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Review

3D Bioprinting in Cancer Modeling and Biomedicine: From Print Categories to Biological Applications

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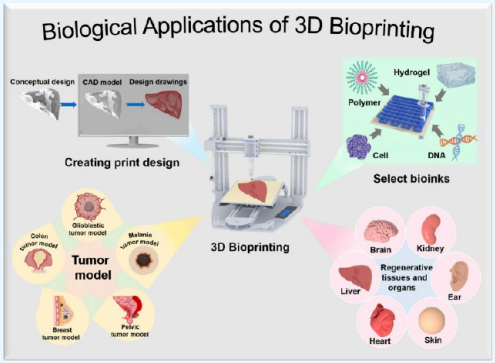
ABSTRACT: The continuous interaction between tumor cells and the local microenvironment plays a decisive role in tumor development. Selecting effective models to simulate the tumor microenvironment to study the physiological processes of tumorigenesis and progression is extremely important and challenging. Currently, three-dimensional (3D) bioprinting technology makes it possible to replicate a physiologically relevant tumor microenvironment and induce genomic and proteomic expression to better mimic tumors *in vivo*. Meanwhile, it plays a crucial role in the prevention and treatment of human diseases, contributing to drug delivery and drug screening, tissue development and regenerative medicine. This paper provides an overview of the categories of 3D bioprinting technology, and the recent advances in the bioinks required for printing. In addition, we summarize the current tumor models based on 3D bioprinting and provide an assessment of possible future biological applications.



1. INTRODUCTION

Cancer is a leading cause of death, and has long been a focus of global biomedical research and practice as associated fatalities increase year on year. Although researchers are learning about the origin of cancer cells, the formation of cancerous tissues, the mechanisms of cancer cell spread and recurrence, and tumor development are not fully understood, which has seriously hampered prognosis and treatment. Cells in normal tissues have a fixed localization, normal proliferation and apoptosis, and a homeostatic cell population. Cancer cells are transformed cells with a range of genes and epigenetic inheritance that enable them to self-renew, proliferate, disrupt *in vivo* homeostasis and growth inhibition, resist apoptosis, induce angiogenesis, and activate invasive migratory mechanisms, resulting in tumor formation.[1] The interaction between tumor cells and the tumor microenvironment (TME) plays a decisive role in tumor progression, metastasis, and therapeutic response.[2] The nature of this microenvironment has been the focus of significant research as it is a key factor that influences evolutionary and ecological processes in tumorigenesis and therapy. There is a pressing need for detailed cancer mechanisms, comprehensive modeling, informed screening of oncology drugs, and analysis of intertumor heterogeneity, microenvironment composition, and metastatic potential. The treatment outcome varies between individuals due to differences in physique, age, environment, lifestyle, and treatment history, suggesting that nonspecific treatments may be ineffective or even dangerous to a significant extent.

Currently, clinical trials represent a final determinant of drug efficacy but these are constrained by ethical and safety considerations. Consequently, the use of *in vitro* tumor models for preclinical studies is particularly important. Traditional tumor models include *in vitro* two-dimensional (2D) models and animal models, whereas emerging tumor models include three-dimensional (3D) models (Figure 1) and organoid models. The 2D model has the benefits of low cost, simple operation and a high survival rate of cells, but the response pattern of tumor cells to drugs in the 2D model differs greatly from that of *in vivo* model. The 2D culture cannot accurately predict the anticarcinogenicity of the drugs,[3] and lacks the cellular communication (cell−cell and cell-matrix) to accurately mimic the natural TME. Although animal models can simulate the *in vivo* environment to a certain extent, they are difficult to operate, costly, time-consuming, and subject to individualized variations. As a result, 3D models have become key to reducing the use of experimental animals in tumor research, tissue engineering and fundamental biology research.

The 3D tumor models include scaffold-based cellular models,[4,5] self-assembly based microengineering,[6] fiber en-

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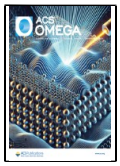

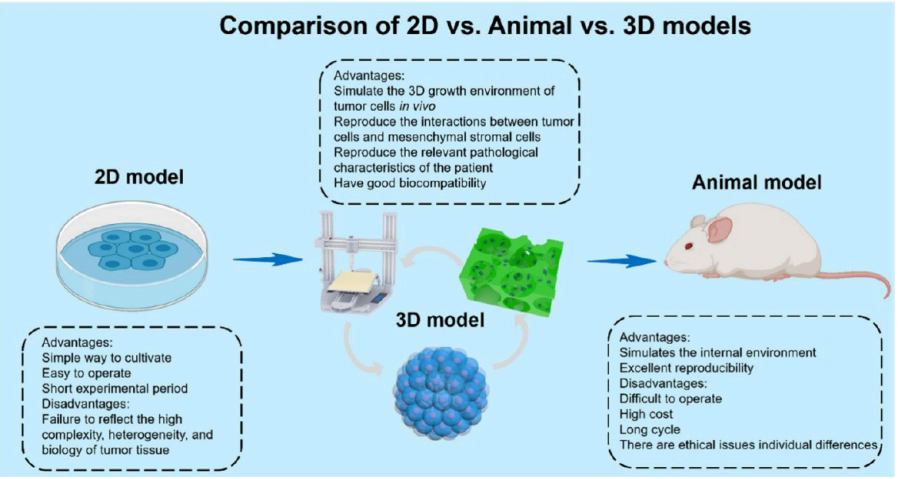

Figure 1. Comparison of 2D culture, 3D and animal models.

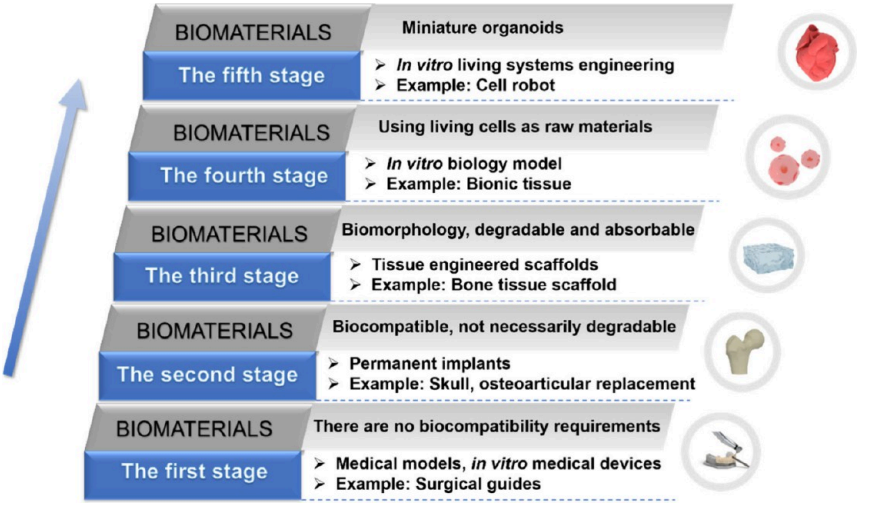

Figure 2. Stages in the development of 3D bioprinting.

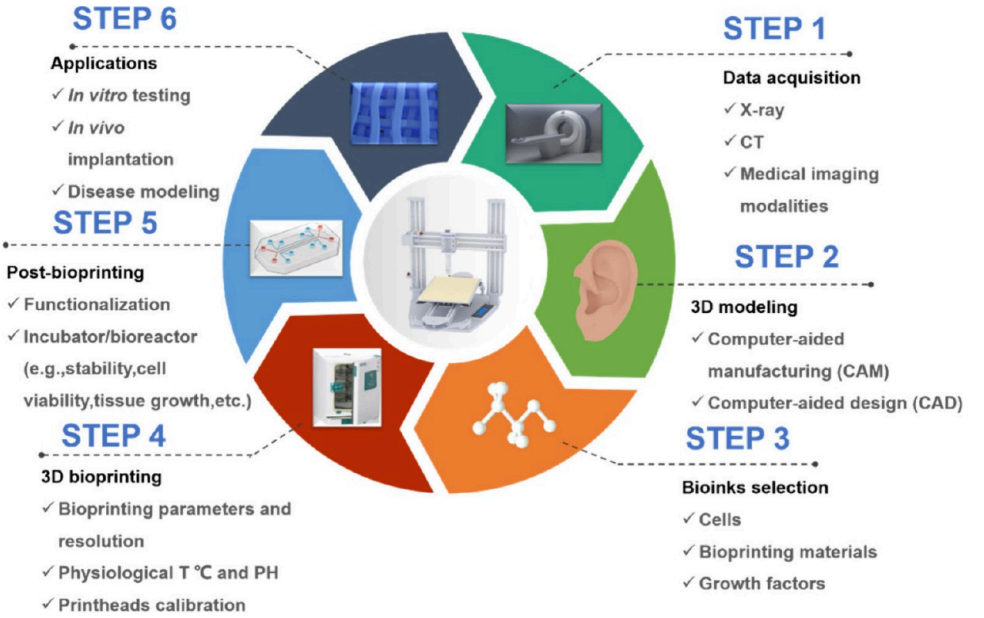

Figure 3. Steps involved in 3D bioprinting technology.

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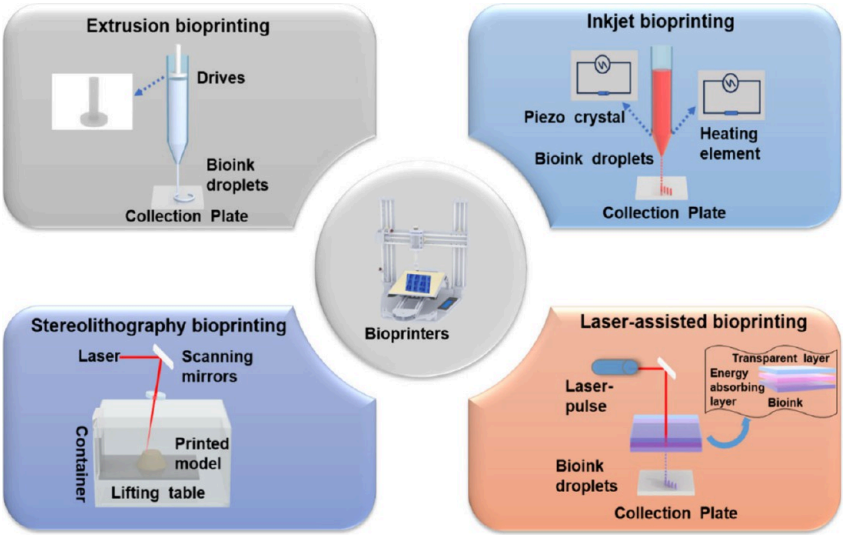

Figure 4. Four principal 3D bioprinting techniques.

gineering, and scaffold-free engineering. The majority of these models are significantly limited in fabricating complex tissue structures with the precision and controllability required to replicate biologically relevant tissues.[7] In 3D bioprinting, the use of different bioinks produces different patterns which can form complex 3D structures with bionic tumor microstructures, facilitating a wide selection of biomaterials to mimic the hardness and ultrastructure of the natural extracellular matrix (ECM). In addition, the use of 3D bioprinting can replicate a physiologically relevant tumor microenvironment, applying a wide range of bioinks with different cell types (tumor and mesenchyme) to construct tumor models, and integrate a sustainable vascular network. Moreover, the 3D microenvironment in the printed tumor structure induces genomic and proteomic expression to better mimic tumors *in vivo*.[8]

Breakthroughs in bio-3D printing technology for regenerating organs and tissues have brought new opportunities to the biomedical field. By incorporating computer-assisted technology, it is possible to improve the surgical outcome and success rate of organ transplantation and provide surgeons with an accurate platform for surgical guidance and simulation. Drug screening *in vitro* allows the selection of appropriate drugs based on individual differences, enabling precise treatment and reducing the number of clinical trials and errors. The new printed organs and tissues can better replace the patient's own condition, reduce the risk of rejection in organ transplantation, and largely avoid the long-term use of immunosuppressive drugs to mitigate rejection.

2. 3D PRINTING TECHNOLOGY, PRINCIPLES AND CLASSIFICATION

2.1. 3D Printing Technology. The concept of 3D printing was first introduced in 1986, and has been developed step-by-step from the first stage with no biocompatibility requirements to the ultimate generation of micro-organs. As illustrated in Figure 2, advances in 3D bioprinting have now enabled fabrication of geometries with preprogrammed structures, containing biomaterials/living cells (collectively referred to as bioinks) by synchronizing the deposition/cross-linking of bioinks that mimic the motorized stage of 3D tissue constructs.[9]

3D printing prepares scaffolds by precise layer stacking using 3D models created from medical imaging data such as computational tomography (CT) and magnetic resonance imaging (MRI). The processing steps in 3D bioprinting include (Figure 3): (1) design and geometric optimization of the 3D printed object according to the printer characteristics using computer-aided design software; (2) 3D modeling in a file format recognizable by the printer; (3) transfer of the file to the software with the creation of the layers to be printed; (4) construction of the model by printing the material layer-by-layer employing a special printing method.[10]

2.2. Principles of 3D Printing. The principle of 3D bioprinting technology refers to the 3D modeling technology in which cells and suitable bioinks are printed according to a designed print pattern through computer-aided design (CAD) software. It is a precise bioprinting method based on the principle of "additive manufacturing", which focuses on the processing of active materials such as cells, bioactive factors and bioinks. This leads to the realization of a new regenerative medicine tissue engineering technology with the goal of reconstructing human tissues and organs in an interdisciplinary field. With the developments of CT, MRI and other medical imaging technologies, high-resolution scanning has led to a significant increase in accuracy, facilitating the advancement of 3D printing technology in modern medicine. Currently, 3D printing can achieve an extremely thin printed product, representing very fine units of measurement. Incorporation with computer design technology for constructing draws on software that circumvents the need for the physical object, enabling a low-cost, efficient and adjustable method applied to cumbersome 3D structures with an extremely fine degree of control.[11]

2.3. 3D Printing Classifications. The tumor microenvironment exhibits complex microstructure ruptures with cancer-associated fibroblasts, infiltrated immune cells, blood and lymphatic networks suspended in the ECM. 3D bioprinting has made it possible to construct tumor models that replicate tumor structures *in vivo*.[8] The technology can be broadly categorized into inkjet bio-3D printing, extrusion bio-3D printing, stereolithography bio-3D printing, and laser-assisted bio-3D printing (Figure 4). These techniques can produce accurate 3D structures through computer-aided

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Table 1. Classification and Advantages and Disadvantages of the Four Printing Technologies

Printing Technology Classification	Advantages	Disadvantages	Application	Ref.
Inkjet-based bioprinting	• Low cost	• Different nozzle sizes, resulting in lower viscosity of bioinks	3D replicas of cartilage, engineered neural tissue, brain tissue, kidney tissue, 3D contractile smooth muscle tissue, skin tissue, tissue barriers, human tissue chips, branching blood vessels, liver and other complex heterogeneous tissue constructs	13−18
	• High efficiency	• Continuous inkjet requiring conductive inks		
	• High resolution	• Low adaptability of printing		
	• High deposition accuracy	• High contamination of ink recirculation		
	• Drop-on-demand and noncontact material transport			
Extrusion-based bioprinting	• Low technological threshold	• Must use photo-cross-linkable biomaterials, and generally need to add a certain amount of cytotoxic photoinitiators	Kidneys, liver, blood vessels, tissue-engineered muscles, intestinal tissues, adipose tissues, organ transplants, dental tissues, vascularized soft tissues, skin structures, engineered neural tissues, brain tissues, kidney tissues, cartilage tissues	12, 19−21
	• Low cost of maintenance	• Embedded in the cells are prone to apoptosis affecting the survival rate of cells		
	• Provides continuous power without being limited by the concentration of bioink	• The printed model is easily deformed.		
	• Wide choice of biomaterials			
	• Allows printing of 3D models with good structural strength			
	• Fast printing of large-sized scaffolds			
Stereolithographic bioprinting	• With high precision	• Limited by the properties of light-curing materials such as brittleness	Cranial grafts, heart valves, skin tissue, bone and cartilage tissue, vascular tissue	22−25
	• Smooth surface of the print object	• Easy deformation		
	• Fast printing speed	• Poor weathering resistance		
		• Poor biocompatibility		
Laser-assisted bioprinting	• High resolution	• Possible transfer of hazardous residues from the energy-absorbing layer during the printing process	Hollow tube tissue structures, skin tissue, bone tissue and other 3D tissue grafts, liver lobule structures	26−30
	• Real-time visual identification and localization of cells and biomaterials			
	• Prevents cell clogging			
	• Controls cell density, microscale distribution and viability of cells			
	• Provides high speed deposition			
	• Degree of precision printing			

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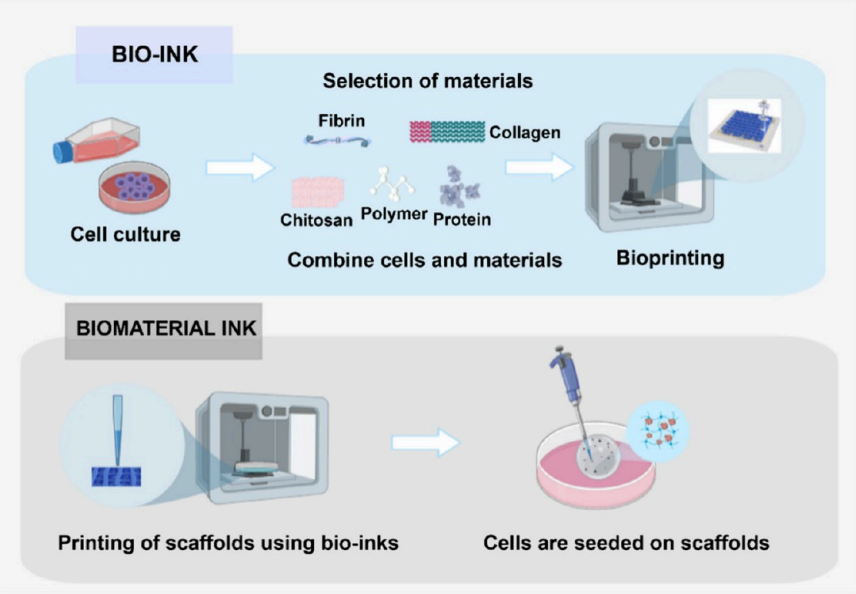

Figure 5. Selection of inks for 3D bioprinting.

design and manufacturing. The bioprinting systems exhibit a different resolution and unique dimensions in printing each layer, as shown in Table 1.[12]

2.3.1. Inkjet-Based Bioprinting (IBB). IBB relies primarily on the deposition of cellular droplets to create natural-like tissues/organs that may be transplanted into the human body to replace damaged ones. According to the mechanism of droplet generation and diffusion, inkjet bio-3D printing can be categorized into continuous, drop-on-demand and electrohydrodynamic inkjet printing. Inkjet bio-3D printing offers high resolution and deposition accuracy during printing.[13] However, the use of different nozzle sizes can result in a lower viscosity of the bioink.

2.3.2. Extrusion-Based Bioprinting (EBB). EBB is the most widely used bioprinting method, which employs a biological system to facilitate bioink deposition under the control of computer modeling data. EBB replaces standard fused printing materials with biomaterials that are extruded as cylindrical filaments from a nozzle by pneumatic or mechanical force to prepare 3D scaffolds with the contribution of cross-linking curing and molding.[31] Depending on the force supplied, extrusion 3D bioprinting can be subdivided into piston, screw and pneumatic extrusion technologies.[32] The passage of bioink through the nozzle can generate a shear force resulting in cell damage and death. Cell survival is possible by carefully controlling the parameters that induce shear (pressure, viscosity of the bioink, size and shape of the nozzle) during the printing process.[12] The advantages of extrusion-based bio-3D printing include the low technological threshold, low cost, continuous power supply that is not limited by the bioink concentration, wide selection of biomaterials, the ability to print 3D models with good structural strength, and the ability to quickly print larger scaffolds.

2.3.3. Stereolithographic Bioprinting (SLA). Light-cured 3D bioprinting uses UV light for layer-by-layer material curing. Liquid photosensitive resins are commonly used curing materials. Photolithography biological 3D printing technology can be categorized in terms of stereolithography and digital light processing. 3D System Corporation launched the first 3D biological printer based on light curing (stereolithography). Following three decades of development, there are a number of new technologies based on light curing, including stereolithography (SLA), digital light processing (DLP), liquid crystal display (LCD), continuous liquid interface production (CLIP), multijet printing (MJP), two-photon 3D printing (TPP), and holographic 3D printing (HGP). Light-cured 3D printing has the advantages of high precision, smooth printed surfaces, and fast printing speed, but is limited by the properties of the light-cured materials such as brittleness, easy deformation, poor weathering resistance, and poor biocompatibility.[22] Photolithography bioprinting employs photo-cross-linkable biomaterials, requiring the addition of cytotoxic photoinitiators that cause damage to cells is greater and must be strictly controlled.

2.3.4. Laser-Assisted Bioprinting (LAB). Laser-assisted bio-3D printing is a technology based on the principle of laser-induced forward transfer of biomaterials through the absorption of laser energy by transparent glass or quartz covered with layers of gold, titanium or platinum (energy-absorbing layers) to the receiving substrate. Laser-assisted bio-3D printers are composed of three main components: (1) a pulsed laser light source; (2) a target or ribbon for printing biomaterials; (3) a receiving substrate to collect the printed material.[26] The energy generated by the laser is cavitated and moves the cell-containing microdroplets to the receiving substrate at speeds in the kilo Hertz range. LAB involves nozzle-less hardware, circumventing cell clogging and allowing control of cell density, microscale distribution and cell viability. The technology provides precise printing and shows significant potential in creating 3D defined precancer and cancer models, contributing to automation, reproducibility and high throughput.[27] However, the LAB technique may transfer harmful residues from the energy absorbing layer during printing.

3. 3D PRINTING MATERIALS

The bioink in 3D bioprinting is used to protect cells from damage sustained during the printing process, and provides geometric support for the constructed 3D model. Bioink is usually composed of biomaterials, living cells and biomolecules (Figure 5).

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The choice of biomaterials should depend on the type of tumor model under construction, the location of the tumor, and the stage of the tumor (primary or metastatic). In order to better mimic the hierarchical structure of natural tissues, studies have been directed at improving printability, focusing on ink viscosity, fast cross-linking and mechanical properties.[7] In addition, different tissues and organs can be printed using 3D bioprinting by choosing the correct bioinks and combining different cells (Figure 6). The TME is relatively complex with

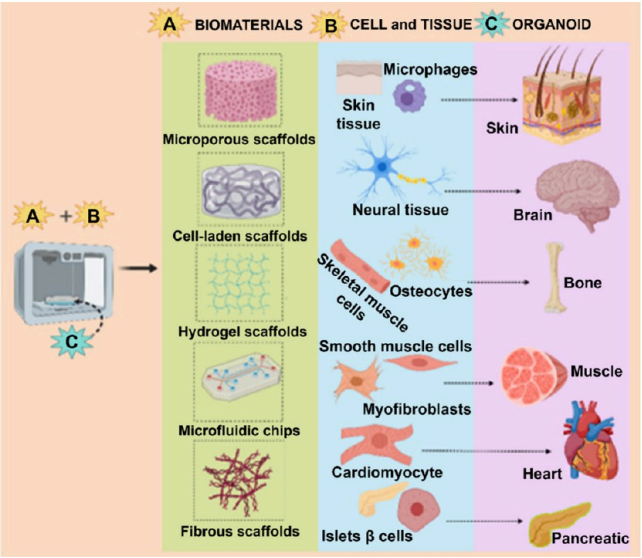

Figure 6. Biomaterials and tissue structures used in 3D bioprinting.

variations in composition and mechanical characteristics for different tumor types and disease stages. Bioinks can be used as a microenvironment to induce tissue formation and maturation,[12] which should be biocompatible and tumor-specific, and preferably include a multimaterial component to provide an effective composition and dynamic environment for tumor growth.[33] Currently, biomaterials used in 3D printing are classified as natural materials, artificial materials, composites, and bioprinting materials containing living cells.

3.1. Natural Materials. Natural biomaterials are widely used in cancer-related research due to their abundance in the tumor microenvironment. Such biomaterials include collagen, chitosan, sodium alginate, gelatin, hyaluronic acid, agarose, matrix gel, fibronectin, decellularized matrix and matrigel (Table 2). Gelatin and collagen-based natural materials can promote cell adhesion, proliferation and differentiation. They have good biocompatibility and biodegradability, low immunogenicity, and can be used as cancer-targeting ligands with high drug-carrying capacity and plasma membrane permeability.[34] Chitosan is the product of removing part of the acetyl group of the natural polysaccharide chitin with multiple biological functions, and shows high activity against various tumors.[35−38] Sodium alginate has the advantages of hydrophilicity, biocompatibility, bioavailability, low environmental impact, and low cost, which makes it suitable for targeted cancer therapies.[39] Hyaluronic acid enables interactions between signaling molecules, forms a pericellular membrane around most cells and regulates cell adhesion, migration and proliferation. It plays a key role in many physiological and pathological conditions,[40] and is considered a key material in pharmacological applications, enabling wound repair and the construction of tumor models.[41]

The basement membrane, mostly found in epithelial and endothelial tissues, is a very important ECM. It can maintain tissue integrity, acting as a barrier for cells and molecules, transmitting signals and maintaining tissue-specific biological functions. Matrix gels are widely used in analyses and models to improve the understanding of tumor biology.[62] Fibronectin is essential for hemostasis, wound healing, inflammation, angiogenesis and several other biological functions. In addition, fibronectin facilitates the attachment of cells to the ECM, forming a fibrous meshwork that is involved in cell migration during tumor metastasis.[63] In recent years, decellularized ECM bioinks originating from animal organs have attracted appreciable attention due to their good biocompatibility. Decellularization is the process of removing cellular components from tissues and organs through the use of chemical reagents and physical and mechanical methods. In this way, biomimetic and biochemical components may be reserved, including ECM fibers, growth factors, and other organ proteins. However, they are characterized by poor printability, poor mechanical properties, low viscosity, and slow cross-linking,[42] and are therefore combined with other materials to prepare bioinks.

3.2. Artificial Materials. Artificial materials are macromolecule polymers synthesized by chemical methods. When compared with natural materials, the structure and mechanical properties of artificial materials are easy to regulate, but there are drawbacks with respect to biocompatibility and biodegradability. Currently, artificial materials are principally based on polyethylene glycol, poly(lactic acid), poly(vinyl alcohol) and poly(caprolactone). These synthetic materials are more advantageous in 3D cultures containing a wide range of cell types, including neuronal cells, osteoblasts, chondrocytes, muscle and kidney cells. The synthetic materials can bind to biologically active components such as matrix metalloproteinase-sensitive peptide linkages or cell adhesion ligands.[64] This can improve the biocompatibility, biosafety and efficiency of synthetic materials in binding drugs, proteins, nanoparticles and microlipids.[65] The cross-linking method is simple and easy to control when applied to synthetic materials.[66] In order to address issues of biocompatibility, biodegradation, brittleness, fracture toughness, and cell adhesion, synthetic materials are generally combined with natural materials in 3D bioprinting to improve the hydrophilicity and connectivity required for the tumor model.

3.3. Composite Materials. A single material has failed to meet the requirements of the product function and performance with the rapid development of 3D bioprinting. Current 3D bioprinting technology employs composite, comingled, or multiphase materials, combining two and more substances with different physical structures or chemical properties when applied to biomedicine. Composite materials can maintain the performance of single components, where optimizing the configuration addresses the deficiencies of a single material.[67] However, it is necessary to consider both the cross-linking agents and method to ensure effective integration of materials with large differences in physicochemical properties. The printability of the bioinks has to be taken into account in the overall preparation process. For example, a sodium alginate-galactosylated chitosan-heparin blend can mimic hepatic extracellular matrix,[54] and galactosylated chitosan grafted

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Table 2. Four Types of Materials and Their Advantages and Disadvantages

Materials	Components	Advantages	Disadvantages	Ref.
Natural materials	Collagen	• Natural materials characterized by low or no toxicity	• Alginate-based bioinks have weak biocompatibility, directly affecting the transformation of cells to tissues after printing	42−48
	Chitosan	• Good biocompatibility	• Collagen-based bioinks exhibit slow forming speed and poor mechanical properties, and need to be modified and mixed with other materials	
	Gelatin	• Promotion of cell-material interactions		
	Sodium alginate	• Ease of processing		
	Hyaluronic acid	• High cellular activity		
	Agarose	• High plasticity		
	Matrix gel	• Good biodegradability		
	Fibronectin	• High stability		
	Decellularized matrix, etc.	• Good anticoagulant properties		
Artificial material	Polyethylene glycol	• Easily adjustable structure and properties	• Poor biocompatibility and biodegradability	49−52
	Polylactic acid	• Good mechanical properties, linkable to bioactive components or cell adhesion ligands	• Lack of hydrophilicity and brittleness	
	Polyvinyl alcohol	• Simple cross-linking method	• Low fracture toughness	
	Polycaprolactone, etc.	• Low requirements for cross-linking agents		
Composite material	Sodium alginate-galactosylated chitosan-heparin	• Maintain the performance of a single component, optimize the configuration, make up for a single material deficiencies	• For materials with widely varying physicochemical properties, the variety of cross-linking methods and approaches makes it difficult to manipulate and consider the printability of bioinks made from composites during the printing process	53−57
	Galactosylated chitosan grafted with polyethylene glycol, etc.			
Biomaterials containing bioactive factors	Growth factors	• Cell survival on co-cultured 3D printed scaffolds is higher than normal 2D culture	• The direct involvement of cells in the printing process is complex	58−61
	Fibroblasts		• The operation is demanding and susceptible to contamination	
	Cancer cells		• High demands on the cells themselves	
	Stem cells, etc.		• The selection of biomaterials for co-culturing with the cells is a difficult issue	

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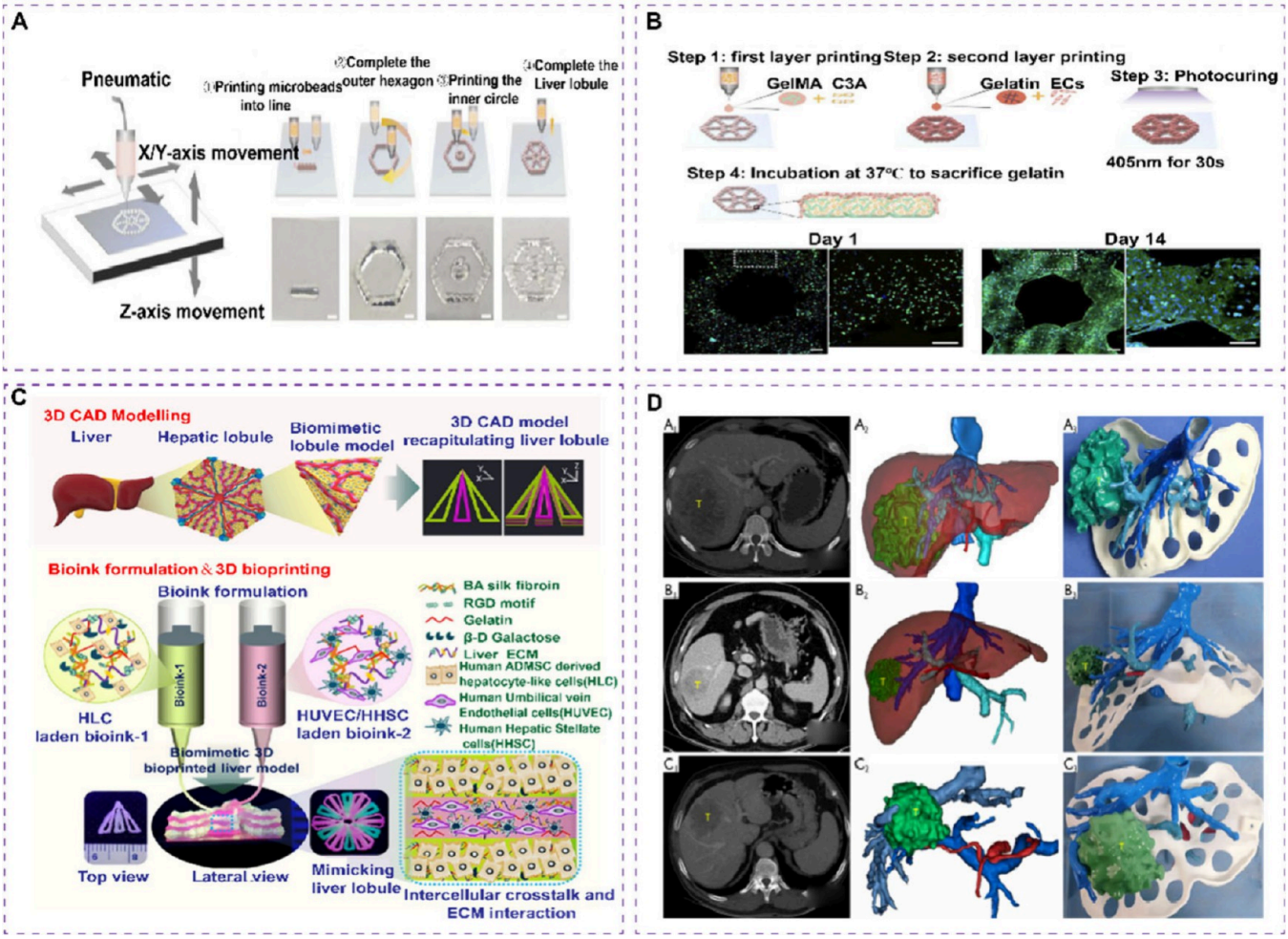

Figure 7. Construction of a 3D printed liver cancer model. (A) GelMA hydrogel beads produced by the DEP system were utilized to produce liver lobulate-like structures. (B) Images of lobular structures in different layers, as well as analysis of C3A cell viability before and after printing. Reprinted with permission from ref 75. Copyright 2023 MDPI. (C) Schematic representation of CAD design, bioink formulation and bioprinting of the liver lobule model. Reprinted with permission from ref 76. Copyright 2022 Amer Chemical Soc. (D) 3D virtual reconstructions and 3D printed liver models created for the three HCC cases using MDCT. Reprinted with permission from ref 77. Copyright 2023 Ame Publishing Company.

with polyethylene glycol has been used as a hepatocyte-targeted DNA carrier.[55]

3.4. Bioprinting Materials Containing Bioactive Molecules. Biomolecules can be incorporated in biological inks. Growth factors refer to a group of proteins or steroid hormones that stimulate cell differentiation, proliferation, survival and tissue regeneration. Some growth factors act on specific types of tissues and are widely used in tissue engineering. These growth factors can be used as additives with scaffold materials or encapsulated in controlled release systems. To date, researchers have used a wide range of 3D bioprinted scaffolds for cell cultures and have demonstrated that cell survival on 3D-printed scaffolds is much better than normal 2D cultures.

4. 3D PRINTING OF TUMOR MODELS

Classical tumor models such as 2D cell culture, 3D tumor spheroids, and tumor organs lack the necessary tumor microenvironmental components. 3D bioprinting can be used to fabricate bionic tissue models by patterning different cell populations in a spatial dimension to replicate *in vivo* the architecture to build models with physiological microstructures and microenvironments, which provides a tremendous advantage for the development of *in vitro* tumor models.[7,68] Currently, several tumor models have been printed using 3D bioprinting, and used to study cell−cell interactions and cell-matrix interactions under physiological conditions.[69−71] Another advantage of 3D bioprinting in building cancer models is that experiments can be conducted for several months which permit longitudinal studies and investigation of the crosstalk between cancerous cells and resident cell populations within the tumor, and cells in the surrounding healthy tissue.[8,72]

4.1. 3D Printed Liver Cancer Model. Hepatocellular carcinoma (HCC) is one of the most common cancers globally, representing a major healthcare challenge. Patients with HCC have a wide range of therapeutic options that include liver transplantation, surgical resection, percutaneous ablation and radiotherapy, as well as trans-arterial and systemic therapies.[73] However, there is a lack of credible and usable *in vitro* models for patient-specific screening of HCC drugs. 3D printed models enable the prediction of individualized patient therapeutic agents. Xie et al. have successfully established patient-derived 3D bioprinted HCC models (3DP-HCC) based on sodium alginate and gelatin bioink.[74] The 3DP-HCC models preserved the characteristics of the parental

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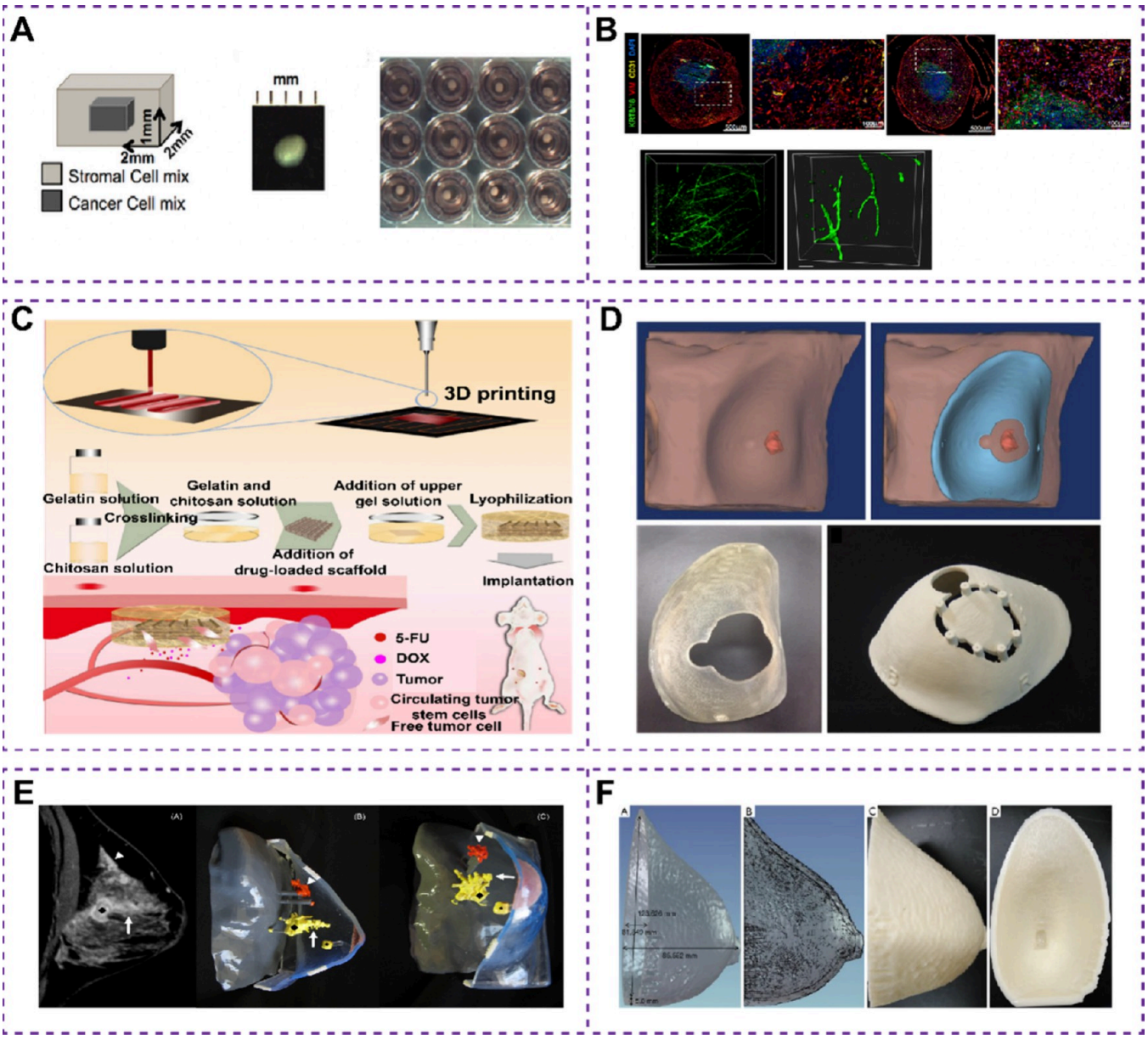

Figure 8. 3D bioprinting breast cancer models. (A) Schematic diagram of 3D bioprinting and photographs of cultured printed tissue in millimeters. (B) Immunofluorescence results of specific proteins of cancer cells and fibroblasts in 3D printed cancer models. Reprinted with permission from ref 81. Copyright 2019 Cell Press. (C) Schematic representation of the construction process of the drug-loaded 3D printed breast cancer model. Reprinted with permission from ref 82. Copyright 2022 Ivyspring Int Publ. (D) Creation of 3D models of the breast tissue and tumor using preprocessed MRI images. Reprinted with permission from ref 83. Copyright 2019 Nature Portfolio. (E) 3D printed breast model from MRI images. Reprinted with permission from ref 84. Copyright 2021 Wiley. (F) Breast model using 3D printing. Reprinted with permission from ref 85. Copyright 2019 Ame Publishing Company.

hepatocellular carcinomas, including stable expression of biomarkers and maintenance of expression profiles. These models can visualize and quantify the results of drug screening. Fan et al. prepared endothelialised hepatic lobule-like structures by printing GelMA hydrogels loaded with hepatocytes and gelatin microspheres loaded with HUVEC using extrusion printing.[75] This provided an effective platform for reconstructing the structural architecture of natural hepatic tumors and the tumor-scale microenvironment (Figure 7A–B). This provided an effective platform for reconstructing the structural architecture of natural hepatic tumors and the tumor-scale microenvironment (Figure 7A–B). In another study, researchers used extrusion 3D bioprinting to print a human blood-supplied liver model using a novel ECM-based bioink to assess hepatotoxicity, providing a robust platform for hepatotoxicity screening (Figure 7C).[76]

Previous studies have shown that 3D printed models (3DPM) of HPC may provide more information than 3D virtual reconstruction (3DVR) and multidetector computational tomography (MDCT). Cheng et al. combined 3D printing with clinical teaching, significantly improving the professional theoretical level of medical trainees, and enhancing clinical thinking and comprehension, with improved teaching applied to HPC.[77] They considered three cases of laparoscopic hepatectomy and created 3DVR and 3DPM models based on MDCT data for each case. The surface of the

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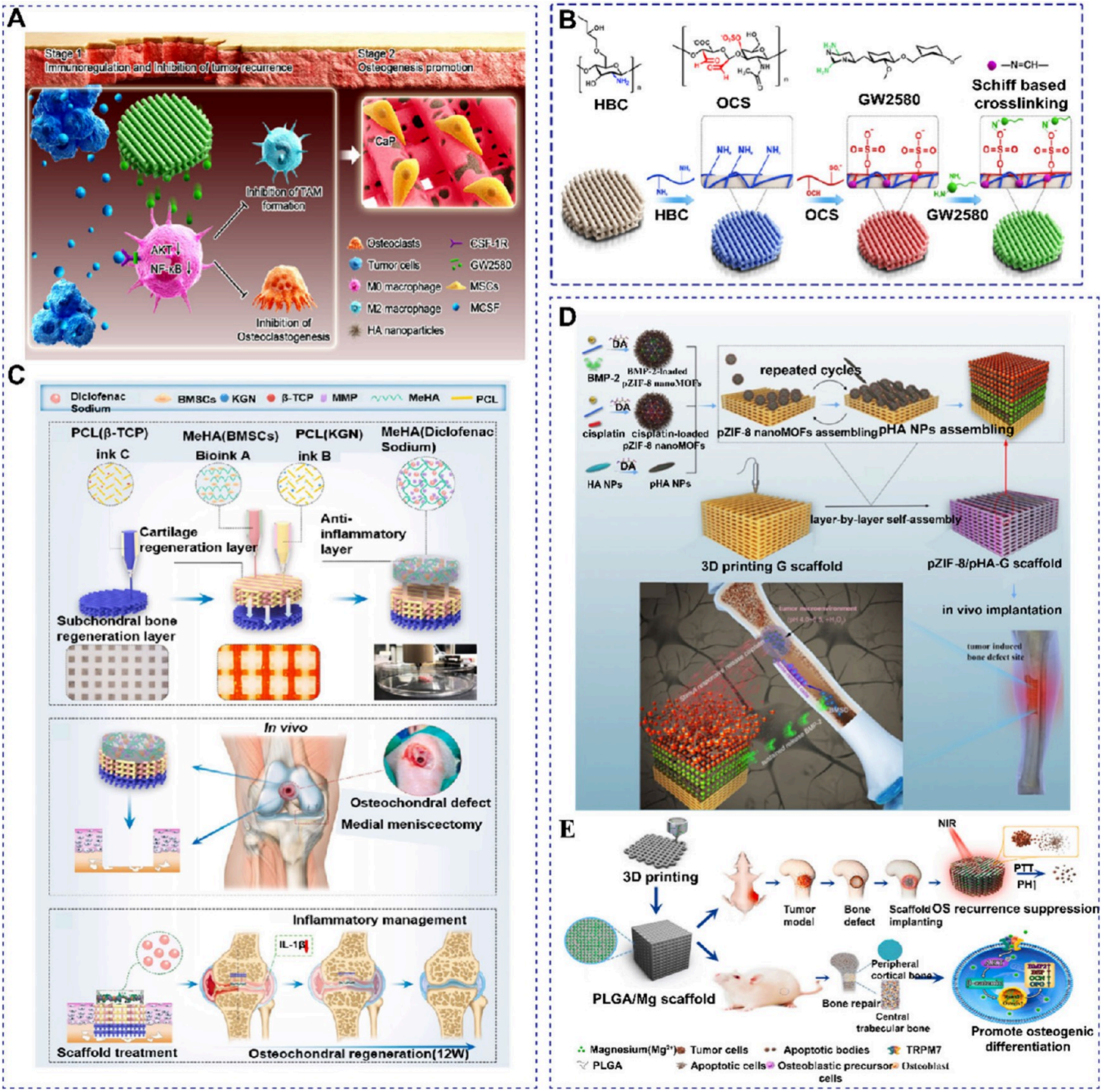

Figure 9. 3D bioprinting bone tumor models. (A) Functions and mechanisms of 3D bioprinted scaffolds modulating the macrophage immune microenvironment for postoperative treatment of bone tumors. (B) Preparation process and related mechanism of scaffold loaded GW2580 inhibitor. Reprinted with permission from ref 87. Copyright 2023 Keai Publishing Ltd. (C) Flowchart of 3D printed scaffold fabrication and therapy for osteochondral defects. Reprinted with permission from ref 88. Copyright 2021 Elsevier. (D) Construction of 3D printed models with anticancer and bone forming properties. Reprinted with permission from ref 89. Copyright 2021 Elsevier. (E) Schematic representation of a 3D printed PLGA/Mg scaffold as an integrated platform for recurrence inhibition and bone regeneration after postsurgical osteosarcoma. Reprinted with permission from ref 90. Copyright 2021 Elsevier.

3DPM was skeletonized and the newly printed models cured with UV lamps following staining in a processing box to finalize a specific 3DPM (Figure 7D). The results demonstrated that 3DPM can indicate the correct tumor location, accurately identify the relationship between the tumor and blood vessels, and design an appropriate surgical strategy.

4.2. 3D Printed Breast Cancer Models. Breast cancers are heterogeneous with different morphological and biological characteristics, and exhibit different clinical behavior and therapeutic responses.[78] 3D printed models can facilitate research into the diagnosis and treatment of breast cancer patients. Dankó et al.[79] established a breast cancer (BRCA) model with 3D bioprinting based on sodium alginate bioink and cultured tumor cells *in vitro* over a long-term. The 3D bioprinted tissue-mimicked scaffolds exhibited a close similarity in terms of drug sensitivity and protein expression profiles. In addition, the 3D bioprinted model represented *in situ* tissue heterogeneity that is characteristic of human breast

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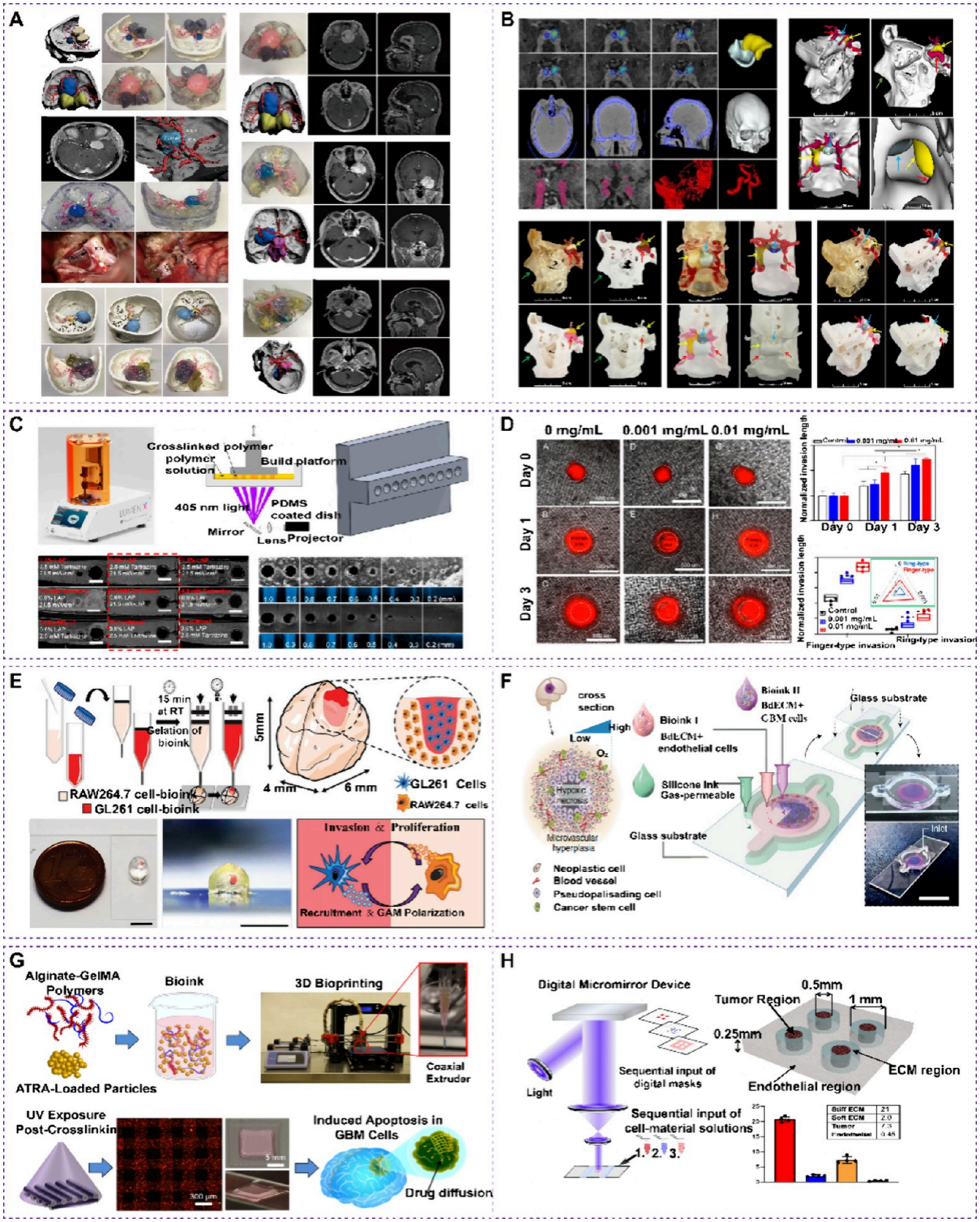

Figure 10. 3D bioprinted brain tumor model. (A) 3D bioprinted models of meningeal tumors at the base of the skull can present the structures of tumors and adjacent normal glands in detail. Reprinted with permission from ref 93. Copyright 2021 μ_BC. (B) 3D bioprinted pituitary tumor model. Reprinted with permission from ref 94. Copyright 2020 Ame Publ Co. (C−D) 3D printed tumor-on-a-chip models for the study of the effect of matrix hardness on glioblastoma invasion. Reprinted with permission from ref 96. Copyright 2023 MDPI. (E) Schematic of the bioprinting process and bioprinted miniature brain. Reprinted with permission from ref 97. Copyright 2019 Wiley-V C H Verlag Gmbh. (F) Bioprinted glioblastoma-on-a-chip. Reprinted with permission from ref 72. Copyright 2019 Nature Portfolio. (G) 3D printed hydrogel meshes of polymer particles loaded with ATRA. Reprinted with permission from ref 98. Copyright 2019 Elsevier. (H) 3D bioprinted GB models with biophysical properties of different regions. Reprinted with permission from ref 99. Copyright 2021 Wiley-VCH Verlag Gmbh.

cancer. A biomimetic bone matrix was prepared using 3D bioprinting to study the interaction between BRCA cells and bone stromal cells (fetal osteoblasts and human bone marrow mesenchymal cells (BMSCs)). It was found that osteoblasts

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ACS Omega 2024, 9, 44076−44100

and MSCs exhibited a growth-promoting effect on BRCA cells, whereas BRCA cells had an inhibitory effect on the proliferation of osteoblasts and BMSCs, which may provide a valuable tool for subsequent studies of breast cancer progression following bone metastasis.[80] Another study designed a tumor model in which the core tumor cell bioink was surrounded by a normal stromal cell bioink. The hydrogel bioink was chemically modified to ameliorate tensile strength during tissue construction (Figure 8A). Immunofluorescence staining results have demonstrated a close interaction between epithelial cancer cells and mesenchymal fibroblasts. Endothelial cells in the bioprinted tumor tissues were visualized and formed an intact network with multiple branches as analyzed by Clarity Technology using light-sheet microscopy (Figure 8B). Finally, the researchers transferred the tissues into mice and found that the cancer cells in the bioprinted tissues maintained their tumorigenic properties and grew as xenografts. The findings showed that bioprinted epithelial and stromal cell types could survive, self-organize and interact to form tissue-like structures.[81]

The 3D printing model can provide a drug loading platform to prevent recurrence and metastasis of breast cancer. Shi et al.[82] developed a polylactic acid-glycolic acid-gelatin-chitosan-loaded anticancer drug scaffold with a good hemostatic effect and suitable potential of hydrogen sensitivity. The multifunctional implantable scaffold was effective in preventing tumor recurrence and metastasis after surgery, which offers significant potential for integrating oncology treatment and postoperative wound healing (Figure 8C). In addition, 3D bioprinting enables complete breast reconstruction combined with CT and MRI, providing an effective means of postoperative treatment. We should note the application of 3D printing technology to breast cancer patients who underwent partial mastectomy after neoadjuvant chemotherapy. The work involved 3D models of the breast tissue and tumor using preprocessed MRI images and provided surgical guides to mark the primary tumor (Figure 8D).[83] In another report, personalized 3D printed breast models were generated using MRI images (Figure 8E).[84] In addition, He et al. used 3D printing to create multifunctional breast body models with tissue-equivalent materials for quality management of multimodality imaging (Figure 8F).[85]

4.3. 3D Printed Skeletal Tumor Models. Bone tumors are one of the most common clinical challenges in orthopedics, and include primary, invasive and metastatic bone tumors. Once these tumors grow and develop in the bone system, they interact with osteoblasts and other environmental cells, ultimately leading to the destruction of the skeleton physical structure. Surgical procedures for bone tumors may lead to permanent defects.[14] 3D bioprinting has the advantage of replacing or repairing damaged tissues and organs, which can be predesigned to accommodate bone defects with different shapes and sizes. In addition, the interconnected perforations created by 3D printing provide conditions for cell activity, nutrient delivery and drug delivery.

Zhao et al. used a 3D printed personalized prosthesis to reconstruct large segmental bone defects after resection of malignant tumors. The porous structure of the prosthesis provided early biostability, and osteointegration occurred at the prosthesis-bone interface in all patients with satisfactory limb function.[86] In another study, a two-stage regenerative 3D-printed scaffold was constructed for postoperative bone tumors, which could modulate the macrophage immune microenvironment and promote bone regeneration (Figure 9A). A biofunctional CPC/hydrogel/GW2580 composite scaffold was prepared by adding hydroxybutyl chitosan (HBC) to the printed calcium phosphate ceramic (CPC) scaffold, and cross-linked with oxidized chondroitin sulfate (OCS) with an incorporation of inhibitor GW2580 (Figure 9B). The pore sizes of the CPC and CPC/hydrogel scaffolds were ca. 500 μm, ideal for regeneration of bone tissue.[87] Liu et al.[88] designed a 3D bioprinted MeHA-PAC multilayer scaffold loaded with BMSCs. The scaffold combined kartogenin and β-tricalcium phosphate (β-TCP), used to repair osteochondral defects in each region. In addition, diclofenac sodium modified matrix metalloproteinase-sensitive peptide MeHA was induced on BMSC-loaded scaffolds as an anti-inflammatory strategy. The results confirmed the viability of 3D bioprinted BMSC scaffolds to inhibit joint inflammation as well as promote repair of articular cartilage defects (Figure 9C).

The application of 3D printing can simultaneously release anticancer drugs and growth factors for antitumor therapy and osteogenesis. Jiang et al.[89] designed a 3D printed implant with alternating polydopamine (PDA) hybridized nanosized zeolitic imidazolate framework-8 (pZIF-8 nano-MOFs) and PDA modified hydroxyapatite nanoparticles (PDANPs) on the surface of 3D printed gelatin scaffolds using a layer-bylayer assembly strategy. The *in vitro* and *in vivo* experiments indicated that the scaffold effectively induced osteogenic differentiation and promoted new bone formation (Figure 9D). A PLGA/Mg porous scaffold was developed for comprehensive treatment following osteosarcoma surgery. The researchers prepared 3D printed scaffolds with a bionic layered porous structure, which promoted bone regeneration. The PLGA/Mg scaffold served to inhibit tumor recurrence under near-infrared light irradiation and effectively repaired bone defects *in vivo* (Figure 9E).[90]

4.4. 3D Printed Brain Tumor Models. The most common brain tumors are brain metastases, meningiomas, pituitary tumors and glioblastomas.[91] The base of the skull serves as the supporting structure of the brain and the main dividing line lies between the intracranial central nervous system and the extracranial head and neck. Many different benign and malignant processes may affect the base of the skull, where the involvement of malignant processes can dramatically alter staging, surgical access and radiation planning.[92] Surgery for skull base meningiomas is typically difficult and complex. Gillett et al.[93] have created accurate 3D anatomical models of pituitary tumors and adjacent normal glands using four different 3D printing techniques based on results from anatomical and molecular imaging (Figure 10A). Guo et al.[94] confirmed the effectiveness of 3D printed models for skull base meningiomas in terms of anatomical reconstruction and simulation of surgical planning. They reported that the 3D printed model could visually reveal the relationships between different structures, including the skull, blood vessels, brain nerves and tumors (Figure 10B). In this way, doctors can choose the appropriate surgical approach by applying the model before surgery, taking care to protect important structures and cutting off the tumor's blood supply during surgery.

Glioblastoma (GB) is the most common and fatal primary central nervous system cancer in adults. 3D bioprinted constructs make it possible to study cells and cell-ECM interactions in a species-matched, high-throughput and reproducible manner.[95] We should note a study that

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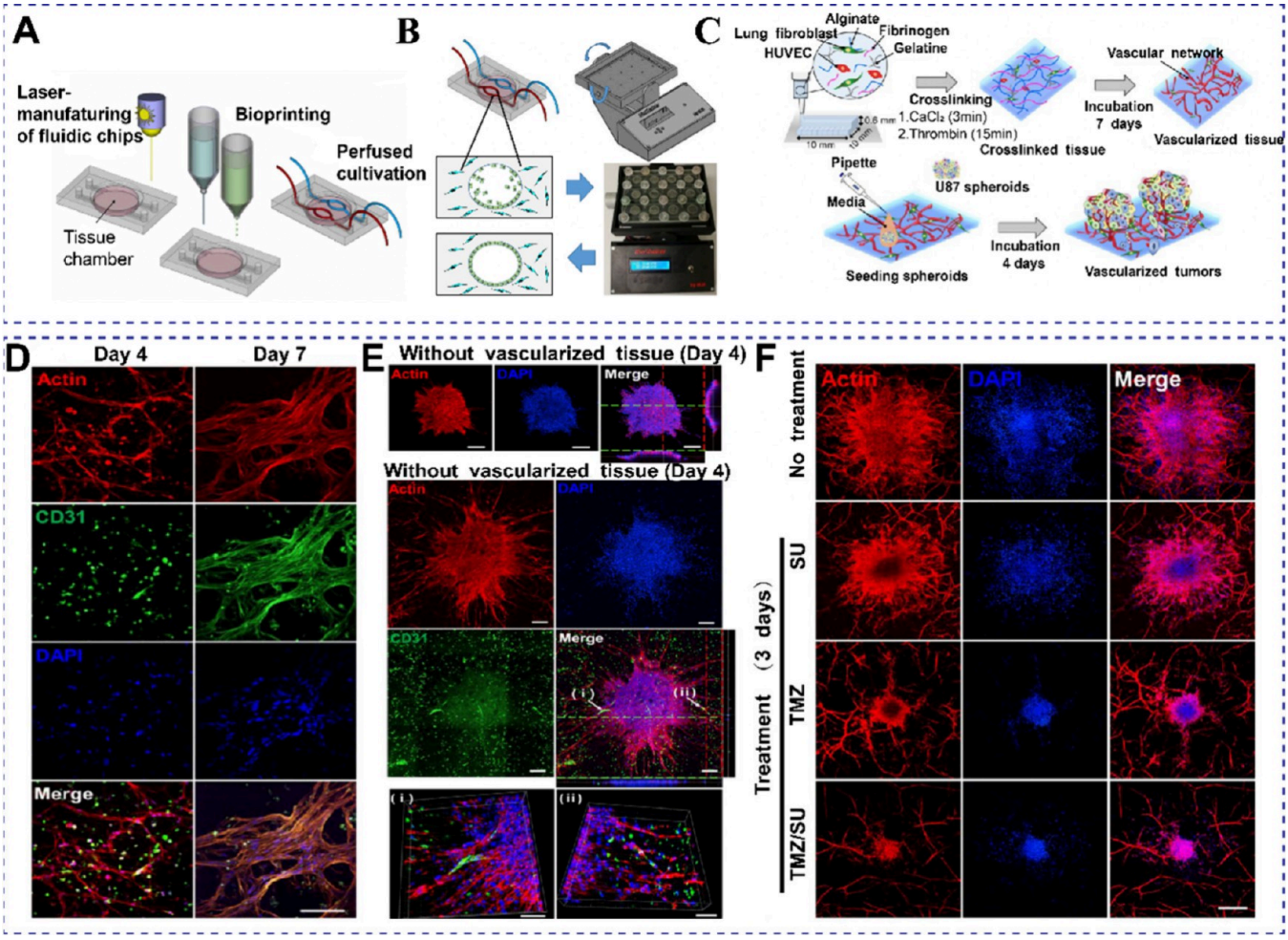

Figure 11. 3D bioprinted vascularized neuroblastoma model. (A) Schematic presentation of printed tumor model into a customized fluidic chip. (B) A programmable 3D orbital vibrator to improve and standardize the collagen and endothelial cell coating of the catheter wall. Reprinted with permission from ref 102. Copyright 2022 IOP Publishing Ltd. (C) Combination of bioprinted vascular layer and MCTS to obtain uniformly sized vascularized tumors. (D) Cross-sectional images of the microvessels in the layer labeled with phalloidin (red), Alexa Fluor 488-conjugated anti-CD31 antibody (green), and DAPI (blue) on days 4 and 7. (E) Laser confocal images showed the effect of vascular tissue without and with vascularization on the growth and morphology of U87 MCTS. (F) Immunofluorescence results of MCTS on vascular tissues treated with TMZ, SU or TMZ/SU for 3 days demonstrated the synergistic inhibitory effect of TMZ and SU on U87 cells. Scale bar = 500 μm. Reprinted with permission from ref 104. Copyright 2020 MDPI.

investigated the effect of matrix hardness on the invasion patterns of human GB using a 3D printed single chip culture platform.[96] The results demonstrated that tumors exhibited two invasion patterns in response to collagenase concentration. A higher collagenase concentration generated a longer invasion length, confirming a strong dependence of tumor behavior on the stiffness of the surrounding matrix (Figure 10C−D). In order to study the role of glioblastoma-associated macrophages (GAMs) in the development and invasion of glioblastoma multiforme, researchers printed mini-brains including GB cells and macrophages. This work has provided a tool to study the interaction between the two cells and assess therapeutic strategies, which contribute to an understanding of tumor biology in evaluating new cancer therapies (Figure 10E).[97] Recombinant glioblastoma was created to mimic the structural, biochemical and biophysical properties of natural tumors using 3D bioprinting with a bioink composed of patient-derived tumor cells, vascular endothelial cells and decellularized stroma from brain tissue. The pathological features and complex ecology of the tumor were reproduced in a zoned cancer-tumor concentric ring structure that maintains a radial oxygen gradient.[72] The results have demonstrated that 3D printed glioblastoma on a chip replicated clinical patient resistance to radiotherapy and Temozolomide treatment, which may assist the development of effective tumor-killing drug effects (Figure 10F).[72] Mirani et al.[98] applied 3D bioprinting to fabricate hydrogel meshes of polymer particles loaded with all-trans retinoic acid (ATRA). The resultant meshes facilitated the slow release of ATRA at a controlled rate, inhibiting the growth of U-87 MG cells, and providing a novel option for the treatment of glioblastoma (Figure 10G). Tang et al. created a bionic three-region glioblastoma model that included tumor, cell-free ECM, and endothelial regions with regional stiffness corresponding to the glioblastoma mesenchyme, pathological or normal brain parenchyma and capillaries. The model achieved flexible, rapid and reproducible patient-specific glioblastoma modeling with biophysical heterogeneity. It can also be used as a tunable system to study glioblastoma mechanisms and screen drug compounds (Figure 10H).[99]

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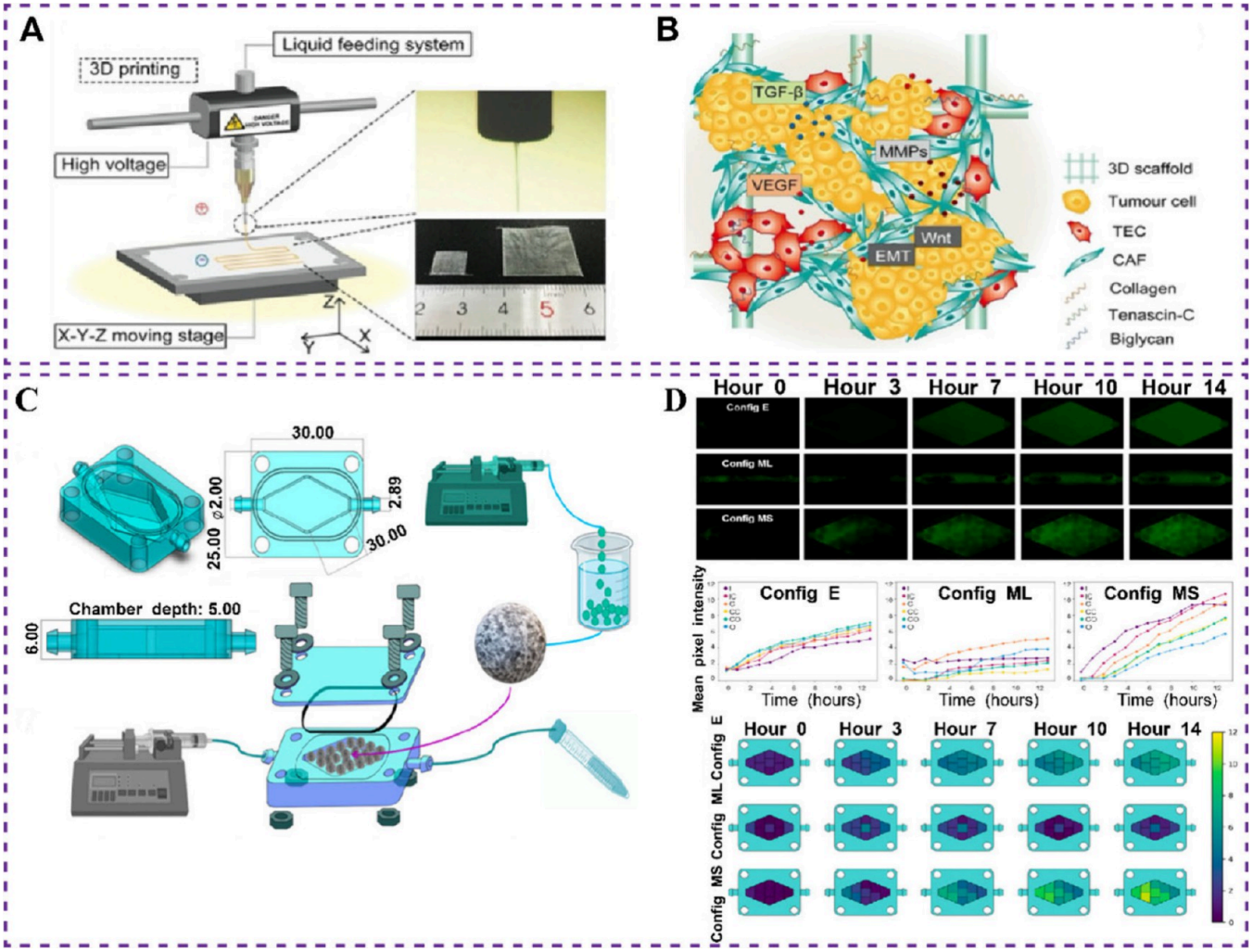

Figure 12. 3D bioprinted colorectal cancer model. (A–B) Schematic illustration of the electrohydrodynamic printing device and 3D tumor tissue, respectively. Reprinted with permission from ref 106. Copyright 2020 Ivyspring Int Publ. (C) Designing the colorectal tumor on a chip. (D) Comparison of culture medium diffusion in three different configurations of the rhomboid chip: Config E indicated an empty culture chamber, config ML indicated a monolithic 3D GelMA hydrogel, and config MS indicated hydrogel microspheres filling the chamber. Reprinted with permission from ref 107. Copyright 2023 MDPI.

4.5. 3D Printed Neuroblastoma Model and Vascularized Tumor Model. Neuroblastoma is an extracranial solid tumor with poor prognosis. It usually occurs in early childhood, most commonly in the abdomen and adrenal gland.[100] Neuroblastomas are distinctly heterogeneous with a diverse range of biological and clinical features.[101] Nothdurfter et al.[102] developed a perfusion and microvascular tumor model that was directly bioprinted onto a customized fluidic chip. GelMA containing fibronectin of multiple cell types has mimicked the TME where embedded endothelial cells promoted spontaneous microvessel formation. Patient-derived neuroblastoma spheroids were combined with the matrix during the printing process, and allowed to grow for over 2 weeks, generating tumor spheroids. Once the spheroids were destroyed, neuroblastoma cells invaded the tumor environment, enabling the bioprinted model of the microvascular neuroblastoma TME to be incorporated on a fluidic chip (Figure 11A and 11B).

Tumor angiogenesis is considered a promising target for limiting cancer progression because tumor-associated blood vessels supply blood and provide metastatic pathways. Therefore, the use of 3D bioprinting to reproduce vascularized tumors *in vitro* plays an important role in understanding cancer pathology and determining the mechanisms of tumor cell proliferation and metastasis.[103] Han et al.[104] investigated a bioprinting method for reproducing TMEs with a controllable sphere size. TMEs were constructed by printing a vascular layer consisting of fibroblasts and endothelial cells in gelatin, sodium alginate, and fibrinogen. Multicellular tumor spheroids (MCTS) of glioblastoma cell line U87MG cells were then constructed on the vascular layer (Figure 11C). Immunofluorescence results have shown that the blood vessels gradually formed and surrounded the MCTS (Figure 11D and 11E). The inhibitory activity of the combined anticancer drug Temozolomide (TMZ) and angiogenesis inhibitor sunitinib (SU) was superior to TMZ alone for perivascular MCTS, suggesting the feasibility of TME for *in vitro* pharmacodynamic testing (Figure 11F). These findings have established that bioprinted vascularized tumors are valuable for understanding tumor biology as well as *in vitro* drug testing.

4.6. 3D Printed Colon Cancer Models. Colorectal cancer is the second most common cancer in adult women and the third most common cancer in men, accounting for 9.2% of cancer deaths worldwide.[105] There is a pressing demand for

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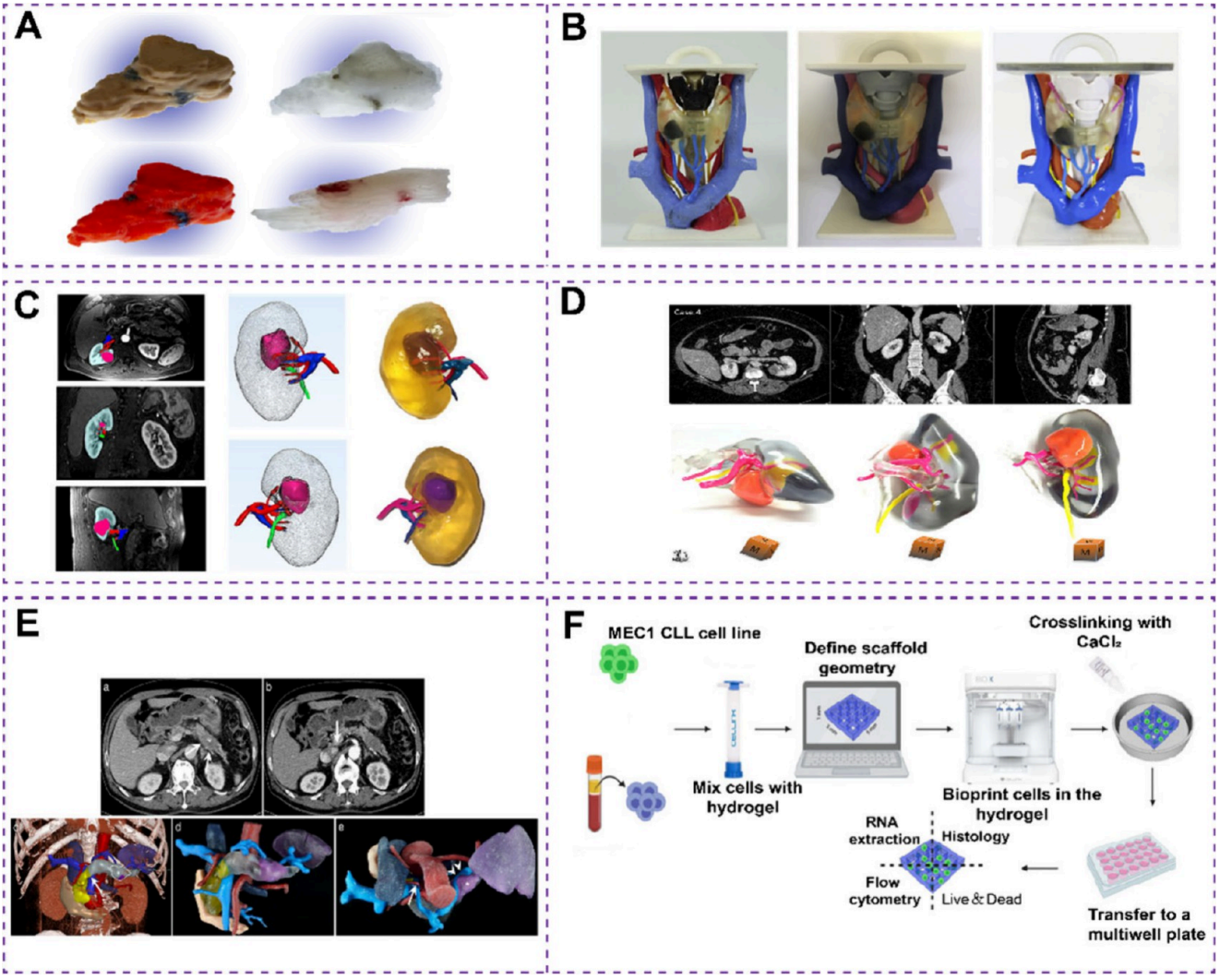

Figure 13. 3D bioprinting of other tumor models. (A) 3D printed prostate cancer model. Reprinted with permission from ref 110. Copyright 2019 IEEE. (B) Thyroid cancer models produced by three different 3D printing techniques. Reprinted with permission from ref 111. Copyright 2019 Pergamon-Elsevier. (C) 3D printed renal tumor model based on MRI data. Reprinted with permission from ref 113. Copyright 2017 Springer. (D) 3D printed renal tumor model based on CT scans. Reprinted with permission from ref 114. Copyright 2016 Springer. (E) 3D bioprinted pancreatic cancer model based on CT scan images. Reprinted with permission from ref 115. Copyright 2023 Springer. (F) Schematic strategy for 3D bioprinted CLL tumor models. Reprinted with permission from ref 116. Copyright 2021 Frontiers Media SA.

new effective treatments for colorectal cancer, and 3D printing can serve as a useful resource for studying tumor progression and drug therapy. Chen et al.[106] used tumor-associated stromal cells to create an *in vitro* 3D tumor model that mimicked the physiological functions of cells *in vivo* (Figure 12A). Colorectal cancer cells, CAF and TECs cells were co-cultured on 3D printed scaffolds (Figure 12B), enabling cell adhesion, differentiation, proliferation and angiogenesis. Normal mesenchymal stromal cells were activated and reprogrammed into tumor-associated mesenchymal stromal cells, constructing the tumor microenvironment. It was found that the activated stromal cells highly expressed various tumor-associated markers, and remodeled the ECM. The metabolic signatures and malignant transformation of the 3D tumor tissue on the scaffold were largely similar to those observed *in vivo*. The 3D tumor tissue was physiologically active and highly drug resistant. This finding indicates that the 3D printed scaffold model is readily applicable in the study of tumor biology and the development of individualized cancer therapy.

In another study, researchers proposed a simple 3D printed tumor microarray system suitable as a platform for drug testing and cancer biology research.[107] The results suggested that combining 3D biofabrication with a tumor microarray platform can provide insight into the different tumor-on-a-chip (ToC) systems cultured in a relatively simple 3D-printed bioreactor and applied in colorectal and other types of cancers (Figure 12C and 12D). Sun et al.[108] have reported an innovative construction of an 3D model of colorectal cancer/colorectal cancer liver metastasis employing 3D bioprinter technology with patient-derived primary tumor cells and bioink. The genomic and histological results have confirmed that the 3D tumor model can effectively preserve the parental tumor biomarkers and mutation profiles. Furthermore, there was a significant correlation between drug responses in the 3D tumor model and the clinical outcomes of neoadjuvant chemotherapy.

4.7. 3D Bioprinting of Other Tumor Models. Tumor metastasis is a multistage sequential process in which tumor cells metastasize from the primary site to distant organs, and

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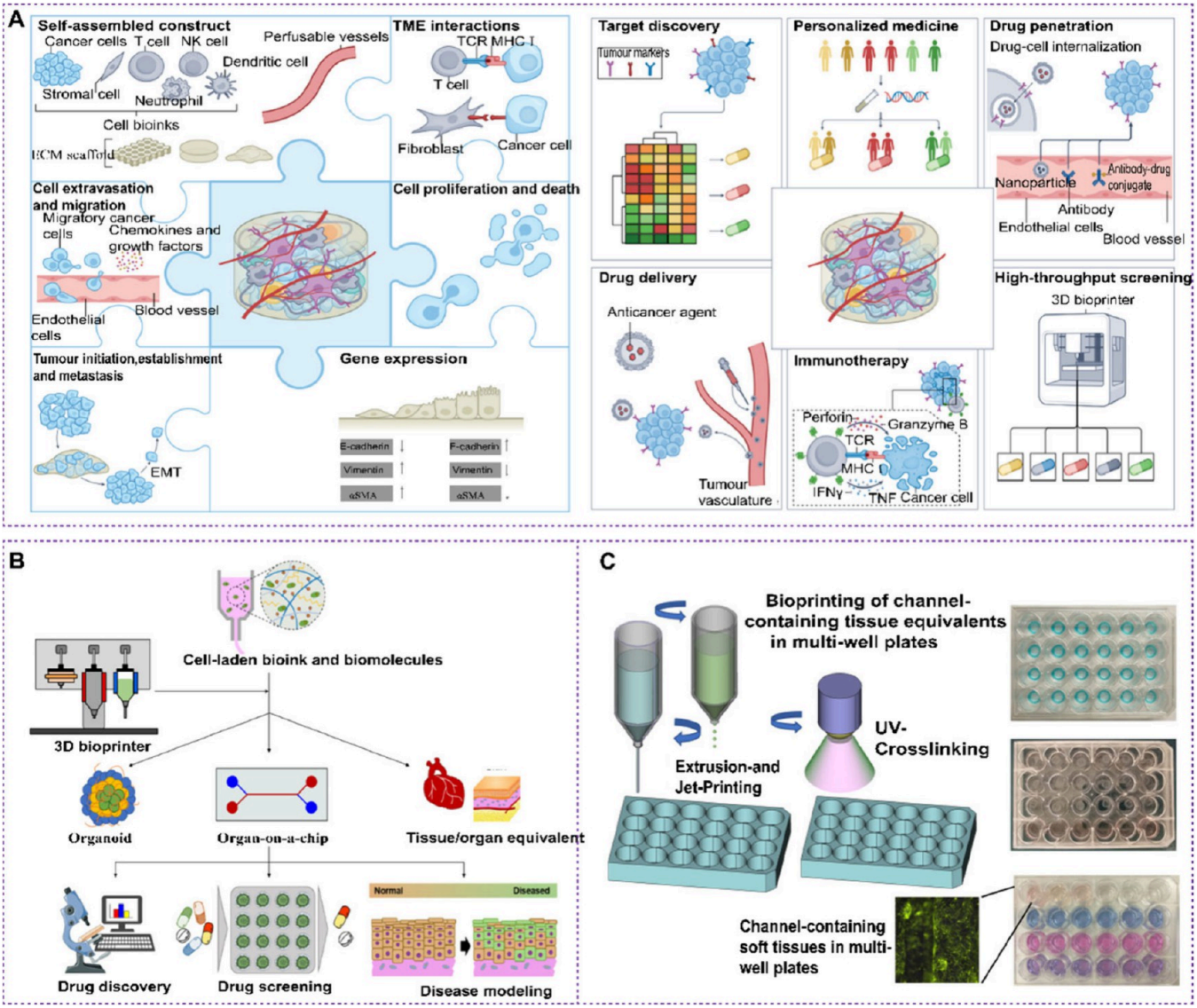

Figure 14. Application of 3D bioprinting in drug delivery and screening. (A) 3D bioprinted models was applied to study cancer cell migration, invasion, cell growth and gene expression, and evaluate drug efficacy. Reprinted with permission from ref 120. Copyright 2022 Nature Portfolio. (B) 3D bioprinting technology enables the creation of 3D cell culture devices that facilitate pharmaceutical applications. Reprinted with permission from ref 121. Copyright 2021 MDPI. (C) Bioprinting of catheter-containing tissue equivalents in multiwell plates to assess drug efficacy and toxicity. Reprinted with permission from ref 122. Copyright 2021 Portland Press Ltd.

subsequently adapt to the foreign microenvironment. The application of 3D bioprinting plays an important role in clinical diagnosis and treatment, and the study of metastasis after tumor resection. In addition to the more common cancer models, there are many additional models that warrant exploration. Prostate cancer is a malignant tumor that affects men and is an important cause of increasing male mortality worldwide. Patients with prostate cancer may develop localized or advanced disease.[109] 3D printing has assumed increasing importance in associated biomedical applications. Physical models of anatomical structures can expand digital representations and facilitate teaching and analysis. In one study, researchers combined 3D histology with 3D printing to create realistic physical prostate cancer models of tissues with hotspots (Figure 13A).[110] The use of medical imaging data and computer-aided technology coupled with 3D bioprinting is emerging as a new approach to inform tumor development and therapeutic strategies. The use of computer-aided technology is particularly relevant in the treatment of locally advanced thyroid cancer (Figure 13B).[111]

Renal cancer is one of the common urological malignancies, and is ranked second among urological malignancies.[112] Selection of a suitable model is urgently required for the study of renal cancer. The use of CT imaging and MRI data in conjunction with 3D printing is currently widely used in determining urologists' preoperative planning decisions. The results of several studies have shown that 3D printed models not only enable effective communication between doctors and patients, but also improve the level of accuracy in the study of kidney cancer. Moreover, the utilization of 3D printing technology enhances the medical education of patients and the scope of the surgery required before operation (Figure 13C and 13D).[113,114]

The use of personalized 3D printed models of pancreatic cancer can augment the anatomical techniques available to doctors in presurgical planning (Figure 13E).[115] Traditional

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2D models cannot accurately simulate the *in vivo* tumor environment, making *in vitro* studies of chronic lymphocytic leukemia (CLL) cells extremely challenging. Sbrana et al.[116] have reported the first use of 3D printing in an *in vitro* CLL model, where the resultant CLL cells survived for up to 28 days. In addition, the researchers have examined the activity, phenotype and gene expression of the CLL cells throughout the culturing, establishing a long-term stable and reproducible 3D culture leukemia model (Figure 13F).

5. 3D BIOPRINTING FOR DRUG SCREENING AND DELIVERY

Drug screening requires large-scale lateral comparisons of physiological activity and drug toxicity of different compounds, and precise molding and local microstructuring of a wide range of materials applying 3D bioprinting.[117] In the past decades, drug screening has largely relied on genetically modified animals, but the use of animal models has raised serious ethical issues and results can vary considerably when it comes to medication due to individualized differences.[118] 2D cellular models do not provide a realistic and comprehensive representation of *in vivo* tissues. Consequently, developing a disease model that can more accurately describe pathophysiological behavior and potential drug response is extremely important for research purposes. The use of 3D bioprinting to simulate complex tumor microenvironments *in vitro* permits a flexible fabrication of multicomponent cell types with fine spatiotemporal control. In addition, *in vitro* tumor models constructed by 3D printing for drug testing offers significant advantages in terms of speed, high-throughput screening, improved mimicry and reproducibility. This approach is particularly important in screening chemotherapeutic drug classes, coadministration and drug target identification.[119] Heinrich et al.[97] applied 3D printing to construct mini-brains composed of glioblastomas and macrophages, and evaluated drug activity targeting glioblastoma. It was shown that macrophages can induce growth and invasion of glioblastoma cells in the cerebellum. The results of a drug efficiency assessment, including the chemotherapeutic agent bis-chloroethyltrosourea (BCNU) and immunomodulatory drugs such as BLZ945 and AS1517499, confirmed the applicability of the 3D printed model to different types of drug screening as a useful tool to assess new cancer therapies.

Drug development is typically time-consuming and subject to significant variability with respect to individual patients. Therefore, effective methodologies are urgently needed to find effective anticancer with accurately predicted clinical value. 3D printed cancer models can be used to establish the molecular mechanisms in cancer progression and therapeutic response, and to characterize proteome and genome biological processes. Moreover, 3D printing can serve as a platform for identifying new molecular targets to study drug efficacy (Figure 14A).[120] Current bioprinting technology has enabled the creation of advanced 3D cell culture devices, such as organoids, organs-on-a-chip, and tissue/organs-equivalents (Figure 14B) that can facilitate new drug discovery, drug screening, and disease modeling for pharmaceutical applications.[121] The traditional approach to drug development has required testing on 2D cultured mammalian cells and subsequent experiments on rodents, where the drug response is physiologically different from humans. The use of 3D bioprinting to generate multiple tissue discs in 24-well plates enables an evaluation of the biocompatibility of novel hydrogel formulations or the assessment of drug efficacy and toxicity (Figure 14C). 3D printing offers a new option for drug research that addresses potential mechanisms for directly assessing drug efficacy in target tissues.[122]

The development of effective drug delivery systems can reduce potential off-target side effects and maximize efficacy.[121] In recent years, 3D bioprinting has received increasing attention as it enables the preparation of drug formulations with precise release, controlled drug dosage and multidrug distribution to meet the therapeutic needs of different patient groups. Using 3D printed strategy for oral solid dosage promotes rapid preparation and optimization of parameters when compared with traditional pharmaceutical methods. Previous study reported the polymer-containing nanocapsules prepared by 3D printing and nanotechnology could tailor the drug dosage and drug release profile.[123] In addition to oral formulations, dermal drug delivery is increasingly used to administer medications, bypassing the first-pass effect of the liver and improving patient compliance. Lim et al.[124] used the 3DM-Castable resin to create personalized microneedle patches, designing a personalized splint based on a model of the patient's hand. These splints have a unique curved surface that matches the contours of the patient's fingers and can be used to immobilize the injured fingers and assist in the delivery of pain medication via microneedles.

6. OTHER APPLICATIONS OF 3D PRINTING IN BIOLOGY

Precision medicine refers to a customized treatment of disease which focuses on the genetic, environmental and lifestyle factors of an individual patient, and includes tailored medical decision-making and treatment. This approach aims to offer healthcare services for each individual, minimizing complications due to factors such as genetics, environment and lifestyle.[125] In terms of current biomedicine, 3D printing has untapped potential in tissue development, regenerative medicine, and prevention and treatment of human diseases, in addition to applications in tumor models and drug research.

6.1. Bioprinted Models Applied to Tissue Development. The human body consists of four main tissue types: neural, epithelial, muscular, and connective. Each tissue has specific histological characteristics. 3D bioprinting enables a customization of functional organs through using multiple biomaterials and techniques.[126] 3D bioprinting technology combined with cell targeting has been used to a limited extent in tissue regeneration, providing an alternative to cell conduction-based tooth regeneration. Yuan et al.[127] utilized bioprinting to enable dental production of periodontal ligaments integrated into the natural alveolar bone in a rat model.

A successful rehabilitation of vascular diseases usually relies on surgical repair of damaged vessels. Currently, the main treatment requires transplantation of the patient's skin or donor skin. However, this approach is subject to limitations associated with individual differences and rejection reactions, and is prone to immune reactions.[128] Research is now directed at developing artificial blood vessels as an alternative to the traditional methods of repairing damaged blood vessels. Integration of blood vessels is one of the most important challenges in tissue printing as blood vessels should exhibit endurance and flexibility to withstand repeated dilatation and contraction. The use of 3D bioprinting allows for more biomass structures, improving biodegradability and thrombus

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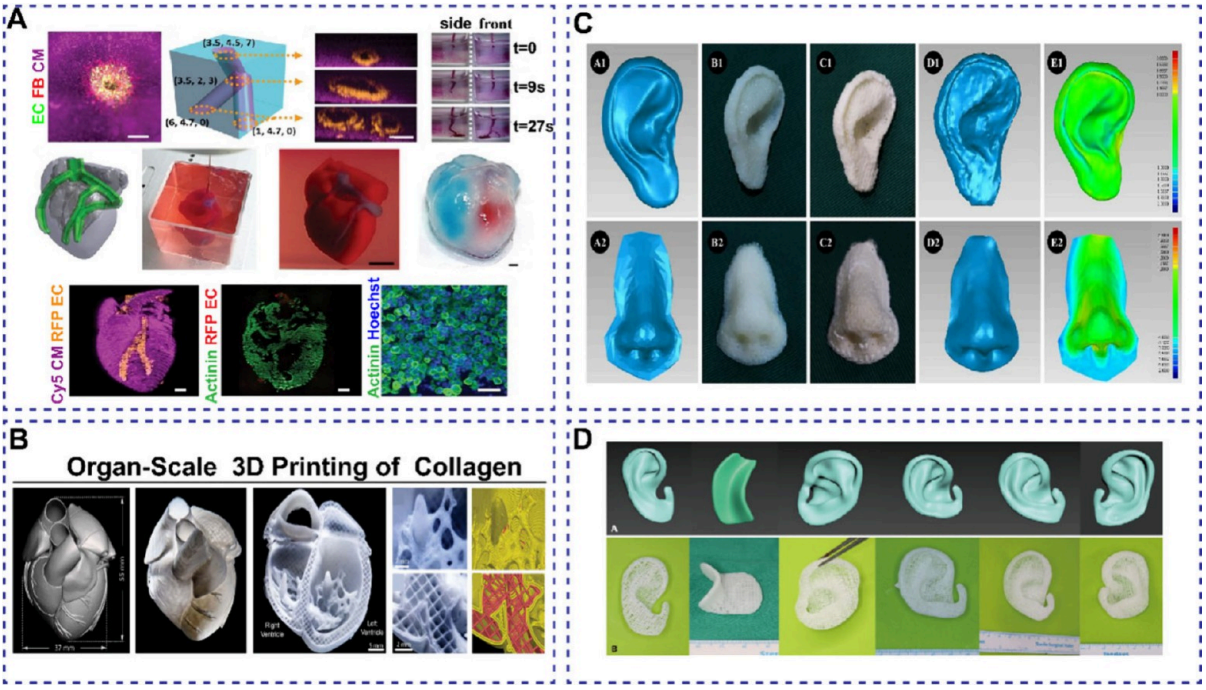

Figure 15. 3D bioprinting in regenerative medicine. (A) 3D printed thick vascular tissue. Reprinted with permission from ref 133. Copyright 2019 Wiley. (B) 3D bioprinted heart based on collagen composition. Reprinted with permission from ref 134. Copyright 2019 Amer Assoc Advancement Science. (C) Preparation and morphological analysis of human ear and nose scaffolds. Reprinted with permission from ref 135. Copyright 2018 Amer Chemical Soc. (D) Schematic of 3D bioprinted ear model. Reprinted with permission from ref 136. Copyright 2023 Yonsei Univ Coll Medicine.

formation and stenosis.[10] Hann et al.[129] successfully developed 3D-printed flexible small-diameter damaged blood vessels. The results demonstrated that the 3D-printed blood vessels exhibited the appropriate mechanical properties. The cells in the fibrin layer proliferated appreciably over time to form a stable structure, which can be used as a therapeutic platform for treating vascular diseases.

In addition, 3D printing technology enables the construction of muscles. It has been reported that 3D bioprinting can develop four different components to mimic a complete muscle-tendon unit (MTU).[130] The researchers used thermoplastic polyurethane (PU) and C2C12 cellular hydrogels as bioinks to achieve elasticity and muscle development, and PCL and NIH/3T3 cellular hydrogels to ensure stiffness and tendon development. The findings suggest that bioprinted intact tissue structures have region-specific biological and mechanical properties where artificial adipose tissue structures can be used for soft tissue reconstruction in orthopedic and prosthetic surgery.

6.2. 3D Printing in Regenerative Medicine. Regenerative medicine techniques aim to repair and regenerate hypofunctional organs to an immunosuppression-free state in order to minimize complications and toxicity. Currently, there are many innovative strategies, notably the use of cell-free scaffolds from acellular cells as organ templates,[131] but this approach has a number of disadvantages. Currently, 3D bioprinting has been utilized in regenerative medicine to meet the demand for suitable transplanted tissues or organs, which represents a more complex process than nonbiological 3D printing.[132] A study has considered the printing of perfusable and vascularized cardiac patches. These patches were taken from the patient's greater omental tissue for biopsy, where the ECM was processed into personalized hydrogels and the cells were differentiated into cardiomyocytes and endothelial cells. The cardiac parenchymal tissue and blood vessels were further developed by combing these two cell types with hydrogels. The resultant elongated cardiomyocytes with numerous actinophorin stripes suggested a successful printing of a naturally constructed cellularized human heart.[133] These results serve to demonstrate the potential of 3D printing in personalized tissue and organ engineering, or for drug screening applied to appropriate anatomical structures and patient-specific biochemical microenvironments (Figure 15A). In one study, researchers used 3D-printed collagen to reconstruct human heart components. The results have indicated that the 3D-printed heart accurately replicated patient-specific anatomy as determined by microcomputed tomography scans (Figure 15B).[134]

Due to the limited availability of autologous cartilage and the complex modeling and clinical skills required for auricular reconstruction, 3D bioprinting plays a critical role in producing artificial ears. Xia et al.[135] have constructed precise hydrogel scaffolds in the shape of the human ear and nose based on 3D bioprinting technology, which ensured precise control of the internal pore structure and external shape. The scaffolds retained the shapes of ears and nose with a similarity greater than 90% when compared to that of the original digital model (Figure 15C). The application of 3D bioprinting is significant as a new method of ear reconstruction where researchers use a mirroring and segmentation process to reconstruct a 3D geometric ear model based on CT data from the patient. A 3D implant has been created using the 3D printing system for use in reconstructive ear surgery that warrants future medical development (Figure 15D).[136]

3D bioprinting has also been used to create different eye models. This has involved computer-aided design to produce

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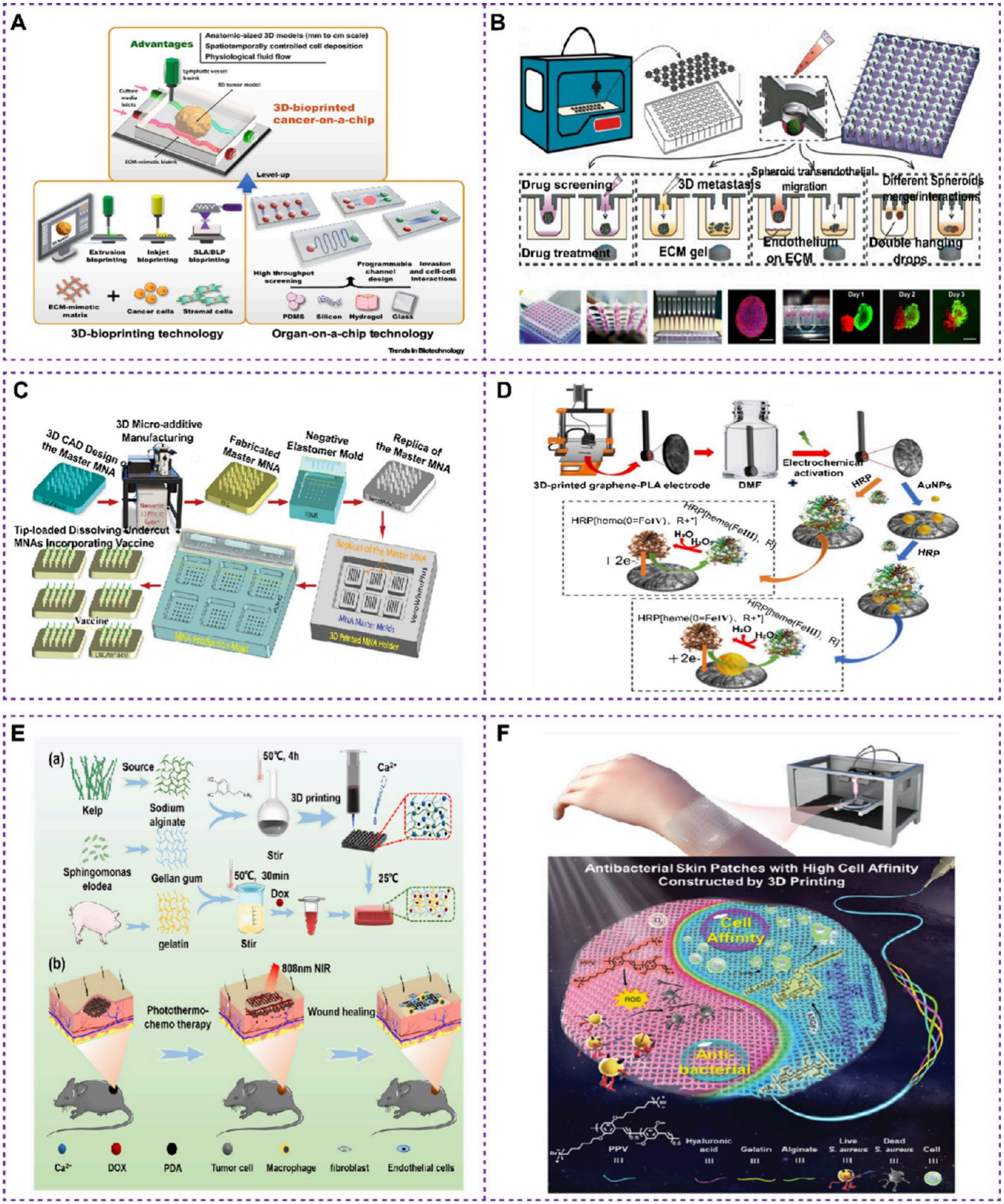

Figure 16. Combined application of 3D bioprinting with other technologies. (A) 3D bioprinted tumor microarray model. Reprinted with permission from ref 139. Copyright 2022 Elsevier. (B) 3D printed suspension dropper for tumor spheroid research. Reprinted with permission from ref 140. Copyright 2019 Nature Portfolio. (C) 3D printing combined with microneedle mold manufacturing strategy. Reprinted with permission from ref 141. Copyright 2020 Elsevier. (D) Applications of 3D bioprinting combined with biosensors. Reprinted with permission from ref 142. Copyright 2020 Elsevier. (E) 3D bioprinting combined with photothermal chemotherapy modality for wound healing. Reprinted with permission from ref 143. Copyright 2022 Royal Soc Chemistry. (F) Schematic of 3D printed high cell affinity antimicrobial skin patch. Reprinted with permission from ref 144. Copyright 2022 Royal Soc Chemistry.

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seven prosthetic eye models, injecting hydrogel into the vitreous cavity and generating corneas of different thickness (200−800 μm). Moreover, intraocular pressure (IOP) measurements were conducted on each eye model with a positive correlation between corneal thickness and IOP.[43] The findings indicate that the 3D printed technique is a promising alternative for eye models.

6.3. Combination of 3D Bioprinting with Other Technologies. The combination of organ-on-a-chip platforms with a portfolio of additive manufacturing methods is considered as a viable technology. Previous reports have demonstrated the latest advances in 3D printed cancer chip platforms with considerable high-throughput analysis and comprehensive design guidelines.[137,138] This hybrid platform represents a new generation of highly complex 3D tumor models, offering improved biomimicry and prediction of therapeutic performance (Figure 16A).[139] When compared with traditional monolayer cell cultures, 3D tumor spheroids can effectively reproduce the structure and physiology of solid tumors, and have become an essential *in vitro* model in oncology research. Zhao et al.[140] employed 3D printing to generate tumor spheroids in multiwell plates. A 3D-printed hanging drop dripper has been used in tumor sphere studies (Figure 16B). These spheroids can be inoculated directly onto 96/384-well plates, avoiding the cumbersome fabrication process from a micromechanical system and readily verifying tumor malignancy using transmission electron microscopy. This approach can provide a useful workflow to accelerate *in vitro* simulation of tumor models.

Microneedle arrays (MNAs) represent an effective tool originally designed to engineer the skin microenvironment for diverse immunization strategies (Figure 16C). In a previous study, researchers have reported novel solubilized undercut MNAs, demonstrating application in multicomponent skin vaccination.[141] By combining 3D laser lithography, micro-additive fabrication is possible with unique geometrical capabilities and nanoscale resolution, allowing a micromolding of well-characterized materials. 3D printing also finds viable application in electrochemistry, where it has been used to create customized electrodes as a biosensing platform, and in energy generation and storage devices. Marzo et al.[142] have created a direct electron transfer enzyme biosensor for hydrogen peroxide detection by 3D printing a graphene/polylactic acid electrode with immobilized horseradish peroxidase (HRP) (Figure 16D).

In addition, 3D printing can be combined with a photothermal effect for wound healing. Xu et al.[143] designed sodium alginate (SA)/gelling gel (GG) /polydopamine (PDA) nanoparticle multiphase hybridized hydrogel scaffold for sequential photothermal treatment and chemotherapy to inhibit melanoma recurrence in wound healing. The PDA nanoparticles were generated *in situ* in the hybridized bioink, generating scaffolds with excellent photothermal effects. The chemotherapeutic drugs were wrapped on the surface of the hydrogel, which accelerated drug release triggered by a photothermal effect, achieving photothermal chemotherapy that inhibited the proliferation of tumor cells and recurrence after surgical resection (Figure 16E). In a study of skin trauma repair, researchers developed a printable bioink consisting of the gelatin, SA, hyaluronic acid, and a photoactive cationic conjugated poly(phenylenevinylene) derivative for artificial skin patches. The patch has an integrated antimicrobial capacity, the ability to promote tissue regeneration and rich microstructural styles, making it a more suitable skin substitute for treating skin trauma (Figure 16F).[144]

7. CURRENT AND FUTURE PERSPECTIVES

7.1. Printing of Tumor Models. In the past decades, a number of studies have served to develop and validate different 3D cancer models, ranging from monoculture spheroids to complex perfusable on-chip cancer models and bioprinted heterogeneous tumor models. Current advances in 3D bioprinting have gone some way in developing 3D cancer models with bionic tumor microstructures and tissue-specific tumor microenvironments for different cancer types. These tumor microenvironments exhibit physiologically similar behavior to tumors *in vivo*, and the bioprinted cancer models have been verified with various chemotherapeutic regimens, opening new avenues in personalized drug research. However, this platform is still in its infancy and requires appreciable development and adaptation to become a commonly used laboratory tool. Most 3D bioprinted cancer models do not take into account the direct or indirect interaction of cancer cells with the immune cell population, so the various tumor targets currently studied under 3D culture conditions fail to manifest themselves in tumor formation pathways.[68]

7.2. Drug Development. The growing demand for customized pharmaceuticals and medical devices in recent years has led to a rapid increase in the impact of additive manufacturing. 3D printing is recognized as one of the most revolutionary and powerful tools in the precise manufacture of individually developed agents, tissue engineering and disease modeling.[145] The *in vitro* tissue models for drug screening generated using 3D printing can accurately locate biomaterials and living cells, reconstruct complex model structures, and reproduce cell−cell and cell-matrix interactions *in vivo* tissues and the gradient distribution of various biochemical factors. Consequently, the results of drug screening more closely match the actual physiological characteristics of *in vivo* tissues. Drug delivery systems based on 3D bioprinting enable a continuous and precise distribution of drug concentration gradients and personalized doses, diverse shapes and complex structures with respect to drug formulations. The application of multi-component combination, multimode release and multidimensional control of drug preparations increases the scientific basis for precise and complex drug preparations, where 3D bioprinting can provide basic technical support for the combination of drugs and individualized medical treatment.[146]

7.3. 3D Bioprinting for Biomedicine. 3D bioprinting is attracting increasing attention for medical applications because of its ability to print bionic organs and tissues quickly and efficiently. In recent years, 3D bioprinting, as a front-end technology in the biomedical field, has been applied to print designed tissues and regenerative organs by combining cells or biologically active factors to regenerate a number of organs, such as the heart, liver, skin, ears and nose. The development of biomaterials with desirable biocompatibility, cell viability, and mechanical properties to print novel biomaterials for regenerative organs that can accurately mimic the environment inside the human body is an urgent need for 3D bioprinting technology in the biomedical field. Currently, researchers are constantly advancing in the research and development of biomedical applications, as well as the design of new printing systems.[103]

7.4. Bioink Selection. 3D printing has certain drawbacks, such as the design of cells and biomaterials to function after

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implantation and *in vivo* integration issues. The choice of bioink is critical, as it must adapt to cell growth on the printed model and achieve suitable printability. Many of the materials used can result in poor cell interactions and harmful differentiation of stem cells. In addition, during the printing process, the ink may clog the nozzles, leading to unshapable or difficult and time-consuming model printing. The 3D printed models must exhibit sufficient mechanical strength and stability to facilitate transplant into the living body.[10]

8. CONCLUSIONS

3D bioprinting combines cell biology, neurobiology, pharmacology, personalized medicine and regenerative medicine. It provides a broad research platform for biology researchers and serves as a valuable technical tool for surgeons. However, there is still a growing demand for 3D printing techniques and materials to meet the challenges in building models with speed and accuracy, stability and functionality. Although 3D bioprinting has made a series of improvements in resolution and printing speed, these challenges are compounded by issues such as the printability of the selected materials, the compatibility of print parameters between materials, selection of the right ratio between bioinks, and the survival rate of cells at printing or at the end of printing. On the other hand, for the 3D printers themselves, the correct printing time and printing speed also have a significant impact. One of the challenges is how to minimize the negative impact on cell viability at the exact time, and whether to maintain a balance between printing speed and cell viability. Undoubtedly, both in *vitro* simulation and in *vivo* implantation need to satisfy the characteristics of the human body *in vivo* itself, and the selected bioinks should be chosen according to their own requirements. The technology faces many challenges, requiring the collaborative effort of researchers from different disciplines to overcome current difficulties and realize the benefits of 3D printing. Targeted research will undoubtedly extend the range of 3D bioprinting applications with important contributions to advanced medicine.

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ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
TME	Tumor microenvironment
ECM	Extracellular matrix
IBB	Inkjet-based bioprinting
EBB	Extrusion-based bioprinting
SLA	Stereolithographic bioprinting
LAB	Laser-assisted bioprinting
CT	Computational tomography
MRI	Magnetic resonance imaging

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