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Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling

Priyanshu Shukla[1], **Sriya Yeleswarapu**[1], **Marcel A Heinrich**[2], **Jai Prakash**[2,*] **and Falguni Pati**[1,*]

[1] Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Kandi, Sangareddy 502284, Telangana, India
[2] Department of Biomaterials, Science and Technology, Faculty of Science and Technology, University of Twente, Drienerlolaan 5, 7500AE Enschede, The Netherlands
[*] Authors to whom any correspondence should be addressed.

E-mail: falguni@bme.iith.ac.in **and** j.prakash@utwente.nl

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Abstract

The tumor microenvironment (TME) typically comprises cancer cells, tumor vasculature, stromal components like fibroblasts, and host immune cells that assemble to support tumorigenesis. However, preexisting classic cancer models like 2D cell culture methods, 3D cancer spheroids, and tumor organoids seem to lack essential TME components. 3D bioprinting offers enormous advantages for developing *in vitro* tumor models by allowing user-controlled deposition of multiple biomaterials, cells, and biomolecules in a predefined architecture. This review highlights the recent developments in 3D cancer modeling using different bioprinting techniques to recreate the TME. 3D bioprinters enable the fabrication of high-resolution microstructures to reproduce TME intricacies. Furthermore, 3D bioprinted models can be applied as a preclinical model for versatile research applications in the tumor biology and pharmaceutical industries. These models provide an opportunity to develop high-throughput drug screening platforms and can further be developed to suit individual patient requirements hence giving a boost to the field of personalized anti-cancer therapeutics. We underlined the various ways the existing studies have tried to mimic the TME, mimic the hallmark events of cancer growth and metastasis within the 3D bioprinted models and showcase the 3D drug-tumor interaction and further utilization of such models to develop personalized medicine.

1. Introduction

Cancer is a multifactorial disease caused by unregulated and unchecked cellular division due to genetic mutations. Tumor cells recruit, collaborate, and work with multiple other cells to promote tumor progression and metastasis, forming a 3D tumor niche, more commonly known as the tumor microenvironment (TME) [1]. According to IARC-WHO Global Cancer Observatory, the global cancer statistics for 2020 have shown an increased cancer burden at 19 292 789 new cases in 2020 alone, with 9 958 133 cancer-related deaths for the same year. The frequently reported types of cancer (cancer incidence (percentage of new cancer cases out of the total reported cancer cases (I)/cancer mortality (percentage of deaths due to specific cancer type out of total deaths reported due to cancer (M)) were reported as breast cancer (I: 11.7%/ M: 6.9%), lung cancer (I: 11.4%/ M: 18%), colorectum cancer (I: 10%/ M: 9.4%), prostate cancer (I:7.3%/ M: 3.8%), stomach cancer (I: 5.6%/ M: 7.7%), liver cancer (I: 4.7%/ M: 8.3%). (IARC-WHO GCO 2020 data https://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf).

The past few decades have shown tremendous research and development in the field of cancer biology with the help of a variety of different cancer models ranging from traditional 2D monolayer cell cultures [2] to *in vivo* models, which both form crucial elements in the efficient development of novel therapeutics in laboratories worldwide. In recent years these conventional practices have been supplemented with 3D cell culture to study cancer cells in a realistic three-dimensional setting, with cells growing as observed *in vivo* [3]. The introduction of 3D

cell culture was essential for the drug development process as, despite several drugs clearing pre-clinical trials, most of the drugs fail to pass through clinical trials. A few reasons for this were the absence of three-dimensional growth of cancer cells with enhanced response to chemotherapeutic drugs, their gene and protein expression varying in 2D conditions versus the *in vivo,* and a lack of cell culture conditions accurately mimicking the natural tumor microenvironmental complexities. This would ultimately lead to false-positive drug response in preliminary cell culture experiments, proving to be a time-consuming and costly process. Therefore, 3D cell culture models now serve as a steppingstone before preclinical and clinical studies to predict anti-cancer drug efficacy and toxicity more accurately [4]. 3D cell culture models serve as 3D platforms for basic cancer research, cancer diagnosis, and rapid drug screening. Despite the availability of an extensive array of cancer models currently used for cancer research and anti-cancer drug development, most models can still not fully mimic the complexity found within the TME, which encompasses various extracellular matrix (ECM) proteins, multiple cell types, and has its microarchitecture and mechanobiology [5]. While several cancer models such as spheroids try to implement TME-specific ECM or several cell types, they often lack spatiotemporal arrangement of cells and fail to replicate intratumoral heterogeneity, causing the drug studies done on such models not to have a biological relevance or high degree of drug response predictability [6]. Hence, a need exists to address the gap of replicating tumor heterogeneity within a single cancer model in a controlled fashion.

3D bioprinting technology has recently gained fame in tissue engineering and regenerative medicine by allowing computer-aided designing and 3D assembling of tissue constructs using biomaterials as a scaffold material [7]. 3D bioprinting allows users precise control over the deposition of a cell-laden biomaterial, often referred to as bioink, into specific patterns to form a bioprinted construct that can mature in conventional cell culture conditions. The bioink usually is a printable cell-laden material with composition differing according to tissue or organ of interest. Hydrogel-based bioinks, natural or synthetic derived, are biocompatible materials as they can retain a large amount of water and provide a hydrated microenvironment for cells in printed construct [8]. In addition, different bioprinting techniques allow the assembly of multiple cells together suspended within different biomaterials, creating a heterogeneous tissue-like construct, as seen in the TME [7].

Consequently, 3D bioprinting is a powerful tool that can bridge the gap between the erroneous *in vitro* drug response and the *in vivo* conditions for biological studies, anti-cancer drug screening, and the development of cancer therapeutics [7]. Although 3D bioprinted cancer models cannot fully function as the only tool to evaluate novel therapeutics due to their reproducibility and time consumption limitations, there are already some inspiring proof-of-concept studies mimicking the *in vivo* microenvironment, which showcased the evaluation of various chemotherapeutic drugs or chemoradiation. This review presents the advancements in developing 3D cancer models, ranging from different TME biomimetic cancer models to their applications. Further, we discussed the status of 3D bioprinted cancer models, their advantages, caveats, and future work direction in cancer modeling. Our goal with this review paper is to shed light on the importance of tumor microenvironmental cues in established cancer models and showcase how these models are currently being fabricated in a more relevant manner. Critical insights into the existing studies can ultimately help develop models closer to *in vivo* tumors and hence be adapted in a clinical setting to help researchers, clinicians, and oncologists for the betterment of cancer patients' healthcare and the development of personalized medicine.

2. TME

In the early 1800s, scientists put forward the seed and soil hypothesis, where TME was referred to as 'fertile soil' required to nurture the 'seeds' or the tumor cells [9]. The TME is now acknowledged as a critical player influencing cancer progression and drug resistance in patients [10]. Within the TME, interactions between various tissue microenvironment components, including the vasculature and dysregulated immune responses, form a tumor niche that has been extensively discussed elsewhere [9–12]. The tumor niche is critical for tumorigenesis and initiates the cascade of invasion, metastasis, and drug resistance (figure 1). The TME is continuously changing and evolving with the changes in different tumor progression stages to allow the cancer cell population to thrive and grow [11–13]. Various components make up the TME, such as stromal cells, fibroblasts, endothelial cells, and innate and adaptive immune cells. It is the crosstalk between them that helps promote cancer progression [14, 15].

2.1. Tumor extracellular matrix & tumor stroma

The ECM around and within a tumor needs remodeling and reorganization for cancer progression and development [16]. Over the last few decades, the ECM has been widely implicated in tumor fate and has been known to actively participate in crosstalk with tumor cells to change the normal homeostatic microenvironment to an acidic, tumor-supporting microenvironment [17]. ECM constitutes a collective mixture of structural proteins like collagens, glycoproteins like laminin, fibronectin, proteoglycans like decorin, and other factors discussed in detail in previous reviews elsewhere and summarized in table 1

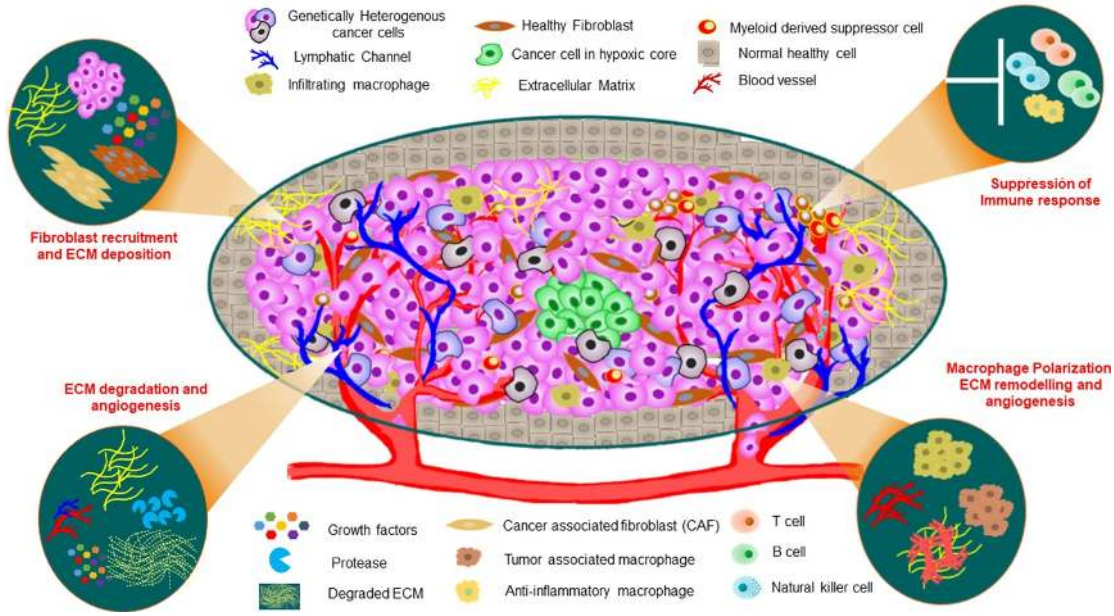

Figure 1. Illustration of a tumor niche with multiple TME components. The tumor niche serves as the background for the dynamic interplay of various components of the TME, such as healthy cells, various stromal cells, cancerous cells, immune cells, and ECM proteins, all promoting pro-tumorigenic activities of cancer cells such as fibroblast activation, ECM modulation, immunosuppression, and angiogenesis to drive the process of tumor progression.

Table 1. ECM components secreted by cancer cells and their function in maintaining the TME.

Cancer cell-derived ECM proteins	ECM Protein subtypes	Functions in tumorigenesis
Collagens	Collagen 1-12, 15, 16, 18, 19, 22, 24	• Collagen deposition occurs at the tumor site due to overexpression of linear, thick, and excessively crosslinked collagen fibers. • Overexpression leads to increased local stiffness and inefficient targeted drug delivery [27].
Glycoproteins	Laminin α4, β1, β2, γ2	• Mutations in laminins cause the failure of the basement membrane assembly. • Overexpression is seen in cancer cells leading to invasive behavior and poor prognosis [28].
	Fibronectin 1	• Cancer cell-secreted fibronectin is necessary for collagen deposition and interaction with integrin, an essential ECM protein. • The expression of fibronectin helps in overall cancer cell survival and chemoresistance [29].
	Hyaluronan	• Increased expression of hyaluronic acid is seen in the tumor microenvironment. • Overexpression leads to poorer prognosis and increased malignancy rates as it mediates EMT [30].
Proteoglycans	Decorin	• Increased expression in cancer cells generally; expression levels differ according to cancer type [31].
ECM Regulators	LOX, LOXL2, 4	• LOX family of proteins acts as a significant modulator of tumor ECM. • Elevated LOX activity leads to increased collagen deposition and invasiveness of tumor cells [32].
Secretory factors	TGFβ2	• Known to inhibit tumor growth at an early stage but promotes tumor cell invasiveness and metastasis at later stages of tumorigenesis [33].

[9, 11, 16–18]. Stromal cells secrete their own ECM proteins in conjunction with cancer cells to form a tumor-supporting microenvironment [12, 19]. The stromal component of a tumor provides the tumor with its structural identity and facilitates the signaling between cancer cells and other components of the

tumor, such as cancer-associated fibroblasts (CAFs) and endothelial cells [9, 20]. CAFs are known to produce and remodel the ECM surrounding the tumor [21, 22]. Normal fibroblasts within the tissue are recruited by the cancer cells detached from the tumor mass to form CAF phenotypes [23]. In addition, they secrete various signaling molecules like mitogenic fibroblast growth factor and insulin-like growth factor 1, and TGF-β, all of which significantly influence tumor cell migration, invasion, and epithelial-to-mesenchymal transition (EMT) processes [24]. Tumor stromal composition differs according to tissue type, leading to different tumor progression routes [23–26].

2.2. Tumor vasculature

The vascular network associated with the tumor is different from the vasculature seen within normal tissues in homeostatic conditions [34]. The blood vessels formed due to angiogenesis toward the hypoxic tumor face unregulated proliferative cancer cells all around it, leading to stress over these vessels causing slow blood flow within these structures [35]. These vessels are also known to have perforations and are called leaky vessels, and because of aberrant signaling, the vessels within tumors show disorganized bifurcation and heterogenous lumen [36]. Endothelial cells within the blood vessels play an important role within the TME. These cells respond to growth factors secreted by the tumor and initiate angiogenesis [37]. Pericytes are another cell type within blood vessels that support the tumor vasculature [38].

2.3. Tumor immune microenvironment

Another well-known hallmark of cancer is a dysregulated immune system that helps cancer progression and survival of cancer cells [39]. The TME tends to have different immunological cell populations in crosstalk with cancer cells at various tumor growth stages, starting from the primary tumor growth, invasion, and metastasis. Tumor genotype is also known to affect the landscape of tumor immune microenvironment (TIME), with crucial mutations leading to an increased expression of tumor-origin cytokines and chemokines that ultimately affect immune cell infiltration [40] and increased tolerance of immune cells to tumor cells, eventually leading to an immunosuppressive TME [39, 40]. A state of anergy arises at the tumor site due to a disbalance between immune regulatory or suppressor cells and the accumulation of immunosuppressive cytokines and other chemokines [41]. The TIME has a diverse immune cell population, and its detailed profile and functions have been discussed in previous reviews extensively [42, 43]. Many tumor immunology studies showcase the T-lymphocytes and macrophages of protumorigenic phenotype as key players within the TIME, with T-cell exhaustion or dysfunction and an immunosuppressive microenvironment coupled with tumor-origin cytokines working in tandem with advancing tumor stage and metastasis [44–46]. In summary, the TIME is subject to dynamic spatiotemporal changes, and each event has to be considered while working on immunomodulatory therapeutic approaches.

3. Conventional approaches to tumor modeling & their limitations

Two-dimensional monolayer cancer cell culture and *in vivo* or xenograft animal models are the two most widely used conventional platforms to study cancer biology and preclinical validation of potential anticancer drugs. 2D cell culture systems provide distinctive advantages such as the ease of availability of cell lines, cost-effectiveness, defined protocols, and high reproducibility. However, cancer cells growing *in vitro* fail to offer spatially and temporally controlled extracellular environments [47, 48]. There is a lack of organization and architecture and a low frequency of cellular signaling within monolayer cell cultures. This makes the 2D cancer cell culture less reliable for studying complex processes within TME, such as cell-to-cell communication, the phenomenon of invasion and metastasis, and accurate response toward a potential drug candidate, often leading to the drugs failing to show good results during animal studies [48]. Animal models such as the immunodeficient mice model and patient-derived xenografts (PDX) *in vivo* models are necessary to validate any therapeutic options and any associated toxicities as they provide conditions for studying tumors in mammalian physiology with three-dimensional TME and have proven to be highly reproducible systems [48–50]. PDX models allow clinicians to model, propagate, and experiment on patient tumor samples within a mammalian-origin tissue under physiological conditions [51, 52]. However, the generation of PDX models can take anywhere between 4 and 8 months, with a low engraftment success rate, rendering the motivation to design or establish a successful therapeutic regimen in time for the patient redundant. Another limitation of immunodeficient mice models is that they cannot recapitulate the immune response generated by a human immune system. The histological difference must be considered, especially when modeling specific subtypes of cancers. In addition, animal models are expensive to maintain until the completion of experimental studies and subject to ethical concerns [4].

Over the years, researchers shifted to experiment with more rapidly developed, cost-effective model systems- 3D cancer spheroid and organoid models. 3D cell culture of multicellular tumor spheroids (MCTS) and the highly organized and heterogenous Organoid-based cancer models allow for biomimetic cell-to-cell and cell-to-matrix signaling. These

3D models can generate the cancer cells in a gross tumor microarchitecture with a necrotic core surrounded by a hypoxic zone of tumor cells [53]. 3D organoid models allow researchers to assess the complex interactions of cancer cells with other TME components and evaluate complex processes such as angiogenesis, hypoxia, and *in vivo* cancer stem cell behavior without the complexities of generating *in vivo* models. 3D cancer organoids have been used for a wide range of applications, from studying tumor biology to drug testing and screening studies for multiple tumor types, for example, liver cancer [54–56], pancreatic cancer [57–59], brain cancer [60], prostate cancer, [61] stomach and colorectal cancer [62, 63], gastrointestinal cancer [64], bladder cancer [65], stomach cancer [66], and renal cancer [67]. The development of personalized patient-specific tumor organoids allows researchers to carry out various genomics, proteomics, and anti-cancer drug studies specific to patient cells [68, 69]. There are certain challenges concerning 3D cancer spheroid models. Firstly, not all cancer types follow the principle of spheroid formation as observed in leukemia cells or other non-solid cancers; hence spheroids cannot be used to model all types of cancer in certain conditions. Secondly, even with the evolution of 3D culturing methods, the cancer spheroids cannot wholly replicate complex microarchitecture and the interaction with other physiological structures—for example, the blood-brain barrier, making it challenging to conduct drug studies on 3D glioblastoma (GBM) *in vitro* spheroid models. Recently developed tumor organoids can mimic the feature of tumor vasculature by coculturing endothelial cells and tumor cells, but this does not give researchers a clear idea of drug transport to the tumor site and how the leaky vessels affect drug studies *in vivo*. Finally, the 3D tumor spheroids and tumor organoids often lack the total cellular population of stromal and immune systems, which is an incomplete model used for anti-cancer drug assays [70].

Cancer-On-Chips arose as an amalgamation of microfluidic technology and microfabrication with application in disease modeling. Cancer-on-chips started with the aim of early cancer detection and drug screening studies for therapeutics with the cancer cells cultured under a dynamic flow of media to imitate the *in vivo* conditions of blood flow in capillaries, which served as a massive advantage over static 2D cell culture models, especially for the drug development process [71]. Initially, the microfluidics chip designs allowed for a 2D layout with dynamic media perfusion, but new biofabrication techniques allow for depth and width, ensuring that cancer cells can be cultured in three dimensions and given the necessary tumor microenvironmental cues [72]. Cancer-on-chips now serve for disease diagnosis and studying cancer pathophysiology while providing a fluid flow that gives shear stress to cells, helps establish an oxygen gradient, and assesses tumor progression in real-time [73]. Most cancer-on-chips are fabricated with the help of the photolithography technique and used for cancer modeling in static or perfusion-based culture systems to study the progression of different cancers such as lung cancer [74], bladder cancer [75], metastasis model [76], a vascularized cancer-on-chip model [77]. These cancer-on-chips have also been extended in their application to assess the effect of immune cells on melanoma cancer cells [78] and study the role of mesenchymal stem cells in breast cancer metastasis to bone tissue [79]. They provide an irreplaceable alternative to conventional *in vitro* models by enabling multiplex experimentation with different anti-cancer drug concentrations in different microenvironmental cues on the same chip.

With increasing focus on targeting TME cues as a therapeutic option, it is essential to have cancer models that can replicate the different building blocks of the TME, keeping in mind the *in vivo* growth of tumors over a long duration of time and the chain of biological events in and around the tumor, that all ultimately link up and drive the tumor progression. Cancer models that can accurately recapitulate tissue-specific, stage-appropriate human tumors complete with consideration to its TME would best reflect the physiological response to anti-cancer therapeutics and serve as an indispensable bench-to-bed translational tool. The following sections will discuss the different approaches to mimic the TME via 3D bioprinted based cancer modeling.

4. 3D bioprinting technology (3DBP) for tumor modeling

The major drawback of preexisting cancer models lies in replicating exact human physiological conditions and associated functionality within these 3D tumor models [80–82]. Fabrication of native tissue-like constructs as tumor models with identical cellular and ECM compositions can significantly increase the relevance of such disease models being used to study molecular and biological mechanisms of cancer biology [83]. 3D bioprinting can fabricate a biomimetic tissue model by patterning different cell populations in the spatial dimension to replicate *in vivo* microarchitecture [84–86]. 3D bioprinted constructs have physiological microarchitecture and microenvironment, which can define the functionality of the lab-engineered tissue [7, 87].

With advances in rapid prototyping and additive manufacturing, researchers in the past few decades have dived into using different 3D bioprinting techniques for biofabrication of 3D *in vitro* cancer models that mimic the complexity and heterogeneity of a human tumor, making the experimental results of the preclinical studies more relevant [88]. These 3D bioprinted cancer models serve as bridges between *in vitro* and *in vivo* and can be used as preclinical drug

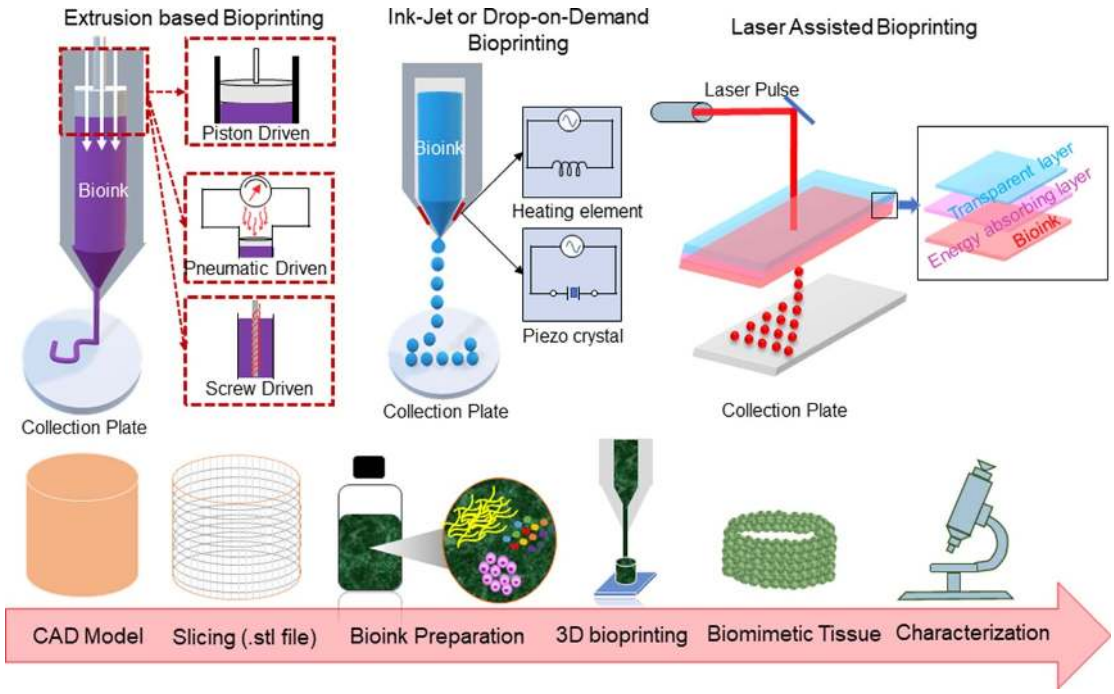

Figure 2. Schematic of different 3D bioprinting modalities and techniques used for 3D modeling of biological tissue. Extrusion-based bioprinting, inkjet or drop-on-demand (DOD) bioprinting, and laser-assisted bioprinting (LAB) modalities are used to actualize a computer-generated CAD drawing of the target tissue. The CAD file is used to fabricate or print cells suspended in different biomaterials within a bioink. Cell-laden bioink is patterned into desired tissue microarchitecture to form a biomimetic structural and functional construct, which over a culture period is then subjected to various biological characterizations to ensure tissue functionality.

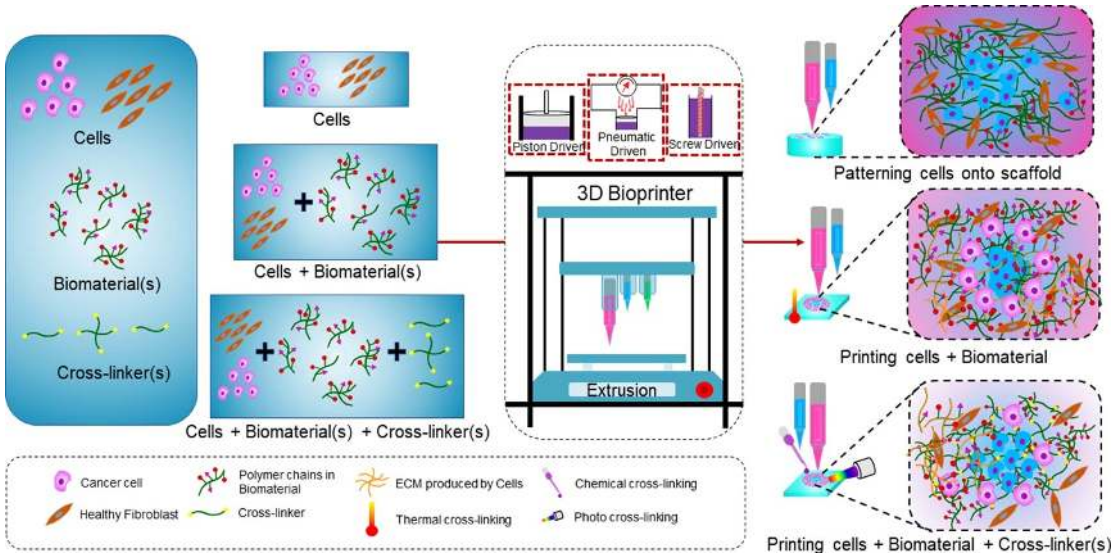

Figure 3. Illustration of the strategy to develop *in vitro* 3D TME using extrusion-based 3D bioprinting technology.

screening platforms. Various 3D bioprinting techniques, as showcased in figure 2, are used to fabricate cancer models or tissue analogs. The choice of bioprinting modality depends on how cell-supportive the printing process is once the biomaterials for the bioink are selected, and the construct should have desired mechanical property post-printing [87, 88].

The most common bioprinting modalities used for cellular printing are inkjet-based, extrusion-based (figure 3), direct light patterning-based bioprinting, and laser-assisted bioprinters (figure 4) [89–92]. Broadly categorizing, two strategies can be used to introduce the cellular component using any of the aforementioned 3D printing modalities. The first strategy is commonly termed as 'two-step' biofabrication strategy, which involves firstly 3D printing a scaffold made up of biocompatible polymer using any of the different printing techniques or

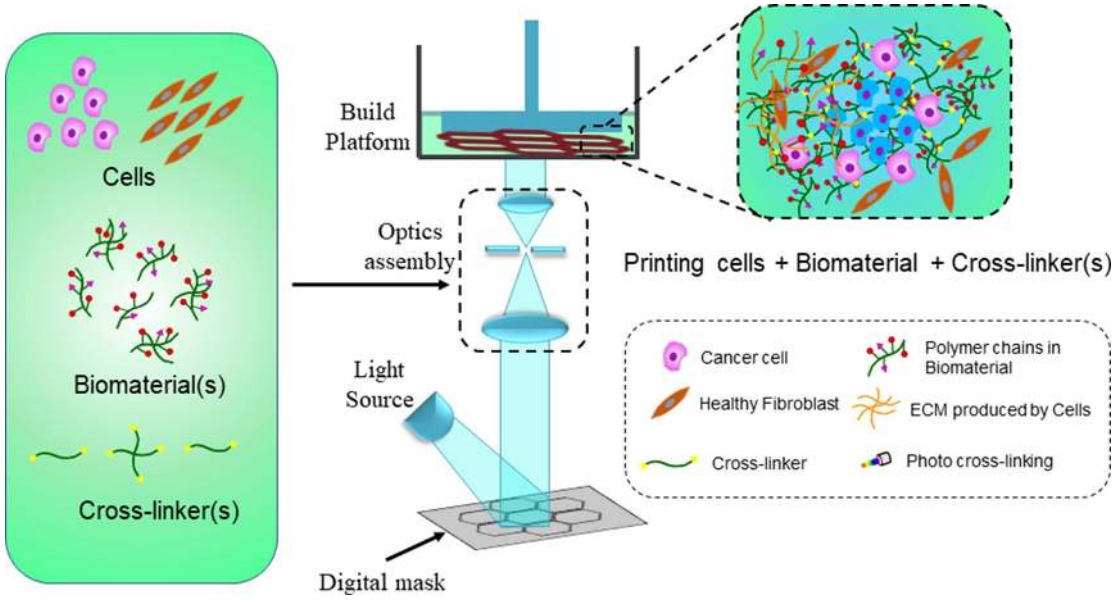

Figure 4. Illustration of strategy to develop *in vitro* 3D TME using direct light patterning (DLP) based 3D bioprinting technology.

bioprinter, including crosslinking step if any and then secondly followed by seeding cells onto it in the form of top seeding and culturing them to develop into a 3D printed cancer model. The generation of 3D printed scaffolds allows researchers to fabricate the scaffold in a predetermined manner and seed cancer cells taken directly from patients or a primary cell line for more accurate drug screening and efficacy studies [88].

In comparison, the 'one-step' biofabrication technology involves using a cell-laden bioink printed directly on a platform to form a high-resolution bioprinted model as a scaffold-free model or within a cancer-on-chip setup (figure 5). With many advantages, such as spatial control over depositing multiple cells, with varied cell- densities, it became possible to deposit multi-materials in a well-defined desired architecture, which eventually facilitates the 3D bioprinting of tumor-like replicas [93, 94]. Most often, an increased preference for extrusion-based 3D bioprinting for cancer modeling has been observed due to their cost-effectiveness and freedom of choice of a wide range of materials that can be used to fabricate relevant cancer models. 3DBP cancer models have been used extensively to study cell-cell interactions, cell-matrix interaction, their crosstalk, varied gene expressions, and invasion characteristics in physiological conditions, all discussed in detail in the following sections [95]. Another advantage of using 3DBP to fabricate cancer models is that it can be used to conduct long-term experiments for months on end, allowing longitudinal studies [90–95]. Crosstalk between cancer cells and the resident cell population within the tumor niche and cells in surrounding healthy tissues can be well studied in a 3DBP cancer model since the bioprinting technique allows users to print coculture models [90, 93, 94]. Newly discovered or designed drugs for anti-cancer treatment can be screened and tested on these *in vitro* cancer models in a high throughput manner to appreciate the realistic effect of the drugs in a 3D environment found in the body. Many unknown pathways can be well understood, which will lead to better therapeutics for patient care. With 3D bioprinted cancer models, researchers have created a cost-effective, reliable, and precise disease model that allows for better clinical significance results, leading to better disease management [90, 93, 94]. Box 1 summarizes the key advantages offered by 3D bioprinting technology for 3D cancer modeling.

4.1. Biomimetic bioinks for 3D bioprinted (3DBP) cancer modeling

Materials for 3D culturing of cells and scaffolds used for 3D cancer modeling play an indispensable role in attaining desired native TME from their biological constituents to their microarchitecture. The key to the biofabrication of a successful cancer model lies in the physical and chemical nature of the biomaterial being used either as a scaffold material or as a cell-suspension material that is the 'bioink' [84]. Biomaterials used for 3D cell culture applications can generally be categorized into natural polymer-based and synthetic polymer-based biomaterials. Natural biomaterials are composed of naturally derived polymers like Matrigel, collagen, gelatin, and alginate that carry the natural ECM-like properties and allow for a high degree of biocompatibility to encapsulated cells. The origin of biomaterial plays an important role when concocting a bioink for cancer research; for example, collagen, hyaluronic acid, and gelatin are isolated from mammalian sources, while biomaterials

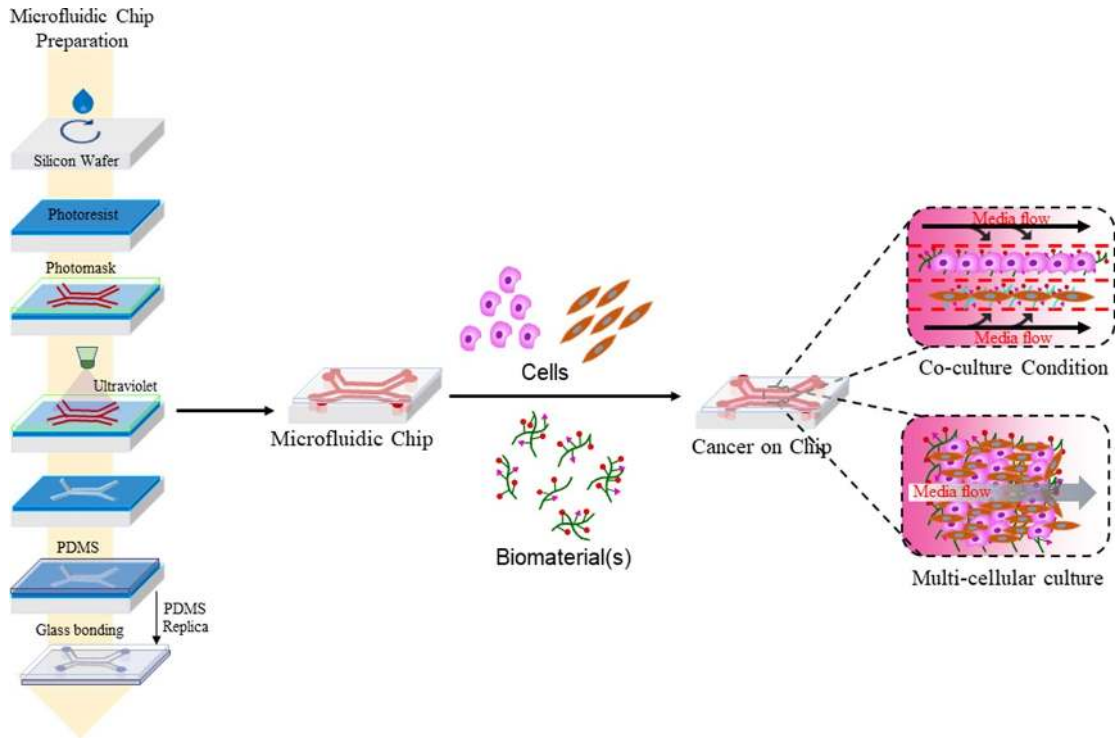

Figure 5. Illustration of a fabrication strategy for microfluidic chips and further development of *in vitro* 3D TME or Cancer-on-chip.

Box 1. Key advantages of application of 3D bioprinting technology for 3D cancer modeling.

Bioprinting allows the patterning of different bioinks in a user-controlled manner to form complex 3D constructs with biomimetic tumor microarchitecture.

A wide range of biomaterial can be opted to mimic native ECM stiffness and ultrastructure, to replicate physiologically relevant TME.

Multiple bioinks with different cell types, both tumor & stromal, can be used for printing functional tumor constructs.

Various bioprinting strategies allow the integration of perusable vascular networks within 3D cancer models, often not seen in 2D culture or spheroids.

3D microenvironmental cues within 3D bioprinted tumor constructs induce genomic and proteomic expression similar to *in vivo* tumors.

Fast and inexpensive biofabrication methods of 3D bioprinted cancer models allow for adapting these models in clinics as diagnostic tools for rapid testing and analysis of patient tumor samples.

Preliminary experimentations with 3D bioprinted *in vitro* models reduce animal experiments, saving resources and time.

3D bioprinting allows developing a translational platform that can lead to faster validation of potential drugs pipelined for preclinical and clinical trials.

like alginate and chitosan are from non-mammalian sources. On the other hand, synthetic biomaterials are composed of lab synthesized polymers like polylactic acid, polyglycolic acid, hydroxyapatite and provide features like matrix stiffness, cell alignment, and other mechanical cues necessary to maintain the 3D scaffold or bioprinted construct for a longer duration as compared to natural biomaterials-based 3D scaffolds which show an increased rate of biodegradation [96]. Cell-laden biomaterials or hydrogel-based bioinks are patterned using 3D bioprinters modalities to form functional constructs [87]. Due to advances in the biofabrication field, both natural and synthetic biomaterial-based bioink can be printed simultaneously via hybrid printing [88].

The selection of biomaterial for bioprinting a cancer model requires a familiarity with the tumor ECM components specific to the tumor type and origin of tissue. The tumor ECM constitutes a collective mixture of structural proteins, glycoproteins and proteoglycans, and other factors, as summarized in table 1 and their expression within tumor stroma

varies in composition according to the tissue of origin and tumor stage [16, 17]. Therefore, bioink made up of natural biomaterials like collagen is a popular biomaterial choice as it is the most abundant protein found in animals and can mimic the natural ECM [97]. In addition, it has been well studied that malignant tumors tend to secrete collagens extensively, leading to stiffness and mechanotransduction signaling, EMT, migration, and metastasis. Therefore, natural biomaterial-based bioink such as gelatin and Matrigel with collagen as a significant component can closely mimic *in vivo* conditions. Recently, tissue-specific decellularized ECM (dECM) based hydrogels have been gaining momentum since it provides all the requirements needed from a biomaterial- native architecture, native growth factors, tissue-specific signaling molecules, all together providing similar conditions to those found in native tissue *in vivo* [98].

The biomaterial selection for a bioink should depend on the type of tumor model being fabricated, tumor location (hard or soft microenvironment), and the tumor stage (primary stage or metastatic stage) that needs to be recapitulated. The mechanical properties of a biomaterial, such as its Young's modulus and compression modulus, would indicate the stiffness of bulk hydrogel or bioprinted construct, and this would help mimic the 3D tumor elasticity or rigidity, ultimately establishing a native tumor niche. Bioinks based on synthetic biomaterials are often used to mimic the solid TME. These biomaterials can offer an ultrastructure that supports cell adhesion and spreading and can be modified using peptides like the RGD or Arg-Gly-Asp amino acid sequence that naturally enhance the biocompatibility of synthetic materials. These bioinks can be used to print scaffolds that mimic bone TME and serve as *in vitro* bone metastasis models [81, 96, 99]. As different factors like biophysical cues, availability of native growth factors, and the natural polymer microarchitecture are to be kept in mind, researchers often opt for bioinks made up of composite biomaterials such as PEGDA or GelMA for 3D bioprinting to balance biological activity and mechanical strength within the printed construct. The matrix material for the bioink can further be tuned to the biophysical requirements of tumor cells by strategies like using different synthetic biomaterials in combination or by using a higher concentration of crosslinkers to increase the matrix stiffness.

The intrinsic properties of biomaterials are crucial for designing bioinks for 3D printing or bioprinting of cancer models. It is essential to consider various parameters of a bioink that is to be chosen for 3D bioprinting a cancer model, like its physical properties such as thermoresponsive nature, swelling, and deswelling behavior, as well as degradation property. Another essential parameter to be optimized for a bioink to be chosen for 3D printing application is its rheological properties. It includes the chosen biomaterial's viscous property in response to shear rate, associated linear viscoelastic property in response to shear strain sweep, as well as gelation kinetics. An ideal bioink would exhibit shear-thinning behavior to be easily micropatterned into a 3D architecture with minimum shear strain so that the printing process is cell supportive and the bioprinted construct shows high cell viability post-printing.

Most of the research on biomaterials is currently focused on designing scaffolds that provide native tumor microarchitecture, strengthening the stability of hydrogels, and synthesizing novel biomaterials. Therefore, to fabricate a tissue-specific 3D bioprinted tumor model, cancer cells can be encapsulated within its native tissue dECM-based bioink, guaranteeing a physiologically similar microenvironment and accurate responses. The adage 'form follows function' indicates the importance of biomaterials and the bioink compositions, both of which go hand-in-hand with the design consideration of tissue architecture. Before applying 3D bioprinting technology to develop a biomimetic tumor model, it is crucial to predetermine the choice of biomaterial and the different cell types that need to be incorporated within the bioink. The biomechanical property of the biomaterials altogether, in due course, is decisive to the functionality of bioprinted structures and, therefore, long-term stability, biocompatibility, and degradation studies of novel biomaterials and new bioink formulations need to be studied before being used as a 3D scaffold or bioink. Researchers also need to narrow down the best-suited bioprinting modality for a particular bioink to yield high cellular viability within the printed construct. Similarly, the printing technique or the printing strategy needs to be selected to simultaneously provide a high-resolution construct that would mimic the intricacies of a tumor niche.

4.2. Mimicking TME by 3D bioprinting

The TME has a complex microarchitecture compromising CAFs, infiltrating immune cells, the blood, and lymphatic vascular networks, all suspended within the ECM that can be soft or stiff depending on the type of tumor (figure 1). 3D bioprinting technology provides researchers with the opportunity to replicate *in vivo* like tumor architecture down to micro levels of about 100 μms ultrastructure, to lay down different types of cells and ECM materials found in a TME in a pre-decided manner. Researchers can study tumor-tissue interaction within cost-effective, rapidly fabricated detailed designs that maintain physiology with the help of biofabrication technology which was previously not possible in conventional cancer models. The development of 3D bioprinted cancer models allows monitoring cancer cells in real-time and tracking cell behavior while maintaining the printed construct over a long time. 3D bioprinted cancer models serve as a better *in vitro* model for rapid drug testing and optimizing dosages that can later be taken forward as an appropriate platform for preclinical

and clinical trials for high throughput and rapid drug testing. 3D bioprinted cancer-on-chips can give an edge to commercially manufactured microfluidic chips by providing the feature of personalization within a biomimetic 3D TME system for an accurate diagnosis and further study of cancer and discovering new anti-cancer drug targets and development of novel therapeutics.

4.2.1. 3DBP models mimicking 3D tumor stroma

3D bioprinting technology has been used to fabricate *in vitro* cancer models with relevant tumor physiology, which refers to the complex tumor microarchitecture [103]. Most solid cancers show a unique tumor microarchitecture with a hypoxic core of tumor cells and cancer stem cells, surrounded by a ring of normoxic tumor cells and neoplastic cells at the periphery. The stromal cell arrangement around the cancerous lesion affects the cancer progression and invasion. It is vital to consider while developing therapeutic options targeting stromal components such as the delivery of anti-cancer drugs within the whole of the tumor, or how the stiffness of the tumor might be a hindrance during chemotherapy or stromal cells might even be increasing the chemoresistance of cancer cells within the tumor. With the advantage of multiple bioinks and various bioprinting modalities, researchers have fabricated different tissue-specific 3D cancer models with the initial aim of developing a 3D model and then characterizing the cancer cell behavior in 3D [90, 93, 94]. Here, we will discuss some *in vitro* 3D bioprinted models and a few examples of 3D bioprinted microfluidic cancer-on-chips that have recapitulated some of the native tumor physiological features. One of the first studies in the 3D bioprinted cancer modeling field was done by Zhao *et al* [80] using extrusion-based bioprinting of cervical cancer cells. The group fabricated a 3D bioprinted multilayered grid construct of HeLa cells encapsulated within a gelatin/alginate/fibrinogen (GAF) hydrogel. This study was the first to show the difference in cellular behavior in 2D versus the 3D bioprinted system; however, it only involved a monoculture of cervical cancer cells. This 3D bioprinted *in vitro* cervical tumor model showed increased cellular proliferation and spheroid formation, invasion marker expression, and higher drug resistance in cells within the 3D grid construct than cells grown in 2D monolayer culture [80]. GBM is aggressive cancer that affects the central nervous system. Patients often relapse after complete cycles of chemoradiation therapy and express drug resistance during the chemotherapeutic regimen. To study the association of immune cells as a stromal component in TME, Heinrich *et al* [85] fabricated a 3D bioprinted GBM cancer model. This 3D bioprinted model replicated part of GBM TME by developing brain models with GBM cells and macrophages separately and printing a model with a coculture of GBM-associated macrophages and GBM cells using GelMA based bioink. The group was able to mimic various paracrine, juxtracrine, and autocrine signaling pathways activated between cancer and stromal immune cells and monitored them in real-time. The genomic results obtained from these 'mini-brains' were compared with clinical data obtained from 150 GBM patients showcasing the potential of adapting such 3D printed models in clinical research [104].

Similarly, in past years, brain tumor physiology has been replicated on various 3D-printed GBM chips. A recent example of 3D bioprinted GBM-on-chip that recapitulates the cancer-stroma mimicking tumor microarchitecture was developed by Yi *et al* (2018). The group fabricated a silicon-based chip. Two bioinks were bioprinted in concentric circles; an inner core made up of porcine decellularized brain matrix encapsulating patient-specific cancer cells and an outer region of endothelial cells suspended within GelMA hydrogel. This layout was physiologically similar to hypoxic tumors observed *in vivo* and, on maturation, showed the development of an oxygen gradient [93]. The effect of oxygen gradient showcases the importance of designing cancer models to attain a physiologically realistic TME. This study showed static culturing of GBM cells on the chip; however, tumors are exposed to dynamic blood flow via leaky vessels *in vivo*. Therefore, the introduction of dynamic culture conditions in cancer models can be considered an essential modification within these cancer-on-chips so that they can perform as mini-bioreactors.

Breast cancer and its various subtypes have been widely studied using hydrogel scaffolds that mimic TME features like matrix stiffness and ECM composition. 3D breast cancer models have helped researchers understand this highly invasive cancer, particularly the metastatic progression to bone, cancer stem cell behavior, and therapeutics [105]. 3D bioprinting technology has bought breast cancer research a step forward by allowing rapid biofabrication of the breast TME to serve as an *in vitro* platform. One of the earliest studies by Zhou *et al* [99] showed a coculture bioprinted breast cancer model where osteoblasts or mesenchymal cells were encapsulated within GelMA hydrogel. On top of this, breast cancer cells were seeded to mimic breast-bone metastatic conditions [99]. Another study used laser-assisted bioprinting to fabricate a 3D bioprinted breast cancer construct laden with one cell type (figures 6(2A–C)). Kingsley *et al* [92] showcased the development of monoculture tumor spheroids by culturing LAB-printed microbeads arranged in a rectangular mat [92]. An example of a heterocellular bioprinted tumor model would be the breast cancer model recently fabricated by Langer *et al* (2020). They used extrusion bioprinting to print patient-derived breast cancer cells into a stromal microenvironment with mammary fibroblasts and endothelial cells. The

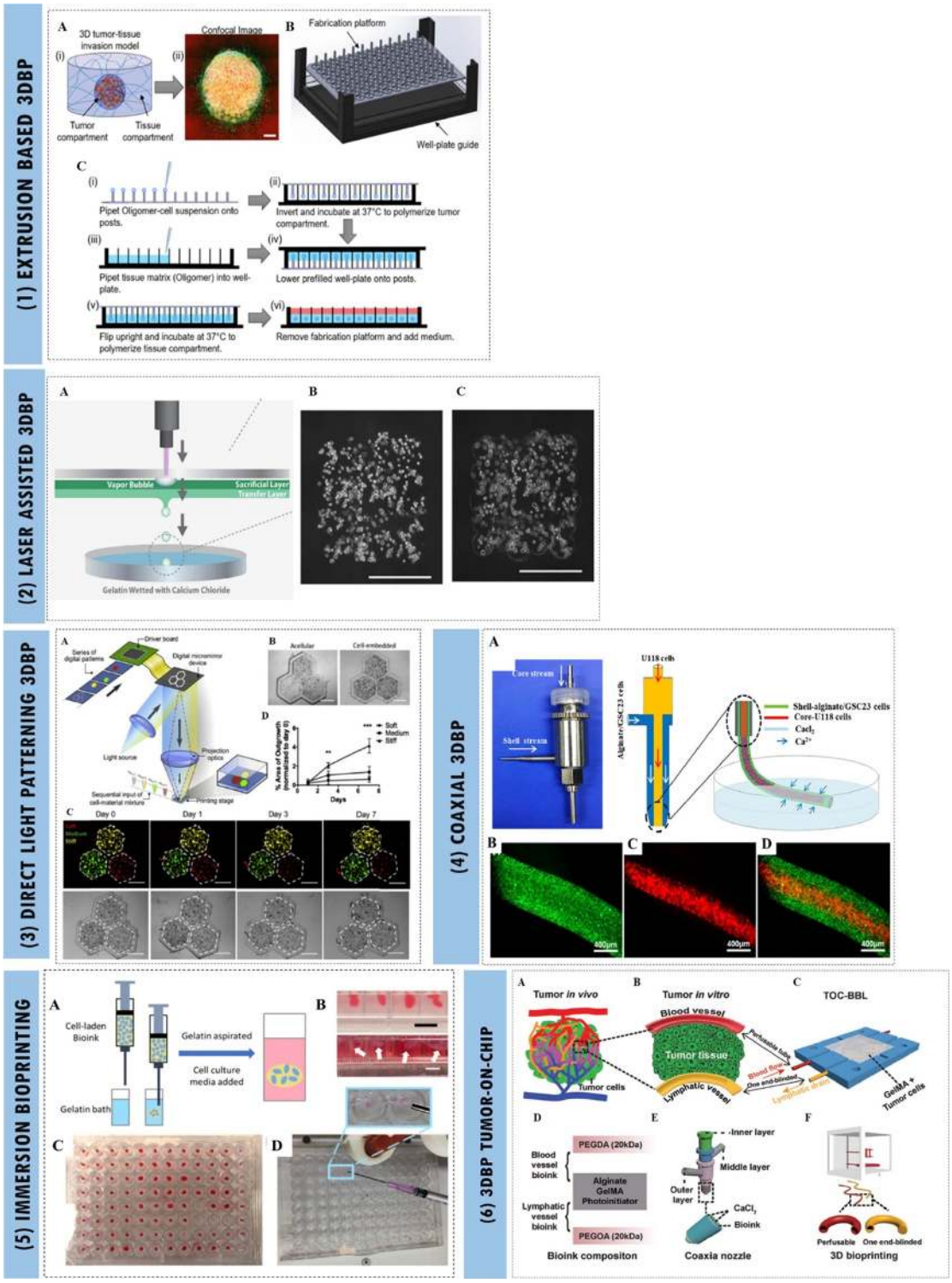

Figure 6. Various examples of 3D bioprinted cancer models fabricated using different bioprinting modalities and techniques like (1) Extrusion Based Bioprinting. (A)–(C) Fabrication and validation of a novel high throughput 3D bioprinted pancreatic cancer invasion model by Puls *et al* [90]. (2) Laser-Assisted Bioprinting (LAB). (A)–(C) Kingsley *et al* [92] presented a printing approach using breast cancer cell-laden microbeads and laser direct writing to fabricate the 3D cancer model by controlling the spatial spread and size of tumor spheroids in the form of microbeads [92]. (3) Direct Light Patterning based Bioprinting. (A)–(D) Ma *et al* [91] fabricated a cirrhotic liver model using multiple biomaterials to create different stiffness zones [91]. (4) Coaxial 3DBP. (A)–(D) Wang *et al* [100] presented a coaxial bioprinting approach to fabricated glioma models using 3D bioprinting [100]. (5) Immersion-based Bioprinting. Maloney *et al* (2020) fabricated a 3D bioprinted liver cancer model (A) using patient tumor-derived cells with the help of a support gelatin bath, leading to organoid development as shown in (B) within 96 well plates, scale bar = 5 mm. Figure (C) and (D) shows crosslinked bioink (red color) immersed in a gelatin bath [101]. (6) Hybrid System with bioprinting and microfluidic technology. (A)–(F) Fabrication of biomimetic *in vitro* model of tumor-on-chip with bioprinted blood and lymphatic vessel pair (TOC-BBL) by Cao *et al* [102]. Reproduced with permission from Puls *et al* [90]; Kingsley *et al* [92]; Ma *et al* [91]; Wang *et al* [100]; Maloney *et al* [101]; Cao *et al* [102].

group could also bioprint pancreatic cancer models using patient tumor-derived cells from PDX models [94]. This study incorporated multiple stromal cells and defined microarchitecture and can be further developed as a cancer model used as a drug screening platform to analyze tumor-stromal targeting drugs.

Pancreatic cancer is a highly invasive malignant tumor growth within the pancreatic duct with complex tumor anatomy. The patients usually have a low survival rate because of late detection of the disease, and drug resistance to chemotherapeutics makes treatment difficult. Many studies have been done using PDAC 3D organoid modeling; however, recapitulating the TME using 3D bioprinting has not been widely explored. Recently, a 3D bioprinted invasion model fabricated by Puls *et al* [90] recapitulated the TME by using Oligomer hydrogel, a type 1 collagen-based biomaterial with biomimetic matrix stiffness within which pancreatic cancer cells were encapsulated along with CAFs forming a tumor compartment surrounded by stromal compartment (figures 6(1A–C)). This printed construct had a similar layout observed within a pancreatic tumor microarchitecture. This heterogenous bioprinted model helped study EMT and invasive phenotype of patient-derived pancreatic cancer cells in the presence of CAFs and served as a drug screening tool [90]. This study via co-culture of cancer cells and CAFs was only one step towards replicating tumor microarchitecture and could have been advanced by incorporating more stromal cell types.

4.2.2. 3DBP models mimicking tumor vasculature

An important hallmark of cancer is the ability of cancer cells to secrete angiogenic factors and promote the formation of a vascular network via angiogenesis to provide the cancer cells within a tumor, with essential nutrients and oxygen, and help cancer cells to metastasize to secondary tumor sites. Therefore, cancer organoids carrying only endothelial cells in coculture conditions have been widely used as tumor models for research, as discussed in previous sections. However, researchers gradually recognized the need to fabricate a heterogenous cancer model that could support multiple cell populations apart from endothelial cells to mimic *in vivo* TME.

Several 3D cancer spheroids and organoid models fabricated in the last decade have been able to show the process of angiogenesis being initiated at a genetic level; however, only a few studies using cancer-on-chips and well-designed 3D bioprinted cancer models have been able to show formation or development of vascular networks within the tumor construct as a separate entity within the model. As an example, the 3D bioprinted glioma model fabricated by Wang *et al* [100] using coaxial bioprinting (figures 6 (4A–D)) showed increased expression of genes- MMP2, MMP9, VEGFR2 within the bioprinted microfibre construct carrying GSC2 glioma stem cells that were cocultured with U118 glioma cells. These genes are associated with increased malignancy of tumors, invasion, angiogenesis, and metastasis. The group hypothesized that these angiogenic factors were being secreted by GSC2 glioma stem cells only in the presence of U118 glioma cells within the 3D hydrogel scaffold [100]. An extension of this study in the future could have been a more extensive study of the 3D bioprinted microfibres with a prolonged expression of EMT genes and visualization of the formation of capillaries or vascular networks in the long-term culture of the printed construct.

Concerning 3D bioprinted models that could exhibit vascular networks, a study by Kolesky *et al* [106] presented a method to fabricate vascularized constructs with multiple cell types. The group succeeded in fabricating a 3D bioprinted hepatocellular carcinoma model complete with vascular networks. The group used multiple nozzles to print multiple bioinks- HepG2 hepatocellular carcinoma cells and human neonatal dermal fibroblasts encapsulated within GelMA hydrogel, and endothelial cells are laden within the sacrificial or fugitive bioink called Pluronic F127, which led to the formation of a bioprinted heterogenous model complete with vascular channels lined with endothelial cells [106]. This study paved the way for developing stable vascular networks within a tissue construct using multiple biomaterials with different crosslinking properties.

A well-characterized study of a vascularized 3D tumor model was shown via the 3D bioprinted breast tumor tissue fabricated by Langer *et al* [94], who were able to show the formation of a vascular network within their bioprinted tumor tissue. It was possible because the stromal compartment laden with endothelial cells (CD31+ HUVECs) and human mammary fibroblasts (Vimentin positive) allowed for the development of endothelial networks. The group used a recently developed CLARITY technique combined with light-sheet microscopy to visualize continuous networks, if any present, within the bioprinted constructs. Intact capillary networks with multiple branch points were transversing throughout the bioprinted tumor tissue [94]. This study provided visual proof that endothelial cells directly interact with cancer cells within the 3D bioprinted tumor tissue and are capable of spatial organization and forming continuous vascular networks in the presence of other stromal cells and appropriate growth factors.

The importance of stromal cells in vascular network development within a 3D bioprinted cancer construct was demonstrated by Han *et al* [107]. The group came forward with a new approach for effectively obtaining a well-vascularized cancer model within 14 d of culture. They combined MCTS-forming technique with 3D bioprinting technology to fabricate a 3D bioprinted vascular tissue construct of endothelial cells and lung fibroblasts on which they seeded U87 glioma cell line-derived spheroids.

The study showed the development of vascularized MCTS in the presence of lung fibroblasts through this approach. Consequently, neoangiogenesis within the seeded MCTSs leads to increased tumor spheroid size, with the cancer cells exhibiting an EMT-like phenotype and more invasive morphology [107]. Although the cell types differed according to tissue origin, the vascularization strategy was practical for developing pre-vascularized constructs.

A significant limitation in mimicking the tumor vascular network within the 3D cancer models is that only tri-culture of tumor cells, fibroblasts, and endothelial cells within a cancer spheroid does not lead to perfusable blood vessel formation. Another factor to consider during the fabrication of tumor vasculature is that the blood vessels within the tumor are physiologically different as their development is aberrant compared to normal blood vessels. In literature, often referred to as leaky vessels, they face more compressive force due to surrounding, rapidly dividing cancer cells, causing a slower blood flow through them. These unique characteristics have made mimicking well-formed tumor vessels using 3D bioprinting a difficult feat. However, several 3D bioprinted models have made it easier to study tumor cell behavior and angiogenesis in the form of capillary networks with the help of normal human endothelial cells.

Similarly, most cancer-on-chip studies show EMT phenotype gained by cancer cells and rarely develop perfusable vascular networks within the cancer model with the native intraluminal flow. However, a study by Cao *et al* (2019) used bioprinting technology to develop a tumor-on-chip within which a pair of bioprinted vascular networks and lymph nodes were fabricated (figures 6(A)–(F)). The Tumor-On-Chip with 3D bioprinted blood and lymphatic vessel pair (TOC-BBL) was fabricated using multiple nozzles, coaxial method of bioprinting, with the flow rate of bioink and crosslinking being regulated. Their bioprinting strategy led to the formation of perfusable vessels encapsulated within the hydrogel slab laden with MCF-7 cells, each with different permeability rates [102]. Meng *et al* [108] successfully developed a cancer-on-chip setup with a pre-endothelialized microchannel around tumor droplet placed in the fibroblast zone, and 3D printed growth factor releasing capsules around the tumor-vessel zone. This proof-of-concept study created a metastatic model with a perfusable blood vessel and dynamic culture of cancer cells by controllable release of EGF and VEGF containing 3D printed capsules. This study used 3D printing application and cancer-on-chip technology to recreate the invasion and angiogenesis in 3D and can be applied for further drug release studies and metabolism studies [108].

Numerous studies in tissue engineering and regenerative medicine report mimicking vascular networks using 3D scaffolds and 3D bioprinting technology. From these existing studies, several strategies can be adapted to use next-generation 3D bioprinting technology for the fabrication of vascular tubes within 3D cancer models, such as molding techniques shown previously by Vollert *et al* [109] that can be in future manipulated to provide an essential and intricate feature of tumor vasculature to study angiogenesis [109]. Another molding technique shown by Miller *et al* [110] can also be used to 3D print dissolvable vascular channels, which can be utilized to create a large-scale perfusable vascular network, and cancerous tissue can be 3D bioprinted around that mimicking blood flow within normal or tumor tissues [110]. Advances in 3D printing technology, when coupled with other strategies of inducing vasculogenesis or angiogenesis [111], can create much more delicate blood capillaries in the range of 100 −10 μm that can replicate leaky vessels found around tumor tissue too. Vascularization is a crucial component of the tumor niche; therefore, more studies are needed to recapitulate this feature in future 3D bioprinted cancer models and within cancer-on-chips.

4.2.3. 3DBP models mimicking tumor EMT and invasion

Tumor progression is usually followed by the EMT of cancer cells within a tumor and consequently metastasis. 3D bioprinted cancer models can be used as invasion models and serve as an excellent tool to study cancer progression and visualize EMT and metastasis in real-time. One of the first 3D bioprinted models, a cervical cancer model fabricated by Zhao *et al* [80], showed the comparative differences between the expression of the invasion markers MMP2 and MMP9 in 3D printed constructs compared to 2D *in vitro* culture of HeLa cells [80]. Zhu *et al* [112] showed the metastatic potential of breast cancer cells to bone tissue. The group 3D bioprinted a hydroxyapatite nanoparticle suspended in PEG/PEG-DA (printable resins) to form a nanocomposite matrix laden with bone marrow stem cells, followed by MDA-MB-231 and MCF-7 breast cancer cells seeded on top of this composite material to study breast cancer-bone metastasis. The cells favored spheroid formation within the construct and exhibited proliferation and metastatic progression of cancer cells. They also found that the cancer cells showed chemoresistance to fluorouracil in 3D [112]. This type of bioprinted model can help predict the invasive and metastatic behavior of cancer cells and closely observe the remodeling of the secondary site of the tumor to form a suitable niche for metastasized cells. Similarly, apart from the three-dimensional tumor microarchitecture, other tumor microenvironmental cues play an important role in promoting the invasion and migration of cancer cells. Ma *et al* [91] used direct light patterning-based 3D bioprinting to fabricate a liver cirrhosis model to mimic hexagonal

lobules with surrounding inter-lobule fibrous septa (figures 6(3A–D)). The group used HepG2 hepatocellular carcinoma cell-laden dECM-based bioink with tunable matrix stiffness using GelMA hydrogel to construct a 3D bioprinted liver model. They patterned the liver cancer tissue platform with varied scaffold stiffness and provided the cancer cells with a cirrhotic-like mechanical environment. Consequently, the HepG2 cells showed reduced liver-specific gene expression and higher invasive and migration potential toward the stiffer scaffolds on genetic and phenotypic levels [91]. This study used matrix stiffness, a biophysical tumor microenvironmental cue of liver tissue that plays a vital role in tumor progression and invasion.

GBM chemoradiotherapy often fails due to chemoresistant cancer stem cells, and the tumor relapses even after surgical intervention. Several biofabricated glioma cancer models have been used to understand these processes better in a 3D microenvironment. The 3D bioprinted glioma model fabricated by Heinrich *et al* [85] also showed that glioma cells within these bioprinted mini-brains exhibited EMT features with increased vimentin and nestin a gene expression loss of expression of E-cadherin, indicating that the cancer cells were migrating and invading. The TME conditions created by the coculture of glioma cells and macrophages provided appropriate growth factors and signaling pathways, making the data generated by this model potentially be applied in preclinical settings [104]. The novel 3D bioprinted GBM-on-chip developed by Yi *et al* [93] was able to recapitulate a vascular TME, and the group was able to show cancer migration and invasion. They bioprinted endothelial cells encapsulated within GelMA hydrogel on the periphery of a core of patient tumor-derived GBM cells encapsulated within brain dECM. It was observed that cancer cells were invading from the peripheral region of the tumor core toward the outer region with higher oxygen levels and the region with endothelial cells. The zonation with different cells provided a primitive attempt to develop a vascularized *in vitro* 3D cancer-on-chip model. The group could easily visualize cancer progression and the EMT-like phenotype of patient tumor cells [93].

A unique 3D bioprinted pancreatic cancer invasion model was fabricated by Puls *et al* [90] using patient-derived pancreatic tumor cells and CAFs together in a collagen-based biomaterial with realistic matrix stiffness forming a tumor compartment surrounded by surrounding stromal compartment. Post-bioprinting within the printed construct, the group observed enhanced pancreatic cancer cell invasion in the presence of CAFs, and the cells showed matrix remodeling that leads to invasion and migration. This study explored the EMT phenomenon occurring in different cellular phenotypes- in established pancreatic cancer cell lines and patient-derived cancer cells within a 3D bioprinted cancer model [90]. Langer *et al* (2019) were also able to apply their 3D bioprinting protocol to fabricate a heterogenous 3D pancreatic cancer model. The cancer compartment within the 3D bioprinted tumor tissue was made up of cells from a primary cell line derived from patient tumor specimen 'OPTR3099'. In contrast, the stromal compartment consisted of pancreatic stellate cells (PSCs) and HUVECs. The KRT8/18 positive pancreatic cancer cells migrated and invaded the stromal compartment, where these cancer cells and CD31 positive endothelial cells closely interacted with each other. The group also observed that vimentin-positive PSC cells had migrated to the tumor core, and these fibroblast-like cells are known to promote dense desmoplastic in pancreatic tumors. Similarly, the group was also able to visualize proliferating Ki67 positive cancer cells restricted to the core of tumor tissue and present in the surrounding stromal compartment because of the invasive phenotype of pancreatic cancer cells [94].

Migration and invasion within 3D bioprinted cancer models are better characterized by genetic profiling and visualization of such processes using CLARITY or real-time cell tracking of multiple cell types using confocal microscopy. For 3D bioprinted cancer models to serve as invasion models, it is essential to remember that invasion, migration, and EMT are processes that involve multiple cell types, different ECM proteins at each stage of tumorigenesis as well as signaling molecules or growth factors secreted by a tumor or surrounding stromal cells. Therefore, to replicate these conditions, different biofabrication techniques should be used to 3D bioprint multiple heterogeneous cell-laden bioinks to model tumor invasion model, which can then be used for drug screening and drug response studies mainly to target early to mid-stage cancers.

4.2.4. 3DBP models showcasing drug-tumor interactions

The importance of three-dimension cannot be denied while performing anti-cancer drug studies. Cancer cells are also known to react differently to chemotherapeutic drugs in 3D models and show more chemoresistance than cells in 2D culture. The false-positive behavior of cancer cells in 2D cultures toward anti-cancer drugs makes 3DBP cancer models much more significant preclinical models for drug screening and the development of personalized medicines [104]. 3DBP cancer models could be an alternative to false-positive *in vitro* drug assays and animal models, which are histologically and physiologically different from humans in a research laboratory. In keeping with varied cancer cell responses to chemotherapeutic drugs in 3D as compared to monolayer culture, a bioprinted cervical cancer model fabricated by Zhou *et al* (2014) showed that HeLa cells within the printed construct developed chemoresistance to the drug paclitaxel and showed distinct expression markers as

compared to the cancer cells grown 2D *in vitro* culture conditions [80]. The study was not a long-duration study and was only a first step toward the difference in drug response across different culture conditions. Similarly, the 3D pancreatic cancer invasion model by Puls *et al* [90] demonstrated how their model could be used as a high throughput, high content drug screening platform. The group was able to study the effect of the anti-cancer drug Gemcitabine on both the pancreatic cancer cells and surrounding CAFs within a 3D scaffold model. This study showcased an innovative approach to the rapid fabrication of cancer models for drug screening [90].

Chemoresistance has been well noted within GBM patients making their treatment ineffective. A precedent was set by Dai *et al* [113], who used 3D-printed cancer models to study drug cytotoxicity and chemoresistance. The group fabricated a 3D-printed glioma stem cell model using a porous GAF hydrogel matrix upon which they seeded SU3 glioma stem cells and U87 glioma cells. They could distinctly see that the cancer cells in the 3D model exhibited chemoresistance to temozolomide compared to cells grown in monolayer culture conditions [113]. Therefore, three-dimensional microenvironmental cues are essential to studying physiologically relevant anti-cancer drug activity. One of the first studies that validated this observation on a 3D bioprinted glioma model was shown by Wang *et al* [100]. The group used the coaxial bioprinting approach to print sodium-alginate hydrogel-based microfibers with core–shell zones carrying GSC23 human glioma stem cells and U118 human glioma cells. This fabrication approach mimicked the glioma microenvironment, and U118 cells isolated from within the core of microfibres after 15 d of culture were incubated in chemotherapeutic drug temozolomide for 48 h. The glioma cells showed dose-dependent decreased cell viability and higher methylation status of MGMT promoter correlating to high drug resistance within the cells compared to previous studies of the same cells cultured *in vitro* [100]. As another example, Heinrich *et al* [85] screened various types of drugs like carmustine (BCNU), standard chemotherapy for GBM, as well as immunomodulatory drugs AS1517499 and BLZ945. Dose-dependent inhibition was seen in the 3D bioprinted model and 2D monolayer culture when treated with BCNU. Still, cancer cells in 3D mini-brains with tumor and macrophage coculture showed more chemoresistance than those glioma cells cultured in 2D culture conditions. Furthermore, each drug showed a different set of genes being upregulated post-treatment, indicating the efficacy and need for targeted therapy when targeting complex cancer like GBM [104]. Chemoresistance is a well-known phenomenon, and Langer *et al* [94] showcased the same in their 3D bioprinted model fabricated using patient tumor tissue and PDX tumor tissue, complete with all stromal cells and blood vessels. The viability of proliferative cancer cells did go down with treatment of Gemcitabine but only with increased dosage or concentration, indicating the importance of drug screening 3D platforms with all the tumor microenvironmental cues for more relevant results [94].

The importance of vascularization for conducting relevant drug studies has been showcased on recently fabricated cancer-on-chips. Since the GBM-on-chip developed by Yi *et al* [93] perfectly mimicked tumor microenvironmental conditions seen in GBM, including the endothelial cell, the group was able to create 3D bioprinted patient-specific chips that could predict drug response as well as any arising drug resistance to chemoradiation as well to the drugs temozolomide, cisplatin, KU60019, and O^6-benzylguanine. The data generated for patient samples were correlated clinically. The chip served to identify the best possible chemotherapeutic options for patients and proved to be a massive step toward personalized medicine [93].

Some of the 3D bioprinted cancer models have been summarized in table 2, which showcases the various bioprinting techniques that have been used to mimic TME features.

5. Future perspectives

In the past decade, tremendous research has been done on developing and validating different 3D cancer models ranging from monoculture spheroids to complex perfusable cancer-on-chip and bioprinted heterogeneous tumor models. The current advances in 3D bioprinting technology allow for the development of 3D cancer models with biomimetic tumor microarchitecture specific to different cancer types and tissue-specific TME that show physiologically similar behavior to *in vivo* tumors. These bioprinted cancer models have also been validated using different chemotherapeutic treatment options, and several studies have also presented advanced bioprinted cancer models using patient tumor cells, which opens up an avenue for personalized medicine research. Figure 7 depicts the workflow of developing personalized 3D bioprinted cancer models and their applications in a clinical setting.

However, this platform is still in its initial phase and needs to be further developed and adapted as a user-friendly tool commonly used across laboratories. Box 2 enumerates various limitations and challenges concerning cancer modeling using 3D bioprinting processes. The importance of TME is reflected in the fact that changes in the microenvironment at the site of the neoplastic lesion, apart from the genetic changes within the neoplastic cells, promote tumorigenesis, tumor invasion, and metastasis. Future 3DBP cancer models need to be tumor stage-specific based on this principle. The associated TME modeling should be done with the help of appropriate biomaterials and associated cell populations

Table 2. Summary of 3D bioprinted cancer models developed in recent years.

3DBP Cancer models	Bioprinting modality	Biomaterial	Cells	Biomimetic Tme features	Key results	Reference
CERVICAL CANCER	Extrusion based bioprinting	• Gelatin • Alginate • Fibrinogen	HeLa cervical cancer cells	✓ Biomimetic tumor architecture ✓ Drug-Tumor Interaction	• 3D cellular spheroids by the eighth day in 3DBP constructs. • Higher expression of invasion proteins like MMP2 and MMP9 in 3D. • Chemoresistance to paclitaxel in 3D.	[80]
GLIOBLASTOMA	Extrusion based bioprinting	• Gelatin • Alginate • Fibrinogen	• SU3 glioma stem cells • U87 glioma cells	✓ Biomimetic tumor architecture ✓ Drug-Tumor Interaction	• Higher CSC marker Nestin and VEGF expression in SU3 cells and differentiation potential of the cells (β-tubulin III) in 3DBP constructs. • Chemoresistance to temozolomide in 3DBP constructs.	[113]
	Extrusion based bioprinting	• Brain decellularized extracellular matrix • GelMA	• U87 glioma cells • Patient-derived GBM cells • Human primary umbilical vein endothelial cells (HUVECs)	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• Silicon-based chip with tumor zonation. • 3DBP constructs with hypoxic conditions as observed *in vivo* • Development of an oxygen gradient. • Static culture conditions.	[93]
	Coaxial extrusion-based bioprinting	Sodium alginate	• GSC2 glioma stem cells • U118 glioma cells	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• Bioprinted microfiber constructs showed increased expression of EMT and metastasis markers • Pre-angiogenesis microenvironment shown	[100]

(Continued.)

Table 2. (Continued.)

3DBP Cancer models	Bioprinting modality	Biomaterial	Cells	Biomimetic Tme features	Key results	Reference
	Extrusion based bioprinting	GelMA	• RAW264.7 mouse macrophages • GL261 mouse glioblastoma cells	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• 3DBP model showcasing the GAM-GBM interaction • Tumor progression and invasion, macrophage recruitment, and polarization shown • Rapid drug screening using 3DBP constructs	[104]
	Extrusion based bioprinting	• Gelatin • Alginate • Fibrinogen	• U87 glioma cells	✓ Biomimetic tumor architecture ✓ Drug-Tumor Interaction ✓ Features of Tumor angiogenesis	• 3D bioprinted vascular tissue construct • Top seeding of U87 glioma cell line-derived spheroids. • Static culture conditions	[107]
BREAST CANCER	Stereolithography 3D bioprinter	• GelMA • Nano-hydroxyapatite particles	• MSCs • MDA-MB-231	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion	• Metastatic progression observed in a co-culture model	[99]
	Multi-nozzle extrusion-based bioprinting	• Alginate • Gelatin	• MDA-MB-231 • IMR-90 fibroblast cells (CAFs)	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion	• Interaction of fibroblasts and breast cancer cells • Formation of MCTS seen over the long-term culture period • Long term Static culture up to 30 d	[114]
	Extrusion based bioprinting	• Methacrylated hyaluronic acid • Methacrylated gelatin	• 21PT primary tumor breast cancer cells • ADMSC primary human cells	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• Interaction and migration of ADMSCs and cancer cells shown • Increasing chemoresistance to doxorubicin in co-culture conditions.	[115]

(Continued.)

Table 2. (Continued.)

3DBP Cancer models	Bioprinting modality	Biomaterial	Cells	Biomimetic Tme features	Key results	Reference
	Extrusion based bioprinting	• Sodium alginate • Gelatin	MDA-MB-231, OPTR3099 primary pancreatic tumor cell line, MSCs, Human pancreatic stellate cells (PSCs), Human subcutaneous preadipocytes (SPA), Human Mammary Fibroblasts, HUVECs	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction ✓ Features of Tumor angiogenesis	• Scaffold-free model to mimic tumor-stromal interactions. • Recapitulation of *in vivo* tumor microenvironment and vasculature. • Development of personalized cancer models.	[94]
	Extrusion based bioprinting	• Matrigel • Gelatin-alginate • Collagen-alginate	• MCF10A • MCF-7 • MDA-MB-231 • MCF10A-NeuN	✓ Biomimetic tumor architecture ✓ Drug-Tumor Interaction	• Co-culture of cancer cells and endothelial cells. • Chemoresistance to paclitaxel in 3D shown	[116]
	Extrusion based bioprinting using coaxial nozzles	• GelMA • Alginate • PEGDA	• MCF-7 • HUVECs	✓ Biomimetic tumor architecture ✓ Drug-Tumor Interaction ✓ Features of Tumor angiogenesis	• Bioprinted cancer-on-chip with blood and lymphatic mimetic structures. • Drug studies in microcirculation conditions are shown.	[102]
	Laser direct-write (LDW) bioprinting	• Alginate • Gelatin	• MDA-MB-231 • CCE Mouse Embryonic Stem Cells (mESCs)	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion	• Bioprinted 3D cellular aggregates formed spheroids and embryoids within controlled dimensions	[92]

(Continued.)

Table 2. (Continued.)

3DBP Cancer models	Bioprinting modality	Biomaterial	Cells	Biomimetic Tme features	Key results	Reference
HEPATOCELLULAR CARCINOMA	Direct light patterning-based 3D bioprinting	• GelMA • Liver Decellularized Extracellular Matrix	HepG2	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion	• Biomimetic cirrhotic-like mechanical environment • Influence of biophysical tumor microenvironment and stiffness shown. s	[91]
	Extrusion based bioprinting	• Collagen Type 1 • Matrigel • Hydroxypropyl Chitin (HPCH)	• Human Peripheral Blood Mononuclear Cells (PBMC) • PUMC-HUVEC-T1 • Human hepatocellular carcinoma SMMC-7721 cell line	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• *In vitro* hepatoma cancer-on-chip model • Two co-culture models were fabricated- 3D tumor cell clusters with HUVECs & with PBMCs.	[117]
PANCREATIC CANCER	Extrusion based bioprinting	Collagen	• Pancreatic adenocarcinoma cell lines PANC-1, BxPC-3 • Patient-derived PANC 10.05 tumor cells • Cancer-Associated Fibroblasts (CAFs)	✓ Biomimetic tumor architecture ✓ Showcased Tumor EMT & Invasion ✓ Drug-Tumor Interaction	• Distinct 3D tumor and stromal compartment. • CAFs and cancer cells interactions were studied within matrices with different stiffness. • A platform for multiplex assay for HT-HC drug screening	[90]

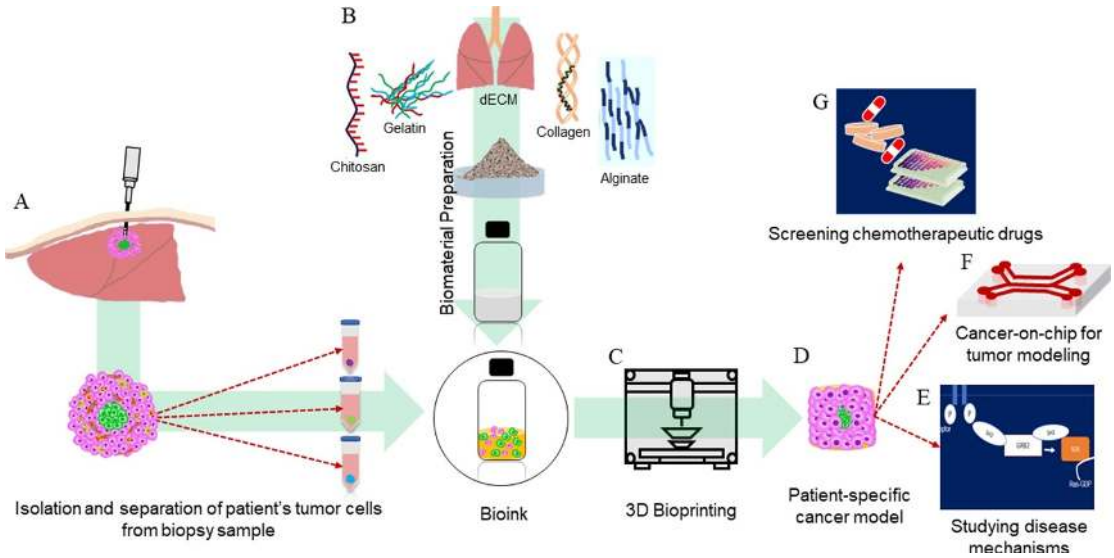

Figure 7. Development of biomimetic patient-specific 3D cancer models using 3D bioprinting. (A) The cellular source of patient-specific cancer models can be any established primary cell lines or using isolated cells from patient tumor biopsy sample. (B) Patient cells are suspended within natural or synthetic based biomaterials based on the choice of bioprinting technique. (C) Models are printed using best suitable bioprinting modality to ensure optimum cellular viability within the patient-specific cancer model. (D) Key applications of patient-specific 3D bioprinted cancer models range from (E), (F) serving as a disease model to study the disease pathology to finding their application in the development of personalized anti-cancer therapeutics and being developed as a diagnostic tool in the form of a cancer-on-chip to be used in a clinical set-up.

Box 2. Challenges to existing 3D bioprinting technology for 3D cancer modeling.

Optimization of biomaterial for bioink and best-suited bioprinting modality (i.e. 3D bioprinter) and standardization of printing protocols for modeling a specific type of cancer remains to be addressed.

Developing a perfusable, vascularized 3D bioprinted construct is still a challenge and requires more experimentation to prove it viable for long-term drug studies.

3D bioprinted constructs require more time to mature and show similar appropriate cellular and tissue functionality.

Handling and maintaining fine structures without developing associated hypoxia or necrosis within the 3D bioprinted tissue construct remains a challenge.

Downstream analysis for 3D bioprinted constructs requires time, specialized types of equipment, and technical skills.

Imaging thick 3D bioprinted constructs and experiments involving the isolation of cellular and genetic components from a multicellular 3D construct are exhaustive processes in clinical settings.

Acceptance of 3D bioprinted construct for pharmaceutical industrial applications and commercial manufacturing is a significant concern.

Ethical and regulatory issues concerning patient-specific 3D bioprinted models will need a monitoring committee.

within the tumor niche at that tumorigenic stage. A bioink that can completely replicate the tissue matrix is yet to be developed. The closest researchers have been developing dECM-based bioink, which loses critical structural proteins during its synthesis and preparation. Therefore, this is one of the goals for future works to develop a single or a combination of bioinks that can replicate the native tissue composition and microarchitecture. Design consideration like this would yield a better 3D *in vitro* cancer model, giving researchers a clearer idea of molecular signaling and phenotypic changes across the spatiotemporal dynamic tumor and enabling 3DBP cancer models to be used as an applicative tool for modeling tumor progression and therapeutic studies. The main aim of future developments should also be to reproduce tumor heterogeneity, tumor microarchitecture, and tumor vascularization. There is an evident lack of perfusable vascular networks or vasculature in existing 3D bioprinted cancer models. It can be achieved if researchers combine techniques like *in vitro* angiogenesis and vasculogenesis with current organoid technologies and 3D bioprinting technology for a perfusable 3D vascularized tumor model. Once these TME features have been replicated in a 3D model, analysis of these different features

as a singular entity or their interactions with each other would require a multitude of imaging and processing systems. With 3D bioprinted constructs, imaging requires closer attention, especially over an extended culture period with high cell densities. Different techniques need to be used to get an overall picture of cellular interactions and microarchitectures present within thick, cell-laden 3D constructs. Therefore, a complex and realistic 3D bioprinted cancer model requires multimodal characterizations and experts from different fields to get the most out of generated data.

A shift of focus is required to introduce more immune system components in 3D cancer models. Most 3DBP cancer models have not incorporated or accounted for direct or indirect interactions of cancer cells with the immune cell populations. A closer look at tumor immune microenvironmental components and their interaction can help researchers decode various implicated targets within a tumorigenic pathway that have not been studied thoroughly in 3D culture conditions. Interaction of tumor organoids and immune cells suspended in different bioinks and 3D bioprinted in a patterned manner upon a cancer-on-chip could help attain a biomimetic, highly functional TME offering a chance to test next-generation immunotherapy drugs and therapeutics in a preclinical setting. The journey from drug screening to drug validation to developing targeted anti-cancer therapeutics involves multiple drug experiments. A high-throughput and high-content 3DBP tumor model would increase the efficacy of experimentation on limited patient tumor samples. They offer a shot of being developed as a commercialized platform to analyze more drugs for personalized therapeutics. Therefore, they would indirectly bring down the cost of investigation of drug response studies and maintenance of animal models, ultimately decreasing the cost of therapeutics available to the general public. In addition, oncologists and clinicians can use patient-specific 3DBP cancer models to generate novel pharmacogenomic and pharmacokinetic data in collaboration with hospitals or health center sites for collecting clinical specimens.

Patient-specific drug response can be influenced by multiple factors ranging from genotypic variations in patient cells to chemotherapeutic drug-resistant cancer stem cells that can evade anti-cancer drugs, making the need for a high-end drug screening platform readily available to observe and study patient cell response. We hope that researchers can start looking into the avenue of personalized medicine or individualized treatment using 3D bioprinting technology as a tool to obtain personalized 3D tumor models for high throughput drug screening and ultimately yield successful clinical trial results. Technical challenges involving 3D bioprinting technology need to be addressed. Printing techniques need to be engineered to support multi-bioink dispersion for printing complex microarchitectures with multiple cell types to address spatial tumor heterogeneity. A standardized printing protocol also needs to be established to formulate bioinks so that various novel bioink combinations can be rapidly tested and printed to accelerate the process of 3D bioprinting of *in vitro* tumor models and 3D bioprinted microfluidic chips. Integration of 3D bioprinting and organoid technology can vastly enhance the applications of biofabricated 3D tumor models, complete with all the spatiotemporal design considerations, biomimetic mechanical cues, and multiple tissue-specific cell-laden constructs, including stem cells made up of various biomaterials. Most of the current 3DBP cancer models have been cultured in static conditions instead of dynamic culture conditions, and this practice needs to be addressed with the help of the introduction of microfluidic technology. Culturing 3DBP cancer models in a bioreactor under dynamic flow conditions by supplementing the constructs with growth factors and other bioactive molecules is necessary to attain cancer models that would ultimately imitate the *in vivo* TME and provide a more accurate drug response.

6. Conclusion

A well-established 3D cancer model can provide researchers with clinically relevant data covering cancer genomics and proteomics and generate effective therapeutic options to target cancer in its entirety [118]. Despite rigorous experimentation with currently available cancer models, the limitations associated with each of the models are reflected in false-positive results during *in vitro* studies leading to fewer potential anti-cancer drugs passing the *in vivo* assessment and even lower drug candidates entering the clinical trial pipeline; ultimately with the entire process is rendered unproductive due to low success rates in clinical trials. The FDA's low drug approval rates prove to be a bane for pharmaceutical companies as a huge cost is incurred from initial drug screening to drug studies. 3D bioprinted cancer models represent tumor microarchitecture and functionality to a vast degree and therefore serve as ideal 3D *in vitro* models compared to preexisting conventional cancer models, with a higher success rate in drug screening and validation. The advantages of current bioprinted cancer models are, however, shadowed by the limitations of optimization of the protocol for the development of a high throughout cancer model with the variables of biomaterial and different bioprinting techniques strategies best suited for particular cancer research and the scope of the model being used as a tool in translational research practically. The inclusion of a multidisciplinary approach toward developing and validating 3D bioprinted cancer models needs to be employed to attain a preclinical *in vitro* 3D cancer model, which

can be possible by incorporating 3D bioprinting technology coupled with tumor organoid models on a lab-on-chip. These preclinical *in vitro* cancer models will find their application relevant to testing new generation anti-cancer drugs and immunotherapy, leading to a revolution in precision medicine.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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ORCID iDs

Priyanshu Shukla https://orcid.org/0000-0001-5213-3794
Falguni Pati https://orcid.org/0000-0002-3588-1800

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