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Bioprinting the Cancer Microenvironment

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Abstract

Cancer is intrinsically complex, comprising both heterogeneous cellular compositions and microenvironmental cues. During the various stages of cancer initiation, development, and metastasis, cell–cell interactions (involving vascular and immune cells besides cancerous cells) as well as cell–extracellular matrix (ECM) interactions (e.g., alteration in stiffness and composition of the surrounding matrix) play major roles. Conventional cancer models both two- and three-dimensional (2D and 3D) present numerous limitations as they lack good vascularization and cannot mimic the complexity of tumors, thereby restricting their use as biomimetic models for applications such as drug screening and fundamental cancer biology studies. Bioprinting as an emerging biofabrication platform enables the creation of high-resolution 3D structures and has been extensively used in the past decade to model multiple organs and diseases. More recently,

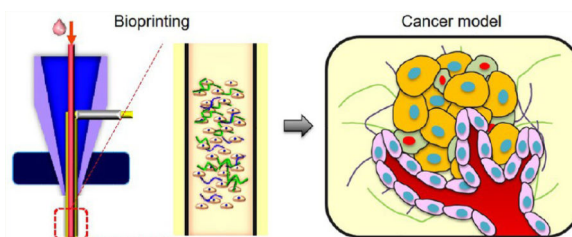
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this versatile technique has further found its application in studying cancer genesis, growth, metastasis, and drug responses through creation of accurate models that recreate the complexity of the cancer microenvironment. In this review we will focus first on cancer biology and limitations with current cancer models. We then detail the current bioprinting strategies including the selection of bioinks for capturing the properties of the tumor matrices, after which we discuss bioprinting of vascular structures that are critical toward construction of complex 3D cancer organoids. We finally conclude with current literature on bioprinted cancer models and propose future perspectives.

Graphical Abstract



Keywords

cancer biology; cancer model; bioprinting; vascularization; drug screening

INTRODUCTION

Cancer is one of the major causes of morbidity and mortality, accounting for around 14 million new cases and 8.2 million deaths in 2012 worldwide.¹ It is further expected that the annual cancer incidences will rise from 14 million in 2012 to 22 million within the next 20 years,¹ leading to significantly increased healthcare costs and the great need to better understand cancer to improve therapy. The most prevalent types of cancer (e.g., breast, colon, stomach, liver, and lung) share common features, each with a unique microenvironment and a hypoxic core surrounded by a dense tissue.^{2–4} Cancer cells are otherwise healthy cells that upon acquiring genetic mutations develop capabilities specific for cancer such as displaying invasive behavior, resisting cell death, evading growth suppression, experiencing uncontrolled replication, showing sustained growth, and triggering abnormal angiogenesis.⁵ This last capability constitutes a limiting step for tumors to be able to fully develop and evade hypoxia (only cells within a 200 μm range from a blood vessels experience sufficient oxygen levels⁶). The cancer microenvironment is highly complex (Figure 1). For example, a vast majority of cancers originate from mutated epithelial cells (developing carcinomas).^{5,7,8} The tumor then grows as it interacts with its microenvironment, ranging from the surrounding stroma composed of both cells (mainly endothelial cells and fibroblasts) and the extracellular matrix (ECM). The tumor cells modulate their proliferation and migration by producing pro-angiogenic factors (e.g., vascular endothelial growth factor, VEGF) and promoting their interactions with the stromal cells and the surrounding inflammatory cells (macrophages, neutrophils, and mast cells).⁹

The tumor microenvironment, including both chemical cues (growth factors and cytokines) and biophysical cues (interstitial pressure and matrix mechanics), is extremely complex and plays a major role in the progression and metastasis of the tumor as it evolves over time.^{9,10} This microenvironment is highly dynamic with distinctive key features (e.g., cellular and ECM compositions, matrix stiffness, and degree of vascularization) present at each of the different stage of the disease.^{11,12} Therefore, it is of primary necessity to be able to decompose all the key elements interacting with the cancer cells and then understand how, why, and when they become implicated in the tumor growth and migration. In addition, there is a high variability between the cancer types (i.e., intertumor heterogeneity) as well as within each tumor (i.e., intratumor heterogeneity).^{13–16} This heterogeneity leads to an extremely high complexity and variability requiring personalized care for the patient.¹⁷ To fully grasp the complexity of the origins of cancer, its development, metastasis, and interactions between the various key players in the tumor microenvironment, as well as screening of various anticancer drugs, it has been increasingly realized that in vitro engineered human cancer models are strongly desired, as the conventional animal-based xenograft cancer models do not necessarily recapitulate the human physiology and drug responses.^{18–20} To address these challenges, three-dimensional (3D) cancer models are anticipated to precisely mimic the in vivo tumor microenvironment in human patients by recapitulating the proper tumor cell/ matrix composition and properties that match both the type and stage of the disease, and therefore provide accurate mechanistic studies as well as a tool for personalized anticancer therapeutics screening.

Here we review the conventional methods to generate in vitro 3D cancer models through tissue engineering approaches. We further state the limitations of these methods and introduce the 3D bioprinting technologies that promise to produce complex tissue constructs at high spatial precision and compositional accuracy. We finally discuss the application of 3D bioprinting to fabrication of biomimetic cancer models and envision future perspectives.

CONVENTIONAL APPROACHES FOR CONSTRUCTION OF IN VITRO 3D CANCER MODELS

State-of-the-art in vitro cancer models range from two-dimensional (2D) monolayer to 3D (co)cultures using conventional tissue engineering approaches (Figure 2). Planar cancer models have a long history, as early as the 1950s (Figure 2A i);^{21,22} however, it was quickly recognized that these models exhibit strong discrepancies specifically drug responses when compared to the in vivo counterparts. The differences arise from the fact that these 2D models lack the functional 3D tumor microenvironment leading to insufficient cell–cell and cell-ECM interactions.^{23–26} Moreover, during the culture process on plastic surfaces cells tend to be selected for their phenotypes that promote their adhesion to stiffer substrates.²⁷ As a result, the applications and efficacy of 2D cancer models have been limited.

In an attempt to mitigate these drawbacks that 2D monolayer cultures present, in vitro 3D cancer models have been subsequently developed mainly based on spheroid (co)cultures and those based on the utilization of 3D matrices (Figure 2A ii-iv).²⁸ Aggregates of tumor cells, usually referred to as multicellular tumor spheroids, can be generated by seeding the cells

onto a nonadherent surface such as agarose and polydimethylsiloxane (PDMS).^{28,29} When these substrates are processed to possess confined features of microwells, uniform spheroids can form inside these microwells that prevent the cells from adhering to the walls and hence self-aggregate.²⁹ Other methods for spheroid generation include inducing aggregation of tumor cells at the bottom of “hanging drops”^{30,31} and through the use of a constantly stirred spinning flask.³² The cells that aggregate in spheroids tend to display similar features as their in vivo counterparts (Figure 2B), where a necrotic core will form at the center of the spheroid upon the growth of the aggregate will start because of the lack of nutrients and oxygen.³³ The multicellular tumor spheroids with improved functionality have been used for studies of fundamental cancer biology³³ as well as drug screening and validation.^{34–36} Although more advanced compared to the 2D cultures, the 3D models based on multicellular tumor spheroids still lack the major ECM component of the tumor microenvironment.^{9,37}

To this end, 3D matrices mimicking the cancer ECM, either based on hydrogels (Figure 2A iii) or porous scaffolds (Figure 2A iv), have been combined with tumor cells to construct more biomimetic cancer models.^{38–42} Multiple types of matrices have been used for this purpose. The biomaterials for matrix reconstitution can be derived from natural polymers such as collagen,⁴³ fibrin,⁴⁴ and Matrigel^{45,46} to form a variety of hydrogels (Figure 2C i). In particular, the latter is commonly used as matrix in the context of cancer modeling as it is harvested from Engelbreth–Holm–Swarm (EHS) mouse sarcoma and thus intrinsically features important properties of the tumor ECM.⁴⁶ Indeed, the use of these hydrogel matrices with precisely controllable stiffness has shown promising results in recapitulating the in vivo tumor behaviors.²⁷ On the other hand, the 3D scaffolds can also be made from synthetic polymers that present better control over their chemical and physical properties including polyethylene glycol (PEG), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymer poly(lactic-co-glycolic acid) (PLG), among others.^{47,48} These scaffolds, usually processed to be porous, can be populated with tumor cells to fabricate cancer models possessing hypoxic cores (Figure 2C ii).³⁸ The 3D cancer models generated using porous scaffolds have also been shown to indicate enhanced performance (e.g., proliferation, growth factor and cytokine secretion, and vascularization) than the 2D monolayer cultures and 3D tissues embedded in Matrigel both in vitro and after in vivo implantation (Figure 2D).³⁸

BIOPRINTING AS AN INNOVATIVE TECHNOLOGY TO BUILD BIOMIMETIC CANCER MODELS

Major challenges associated with current 3D in vitro models include their oversimplified structures and limited vascularization potential. For example, the multicellular tumor spheroids or 3D scaffold-based tumors will be size-limited due to the lack of vascularization and thereby will likely faithfully model the genesis of tumors but not the later stages of their development. Moreover, most of those models lack well organized spatial distribution of tumor cells and ECM compositions. Recently a strong interest toward the utilization of 3D bioprinting technologies has arisen to solve these issues with an aim of constructing more biomimetic 3D in vitro cancer models.⁴⁹

Bioprinting Techniques

The general term of bioprinting refers to multiple strategies that are capable of dispensing biological components such as biomaterials (i.e., the bioinks) and cells in a spatially defined manner.^{50,51} Although 3D printing was developed decades ago, only until recently was this versatile technology adapted to the field of biomedicine in fabricating sophisticated biological structures through the use of biocompatible materials.⁵² The common strategies of bioprinting include inkjet printing,^{53,54} extrusion-based printing,^{55,56} laser-assisted printing,^{57,58} and stereolithography.^{59–61}

Inkjet bioprinting is based on a well-established drop-by-drop deposition mechanism enabled by thermal or piezoelectric actuation (Figure 3A).^{50,51} In a typical setup the cartridge is filled up with the (cell-laden) bioink, and the droplet ejection through the printhead synchronized with a motorized stage driven by a computer program lead to deposition of the droplets at specific locations to build the 3D architecture. Inkjet bioprinting features relatively low cost, fast speed, and high viability of cells in the deposited structures.^{62,63} In comparison, the extrusion-based bioprinting relies on propelling the bioink through the nozzle using pneumatic or mechanical pressure (Figure 3B),^{50,51} which affords low cost and medium-to-fast deposition speed. In the case of laser-assisted bioprinting or laser-induced forward transfer (LIFT) it requires a donor layer that responds to laser stimulation by absorbing the focused energy and local generation of high-pressure bubble that pushes a droplet of the donor bioink onto the collecting substrate (Figure 3C).^{50,51} However, this method suffers from high cost and bulky instrumentation, as well as limited choice over the bioinks. Another versatile 3D bioprinting technique capable of patterning cell-laden hydrogels has been adopted from the stereolithography that was conventionally used for materials fabrication. In this strategy, a digital mask generated by an array of programmed mirrors is projected to a bioink reservoir to selectively photo-cross-link patterns of interest in a layer-by-layer manner; along with the vertical movement of the stage 3D objects containing prescribed architecture can be produced at high fidelity (Figure 3D).^{59–61,64,65} Stereolithography has multiple advantages including high resolution (up to a few micrometers) and a fast bioprinting speed.

All these bioprinting methods enable a highly improved control of cell distribution within the 3D space compared to conventional approaches. Moreover, they permit the creation of large-scale constructs possessing well-organized architecture and arbitrary shapes, hence providing excellent flexibility and ability to reproduce the complex cancer microenvironment that mimic that of their counterparts in vivo.

Designing the Bioinks

The composition of the tumor ECM is highly complex, consisting of a wide variety of extracellular proteins and molecules that constitute the tumor microenvironment together with tumor and stromal cells.^{9,12,23,26} Studies have also revealed that the composition of the tumor matrix is unique for tumor type and stage and exhibits strong heterogeneity between patients, which is critical in maintaining the phenotype of the tumor in vitro.⁶⁶ In addition, the matrix stiffness plays a nontrivial role in regulating tumor behaviors including their initiation, progression, and metastasis.^{67–71} Therefore, the bioinks used for fabricating the

tumor models should be carefully selected or designed to satisfy the needs of cancer bioprinting.

The bioinks used for bioprinting, typically in the form of hydrogels, should possess specific characteristics including printability, cross-linking mechanisms, and biocompatibility.^{50–52,72–75} Although immediate printability of the bio-printed tissue constructs may depend on the physical properties (e.g., viscosity) of the bioink itself, subsequent stabilization of the structures relies on additional cross-linking steps.^{52,76} In general, two types of cross-linking strategies can be applied, that based on physical cross-linking through ionic interactions such as in the case of alginate (gels by Ca^{2+} ions),^{77,78} and those hydrogel precursors able to form covalent bonds such as methacrylated forms of the polymers that are photocross-linkable.^{79–82} The mechanics of the resulting hydrogel matrices post-bioprinting can further be finely tuned within a wide range covering the entire spectrum of tissue stiffness and elasticity by changing the concentration of the polymers making up the bioink or cross-linking density.^{50,83,84}

Existing bioinks have been derived from myriad classes of biomaterials including those of both synthetic (e.g., PEG and its derivatives, pluronics) and natural (e.g., collagen, hyaluronic acid, fibrin, alginate, gelatin) origins.^{50–52,85,86} The major advantage of natural biomaterials lies in their excellent bioactivity such as biocompatibility and presence of intrinsic cell-adhesion ligands (e.g., RGD); however, the usually weak mechanical properties and limited freedom of customization have been their consistent drawbacks.^{52,87} On the contrary, the properties of synthetic biomaterials can be easily tuned over a large range, which include their mechanical properties, degradation, and bioactivity.^{47,88,89} It is expected that by rational combination of the natural and synthetic biomaterials for use as the bioink optimized for each cancer type, maximal recapitulation of the viability, biological behaviors, and functionality can be achieved for the bioprinted cancer tissues.

More recently, bioinks derived from decellularized ECMs (dECMs) have attracted great attention, which are formed by rehydrating powdered tissue-specific dECMs obtained following the standard decellularization procedures.^{90–93} One unique advantage of dECM-based bioinks compared to other artificial bioink formulations lies in the possibility to apply biomaterials and biochemical cues from the target tissue in the bioprinting process, therefore achieving well-matched compositional complexity between the bioprinted structures and their native counterparts in the body.⁹¹ Although this concept has not yet been applied to cancer bioprinting, we anticipate that when tumor-derived dECM bioinks are used in a tumor-specific manner the performance of the resulting bioprinted tumor tissues will be enhanced due to the ability to maintain the biochemical cues of the microenvironment. In combination with well-defined synthetic biomaterials, the biophysical parameters may be simultaneously incorporated to maximally match the properties of the *in vivo* tumor niches.

Bioprinting the Vasculature

Extensive vascularization is a critical hallmark of cancer and thus a critical component in construction of *in vitro* cancer models.^{11,12,94} Bioprinting has the unique capability to introduce well-organized blood vessel-like structures into engineered 3D tissues. One of the most successful strategies lies in the use of a technique referred to as sacrificial

bioprinting.^{85,86,95–97} In a typical procedure, a sacrificial template is first deposited using the bioprinter in arbitrary patterns resembling the vascular network, after which a hydrogel matrix is casted around the template, and finally the template is selectively removed leaving perfusable channels embedded within the hydrogel. These hollow channels can be further endothelialized to mimic the vascular functionality. Several classes of materials have been adopted as the template to achieve sacrificial bioprinting of vasculature. In an earlier example, Chen and co-workers used 3D printed carbohydrate glass fibers coated with a thin layer of PLGA as the template, which were then embedded into a variety of hydrogels including agarose, alginate, PEG, fibrin, or Matrigel encapsulating fibroblasts; the carbohydrate glass fibers could dissolve by perfusion with the cell culture medium to generate the lumens and allow subsequent seeding of endothelial cells on the surface of the channels (Figure 4A).⁹⁵ Alternatively, hydrogels may also be used as the sacrificial templates. These examples include: (i) Pluronic solution that exhibits a sol–gel transition at reduced temperature, allowing bioprinting at room temperature but dissolution of the Pluronic at a temperature close to 0 °C (Figure 4B);^{31,86} (ii) gelatin solution that presents a sol–gel transition at elevated temperature, enabling dissolution of the template when bioprinted constructs are transferred to an incubator at 37 °C (Figure 4C);⁹⁷ and (iii) agarose solution that forms stiff hydrogel microfibers that can be directly extracted from the hydrogel matrix under mild vacuum (Figure 4D).⁹⁶ In all these cases the obtained interconnected microchannels may be endothelialized to recapitulate the biological functions of blood vessels.

More recently, a novel bioprinting method relying on depositing the sacrificial bioink into a supporting hydrogel matrix has enabled fabrication of highly complex vascular structures within a 3D volume (Figure 4E).^{82,98,99} In this case, a medium consisting of a hydrogel is used as the support to prevent the collapse of the deposited bioink during the bioprinting procedure. The medium is usually shear-thinning, allowing movement of the nozzle and fast extrusion of the bioink into the matrix while self-healing to ensure integrity of the embedding hydrogel. By taking advantage of this technique, vascular structures of arbitrary 3D shapes and architecture can be produced, which are not easily achieved using conventional 3D bioprinting approaches.

Recent advancement in bioprinting has further enabled alternative approaches for direct deposition of hollow tubular structures mimicking the blood vessels. A coaxial extrusion device has been proposed to deliver the bioink in the sheath flow and the cross-linking solution in the core, where in situ cross-linking of the bioink and stabilization of its shape immediately occur upon extrusion to obtain tubular structures possessing hollow lumens.^{100,101} The fabricated hollow tubes are continuous and perfusable. Endothelial cells may also be encapsulated within the bioink to fill the wall thickness of the bioprinted hollow tubes, which overtime will spread and proliferate to achieve biological functions of the blood vessels.

Cancer Bioprinting

Current 3D cancer models present multiple limitations such as batch-to-batch variability, limited control over cell patterning, and low throughput. Therefore, 3D bioprinting as a

versatile platform that enables deposition of volumetric structures and architecture at high precision and fidelity, provides a novel solution to address these issues. However, only few applications of bioprinting in construction of biomimetic cancer models have been demonstrated so far since it has entered this field of research only very recently.

As aforementioned vascularization plays a critical role in growth of most cancer types by supplying sufficient nutrients and oxygen.^{11,12,94} To this end, Yoo et al. created a glioblastoma vascularization model combining the sacrificial bioprinting strategy with multicellular spheroids.¹⁰² A microchannel seeded with human umbilical vein endothelial cells (HUVECs) was bioprinted within a collagen block, after which a spheroid consisting of glioma stem cells obtained from a patient was injected beside the vascular channel to mimic the in vivo scenario (Figure 5A). It was noted that the glioma spheroid gradually remodeled over a course of 7 days showing signs of sprouting and potential vascularization. This model is general, and can be conveniently extended to a variety of other cancer types to study the tumor-vascular niche.

Apart from focusing on the vascularization, studies have further investigated the tumor microenvironment, which is also playing a crucial role in tumor development. For example, Sun et al. encapsulated HeLa cells in a composite bioink of gelatin/ alginate/fibrinogen to directly bioprint a 3D cervical cancer model for drug testing (Figure 5B).^{103,104} It was demonstrated that the bioprinted 3D model of cervical cancer was more realistic than the conventional 2D cultures in terms of cancer cell proliferation, viability, and response to chemotherapy. The deposited network of cancer cell-embedding microfibrinous matrix may be designed to assume any shape and architecture to meet the requirement of different cancer cell types. Using a droplet bioprinter, Demirci et al. created a coculture model using OVCAR-5 ovarian cancer cells and normal fibroblasts in Matrigel. The bioprinted pattern permitted an efficient coculture as well as a perfused system.¹⁰⁵ This 3D droplet-based cancer model enabled a better control of the spatial repartition of the cells as well as the cell density, hence enabling a more reproducible cell patterning and controlled tumor microenvironment.

Immune cells are proven to be another critical component in the tumor microenvironment, which actively participate in the remodeling of local cancer tissues.¹⁰ Kilian et al. developed a coextrusion bioprinting model to analyze the interactions between MDA-MB 231 breast cancer cells and macrophages (Figure 5C),¹⁰⁶ which are known to create a paracrine loop inducing an increased motility for both cell types thereby potentially leading to tumor cell extravasation into the bloodstream.¹⁰ The two cell types were loaded into the individual cartridges of the bioprinter and coextruded in a spatially controlled manner, where the breast cancer cells were embedded in an alginate bioink and delivered through the sheath flow while the macrophages were suspended in a CaCl₂ solution and dispensed via the core to cross-link the outer alginate layer. It was discovered that, the macrophages initially residing in the core microchannel could gradually migrate out to interact with the surrounding breast cancer cells, and such interaction (indicated by the correlation of the two cell types) was dependent on the geometry of the bioprinted macrophage channels within the tumor matrix. This model provides a convenient method to study the interactions of tumor cells with the

surrounding stromal cells in the vicinity. Bioprinting tumor cells within a carefully designed niche also enables further studies on cancer metastasis and migration.¹⁰⁷

CONCLUSIONS AND PERSPECTIVES

Advances in the 3D bioprinting technologies has enabled in the past decade the fabrication of in vitro 3D tissue models with improve biomimetic properties and functionality, by providing the ability to precisely engineer tissue composition, architecture, and vascularization at good fidelity and potentially high throughput. However, it was not until recently that the community started to focus on 3D cancer models, which are strongly desired for fundamental biological studies as well as for more accurate anticancer drug screening to eventually achieve personalized cancer treatment.

Although current bioprinting strategies have evolved with great sophistication, one major future direction that we envision is the combination of various but complementary approaches capable of recapitulating the extremely complex and heterogeneous cancer microenvironment. In addition to the need for vascularization, we further propose the requirement for compositional accuracy of the cancer microenvironment. To this end, it is believed that the recent development of multimaterial bioprinting systems will become very useful tools in advancing this new field of cancer bioprinting.^{85,108,109} For example, recently a hybrid bioprinting platform that integrates a heating unit for extrusion of the polymeric scaffold together with three additional cartridges capable of depositing different bioinks of interest was reported (Figure 6A).¹¹⁰ With this multimaterial extrusion-based bioprinting, several types of functional tissues were produced. In comparison, a multi-material stereolithography-based bioprinting platform has also been recently developed, where sequentially presented digital masks were used to pattern biological structures containing more than one bioinks (Figure 6B).⁶¹ Using a microfluidic printhead heterogeneous patterns and gradient structures may also be directly produced.^{76,111,112} Using these novel multi-material bioprinting systems, it is possible to simultaneously deposit desired cell types critical for recapitulation of the cancer microenvironment at the stage of the disease, such as stromal cells, immune cells, and cancer-associated fibroblasts and microvascular cells, in addition to the cancer epithelial cells. Moreover, cell type-specific ECMs, or artificial bioinks mimicking the ECMs, can be used for encapsulating corresponding types of the cancer-related cells to match the requirements of these different cells to achieve their respective optimal functions.

Maintaining sufficiently high viability and native phenotypes of cancer and cancer-associated cells posts another major challenge that needs to be overcome to facilitate the application of 3D bioprinting in cancer tissue engineering. To achieve personalized cancer modeling, it is of great importance to be able to preserve the cell bioactivity and phenotypic expressions in a way they behave in their native niches. To this end the exposure to the shear stress during the bioprinting process (especially in the case of extrusion-based bioprinting) may lead to reduced viability of the cells. Sometimes even though the viability is not significantly affected, the phenotypes, or functional behaviors of the deposited cells may still alter over the subsequent period of culture as a result of the stress that the cells experience as they are bioprinted.^{113–116} Therefore, the proper selection of the bioprinting parameters,

such as the viscosity of the bioink, the extrusion rate of the bioink, and the moving speed of the nozzle become particularly important in an effort to achieve unaltered cell viability and phenotypes in bioprinted cancer models.

We believe that, with further development of the tumor-specific bioinks with optimized bioprinting conditions, as well as the convergence of these various bioprinting techniques, advances in the field of cancer bioprinting will significantly improve the biological and physiological relevance of in vitro cancer models. In combination with patient-derived somatic, stromal, and cancerous cells these advances will facilitate progress toward truly personalized cancer treatment.

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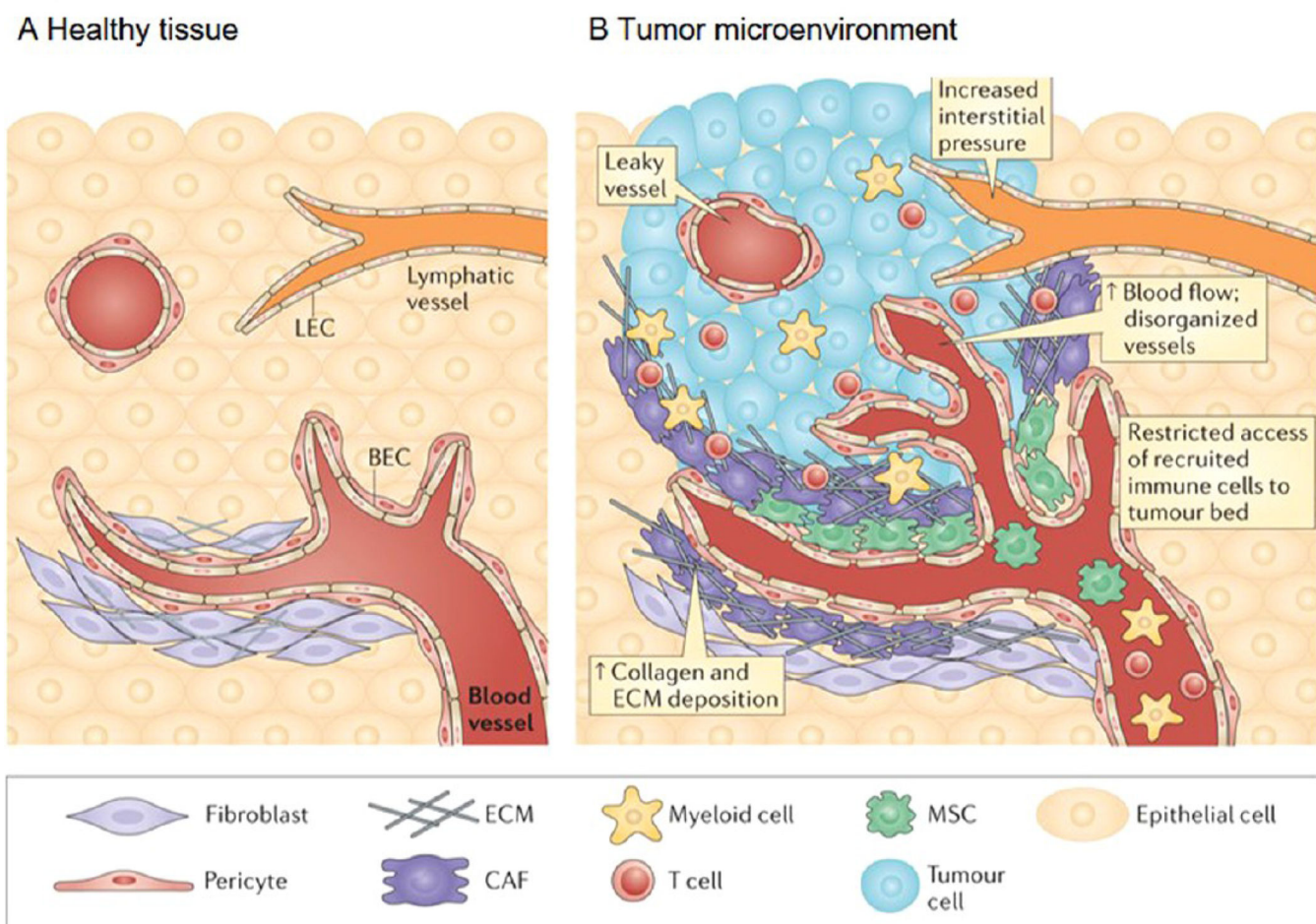


Figure 1.

Healthy tissue and the tumor microenvironment. (A) Stromal cells present in interstitial spaces surrounding the parenchyma of various organs promote tissue integrity by providing growth factors and structural support. Blood endothelial cells (BECs) and pericytes maintain the integrity of blood vessels and ensure the supply of oxygen and other nutrients to the tissue. Lymphatic vessels composed of lymphatic endothelial cells (LECs) drain interstitial fluid. Fibroblasts are constantly remodeling the ECM to cope with mechanical stress within connective tissue. (B) Neoplastic transformation is often accompanied by the formation of a tumor bed and profound alterations in the surrounding connective tissue and stroma, a process that culminates in the establishment of a pathological tumor microenvironment. An imbalance between pro- and antiangiogenic factors results in the formation of aberrant vasculature, characterized by numerous leaky blood vessels. Increased interstitial pressure and inadequate drainage by the lymphatic vessels is also observed. The increased hydrostatic load, along with tumor-secreted molecules (not shown), induces the recruitment of circulating mesenchymal stem cells (MSCs), the activation of cancer-associated fibroblasts (CAFs) and a marked accumulation of ECM. Finally, various chemokines and cytokines in the tumor microenvironment attract activated T cells and myeloid cells to the tumor lesion, but tortuous blood vessels and dense ECM often hinder their access to the tumor nest. Although the makeup of the cellular and extracellular milieu can differ between tumor types

and stages of growth, it is becoming clear that changes in the cellular architecture of the tumor microenvironment can influence tumor growth, metastasis, and drug resistance. Adapted with permission from ref 10. Copyright 2015 Nature Publishing Group.

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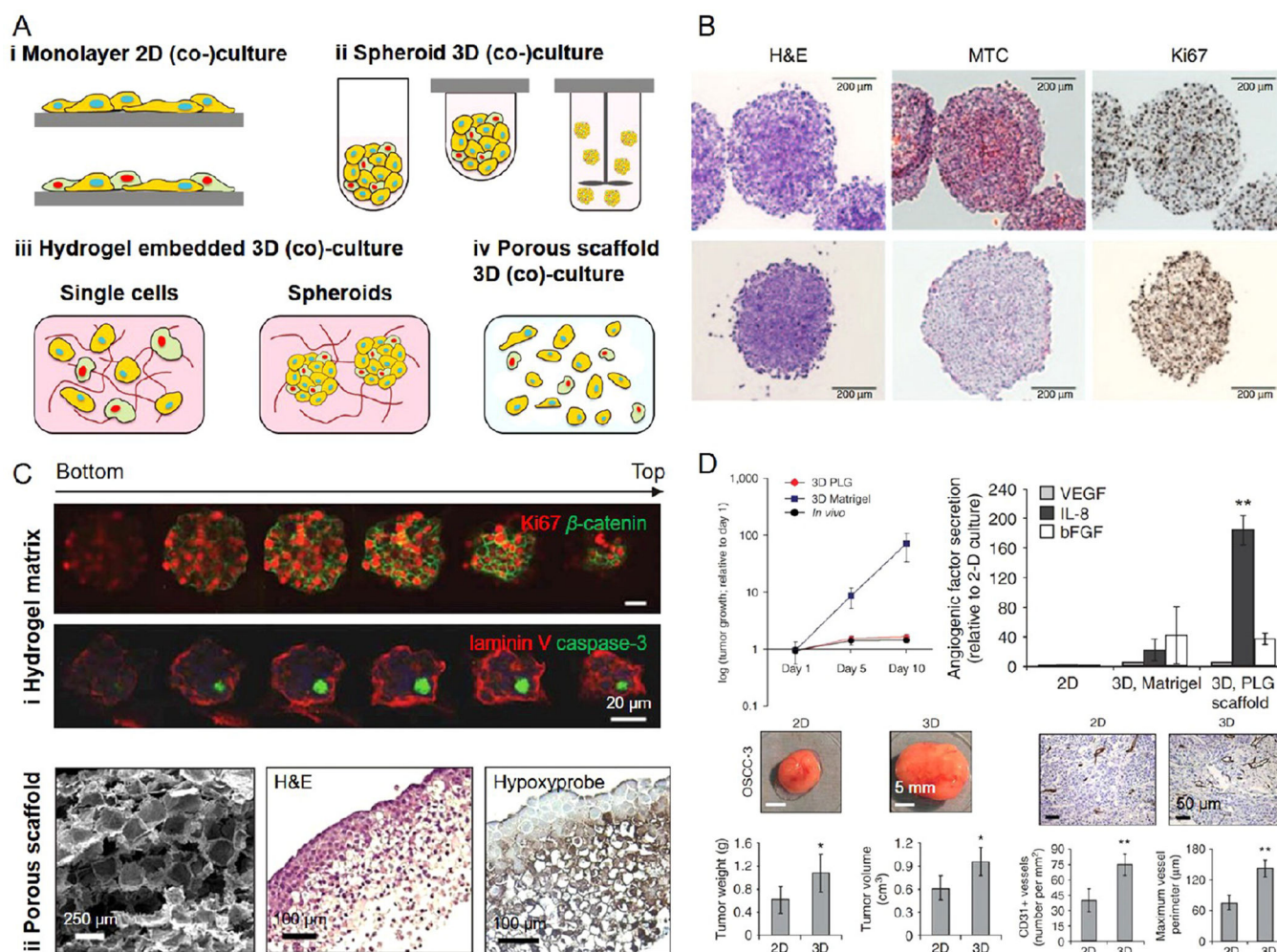


Figure 2. Conventional cancer models

(A) Schematic illustration of cancer models based on (i) 2D monolayer (co)cultures, (ii) spheroid 3D (co)cultures derived from microwells, hanging drops, and spinner flask methods, (iii) hydrogel-embedded 3D (co)cultures, and (iv) porous scaffold-enabled 3D (co)cultures. Adapted with permission from ref 28. Copyright 2014 Wiley–VCH. (B) Multicellular tumor spheroids composed of A375 human melanoma cells were characterized by hematoxylin and eosin (H&E), Mason’s trichrome, and Ki67 staining. Adapted with permission from ref 31. Copyright 2014 Nature Publishing Group. (C) (i) Matrigel-embedded 3D OSCC-3 human oral cancer model stained for Ki67 (red) and β -catenin (green), or laminin V (red) and caspase-3 (green); and (ii) PLG porous scaffold-based 3D OSCC-3 human oral cancer model stained by H&E and hydroxyprobe. (D) Comparisons of tumor growth, angiogenic factor secretion, and vascularization in 3D Matrigel-embedded model, PLG scaffold-based model, and in vivo. Adapted with permission from ref 38. Copyright 2007 Nature Publishing Group.

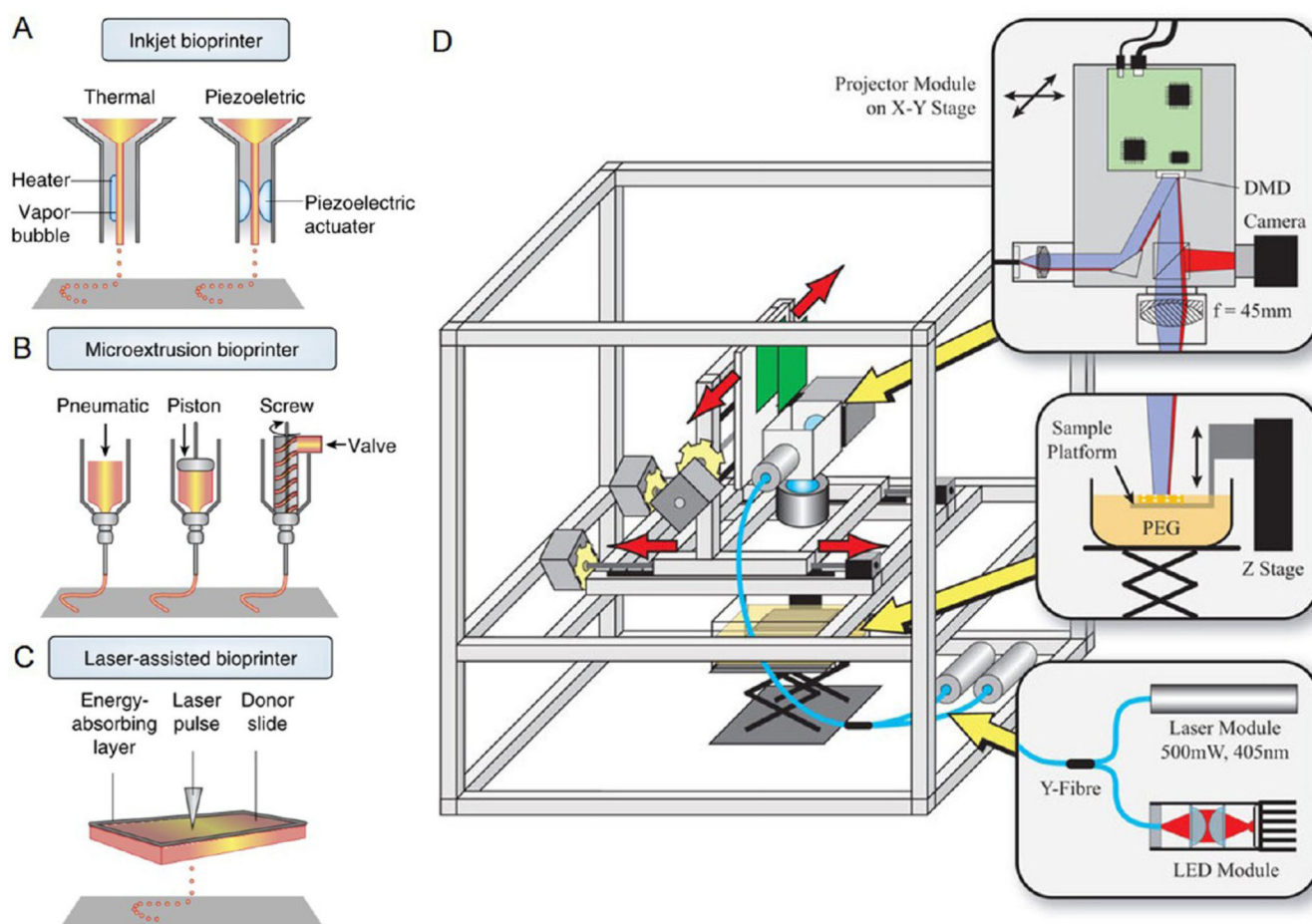


Figure 3. Common bioprinting modalities

(A) Thermal inkjet printers electrically heat the printhead to produce air-pressure pulses that force droplets from the nozzle, whereas piezoelectric printers utilize pulses formed by piezoelectric actuation for droplet ejection. (B) Microextrusion bioprinters use pneumatic or mechanical (piston or screw) dispensing systems to extrude continuous bioinks. (C) Laser-assisted bioprinters use laser focused on an absorbing substrate to generate localized pressures that propel cell-containing bioink onto a collector substrate. Adapted with permission from ref 51. Copyright 2014 Nature Publishing Group. (D) Schematic of the stereolithography-based 3D bioprinter. A frame is used to support the custom precision translation stages and projector system; light from a UV laser then illuminates a digital micromirror device (DMD) projector; a lens is adopted to project the image of the DMD pattern onto the bioink for layer-by-layer cross-linking, which along with vertical movement of the stage generates 3D bioprinted structures. Adapted with permission from ref 65. Copyright 2015 Nature Publishing Group.

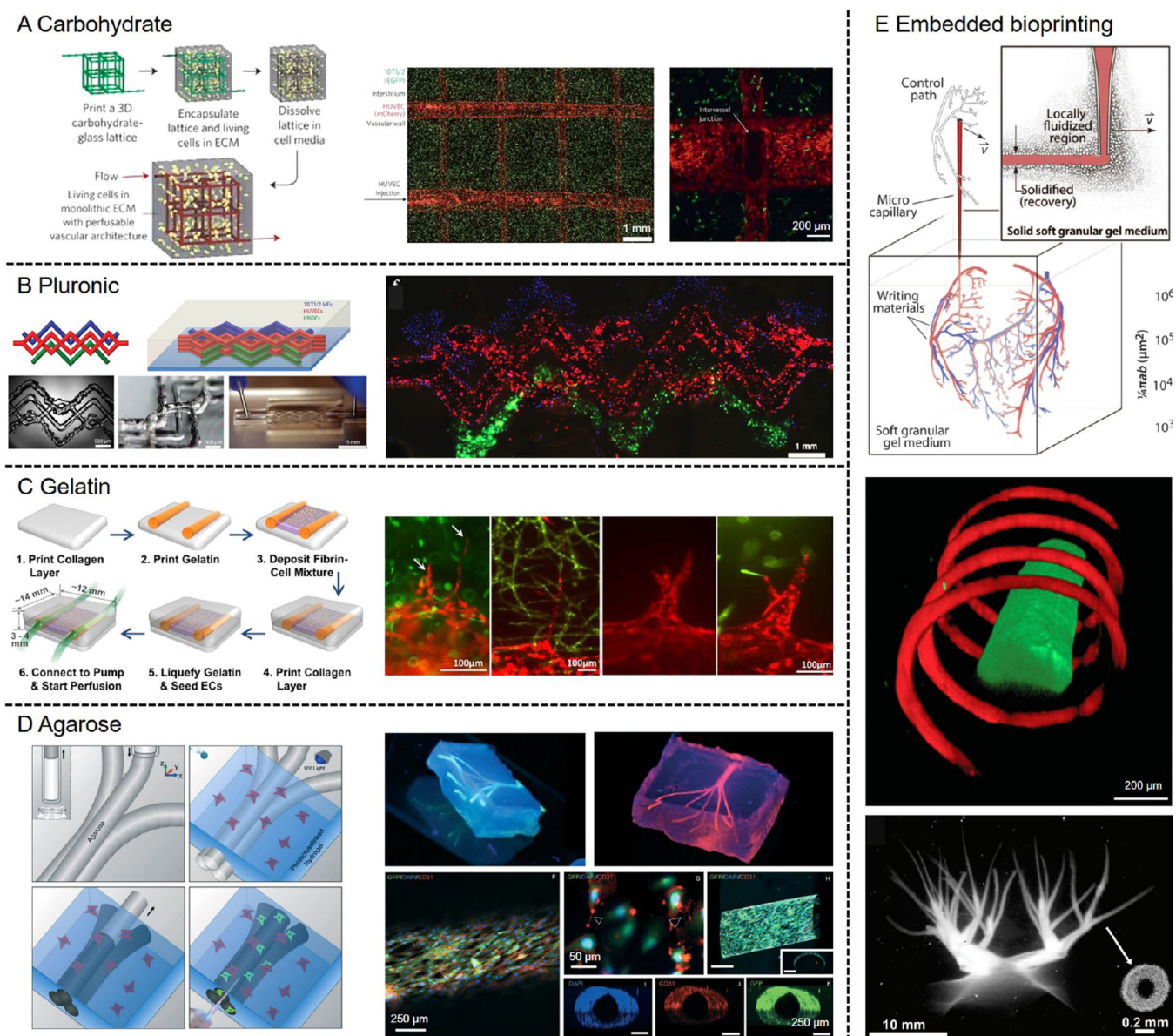


Figure 4. Examples of bioprinted vascular structures

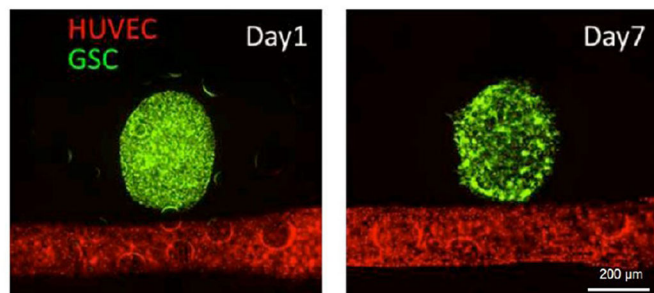
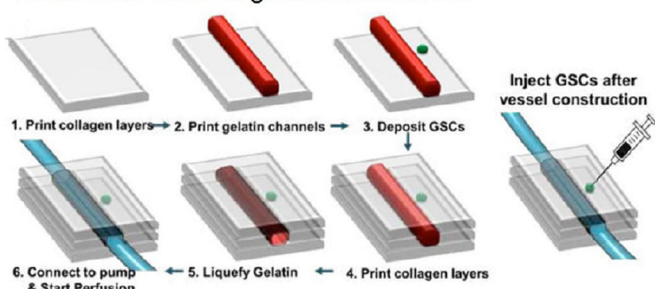
(A) Sacrificial bioprinting by templating against an open, interconnected, self-supporting carbohydrate glass lattice, where endothelialization of channel walls and formation of intervessel junctions could be achieved. The carbohydrate template was dissolved in culture medium. Adapted with permission from ref 95. Copyright 2012 Nature Publishing Group.

(B) Sacrificial bioprinting by templating against pluronic. A heterogeneous engineered tissue construct was produced, in which blue, red, and green filaments corresponded to bioprinted 10T1/2 fibroblast (blue), human neonatal dermal fibroblast cells (green), and HUVECs (red). The pluronic template was liquefied at $<4^{\circ}\text{C}$ and removed by vacuum suction. Adapted with permission from ref 85. Copyright 2014 Wiley-VCH.

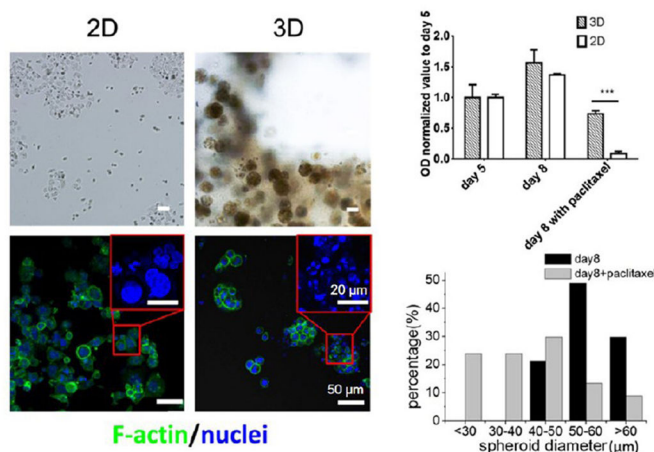
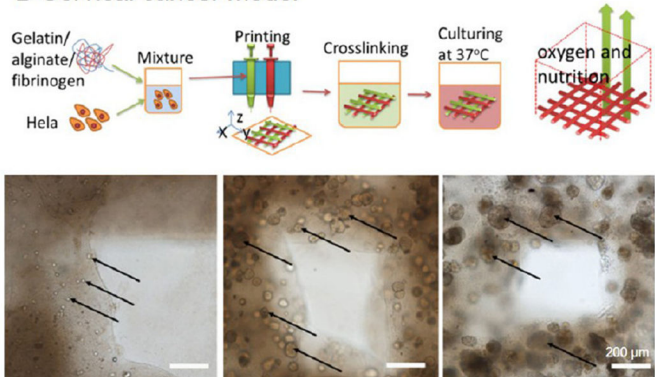
(C) Sacrificial bioprinting by templating against gelatin, where endothelial sprouting into the surrounding matrix was shown to occur. The gelatin template was liquefied at $<37^{\circ}\text{C}$ and removed

spontaneously. Adapted with permission from ref 97. Copyright 2014 Springer. (D) Sacrificial bioprinting by templating against agarose, where the channels could be subsequently endothelialized. The solidified agarose template was removed by pulling or vacuum suction. Adapted with permission from ref 96. Copyright 2014 Royal Society of Chemistry. (E) Schematic showing a microscale capillary tip sweeping out a complex pattern as the bioink was injected into the granular shear-thinning gel medium. Freeform vascular patterns could be generated using this embedded bioprinting approach. Top image adapted with permission from ref 98. Copyright 2015 American Association for the Advancement of Science. Bottom image adapted with permission from ref 82. Copyright 2015 Wiley–VCH.

A Vascularization of glioblastoma model



B Cervical cancer model



C Breast cancer / macrophage co-culture model

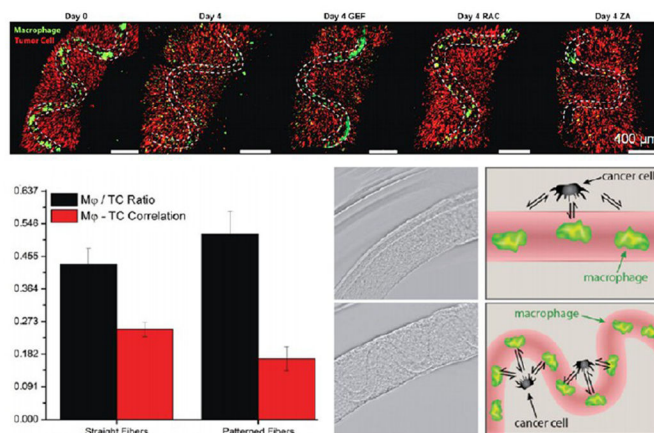
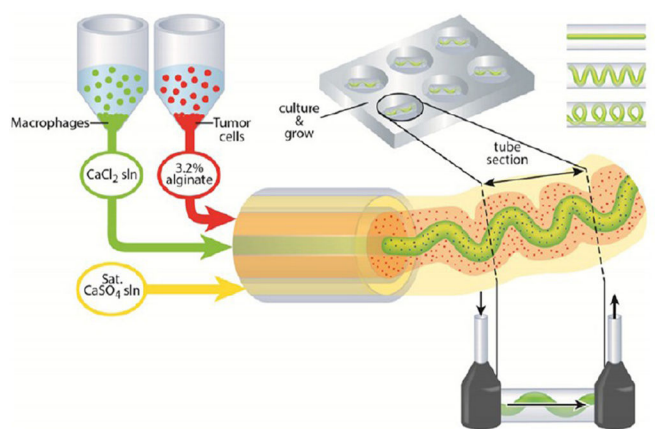


Figure 5. Cancer bioprinting

(A) Construction of a glioblastoma vascular niche by injecting a spheroid of glioma stem cells (green) besides a sacrificially bioprinted perfusable vascular channel (red). Adapted with permission from ref 102. Copyright 2015 Institute of Electrical and Electronics Engineers. (B) Fabrication of a cervical cancer model by direct 3D bioprinting of Hela cell-laden bioink. The cells proliferated and clustered (black arrows) within the bioprinted microfibrillar structure. The bioprinted 3D cervical cancer model showed drug-induced toxicity at higher resistance than the 2D cultures. Adapted with permission from ref 103. Copyright 2014 IOPscience. (C) Bioprinting of a breast cancer/ macrophage coculture model, where the relative geometry of the two cell populations was shown to affect the

tumor-immune cell interactions. Adapted with permission from ref 106. Copyright 2015 Wiley–VCH.

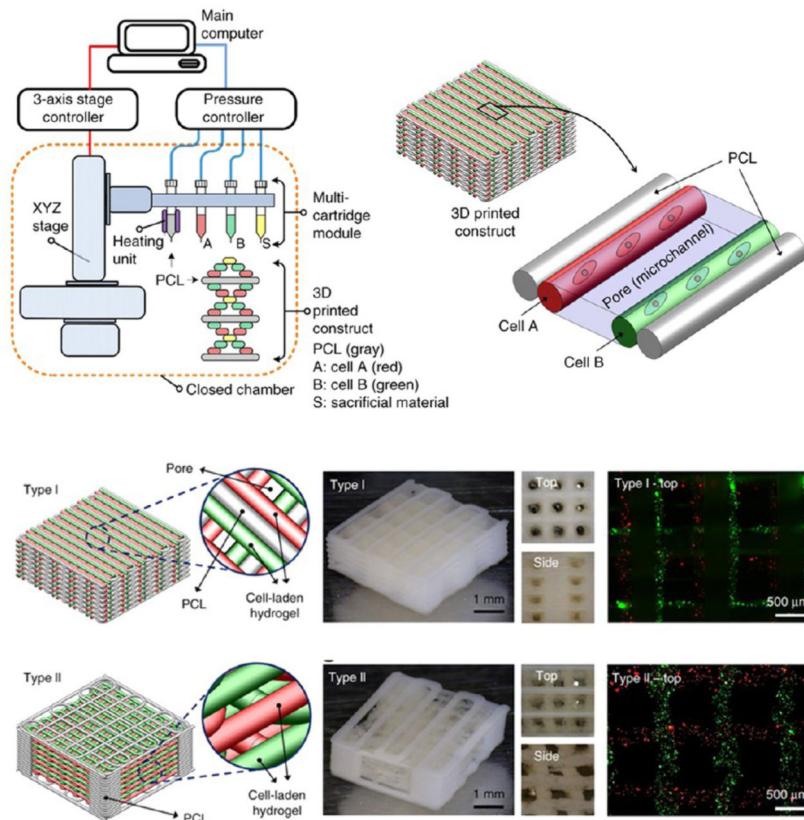
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A Multi-material extrusion bioprinting



B Multi-material stereolithography

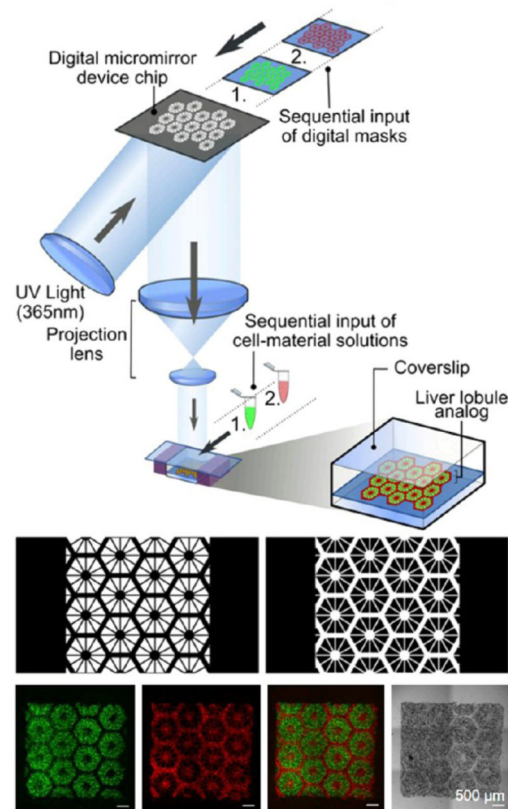


Figure 6. Multimaterial bioprinting

(A) Extrusion-based multimaterial bioprinting system capable of depositing multiple bioinks along with polymeric scaffold for fabrication of large-scale vascularized functional tissue constructs. Adapted with permission from ref 110. Copyright 2016 Nature Publishing Group. (B) Multimaterial DMD stereolithography bioprinting system capable of high-resolution microscale patterning of the complex tissue microarchitecture to achieve functionality. Adapted with permission from ref 61. Copyright 2016 the National Academy of Sciences of the United States.