. The lesions involve structural changes in articular cartilage, subchondral bone, synovial membrane, and periarticular muscles, mainly manifesting as bone friction, morning stiffness, joint motion disorders, and deformities in appearance, which all bring great pain and inconvenience to patients[93,94]. At the same time, OA also causes social burdens[95]. On the one hand, the disability caused by OA and its consequences lead to a reduction in the workforce; on the other hand, the treatment for OA is long-lasting and expensive, which imposes heavy financial burden to the healthcare system[96]. The main treatment principle for OA is to reduce symptoms and slow the progression of the disease, but eventually, more severe OA can only be treated with joint replacement[97,98].

At present, studies on the pathogenesis of OA suggest that it is mainly related to the imbalance in the metabolic homeostasis of cartilage, subchondral osteosclerosis, and synovial inflammation. Synovial is a special type of transparent tissue at the skeletal surface of the joint which facilitates the sliding of the joint surface and reduces friction during movement. One distinctive feature of OA is the loss of articular cartilage, which generally attribute to the imbalance between the anabolism and catabolism of chondrocytes. Aging, obesity, and changes in biological rhythms cause the hypertrophy or apoptosis of chondrocytes, metabolic disorders, and cellular senescence that disrupt cartilage homeostasis, thus triggering OA. Subchondral bone remodeling and bone redundancy formation are important features of OA. Meanwhile, increased subchondral bone angiogenesis and vascular invasion of avascular cartilage are early diagnostic features of OA[99]. Aging plays crucial roles in the development of OA. Chondrocyte loss and cartilage and joint damages occur during aging process, during which senescent cells accumulate in cartilage tissue and form senescence-associated secretory phenotypes through the secretion of cytokines, and eventually lead to pathological changes in articular cartilage aging[100-103].

(A) The role of 3D printing in the treatment and research of bone diseases

Fractures and bone defects due to osteoporosis and tubular arthritis are usually treated with bone grafting, where the structures and functions of bones are reconstructed by customized grafts. Existing autograft and allograft methods have many problems, such as limited supply and dangerous complications. Bioactive materials with strong mechanical properties and biocompatibility are the best alternatives, but often do not integrate well into the recipient site due to the lack of a natural tissue-mimicking structure. Therefore, 3D printing technology is used to produce bones that can more closely resemble their natural counterpart. The high degree of personalization and design freedom make 3D printing well-suited for the manufacturing of bone grafts. The personalized and customized bioprostheses for transplantation becomes a better option to suit the needs of different patients.

The main materials used for bone tissue engineering include metals, ceramics, and biopolymers. Titanium-based scaffolds are frequently utilized as implant materials for treating bone defects. However, the inefficient osseointegration of typical titanium-based implants is frequently observed, which is caused by the misaligned biomechanics and poor bioactivity of these implants. To address this issue, Ma *et al.*[104] used SLM to 3D-print a hybrid scaffold made of Ti-6Al-4V alloy and GelMA with dual bionic properties (GMPT) for bone defect repair. Because of the special dual bionic design, the synthetic GMPT scaffold replicated the microstructure and mechanical characteristics of natural cancellous bone and had superior osteogenic and angiogenic qualities compared to the porous TC4 metal scaffold. Ceramic materials have similar mechanical properties to bones and are used widely in bone tissue engineering. Wu *et al.*[105] developed a novel two-layer scaffold for bone repair using calcium-deficient hydroxyapatite (CDHA) and poly(lactic-glycolic acid copolymer) (PLGA). An optimized mix of CDHA and PLGA with good biocompatibility and negligible cytotoxicity was 3D-printed by DIW to create a bilayer scaffold. Studies in animal models showed successful implantation of the scaffold and significant osteogenic ability within 6 months (Figure 3A and B). Van hede *et al.*[106] used SLA to 3D-print bioceramic-resin composite to fabricate a gyratory scaffold. The scaffold was implanted into rats, and a significant increase in bone volume within the scaffold was observed after 8 weeks, showing excellent bone regeneration properties (Figure 3C). Guillaume *et al.*[107] 3D-printed polytrimethylene carbonate (PTMC)/hydroxyapatite (HA) scaffolds by photo-crosslinking 20 wt% HA nanoparticles with PTMC resin using SLA. In PTMC/HA scaffolds, the SLA process promoted the superficial enriching of HA, which significantly improved bone regeneration and promoted osteogenesis (Figure 3D). Zhang *et al.*[108] used digital light processing (DLP) to prepare large-sized (length >150 mm) HA bioceramics with highly microporous surface structure using urethane acrylate (UA) mixed with poly(ethylene glycol) diacrylate (PEGDA) and HA as a photosensitive composite. Experiments showed that the bioceramics have good

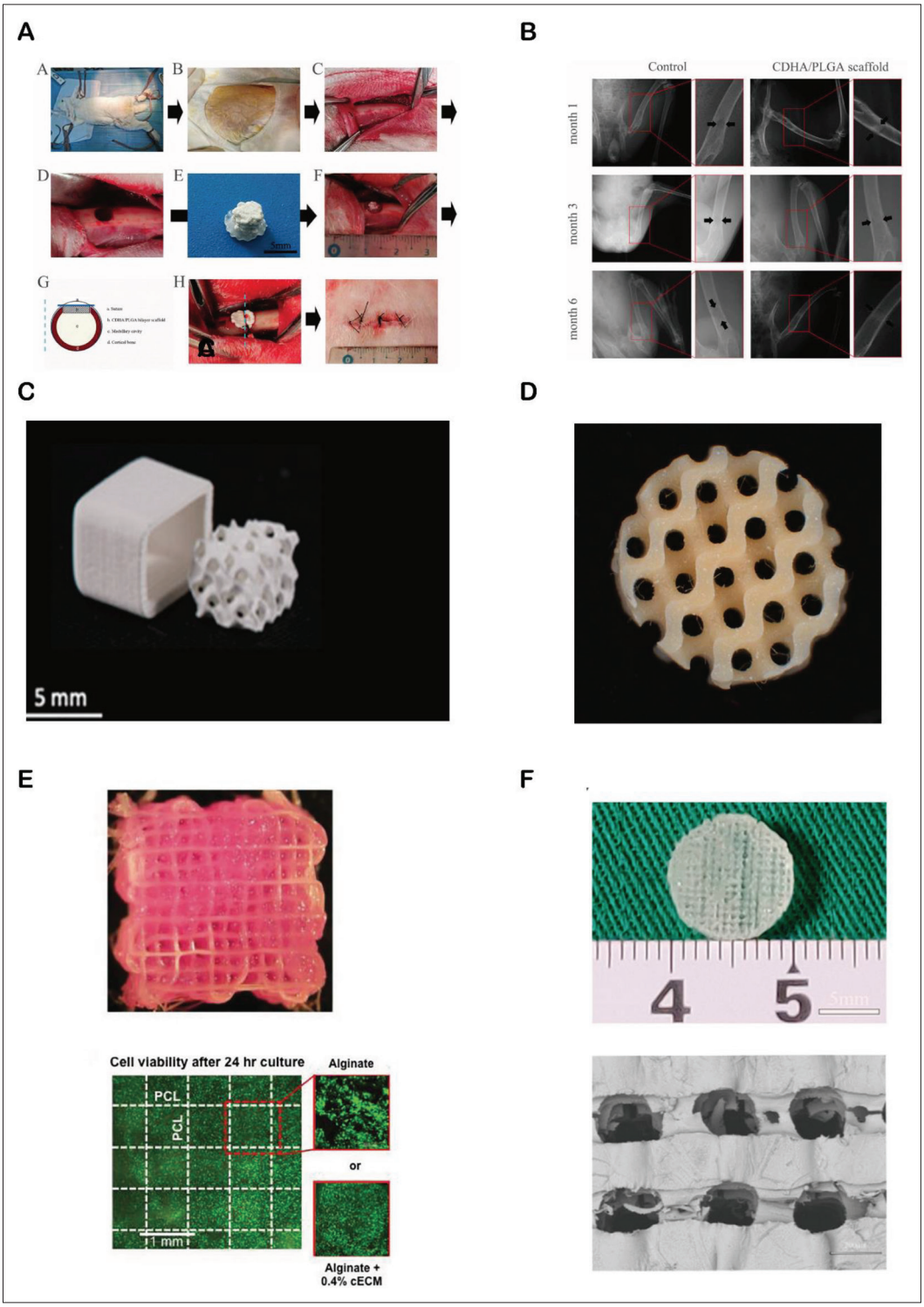

Figure 3. (A) Building the rabbit femur's cortical defect model and implanting a 3D-printed scaffold. (B) Pictures of a rabbit femur taken after surgery at 1, 3, and 6 months. Black arrows point to the bone defect area[105]. Reproduced with permission from IOP Publishing, Copyright © 2021, IOP Publishing. (C) Printing gyroid structures bone supports by SLA[106]. Reproduced with permission from John Wiley and Sons, Copyright © 2021, John Wiley and Sons. (D) Preparation of macroporous scaffolds for PTMC/HA by SLA[107]. Reproduced with permission from Elsevier, Copyright © 2017, Elsevier. (E) 3D-printed PCL networks enhance cECM-functionalized bioink hybrid constructs and cell viability of MSCs[117]. Reproduced with permission from John Wiley and Sons, Copyright © 2019, John Wiley and Sons. (F) Spatial structure and SEM images of 3D-printed PEGDA-GelMA-CSMA hydrogel scaffolds[118]. Reproduced under Creative Commons license.

bone regeneration ability. CS is commonly used in bone tissue engineering, and the mechanical characteristics and biocompatibility of scaffolds can be considerably enhanced by doping CS with ceramic and metal particles. Using FDM technology, Ye *et al.*[109] 3D-printed chitosan acetic acid solution-coated poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/calcium sulfate hemihydrate (PHBV/CaSH) scaffolds. Rat bone marrow stromal cells (rBMSCs) underwent osteogenic gene expression level upregulation by the PHBV/CaSH/CS scaffold, considerably increasing their osteogenic potential. Further proof that the PHBV/CaSH/CS scaffold might successfully encourage the development of new bone came from *in vivo* investigations. Ratheesh *et al.*[110] prepared a double pore size layered scaffold using polycaprolactone (PCL) in combination with melt electrowriting (MEW) and FDM, and the layered scaffold showed improved performance with significantly larger specific surface area and enhanced cell proliferation, which promote cell adhesion and *in vitro* osteogenesis compared to the FDM control scaffold.

Poor osseointegration at the interface following arthroplasty in patients with osteoporosis caused by limited bone regeneration ability typically results in catastrophic consequences such as prosthesis displacement, loosening, and periprosthetic fractures. To improve osseointegration under osteoporosis, Wang *et al.*[111] 3D-printed a hierarchically functionalized porous Ti6Al4V scaffold using SLM. The macroporous structure in this innovative scaffold provided mechanical support, the microporous structure boosted biocompatibility and encouraged cell attachment, and the nanostructure showed biological impacts. Animal studies showed that a significant amount of new bone was produced around and within the distal femur of osteoporotic rats after the biofunctionalized porous Ti6Al4V scaffold was implanted. By inhibiting the Notch1 signaling pathway and increasing the production of anti-inflammatory cytokines, controlled release of epimedium and Mg^{2+} from biologically functionalized porous titanium (PT) significantly improved the polarization of M0 macrophages to M2 type and significantly improved bone metabolism, which improved bone regeneration among PT and osteoporotic bone[111].

The fabrication of targeted drug delivery and release systems for patients with osteoporosis and OA through 3D printing facilitates better recovery and maximizes the efficacy of drugs. Wang *et al.*[112] designed a thermosensitive hydrogel filled with osteoprotegerin (OPG) to suppress excessive osteoclast activity and bone morphogenetic protein-2 (BMP-2) to stimulate osteogenesis. To create a composite scaffold for implantation, the drug-loaded hydrogel was injected into a porous Ti6Al4V scaffold created by 3D printing. The BMP-2 and OPG released from the 3D-printed composite scaffolds sustained for more than 20 days, demonstrating good biocompatibility and encouraging osteogenic differentiation while reducing osteoclast activation. The repair of osteoporotic defects and osseointegration were greatly improved by sustained release of BMP-2 and OPG from the composite scaffolds. Cui *et al.*[113] created an inorganic-organic bioactive system for drug delivery. The system consisted of an electron beam melting (EBM) 3D-printed inorganic porous titanium alloy surface and an organic pozzolanic 407 thermosensitive hydrogel loaded with a new antiosteoporosis drug (technetium methylene diphosphate, 99Tc-MDP). It displayed a sustained drug release profile, improved osteogenic differentiation, decreased osteoclast-associated gene expression, and suppressed osteoclastogenesis. It also demonstrated superior biocompatibility[113]. Li *et al.*[114] used EBM to fabricate a 3D porous titanium prosthetic interface with biomimetic pore size and porosity, after which it was implanted into the distal femur of osteoporotic rabbits, and rapamycin was administered via a transdermal drug delivery system to the implantation site. Ultrasound-mediated rapamycin administration restored cellular activity and prevented potential osteoclast induction and adipose differentiation.

(B) The role of 3D printing in the treatment and research of cartilage diseases

Arthroplasty is one of the best treatments for severe OA. The current gold-standard therapy for OA is total arthroplasty, in which the damaged cartilage and underlying bone are replaced with polymer and metal prostheses. This treatment is now approaching maturity, but still carries the risk of failure and postoperative complications. The advent of cartilage tissue engineering promises a new treatment option. New cartilage tissue engineering methods, such as 3D printing of bioinks that contain cells and bioactive materials, have the potential to produce prostheses with bionic structures and functions, and show great advantages for the development of personalized cartilage implant. It is particularly important to identify bioactive materials and printing strategies that are suitable for cartilage tissue engineering[115]. With the use of DIW, You *et al.*[116] effectively 3D-printed porous cell-loaded hydrogel scaffolds utilizing ATDC5 chondrocyte cells that were encapsulated with sodium alginate. The scaffold promoted chondrocyte proliferation, extracellular matrix (ECM) deposition, and cell survival (85% cell survival) *in vitro*, although it had a significantly lower compressive modulus (20–70 kPa) than human cartilage (700–800 kPa). Rathan *et al.*[117] developed a cartilage extracellular matrix (cECM)-functionalized alginate, which was infused into an FDM-printed PCL network to form a scaffold. The scaffold significantly enhanced chondrogenic potential

and promoted robust chondrogenesis from mesenchymal stem cells with its bionic structures that mimicked natural cartilage (Figure 3E). Guan *et al.*[118] prepared a novel bioink composed of PEGDA, GelMA, and chondroitin sulfate methacrylate (CSMA) to 3D-print scaffolds for cartilage tissue regeneration through the use of conventional FDM-printed PLA porous scaffolds (Figure 3F). The bioink was infused into the 3D-printed PLA scaffold to form an interpenetrating polymer network with strong mechanical properties, good biocompatibility, reduced expression of osteogenic marker genes, enhanced expression of cartilage-specific genes, and deposition of changes in vascular endothelial cells with increased glycosaminoglycan (GAG) levels.

Several studies have shown that the conjunction of electrospun fibers and hydrogels has an important impact on the enhancement of mechanical properties. Visser *et al.*[119] 3D printed high-porosity PCL microfiber scaffolds that were mechanically reinforced with GelMA hydrogels using MEW and showed that the stiffness of the composite scaffold was increased compared to hydrogels or microfiber scaffolds alone (up to 54-fold), and the reinforced GelMA hydrogel had a stress–strain behavior similar to that of healthy articular cartilage. Furthermore, the rigidity of the biodegradable polymers was equivalent to that of articular cartilages in absolute terms, while the mRNA expression of matrix chondrocyte markers was significantly upregulated in the composite hydrogels. Chen *et al.*[120] processed cartilage decellularized matrix (CDM) into a powder form and mixed it with hyaluronic acid solution as an ink to prepare gelatin/PLGA fibers by DIW. The 3D-printed CDM-based scaffold's stiffness and toughness were both increased by the incorporation of fibers. Additionally, the 3D CDM scaffold developed using electrostatically spun fiber reinforcement demonstrated good *in vitro* and *in vivo* biocompatibility and enhanced the repair of cartilage injury in rabbit joints. Bas *et al.*[121] used MEW to fabricate PCL melt electrospun fiber networks combined with star-shaped polyethylene glycol/heparin hydrogels (sPEG/Hep) to form hydrogels with mechanical properties similar to those of natural cartilage and to provide a proper microenvironment for *in vitro* chondrocyte culture and cartilage formation.

The application of 3D printing in bone focuses on bone grafting, orthopedic disease treatment, and drug delivery, and mainly uses metals, ceramic particles and biopolymers as materials. Desired structures are 3D-printed using techniques such as powder bed fusion, material extrusion, and vat photopolymerization. 3D printing in cartilage tissue engineering relies more on material extrusion. Biopolymer, hydrogels, and their composites filled with other functional materials are the commonly used bioinks in these applications. 3D-printed cartilage is expected to be the best alternative to cartilage tissue transplantation. In recent years, significant advancements in the field of bone and cartilage tissue engineering have been attained, but these achievements are still far from real-world clinical applications, likely because engineered tissues usually lack the kind of spatial complexity of real tissues. 3D printing has the unique advantage of controlling the volumetric geometry and internal structure of tissue scaffolds, allowing cells to be arranged according to predesigned patterns to meet the complexity required for tissue engineering. In clinical applications, 3D printing can create specific grafts according to the patient's needs, reducing the time and postoperative risks in surgical transplantation. There has been significant advancement in the development of bioinks for the repair of bone and cartilage up to this point. These inks can facilitate cell proliferation, differentiation, and tissue creation, and they are highly printable and biocompatible. However, the real-world applications of 3D printing in bone and cartilage treatment remain a great challenge as there are many unresolved issues today, such as how to reproduce the regional complexity of natural tissues and how to ensure that a single graft can function properly in a complex bioenvironment. Nevertheless, we believe that 3D printing, with its unique advantages, will be able to solve these problems and be widely adopted in clinical practice to benefit the aging population with orthopedic diseases as research in this field progresses.

3.3. Cardiovascular system

Cardiovascular disease is the most common disorder among the elderly and one of the main causes of death for those over 65 years old[122]. The prevalence of cardiovascular disease is as high as 70% in people aged 60–79 years and rises to 80% in those aged >80 years[123-125].

3.3.1. Heart senescence

The ventricular structure alteration and diastolic dysfunction occur with aging. The significant effects of aging on cardiac structure are characterized as left ventricular myocardial hypertrophy and left atrial dilatation, which increase the incidence of heart failure and atrial fibrillation. Besides, other degenerative lesions of the aortic valve like aortic calcification, which usually contributes to aortic stenosis, increase with age, reaching up to 48% in those older than 84 years old[126]. During aging, cardiac cells undergo remodeling, mainly in the form of reduced number of ventricular myocytes and sinus node pacing cells, which leads to compensatory myocardial hypertrophy. Atrial myocardial fibrosis is closely related to the development of atrial fibrillation. In older adults, fibroblast proliferation and collagen deposition in atrial tissue adversely affect atrial electrophysiology and lower

the threshold for atrial arrhythmias. Many studies have shown that aging can directly or indirectly mediate calcium disorders and contribute to the development of cardiovascular disease[127]. Therefore, aging is marked by the degeneration of all systems, especially the heart, which causes molecular changes in cardiac structure and myocardial cell functions, and in turn leads to a series of cardiovascular diseases[128-131].

3.3.2. Vascular aging

Vascular aging refers to the degenerative alterations of vascular structure and function that occur with age and can be categorized into physiological vascular aging and pathological vascular aging[132,133]. The former is caused by programmed aging of the organism, and the latter is a pathological change caused by environmental factors, nutritional factors and diseases that cause accelerated aging of blood vessels. Vascular aging plays a decisive role in the aging process of human body, which is significantly correlated with the occurrence of cardiovascular diseases and other aging-related diseases in elderly people. Vascular aging at cellular level is characterized as morphological changes in vascular endothelial cells and vascular smooth muscle cells[134,135]. At the histological level, it is manifested as structural and functional changes in the vascular endothelium and middle layer, specifically, the amount of connective tissue, lipid, and calcium content of the subendothelium increases with age, the vascular smooth muscle layer thickens, elastin decreases, and vascular calcification occurs. Molecular studies demonstrated that the nitric oxide production and bioavailability significantly decreased during ageing, accompanied by reduced expression of endothelial-type nitric oxide synthase and its coenzyme tetrahydrobiopterin (BH4), inducing endothelial dysfunction and morphological and structural changes of vascular smooth muscle cells, and accelerating the onset of vascular aging. Telomeres are also involved in vascular aging[136]. Telomere shortening, damage, and decreased telomerase activity lead to senescence and dysfunction of endothelial cells and vascular smooth muscle cells, which in turn lead to vascular aging. Likewise, a series of pathological changes such as systemic and local inflammatory response, endothelial damage, oxidative stress, and mitochondrial dysfunction are underlying vascular pathology, which ultimately damage almost all organs and systems[129,135-138].

3.3.3. The role of 3D printing in the treatment and research of aging-related cardiovascular diseases

The progression of cardiovascular disease usually leads to structural and cellular deterioration of the heart. In the end stage of cardiovascular diseases, replacement of the damaged organ is often the only option to improve the prognosis of affected patients[139]. Current medical treatments usually require clinical implants including autografts, allografts, xenografts grafts, and artificial prostheses[140], such as the saphenous vein grafts and arterial grafts in coronary artery bypass grafting[18,141]. Cardiac implantation methods are mainly limited by donor shortage and immune rejection. In response to this problem, 3D-printed cardiac tissue engineering has emerged as a promising solution. The benefits of 3D printing include the ability to precisely regulate the spatial distribution and structural precision of many components, which makes it possible to successfully mimic the target tissue's inherent structural characteristics, mechanical characteristics, and even functions. Also, 3D printing is often used by cardiovascular surgeons to create patient-specific models to visualize anatomy, which promote a more comprehensive understanding of tissue and organ abnormalities to ensure surgical precision and provide an accurate model for teaching cardiovascular surgery. Ma *et al.*[18] produced complex *ex vivo* vascular models with internal microchannels for thrombosis studies by using DLP 3D printing (Figure 4A). Garekar *et al.*[142] 3D-printed heart models based on information from CT or MRI scans to gain a deeper understanding of intracardiac anatomy. With a combined use of dual-material material jetting 3D printing processes, computer-aided design tools, and CT with high spatial resolution, Maragiannis *et al.*[143] showed that severe degenerative aortic stenosis anatomical and functional characteristics might be faithfully replicated in patient-specific models. These models also made it easier to diagnose the patient's condition (Figure 4B). There have also been major breakthroughs in the recreation of functional devices, such as tissue-engineered heart patches, which have shown great potential as a treatment option for myocardial infarction. Jang *et al.*[144] used DIW triple-jet co-printing technology to print two heart tissue-derived dECM (hdECM)-based bioinks alternately on a PCL support layer and crosslinked them with UV light to fabricate 3D prevascularized stem cell patches. The spatial patterns of double stem cells used in the 3D-printed structures enhanced cell-to-cell communication and differentiation capacity while fostering the functions of tissue regeneration. This stem cell patches displayed improved cardiac performance, decreased myocardial hypertrophy and fibrosis, increased migration from the patch to the infarct zone, which led to new muscle and capillary creation as well as robust angiogenesis and tissue matrix production. Noor *et al.*[145] used DIW-printed personalized hydrogel bioinks to construct thick, vascular, and perfusable cardiac patches that precisely matched the patient's immunological, biochemical, and anatomical features when mixed with the patient's own cells (Figure 4C). Vascular and cardiac scaffolds are one of

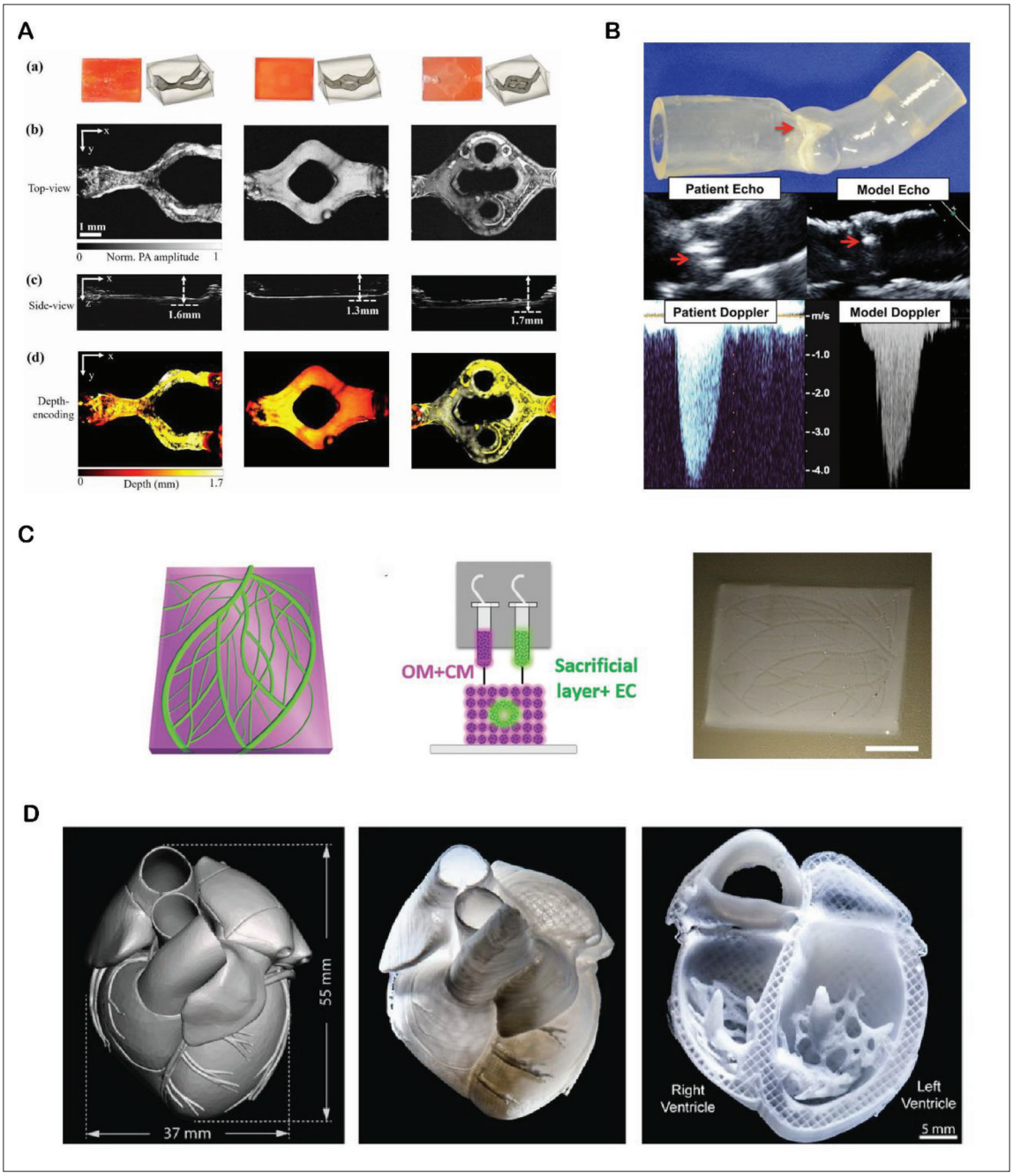

Figure 4. (A) Multiangle photographs of 3D-printed structures of three different types of vascular constructs[18]. Reproduced with permission from IOP Publishing, Copyright © 2022, IOP Publishing. (B) 3D-printed model of the patient (top). Calcification of the aortic valve leaflet (red arrow)[143]. Reproduced with permission from Wolters Kluwer Health, Copyright © 2015, Wolters Kluwer Health. (C) 3D printing of a personalized heart patch. From left to right: a 3D model of the heart patch, printing method, and heart patch with printed blood vessels[145]. Reproduced under Creative Commons license. (D) 3D-printed collagen heart[149]. Reproduced with permission from The American Association for the Advancement of Science, Copyright © 2022, AAAS.

the urgently needed structures for cardiovascular diseases. Wu *et al.*[146] 3D-printed vascular scaffolds with DIW by using gelatin–alginate–montmorillonite nanocomposite bioinks. The inner and outer surfaces of the vascular scaffold displayed networked microporous structures with tensile strength and elastic modulus comparable to those of native arteries. The scaffold aided in the supply of nutrients and cellular infiltration. The hemolysis rate of the scaffold also fulfilled the benchmark for vascular replacement, and the scaffold's rupture pressure was similar to that of

physiological pressure of normal blood vessel. Lee *et al.*[147] 3D-printed biodegradable PLA stents using polymer electrolyte membrane (PEM), and used polydopamine (PDA), polyethyleneimine (PEI), and heparin (Hep) to prevent restenosis and thrombosis by improving anticoagulation and hemocompatibility. Alonzo *et al.*[148] 3D-printed annular hydrogel (gelatin-alginate) scaffolds with microvascular endothelial cells using DIW. These 3D heart-like scaffolds supported long-term cell survival, function, and maintenance of cell phenotype throughout the entire culture period because they were structurally and mechanically stable with highly interconnected pores. The model supported paracrine signaling in addition to heterogeneous cellular contact between endothelial cells and cardiac fibroblasts, cardiomyocytes, and both[148]. Jia *et al.*[19] used a multilayer coaxial extrusion technique to 3D-print a cell-responsive bioink made of GelMA, sodium alginate, and 4-arm poly(ethylene glycol)-tetraacrylate (PEGTA) to enable direct 3D bioprinting for one-step production of perfusable vascular systems with highly organized structures. This hybrid bioink encapsulated stem cell and endothelium in bioprinted structures and supported their growth, producing biologically relevant, highly structured, and perfusable vasculature. Lee *et al.*[149] proposed free-form reversible encapsulation with suspended hydrogels (FRESH) to directly write collagen into bioconstructs of various scales, ranging from capillaries to whole organs. At 20-μm filament resolution, porous microstructures were capable of cellular infiltration and microvascularization. The mechanical strength adequate for the production and perfusion of multi-scale vascular systems and trilobular valves were enabled via pH-driven gelation control. The results showed that the 3D-bioprinted heart accurately reproduced the patient-specific anatomy determined by micro-CT (Figure 4D). Human cardiomyocyte imprinting resulted in coordinated contraction, directed action potential propagation, and wall thickening of up to 14% at peak contraction in the ventricular tissues.

The application of 3D printing in cardiovascular diseases mainly involves cardiovascular phantoms fabricated by material jetting, cardiovascular stents fabricated by material extrusion, and heart patches for treatment fabricated by material extrusion. Materials for bioink mainly include biopolymers such as GelMA and ECM. Live and functional cells are frequently encapsulated and printed together with the biopolymers to simulate certain functions of the cardiovascular system *in vitro*. However, 3D printing of the entire organ remains difficult. Cardiovascular system is a complex anatomical structure. Therefore, to replicate its internal structure and reproduce its function is the greatest challenge in the research and treatment of cardiovascular diseases. Cardiovascular structure restoration is made possible by the careful sorting and placement of cells or tissue blocks in a complex 3D microenvironment with bioprinting technology. Although bioprinting techniques, bioink materials, and post-bioprinting processing are still in their infancy in this area, reproducing heart structures including the myocardium, blood arteries, and heart valves via 3D bioprinting has shown great promises. New bioinks and printers with the ability to manufacture objects with high resolution will advance the science and eventually accomplish the ultimate goal of total organ engineering.

3.4. Digestive system

3.4.1. Application of 3D printing in oral cavity diseases

The tooth loss among the elderly people is mostly caused by periodontal and dental caries. While the gums are aging and shrinking, the exposed area of the cervical root is increased, thereby elevating the risk for caries[150]. Aging also causes severe resorption of alveolar bone, in which case, the supporting structure of teeth is destroyed, causing instability in teeth. Long-term caries and periodontal diseases activate the oxidative damage by free radicals and weaken the repairing ability of the oral cavity, causing tooth loss in the elderly. In summary, tooth loss in the elderly is a systemic process caused by many factors associated with aging[151-154].

Tooth loss adversely affects the life quality and brings great harm to the elderly both physically and mentally. Dental regeneration faces a significant difficulty in replicating the structural complexity and multicellular interactions that resemble their natural 3D counterpart. Due to improvements in 3D printing technology, which enables the creation of structures with patient-specific characteristics, efforts to construct teeth and dental support devices, such as periodontal ligament, alveolar bone, and dental ossicles, have made some progress[155,156]. Although some dental applications of 3D printing are not targeted at the aging population, we focus on the potential applications of these cases in the elderly population, such as 3D-printed crowns and dental implant scaffolds for dental implants in the elderly. It is becoming more apparent that a single material is difficult to meet all the requirements; therefore, the search for more suitable hybrid materials has also become one of the focuses of tissue engineering development[157]. Jeong *et al.*[158] used PEM to 3D-print PCL mixed with HA/β-tricalcium phosphate (β-TCP) into novel scaffolds. It combined bone grafting and implant fixation devices to simplify alveolar bone regeneration and dental implant procedures. The new scaffold had high porosity and good microstructural interoperability. Cell proliferation and alkaline phosphatase assay results were significantly better than those of control scaffolds. The emergence of new hybrid materials is often

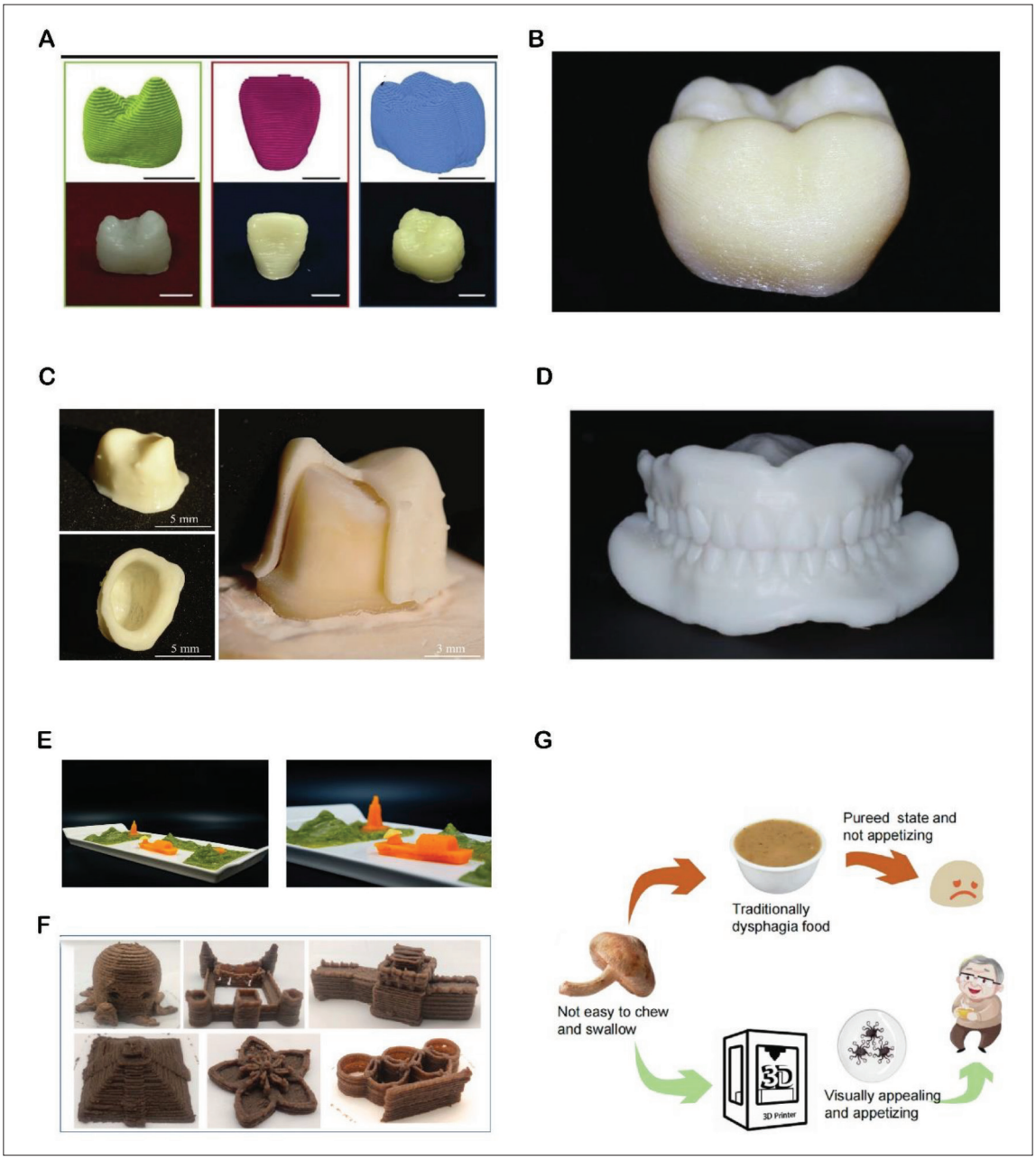

Figure 5. (A) 3D-printed canine incisor and molar crown structures. Cyan, rose red, and indigo represent the crowns of canines, incisors, and molars, respectively[159]. Reproduced under Creative Commons license. (B) 3D printing of dental crown models using photopolymer jetting[160]. Reproduced with permission from Elsevier, Copyright © 2017, Elsevier. (C) SLA-manufactured alumina dental crown[163]. Reproduced with permission from Elsevier, Copyright © 2017, Elsevier. (D) 3D printing of complete dentures using SLA[164]. Reproduced with permission from Elsevier, Copyright © 2023, Elsevier. (E) Image of 3D-printed design combination of peas, carrots and corn suitable for swallowing disorder diet[170]. Reproduced with permission from Elsevier, Copyright © 2021, Elsevier. (F) Sample chart suitable for swallowing disorder diet[168]. (G) 3D printing strategy based on mushroom powder[168]. Reproduced under Creative Commons license.

accompanied by technological updates. Zhao *et al.*[159] used DIW to produce high-precision crowns using multiscale and highly ordered HA frameworks. The smooth printing of "supergravity+" HA nanorod-enhanced hybrid resin-based composites was made possible by designing a nozzle with channels that gradually shrank in response to shear-induced forces. The bending and compression resistance of the crowned structure was superior to the corresponding values of conventional specimens made by molding (Figure 5A). Mai *et al.*[160] used PolyJet 3D printing to fabricate temporary crowns with photosensitive resin and showed that the crowns had better fit and higher marginal accuracy

compared to compression molding and milling techniques (Figure 5B). This result indicated that the technique had a huge potential in endodontic and whole tooth regeneration. Park *et al.*[161] used computational topological design and material injection techniques to 3D-print hybrid human dentin–ligament–bone complexes *in vivo* using PCL and polyglycolic acid (PGA) as inks. The newly formed tissues were grown in the PCL–PGA constructs to form dental osteoid-like tissues, ligaments, and bone structures.

The most popular 3D printing method used in dentistry is vat photopolymerization[162]. Dehurtevent *et al.*[163] used SLA technology and alumina ceramics as raw materials to create a compact 3D crown with controllable shape (Figure 5C). At the same time, many older people are suffering from severe tooth loss due to osteoporosis or oral diseases, which greatly affects their normal life. 3D-printed full dentures provide a new solution to improve the living conditions of the elderly. Deng *et al.*[164] 3D-printed complete dentures with the help of SLA technology combined with traditional process to manufacture final dentures with tooth position accuracy within 150 μm and high production efficiency; therefore, the use of SLA combined with traditional process to manufacture complete dentures is feasible and has significant clinical application (Figure 5D).

Traditional processes of making implants are time-consuming and have the problem of poor fit, but 3D printing effectively reduces the dental surgery time and pain of patients by personalizing the implants according to the characteristics of the patient's lesion. The application of 3D printing in the field of dentistry is becoming more and more comprehensive. Deficiencies that exist at this stage, such as how to make a better combination of mechanical strength and biocompatibility of the denture, are expected to be solved with the continuous innovation of 3D printing technology and materials.

3.4.2. Application of 3D printing in swallowing disorders

The population with swallowing disorders is rapidly increasing with age. Dysphagia is a common disorder affecting approximately 14% of the population over the age of 50 years and up to 40%–50% of residents of senior care facilities[165]. Delayed movement of food mass during swallowing is the characteristic symptom of dysphagia. Oropharyngeal dysphagia causes patients to cough and choke, and food residues to remain in the mouth[166]. Due to the loss of appetite, all these disorders may cause malnutrition, dehydration, and weight loss. In extreme cases, choking may lead to lung infection or even death. To solve these problems, food must be soft enough to chew and safe to swallow. Therefore, it is crucial to develop safe, nutritious and, more importantly, visually appealing diets for this group of people. 3D food printing is capable of creating visually appealing and personalized nutritious foods that are suitable for the dysphagia patients[167]. Liu *et al.*[168] produced nutritious foods with attractive shapes that are compatible with dysphagia people by DIW 3D printing mushroom powder containing hydrocolloids such as xanthan gum (XG) and carrageenan (Figure 5F and G). Most of current 3D food printing studies use processed food powders for DIW 3D printing[169]. However, freeze-dried foods usually lead to loss of nutrients. In contrast, Pant *et al.*[170] 3D-printed fresh vegetables and fruits for dysphagia patients using DIW. These nutritious food inks were prepared in different ways according to the starch, fiber, and water content of the food, which maintained the flavor and nutrition of real foods while ensuring good printability (Figure 5E). Zhang *et al.*[171] used DIW to 3D-print alternative proteins, including plant, animal, insect, and algae proteins, for dysphagia patients. A response surface approach was used to optimize multicomponent protein inks to produce protein-based snacks. Lee *et al.*[172] enhanced the printability and stability of food foam with the help of XG. The food foam was 3D-printed into various shapes to enhance the attractiveness and palatability of food. The 3D-printed food foams were created to facilitate the hydration of patients with swallowing disorders due to the high water content in the ink. Dick *et al.*[173] improved the texture of 3D-printed pork paste by adding hydrocolloids and postprocessing the food by freezing and heating. These 3D-printed food meet international standards for dysphagia diets, and hence is suitable for people with chewing and swallowing difficulties. The cases cited in this review are shown in Table 2.

4. Conclusion and future outlook

In summary, 3D printing has become a viable tool for the fundamental research of aging and aging care. Huge breakthroughs have been made in the area of 3D-printed aging disease models, implants, drug delivery systems, dysphagia diets, and auxiliary devices for the investigation and treatment of various conditions associated with aging in recent years. However, there are still many problems. Traditional cellular and animal disease models have also been explored with more mature solutions. The human body is more complex, and it is difficult to fully replicate the real situation of human diseases with traditional models. With this, 3D bioprinting opens up new opportunities to create models that fully replicate human diseases. However, the exploration of 3D-printed disease models is still largely limited to simple *in vitro* models containing only a single cell type. How to 3D-print a disease model that simulates the physiological characteristics and functions of the real tissue or organ remains a daunting challenge. The research on

Table 2. Summary of applications of 3D printing in aging

Body system	Materials	Cell types	3D Bioprinting technique	Significance	Ref
Nervous system	RGD-GG	Primary cortical neurons of mouse	Material extrusion (DIW)	3D printing brain like structure.	[13]
	GelMA/DA	mNSC	Vat photopolymerization (SLA)	Promote neuronal differentiation of NSCs.	[80]
	GelMA-MENP	/	Vat photopolymerization (2PP)	Deliver neuronal cells and stimulate their differentiation.	[81]
	Eudragit EPO/ POLYOX N80/Pramipexole	/	Material extrusion (FDM)	Personalized dose manufacturing; appropriate drug release curve.	[82]
	PLA/CS	hADMSC	Material extrusion (DIW)	3D-printed levodopa-loaded neural tissue scaffolds to treat Parkinson's disease.	[83]
	Compliant silicone layer	/	Vat photopolymerization (SLA)	Improve patient compliance and satisfaction with non-invasive ventilators (*e.g.*, BIPAP/CPAP).	[87]
Musculoskeletal system	Ti6Al4V/GelMA	rBMSCs	Powder bed fusion (SLM)	Promote osteogenesis and angiogenesis by mimicking the microstructure and mechanical characteristics of genuine cancellous bone.	[104]
	CDHA/PLGA	MC3T3-E1 cells	Material extrusion (DIW)	Good biocompatibility with no cytotoxicity; significant osteogenic capacity.	[105]
	HA/polyfunctional acrylic resins	Rat model	Vat photopolymerization (SLA)	Promote osteogenesis.	[106]
	PTMC/HA	hBMSCs	Vat photopolymerization (SLA)	Enrich surface microlayer structure, significantly improve bone regeneration, and promote osteogenesis.	[107]
	HA/UA/PEGDA	MC3T3	Vat photopolymerization (DLP)	Large structural scale; porous surface; promoted bone regeneration.	[108]
	PHBV/CaSH/CS	rBMSCs	Material extrusion (FDM)	Encourage rBMSC adherence and proliferation while boosting their ability to produce bone; effectively encourage the production of new bone, showing that bone defect repair could be done to its maximum potential.	[109]
	PCL	Osteoblast	Material extrusion (FDM/ MEW)	Significant increase in specific surface area of hybrid scaffold promotes higher osteogenic differentiation.	[110]
	Ti6Al4V/ICA@MOF	Mouse macrophage cells	Powder bed fusion (SLM)	Significantly improve bone metabolism and help improve osseointegration between PT and osteoporotic bone.	[111]
	Ti6Al4V/poloxamer 407	OP-BMSCs	Powder bed fusion (EBM)	Promote osteogenic differentiation while reducing osteoclast activation, significantly improving inward bone growth and osseointegration in osteoporotic defects.	[112]
	Ti6Al4V/poloxamer 407/99Tc-MDP	rBMSCs	Powder bed fusion (EBM)	For individuals with inadequate osteogenesis, 99Tc-MDP-modified inorganic-organic surface can be used as a unique artificial repair interface.	[113]
	Ti6Al4V	OP-BMSCs	Powder bed fusion (EBM)	Delivery of rapamycin effectively promotes osseointegration at the prosthetic interface by modulating autophagy after osteoporosis patients have undergone arthroplasty.	[114]
	Sodium alginate	ATDC5 cell line	Material extrusion (DIW) (3D fiber deposition)	Cell survival rate (85%), cell proliferation and ECM deposition of chondrocytes *in vitro*.	[116]

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Table 2. ***Continued***

Body system	Materials	Cell types	3D Bioprinting technique	Significance	Ref
Musculoskeletal system	cECM/Alginate	hBMSCs	Material extrusion (FDM)	The bionic structure with the same compressive mechanical properties as natural cartilage significantly improves its cartilage potential and promotes the generation of strong cartilage by mesenchymal stem cells.	[117]
	PEGDA/GelMA/CSMA	BMSCs	Material extrusion (FDM)	The scaffold showed good adhesion, proliferation, F-actin and cartilage differentiation with high mechanical strength.	[118]
	PCL/GelMA	Horse cartilage cells	Material extrusion (MEW)	Gel/scaffold composites have a synergistic improvement in stiffness (up to 54 times) when compared to the control.	[119]
	PCL/GelMA/alginate	MSCs and CCs	Material extrusion (FDM)	Equilibrium and dynamic mechanical qualities similar to or identical to those of natural articular cartilage, as well as chondrogenesis mimicking hyaline in co-culture with cells.	[33]
	Gelatin/PLGA/CDM	Rabbit chondrocyte	Material extrusion (DIW)	Significantly enhance the healing of rabbit articular cartilage lesions by significantly enhancing the poor mechanical characteristics of CDM-based scaffolds with good biocompatibility.	[120]
	PCL/sPEG/Hep	Human articular cartilage cell	Material extrusion (MEW)	Similar mechanical properties to natural cartilage for enhanced biological properties.	[121]
Cardiovascular system	PEGDA/GelMA/HAMA	/	Vat photopolymerization (DLP)	*In vitro* model of complex blood vessels with internal microchannels for thrombosis studies.	[18]
	Sandstone	/	Material jetting	Heart anatomy is better understood with the aid of 3D-printed replicas of the organ.	[142]
	VeroWhitePlus RGD835/ TangoPlus FLX930	/	Material jetting	Create patient-specific models to describe the anatomy and function of serious aortic stenosis.	[143]
	PCL/hdECM/vitamin B2/ VEGF	hCPCs	Material extrusion (DIW)	Promote rapid vascularization after patch transplantation to enhance the therapeutic effect of cardiac repair.	[144]
	ECM/Gelatin	iPSCs	Material extrusion (DIW)	Generate thick vascularized tissue that is perfectly matched to the patient.	[145]
	Gelatine/alginate /montmo-rillonite	/	Material extrusion (DIW)	The stent's elastic modulus and tensile strength are comparable to those of natural arteries, and its hemolysis rate satisfies the industry's gold standard for vascular replacement. Its rupture pressure also closely resembles the physiological pressure of natural vessels.	[146]
	PLA/PDA/PEI /Hep	/	Material extrusion (PEM)	Anticoagulation and healthy blood compatibility can help avoid thrombosis and restenosis.	[147]
	Gelatin/alginate	CMs/CFs/ECs	Material extrusion (DIW)	It can be utilized *in vitro* for drug screening and mechanistic research as a model of an organ that resembles the heart and simulates the cardiac environment.	[148]
	GelMA/SA/PEGTA	HUVECs/MSCs	Material extrusion (DIW)	The development of biologically relevant, highly structured, complete blood vessels is caused by novel bioinks that exhibit biochemical properties that are beneficial for cell survival and proliferation as well as changeable mechanical qualities for bioprinting complicated perfusable vascular constructions.	[19]

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	Collagen	C2C12 myoblasts/hESCs	Material extrusion (DIW)	The bioprinted heart faithfully replicates the anatomy of each patient's heart. Synchronized contractions, directional action potential spread, and up to 14% ventricular wall thickening were seen in ventricular tissue imprinted by human cardiomyocytes.	[149]
digestive system	PCL/HA/β-TCP	Saos2 cells	Material extrusion (PEM)	The high porosity and strong microstructure connectivity of the scaffold, when combined with the implant fixation mechanism, outperform those of the control scaffold' based on alkaline phosphatase and cell proliferation findings.	[158]
	HA Nanorods	/	Material extrusion (DIW)	Realization of high-precision crowns with highly ordered HA structures at multiple scales (from atomic to nano to micro to macro).	[159]
	PCL/PGA	hGF	Material jetting	Establish bone, ligament, and tissue structures that resemble tooth cementum.	[161]
	Silicone-replica	/	Material jetting	Better fit and higher edge accuracy.	[160]
	Alumina ceramics	/	Vat photopolymerization (SLA)	Creation of dense 3D alumina dental crown with controlled shape.	[163]
	Photosensitive resin	/	Vat photopolymerization (SLA)	3D printing-based denture tooth displacement accuracy within 150 μm, and SLA shows better accuracy.	[164]
	Shiitake mushroom/AG/XG/KG	/	Material extrusion (DIW)	Use of shiitake mushrooms to produce products for dysphagia individuals, which are also suitable for the elderly.	[168]
	Vegetable/HCs	/	Material extrusion (DIW)	Print out fresh vegetable-based foods that are appealing, nutritious and compatible with dietary standards for individuals with swallowing disorder.	[170]
	Protein/XG	/	Material extrusion (DIW)	Alternative protein is more acceptable to consumers with 3D printing, and is suitable for patients with dysphagia.	[171]
	Pork/HCs	/	Material extrusion (DIW)	Make nutritious, dysphagia-compliant meat products.	[173]
	Food foam/XG	/	Material extrusion (DIW)	Develop food foam with excellent printing performance, which can safely deliver water to patients with dysphagia.	[172]

Abbreviations: AG, arabic gum; BIPAP/CPAP, bi-level positive airway pressure / continuous positive airway pressure; BMSCs, bone marrow stromal cells; β-TCP, β-tricalcium phosphate; CaSH, calcium sulfate hemihydrate; CCs, chondrocytes; CDM, cartilage decellularized matrix; cECM, cartilage extracellular matrix; CFs, cardiac fibroblasts; CDHA, calcium-deficient hydroxyapatite; CMs, cardiomyocytes; CS, chitosan; CSMA, chondroitin sulfate methacrylate; DA, dopamine; DIW, direct ink writing; DLP, digital light processing; EBM, electron beam melting; ECs, endothelial cells; ECM, extracellular matrix; FDM, fused deposition modeling; GelMA, gelatin methacryloyl; HA, hydroxyapatite; hADMSC, human adipose-derived mesenchymal stem cells; HAMA, hyaluronic acid methacrylate; hBMSCs, human bone marrow stromal cells; hydrocolloids (HCs), hCPCs, human c-kit + cardiac progenitor cells; hdECM, heart tissue-derived decellularized extracellular matrix; Hep, heparin; hESCs, human embryonic stem cells; human gingival fibroblast (hGF); HUVECs, human umbilical vein endothelial cells; iPSCs, induced pluripotent stem cells; KG, *k*-carrageenan gum; MENP, magnetoelectric characteristics; MEW, melt electrowriting; mouse neural stem cell (mNSC); MSCs, mesenchymal stem cells; OP-BMSCs, bone marrow stromal cells from osteoporosis; PCL, polycaprolactone; PDA, polydopamine; PEGDA, poly(ethylene glycol) diacrylate; PEI, polyethyleneimine; PGA, polyglycolic acid; PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PLA, polylactic acid; PLGA, poly(lactic-glycolic acid copolymer); PT, porous titanium; PTMC, polytrimethylene carbonate; rBMSCs, rat bone marrow stromal cells; RGD-GG, gellan gum RGD (RGD stands for arginine-glycine-aspartic acid); (sodium alginate) SA , ; SLA, stereolithography; SLM, selective laser melting; sPEG, star-shaped polyethylene glycol; 2PP, two-photon polymerization; UA, urethane acrylate; VEGF, vascular endothelial growth factor; XG, xanthan gum

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new bioink and scaffold is comprehensive and has made big breakthroughs. However, to identify and create materials with physiochemical and physiological properties that are close to the human tissues and meanwhile have good printability still require extensive investigations. Personalized food for the aging population is an outstanding idea empowered by 3D printing, but the relatively high cost and low production throughput have limited the adoption of this technology in most healthcare settings. 3D-printed drugs and drug delivery systems have gone beyond what can be expected from traditional medications. It makes personalized dosage and dosage form a viable option in clinical practice, which could not only reduce the damages caused by the generic formulation but also increase drug efficacy for each individual patient. Nevertheless, price, ethics, regulatory matters, and other medical and nonmedical factors are still big hurdles that prevent 3D drug printing from being adopted in real-world applications. 3D printing still has a long way to go before becoming the go-to technology for aging research and aging care. In our humble opinion, future development in this area should be application-specific and goal-oriented, which means materials, printing platforms, and fabrication process should be developed specifically for each particular application. The highest priority is to develop materials for bioinks that match the physiological characteristics and mechanical properties of human tissue and organs, so that more accurate disease models and transplantable organs could be entirely 3D-printed. From another point of view, although 3D printing has been widely used in the medical field, its applications in the field of rehabilitation medicine have just emerged in recent years. Rehabilitation is one of the important fields of aging research. The use of 3D printing to develop therapeutic and assistive devices, such as hearing aid, limb aid, and disease onset monitoring device, to treat aging-related diseases and restore the activity of daily life of the elderly people is a top priority. 3D printing creates devices that are more convenient, more tailored to the individual, and can perform more functions in a limited space. Future innovation in 3D printing technology should also take fabrication cost and throughput into consideration. Although 3D printing still faces many challenges, we have reasons to believe that it will 1 day become the mainstream technology for addressing aging-related and other medical issues. So far, 3D bioprinting has already made major breakthroughs in nerve regeneration, heart transplantation, bone and cartilage regeneration, and geriatric care. It has helped us gain a deep understanding of geriatric diseases and brought hopes for new treatment and rehabilitation regimes for aging-associated conditions. Despite many challenges, 3D printing has great prospects of benefiting a broader aging population by empowering personalized aging care, thereby promoting active and confident aging, and improving quality of life of elderly people.

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Conflict of interest

The authors declare no conflict of interest.

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