Received: 23 July 2023 | Accepted: 11 September 2023

DOI: 10.1002/cai2.102

CANCER INNOVATION

REVIEW

A comprehensive review of 3D cancer models for drug screening and translational research

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Funding information
MHRD, Grant/Award Number: MHRD IMPRINT (4291); SERB, Grant/Award Number: CRG/2020/005069; Indian Institute of Technology, Hyderabad, Grant/Award Number: IITH/BME/SOCH3; Ministry of Education, Grant/Award Number: MoE-STARS/2023/0640

Abstract

The 3D cancer models fill the discovery gap of 2D cancer models and play an important role in cancer research. In addition to cancer cells, a range of other factors include the stroma, density and composition of extracellular matrix, cancer-associated immune cells (e.g., cancer-associated fibroblasts cancer cell-stroma interactions and subsequent interactions, and a number of other factors (e.g., tumor vasculature and tumor-like microenvironment in vivo) has been widely ignored in the 2D concept of culture. Despite this knowledge, the continued use of monolayer cell culture methods has led to the failure of a series of clinical trials. This review discusses the immense importance of tumor microenvironment (TME) recapitulation in cancer research, prioritizing the individual roles of TME elements in cancer histopathology. The TME provided by the 3D model fulfills the requirements of in vivo spatiotemporal arrangement, components, and is helpful in analyzing various different aspects of drug sensitivity in preclinical and clinical trials, some of which are discussed here. Furthermore, it discusses models for the co-assembly of different TME elements in vitro and focuses on their synergistic function and responsiveness as tumors. Furthermore, this review broadly describes of a handful of recently developed 3D models whose main focus is limited to drug development and their screening and/or the impact of this approach in preclinical and translational research.

KEYWORDS
3D models, cancer, drug development, translational research, tumor microenvironment elements

Abbreviations: 5-FU, 5-fluorouracil; 5-YP, 5-year prevalence; ADSC, adult stem cell; AO, air organoid; BC, breast cancer; CAAs, cancer-associated adipocytes; CAFs, cancer-associated fibroblasts; CRT, chemoradiation; CSC, cancer stem cell; DRIE, deep reactive ion etching; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; FA, fatty acid; FASN, fatty acid synthase; FGFR, fibroblast growth factor receptor; HER, human epidermal growth factor; HGF, hepatocyte growth factor; iPSC, induced pluripotent stem cell; LCOs, lung cancer organoids; NSCLC, non-small lung cancer; PA, peptide amphiphiles; PA-ECM, peptide amphiphiles-extracellular matrix; PDAC, pancreatic adenocarcinoma; PDMS, polydimethylsiloxane; PDOs, patient derived organoids; SSC, slow-cycling cancer; TAMs, tumor-associated macrophages; TME, tumor microenvironment; TOs, tumor organoids; VEGF, vascular endothelial growth factor.

1 | INTRODUCTION

Cancer is primarily caused by genetic and epigenetic changes that enable abnormal cell growth and survival, often involving disruptions in key signaling pathways and broader network distortions within the tumor microenvironment (TME). It's not just one disease but a group of diseases that occur in the same cells. It faces abnormalities in all aspects of cell counting such as cell morphology, protein structure, gene expression, biomolecular function and overall biochemistry. These manipulations do affect the heterogenous tumor pool. Stromal cells influence tumor progression and also undergo morphological and biochemical changes internally. Tumor progression involves many factors, of which cancer cells are only one part. The tumor stroma consists of the extracellular matrix (ECM), which constitutes the mechano-physiological unit of advancing cancer cells. ECM density varies in different cancer types [1] and is an important parameter affecting tumor progression. Tissue heterogeneity, stromal disruption/manipulation, ECM variability and immune/immune cell modulation are major contributors to this micro environmental change. TME elements play a crucial role in tumor formation and are therefore expected to help gain greater knowledge in cancer research. Different cancer research based on hypothetical ideas and research directions have exploited these stromal elements [2, 3] and achieved very remarkable results. The above-mentioned TME components and other quantities such as growth and regulatory factors have been tested to indeed influence different aspects of cancer histopathology (from malignancy to cancer metastasis and its survival) [4–6]. Treating this disruption is difficult because reproducing the TME outside of biological systems is very difficult. Drug development based on the TME is therefore a major target of cancer research. Its primary research began with 2D cell culture plates. Currently, all stages of drug discovery [7] heavily rely on 2D models, which often end up with negligible results. The differences in studies can be well understood by looking at the differences between laboratory results using in vitro 2D models and in vivo experiments, resulting in dramatically different drug discovery experiments and subsequent clinical outcomes. According to early data, pharmaceutical investment in clinical studies of a newly discovered drug is approximately $1.3 billion [8], with poor intercellular communication in in vitro 2D models dominating the cause of failure [9]. Furthermore, the same exposure to nutrients and the limitations of culture space on 2D culture plates lead to in homogenous growth of cancer cells and limit cell duplication, respectively [10, 11]. Apart from it, many cellular properties such as multiplication and proliferation are overemphasized when cells are grown on 2D culture plates. Consequently, the results of metabolic profiling, protein and gene expression can be significantly biased [12, 13]. Additionally, another disadvantage of 2D systems is their low generation rate, thus increasing the need for 3D models. For instance, more than 300 lung cancer cell lines were generated using a 2D culture system, but the feasibility of the system was questioned when the generation rate was as low as 5% [14]. Furthermore, cell death rates vary between 2D and 3D culture systems [15], raising questions about the fidelity of these 2D monolayer models. In vivo models are expensive, difficult to handle, and unrealistic to use where results from 2D systems are unreliable, as reported in some cases [12, 15, 16]. In this regard, 3D models have the characteristics of low cost, high throughput, self-adaption (mechanistic and otherwise), multicellular tumor/heterogeneous tumor culture, and others. 3D models fulfill the need for TME to be reproduced in vitro, morphologically, functionally similar (e.g., gene expression, cell proliferation and differentiation) to that of the parent in vivo source (Figure 1). Collectively, these factors support drug testing and reduce the likelihood of drug sensitivity and resistance due to overestimation/underestimation of inhibitory drug concentrations. Furthermore, despite the clear role of cancer-associated adipocytes (CAA) in cancer progression [17–20], they are neglected in analytical studies such as drug screening and drug discovery. Moreover, because cancer cell cultures do not have physiological characteristics, they

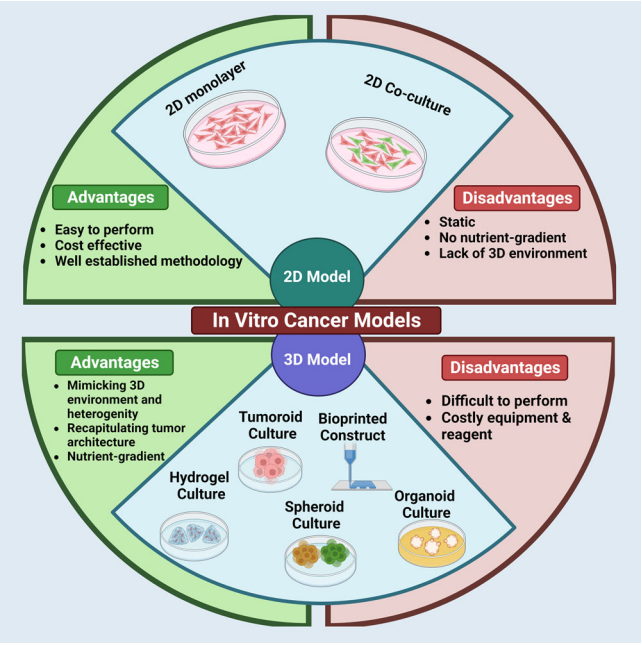

FIGURE 1 Comparison between 2D and 3D cancer models. *Created with BioRender.com.

remain overlooked in those studies. But the distorted cancer physiology needs to be reproduced/studied, and all these factors need to be addressed. Furthermore, the importance of 3D models for angiogenesis and models that follow oxygen availability during cancer cell culture are discussed. Finally, models for efficacy studies, preclinical and clinical trials and other models with the self-adapting matrices are further discussed.

2 | ESSENTIALITY OF STROMAL CELLS IN TUMOR-ORIENTED RESEARCH

Tumors are the incarnation of a group of disordered elements put together. Cellular and molecular interactions have a strong impact on cancer histopathology, with adipocytes and fibroblasts being the most prominent contributors. Studies have found that cancer cells absorb fatty acids (FAs) secreted by the TME containing adipocytes [21]. These FAs are involved in lipid signaling and cancer cell metabolism. The process further promotes metastasis by increasing mitosis and activating cell migration pathways [22]. Among several pathways that promote metastasis, the HIF-1α-regulated metastasis pathway influenced by FAs- is prominent [17]. FAs synthesized by these cells are also utilized by nonmalignant cells of the TME via incorporation into the phospholipid bilayer [21]. This suggests that they have a synergistic effect on tumor progression. These exogenous FAs also play a role in the induction of fatty acid synthesis (endogenous lipogenesis) in cancer cells. Endogenously formed fatty acids are required for membrane biosynthesis and aerobic glycolysis (Warburg effect) and contribute to the rapid division of cancer cells. In contrast to normal cells, fatty acid synthase (FASN), a key enzyme for lipogenesis, was found to be overexpressed in a variety of tumors [23]. A set of other independent studies have shown that increased FASN production correlates with poor prognosis, metastasis and cell survival in cancer [24, 25]. This aspect of tumor histopathology has been overlooked by studies in 2D experimental setups that lack stromal cells such as adipocytes in tumor-oriented experiments. In the case of 2D cancer models, the importance of ECM and TME in studying physicochemical properties cannot be properly studied, whereas in 3D cancer models, the recapitulation of TME and the importance of ECM can be studied. Fibroblasts are undifferentiated connective tissue cells that play an important role in ECM synthesis. Cancer-associated fibroblasts (CAFs) play an important role in the de novo production of ECM [26, 27]. Abundant ECM deposition increases the stiffness of the tumor site, accumulates stress, and forms a protective biophysical barrier. This environment also promotes an increase in tumor-associated macrophages (TAMs) and CAFs, thereby accelerating ECM remodeling and angiogenesis. CAFs also regulate immune responses by secreting proteins and other factors into the TME [28–30], since these cells are mesenchymal, they differentiate through the differential action of different factors secreted in the ECM. Furthermore, CAFs in the TME mediate lipid secretion induced by adipocytes and cancer cells [31, 32]. Fundamentally, cancer cells induce stromal cells, causing the latter to create an altered microenvironment. This interaction-mediated shift in the microenvironment reshapes drug efficacy at the cancer site [33, 34]. Importantly, CAFs have been identified as contributors to cancer progression [27], pathogenesis [35] and tumor drug resistance [36]. These cells play an important role in the prognosis of cancer recurrence [37, 38]. Moreover, the large and significant number of nonmalignant cells (even more than malignant cells) in pancreatic cancer demonstrates the importance of the former [39]. Therefore, the role of stromal cells in tumor evolution implies the necessity of equipping them with malignant cells in cancer research. Tissue heterogeneity, stromal disruption/manipulation, ECM variation and immune/immune cell modulation are major contributors to changes in the tumor microenvironment. Conclusively, cancer histopathology is influenced by multiple environmental and mechanophysiological factors, of which CAAs and CAFs are the most prominent (Figure 2).

3 | TYPES OF 3D CANCER MODELS

Engineering approaches have been used in 3D cancer models to improve and enhance the accuracy of mimicking biomimetic complexity. Elimination of contact with non-bionic tissue culture materials is necessary to achieve good oxygen and nutrient permeability. Approaches to create more biomimetic 3D models of cancer include, but are not limited to: (a) Providing appropriate matrix components in the 3D configuration found in vivo; (b) coculturing cancer cells, endothelial cells and other associated cells in a spatially relevant manner; (c) monitoring and controlling hypoxia to mimic natural levels found in tumors; and (d) monitoring angiogenic factors released by cancer cells in response to hypoxia.

3.1 | Scaffolding model

A scaffold model refers to a framework that incorporates the physical structure, or scaffold, used to grow cancer

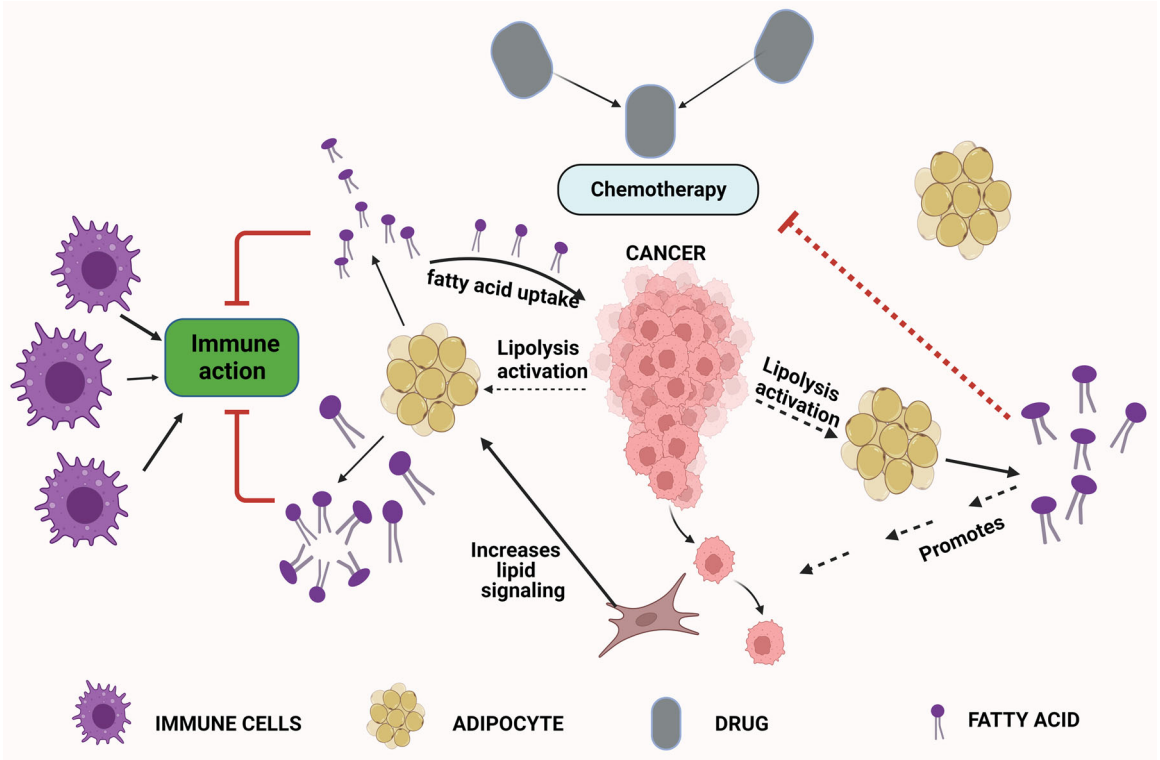

FIGURE 2 Role of CAAs/FAs in cancer progression: The metabolic and molecular events that occur in the tumor site. *Created with BioRender.com.

cells in a 3D environment with computational modeling techniques used to simulate and study the behavior of cancer cells within the scaffold. In 3D cancer models, researchers aim to create an environment that closely mimics the complexity of human tumor growth. It involves the use of scaffolds, often made of biocompatible materials, to provide a physical structure that supports the growth and interaction of cancer cells. These scaffolds can be designed to replicate specific tissue types supported by the TME. However, studying the behavior of cancer within these 3D scaffolds can be challenging. This is where computational modeling comes into play.

Scaffold models combine experimental data and computational techniques to simulate and analyze various aspects of tumor growth and treatment response. The computational component of a scaffold model can involve various approaches such as agent-based modeling, cellular automata, or mathematical models describing tumor growth dynamics and cellular interactions. These models can incorporate factors such as cell proliferation, migration, nutrient and oxygen gradients, cell-cell interactions and response to treatment. Scaffold models in 3D cancer modeling are an active area of research that contribute to advances in cancer biology, drug discovery and personalized medicine. They are powerful tools for studying tumor behavior in controlled and realistic settings, offering valuable insights that can inform clinical decision-making and the development of new treatment strategies. Scaffold models can be divided into solid scaffolds and hydrogels based on their structural orientation.

3.1.1 | Solid scaffold

Solid scaffold refers to a physical structure or matrix that provides support and structure to cultured cancer cells in a 3D environment. The solid scaffold is designed to mimic the ECM found in living tissues, which plays a crucial role in cell behavior and tissue organization. Solid scaffolds serve several important purposes in 3D cancer models:

(a) Mimicking tissue structure: Solid scaffolds provide a framework that resembles the structure and physical characteristics of the tissue in which the cancer is located. This allows researchers to study cancer cells in a more realistic environment than traditional 2D cell cultures.
(b) Cell attachment and migration: Scaffolds offer anchoring points and surfaces for cancer cell attachment, spread and migration. It provides a 3D

space that allows cells to interact with each other and their surrounding environment.

(c) Cell-cell and cell-matrix interactions: Solid scaffolds facilitate cell-cell interactions as well as interactions between cells and ECM. These interactions are crucial for various cellular processes that influence tumor growth and behavior, such as proliferation, migration and signaling.

(d) Nutrient and oxygen diffusion: Scaffolds can be designed to allow diffusion of nutrients, oxygen and waste products within the 3D culture. This helps replicate the physiological conditions and gradients present in living tissue, enabling a more accurate representation of tumor growth and metabolism.

Solid scaffolds used in 3D cancer models are typically composed of biocompatible materials that provide necessary mechanical support and biochemical cues for to cancer cells. These materials can include natural polymers (e.g., collagen, fibrin, alginate) or synthetic polymers (e.g., polyethylene glycol, polycaprolactone), which can be tailored to match the desired properties of the TME.

3.1.2 | Hydrogels

Hydrogels have emerged as a promising tool in cancer research, particularly in the development of 3D models that mimic the TME. These 3D models aim to better recapitulate the complexity and physiological conditions found in real tumors, compared to traditional 2D cell cultures. Hydrogel-based 3D models of cancer remain an active area of research, and progress is being made to increase their complexity, physiological relevance and integration with other technologies such as microfluidics and biomaterial functionalization. These models hold great potential for enhancing our understanding of cancer biology, improving the drug development process, and ultimately facilitating the development of more effective cancer therapies.

3.2 | Scaffold-free models

Scaffold-free models, also known as scaffold-free 3D cell cultures or scaffold-free organoids, are 3D cellular structures that do not rely on external support matrices or scaffolds. Instead, these models allow cells to self-assemble and form complex tissue-like structures. Various advantages of scaffold-free models are illustrated: (a) Recapitulation of tissue architecture—scaffold-free models enable cells to self-organize and form 3D structures that closely resemble the structure and function of native tissues. This better represents the complexity of the in vivo environment compared to traditional 2D cell cultures. (b) Cellular heterogeneity—scaffold-free models allow for the formation of multicellular structures that can incorporate different cell types and mimic those in tissues. This heterogeneity is crucial for studying cellular interactions, disease progression and drug response. (c) Higher physiological relevance—scaffold-free models provide cells a more physiologically relevant microenvironment, including cell-cell interactions, nutrient and oxygen gradients and the development of tissue-specific functions. (d) Drug screening and personalized medicine—scaffold-free models offer a platform for drug screening and testing. They can be utilized to evaluate drug efficacy, toxicity and drug response in a more representative tissue setting. Additionally, patient-derived cells can be incorporated into scaffold-free models to develop personalized medicine approaches.

Scaffold-free models are valuable tools in various research areas, including drug discovery, disease modeling, regenerative medicine and understanding tissue development. They complement scaffold-based models and provide an alternative approach to study complex cellular behaviors and interactions in a more biologically relevant context. Scaffold-free models are broadly classified into spheroids, organoids and tumoroid models.

3.2.1 | Spheroids

These are clustered 3D (spherical) structures formed by the aggregations of like and/or alike cells. Traditionally, tumor spheroids have been formed using magnetic levitation, round-bottomed nonadhesive plates, and hanging drop methods [20], [40–43]. The formation of spheroids in vitro is aided by making the plate surface less adherent and interactive than in vivo systems. Under in vivo conditions, the ECM, different stromal cells (including immune cells, CAAs and CAFs), growth factors and the conditionally formed peritumoral vasculature dwell the same environment as cancer cells and combine to establish the TME. Cells aggregation in tumor spheroids is influenced by these TME elements. 3D concepts in cancer models, including spheroid models, have been under exploration. Nonetheless, they remain underutilized in preclinical and translational research for multiple reasons, including low throughput, high expense, non-reproducible results [44] and poor vasculature [45]. The concept of spheroids demolishes the 2D (monolayer) culture restriction of 2D systems. However, the size inhomogeneity and the variation in the

number of cells in the formed spheroids have long remained its limitations. Different studies proposed and added different solutions [46], but the disadvantages still needed to be mitigated. With advancements, the tumor spheroid model has evolved into a powerful concept that can replicate tumors in vivo not only in physiological morphological analysis but also in preclinical studies. Spheroid models have emerged as a prominent tool for reproducing the TME in vitro. Crucial to TME maintenance, oxygen is always a limiting factor in spheroids and other related concepts in 3D in vitro culture. Oxygen availability remained an issue until 2012, when a group of researchers developed a 3D cancer model in which the walls of the model are oxygen permeable, made of polymeric organic silicon compound polydimethylsiloxane (PDMS), sustaining a continuous spheroidal growth even after 14 days in culture [47]. Another independent research by Seo and colleagues [17] proposed a chip model that allows O_2 to filter through its walls. This model encompasses 1,700 seeding wells in a small 40 mm culture chip. The use of polymeric organic silicon compounds (PDMS) improves the results and provides uniformly sized spheroids. In addition to oxygenation, inappropriate cell aggregation remains *a minor* obstacle to using these models for molecular cancer research. PDMS is transparent and allows real-time tracking of cell cultures. The biocompatibility of PDMS allows the formation of uniform spheroids within amphiphilic wells coated with Pluronic. Collectively, all these factors pave the way to investigate the various roles of different TME cells in the regulation of cancer dynamics. Additionally, this model also showed a high degree of synchrony with a mouse xenograft model [17]. Experimental results from numerous studies truly validate the fact that the histological characteristics of cancer in vivo are conserved in 3D model systems [6, 48, 49].

3.2.2 | Organoids

Organoids are self-organizing stem cell clusters that serve as in vitro reincarnations of human organs [50]. They are structures in vitro with 3D structure and tumor heterogeneity, just like in vivo. Therefore, this concept is well suited to embodying tumor ecosystems in vitro [51]. Experimental studies highlight several aspects of human metabolism and organ development (e.g., brain development) that animal models fail to simulate. Organoids define them because they can develop histologically similar organs outside the organism [52, 53]. This model requires induced pluripotent stem cells (iPSCs) [54] and adult stem cells (ADSCs) [55]. Apart from their advantages, these two methods compensate for each other's shortcomings. iPSCs are created by reprogramming differentiated cells and are capable of producing a wide range of organs and cell types, whereas ADSCs, unlike iPSCs, require only epithelial cells and do not require complex cell dedifferentiation processes [50, 53]. Tumor organoids (TOs) have examined great advancement in their culture and paved the way for the aspects of fundamental research. The goal of this technology is to create cell models of a list of cancer types, namely, pancreatic cancer [56], esophagogastric cancer [57], colon cancer [58], breast cancer [52], and others. Additionally, there are numerous studies targeting organoid cultures that mimic patients' responses to chemotherapy [59, 60] and chemoradiotherapy [61, 62]. Furthermore, a recent study published in Cell Reports [63] focused on identifying molecular and clinical determinants of tumor samples from over 1000 determined patients. The scientists used their organoid platform to monitor various responses for pan-cancer drug screening. Large-scale production screening platform aspires to be used in precision medicine. Furthermore, the organoid platform is geared towards applications in translational research, including molecular profiling and other studies holding therapeutic importance, with a focus on precision medicine. Numerous drug screening studies have been conducted using organoids [64, 65]. Sachs and colleagues [66] did form breast cancer (BC) organoids by eliminating inefficiency hurdles to well-adapted organoid protocol. It was performed by adding neuregulin 1 (ligand for human HER [a human epidermal growth factor]), tyrosine kinase 3 and 4, which mediate long-term expansion of organoids. The experiment was led by the administration of drugs targeting the HER signaling pathway, ultimately yielding complementary results. The presence of differential organoid populations was confirmed by several relative IC_{50} values. It has been reported that cultured organoids are sensitive in the presence of high HER2 expression, whereas in HER2-deficient organoids they exhibit drug resistance. However, few other organoids also defy the classification trend, thus emphasizing functional in vitro testing of organoids. Similarly, there are studies validating that Patient Derived Organoids (PDOs) can recapitulate norms of patient response while being used for pharmacotherapy and may contribute to dedicated research in personalized medicine [67]. Hu et al. [64] generated hundreds of lung cancer organoids (LCOs) and obtained clinically valuable responses from them. LCOs are a type of PDOs that are reliably culturedand can be used in precision medicine with high success rates. A PDO-based drug test (less than 1 week) was performed to simultaneously treat the PDO samples from 21 patients with the anti-lung cancer drugs, gefitinib (Gef) and crizotinib (Cri). The experimental responses were consistent with the genetic mutations and clinical outcomes, demonstrating the reliability of this method.

3.2.3 | Tumoroids

Tumoroid in 3D cancer models refers to 3D culture systems that mimic tumor characteristics and can be used for the study of tumor biology and anticancer treatment evaluation. Tumoroids are formed by aggregating cancer cells or patient-derived tumor cells in 3D culture systems. This can be achieved through methods such as the hanging drop technique, liquid overlay technique, or by hydrogel cell embedding technique. The aim is to create a 3D environment that better recapitulates the structures and cellular interactions within the tumor. The tumoroids exhibit cellular heterogeneity similar to real tumors. They contain a mixture of cancer cells, stromal cells, immune cells and other components of the TME. This cellular diversity allows researchers to study the interactions and dynamics between different cell types within the tumoroids. Tumoroids aim to recreate the complex microenvironment found in tumors. This includes factors such as hypoxia (low oxygen levels), nutrient gradients, ECM composition and cell-cell interactions. By integrating these elements, tumoroids provide a more physiologically relevant platform for studying tumor behavior and treatment response. Tumoroids can be used to evaluate the efficacy of anticancer drugs and identify potential treatment strategies. Tumoroids can also be used to investigate the invasive properties and metastatic potential of cancer cells. By incorporating components that mimic the secondary sites where metastasis occurs, such as specific ECM proteins or organ-specific cell types, researchers can study how cancer cells behave in these environments. Tumoroids in 3D cancer models provide valuable tools for studying tumor biology, evaluating anticancer therapies, and understanding the interactions between cancer cells and the TME. Their ability to recapitulate key aspects of tumors makes them a promising platform for preclinical research and personalized medicine applications (Figure 3 and Table 1).

4 | THE APPROACH OF ADAPTIVE AND SELF-ASSEMBLING BIOMIMETIC CANCER MODELS

In cancer research, 3D models are created to overcome one or more obstacles that prevent researchers from reproducing the cancer in vitro, thereby obtaining more relevant results. Each cancer is unique in its constituent compounds/components, the density of ECM/collagen [1, 76] and the distribution of surrounding stromal cells, which play an important role in cancer invasion [75]. As a result, separate models are needed to simulate various cancer types. Furthermore, factors secreted by stromal cells in the TME play a role in the ability of tumors to withstand drug loading. Melanoma cells express highly activated MAPK signaling due to BRAF mutations and have been shown to be resistant to vemurafenib, a BRAF inhibitor, as Hepatocyte Growth Factor (HGF) is secreted by surrounding stromal cells [77]. HGF promotes the establishment of innate drug resistance in melanoma as well as other epithelial cancers (glioblastoma, colorectal cancer) [78], indicating the importance of the TME in tumor progression. The presence of the ECM is not the only requirement; its composition also contributes to the formation of the TME. Researchers at the Barts Cancer Institute and colleagues developed an ex vivo adaptive and self-assembling model in which ECM tunability and other issues of were addressed, resulting in physiochemical properties and in vivo-like biological signaling [79]. Peptide amphiphile (PA) is a self-assembling component that has been shown to retain the in vivo structure of the ECM due to its ability to generate fibrous hydrogels at the nanoscale level. During synthesis it became apparent that whether PA fibers become less or more aligned is directly influenced by ECM components such as fibronectin. PA fiber formation can be manually adjusted in this model, which primarily produces collagen in vivo system fibers of this size (20×300 nm) [80]. Young's PA-ECM modulus calculations were performed with stiffness as the dominant factor until reaching a range highly similar to that of primary pancreatic adenocarcinoma (PDAC) and PDX, that is, 1 KPa in vivo [81]. Since stem cells are abundant in PDAC, quantitative stemness marker analysis was performed using qRT-PCR. SOX 2 and KLF4 expression levels were found to be within the range of PDX tumor results and significantly higher than values obtained from other models. The fidelity of the self-assembled material improved when the cancer tissue used in this 3D system was transferred to an in vivo system and the same cancer propagation as new cancer cells was observed. Many such models have been developed, but their description is beyond the scope of this review.

5 | EXTENSIVE APPLICATION OF 3D MODELS IN CANCER RESEARCH

5.1 | Cancer angiogenesis

Angiogenesis refers to the formation of new blood vessels from existing vessels and plays a crucial role in various physiological and pathological processes such as embryonic development, wound healing and tumor growth.

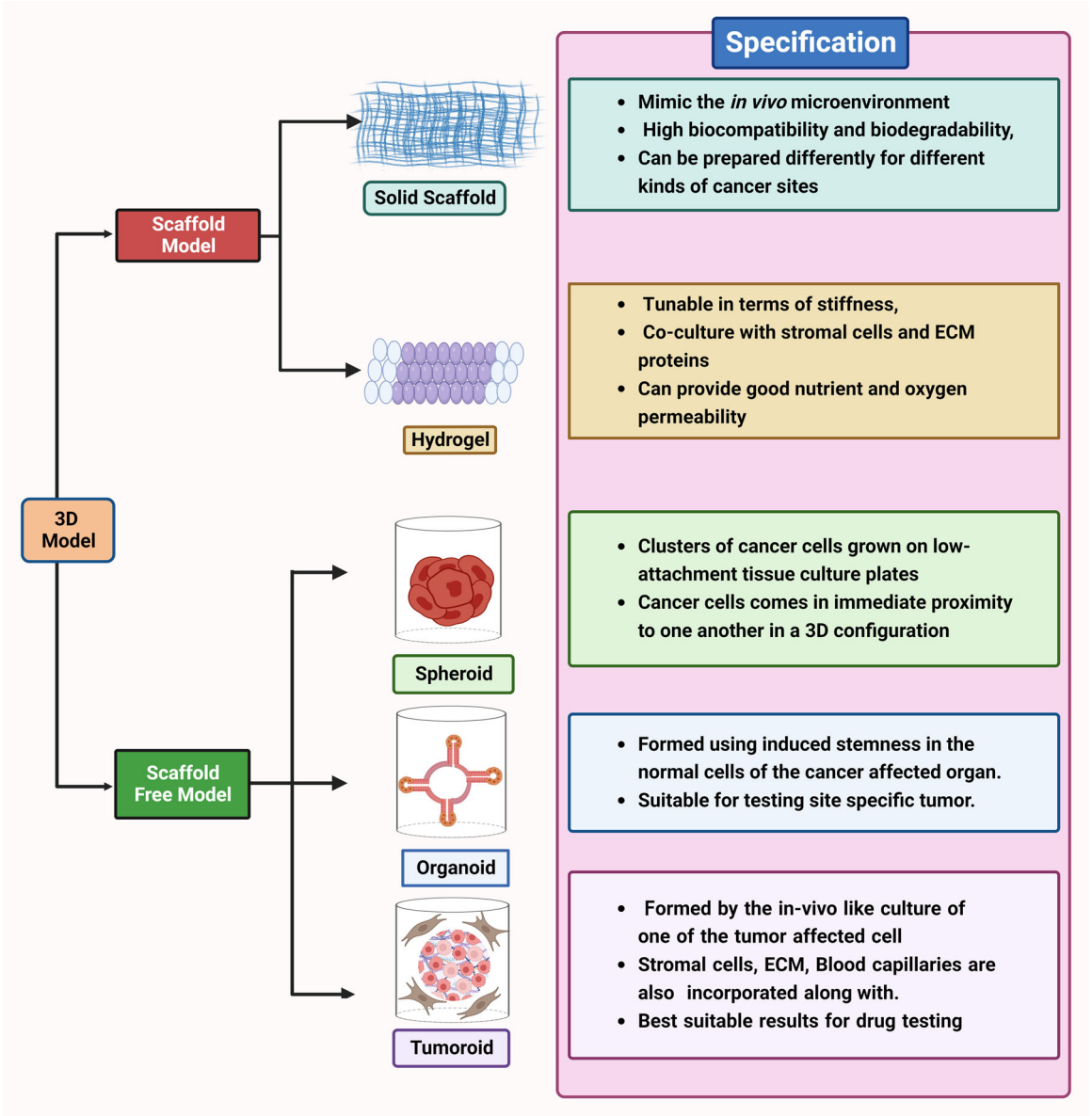

FIGURE 3 Classification of 3D models. 3D models are generally classified in to scaffold models and scaffold-free models. Scaffold models are further classified into solid scaffolds and hydrogels (as depicted in the diagram). Scaffold-free models are classified into organoid models, spheroid models and tumoroid models. *Created with BioRender.com.

Angiogenesis is a prerequisite for tumor metastasis. Certain tumors, such as glioblastoma (GBM), have large numbers of stem cells and are significantly angiogenic [82, 83]. The peritoneal vasculature plays an important role in supplying nutrients, metabolites, or oxygen. It shows the bioavailability of treatments in the cancer immune system [84]. The importance of these components in different bio-fabrication methods and cancer-on-a-chip technologies helps to capture tumor-specific sites and blood vessels that can be observed in vivo [33, 85]. Along with this, the ubiquitous and complex lymphatic network is another important factor to take into account because of its role in metastasis and anticancer drug circulation in vivo [34, 86]. Interestingly, despite its importance, most preclinical in vitro models fail to recapitulate the lymphoid component. Studying angiogenesis in 3D models provides a more physiologically relevant environment, allowing researchers to better understand the complex cellular interactions and microenvironmental factors involved in angiogenesis.

There are several methods for studying angiogenesis in 3D models (Figure 4). Here are some commonly used methods:

(a) 3D cell culture models: Researchers can create 3D cell culture models using techniques such as

TABLE 1 Tumor-microenvironment elements and their impacts on the Tumor.

S. No.	TME elements	Characteristics	Biomolecular mediators	Impact on the tumor	Ref.
1.	Cancer-associated fibroblasts (CAFs)	• Promotes the overexpression of -SMMA and platelet-derived growth factor (PDGF). • Promotes vascularization mediated by cellular communication network factors (i.e., CEN1).	• Growth factors [transforming growth factor (TGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF)]. • Interleukin (IL-6, IL-8, IL-1beta) • Chemokines (Via autocrine and paracrine signaling).	Tumor Metastasis	[68]
			ECM components like collagen and hyaluronic acid	• Desmoplasia • Cumulation of mechanical stress • Protective barrier (biophysical) formation • Tumor metabolic shift	[69, 70]
2.	Tumor-associated macrophages (TAMs)	Favors the TGF-β1 signaling induced EMT transition (Epithelial-Mesenchymal Transition)	• Pro-inflammatory cytokines: -IL-6, IL-1beta, TNF-alpha, IL-10, TGF-beta • VEGF (vascular endothelial growth factor)	• ECM remodeling • Immunosuppressive microenvironment • Angiogenesis • Metastasis (EMT transition) • Inhibitory effect on antitumor T cell response • Overall cancer cell survivability	[71, 72, 73, 74]
3.	Cancer-associated adipocytes (CAAs)	• Fibroblast-like phenotype • De-lipidation • Secretion of inflammatory factors and chemokines	• Pro-inflammatory cytokines. • Chemokines (Prostaglandins, IL-6, IL-8, TNF-alpha etc.)	• Matrix remodeling • Invasion • Cancer Cell proliferation	[22, 32]
			Fatty acids (palmitic acid, stearic acid, cholesterol, oleic acid, linoleic acid, myristic acid)	Lipid-derived metastasis (Act as oncometabolite)	
4.	Peritumoral vasculature	Mediator in cancer-immune cell crosstalk	• Angiogenesis occurs in corresponds to: • IL-1beta-driven signaling (in endothelial cells) • VEGF factor secreted by TAMs	• Escape from immune system surveillance • Supports metastasis	[75]

spheroids, organoids, or tissue engineered constructs. These models allow cells to grow in more natural 3D structures, enabling them to interact and form complex structures that mimic conditions in vivo. By integrating endothelial cells and other relevant cell types, researchers can induce angiogenesis in these models.

(b) Coculture models: Coculture models involve culturing different cell types together to simulate the cellular interactions that occur during angiogenesis. Endothelial cells are often cocultured with other cell types such as fibroblasts, pericytes, or immune cells to create a more realistic microenvironment. These models can be grown into 3D spheroids or scaffold-based systems to promote angiogenic sprouting and vessel formation.

(c) Scaffold-based models: Scaffold-based models provide structural support for cells to organize and form 3D structures. Various natural or synthetic biomaterials can be used as scaffolds to mimic the ECM and provide a suitable microenvironment for angiogenesis. Cells can be seeded on or within these scaffolds, and factors such as growth factors, cytokines, or mechanical cues can be incorporated to induce and guide the angiogenic processes.

These are just a few examples of how angiogenesis can be studied in 3D models. Each approach has its advantages and limitations, and researchers often choose the method that best suits their specific research goals and requirements. Advances in tissue engineering, microfluidics an, bio-fabrication technologies continue to facilitate the development of more sophisticated and physiologically relevant 3D models to study angiogenesis and other complex biological processes. Furthermore, the ECM also plays a significant role in tumor progression. Fibrin, a naturally derived matrix, possesses an analytical role in establishing microenvironments in cell physiology, with adaptive research-relevant cell composition [87]. In summary, a decently controllable 3D platform is essential to support in vitro angiogenesis and related drug testing or research.

5.2 | Prevailing role in cell cytotoxicity and drug efficacy

In vivo studies are used to determine whether 3D model recapitulates the TME, provides significant and realistic results, or is just a concept. Researchers at the University of Fribourg and colleagues used a 3D culture model to study histopathological changes in cancer with or without the involvement of cancer-associated (CA) cells. SW620 and HCT116 colorectal cancer (CRC) cells were investigated for their independent and CA cell-dependent behaviors in

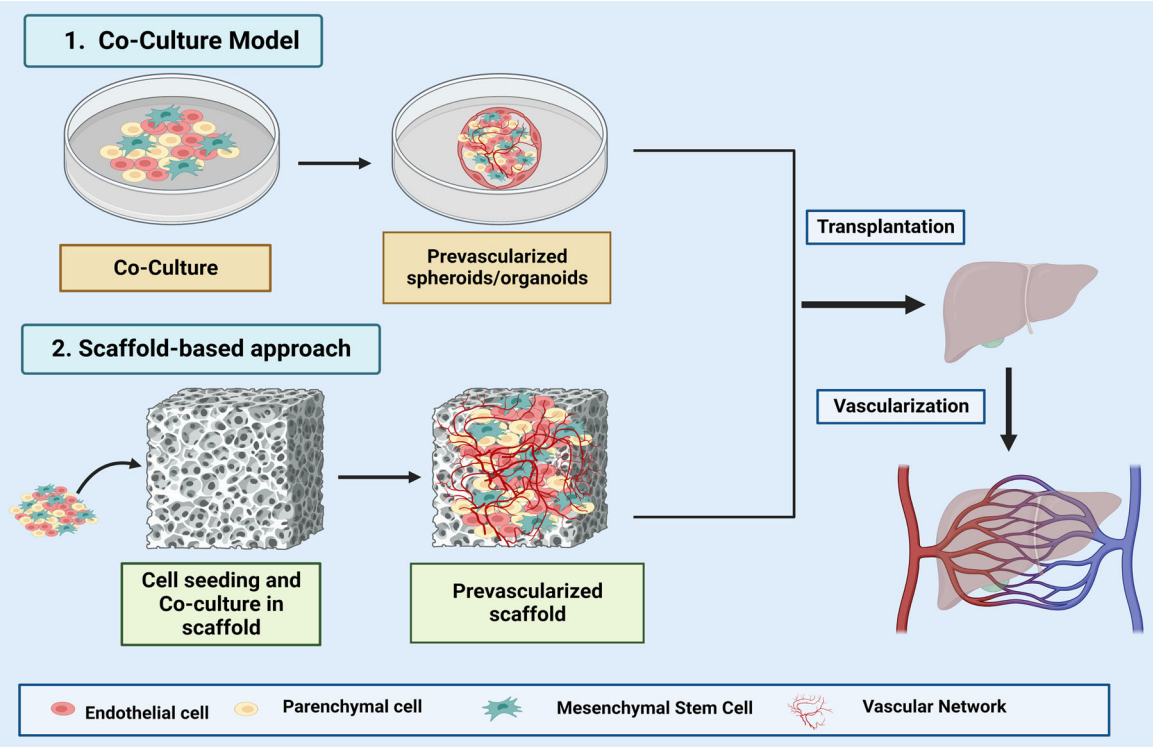

FIGURE 4 Strategies of Angiogenesis in 3D models. *Created with BioRender.com.

cancer invasion, progression and metastasis [88]. The in vivo system is based on the observation that fibroblasts enhance cell migration and invasion in vitro, confirming that fibroblasts affect metastasis. The results of cancer progression affected by endothelial cells in vitro and in vivo models are strikingly similar. Furthermore, the observation of FGFR and SRC as molecular pathways were validated by using Erdafitinib and Dasatinib as respective inhibitors. Because the researchers used a study involving cellular actions, molecular signaling, gene silencing and drug inhibition, and the results obtained were highly synchronized, the 3D model demonstrated the ability to maintain the TME outside of in vivo systems. 3D-based culture models have several limitations, including low throughput, difficulty with duplication and transparency, and lack of real-time analysis. All these limitations are overcome by the discussed new model. Markovitz-Bishtiz et al. [89] developed an easily reproducible model that allows monitoring (the effect of the drug) without the need to transfer culture samples. Identical wells were created using the Bosch process/Deep Reactive Ion Etching (DRIE) approach in system formation, resulting in homogenous spheroids (one per well) throughout the culture system. The uniformity and smoothness of the wells also allowed the use of automated scanning. Automated scanning was unable to support promising studies involving rough wells previously formed using soft lithography techniques. Given the importance of obtaining only homogeneous spheroids in subsequent drug cytotoxicity testing, a number of powerful automated scanning tools for observing spheroids heterogeneity have been developed [90]. Using model cells, spheroid formation could be observed in real time, and growth studies with or without drugs could be conducted that is, the role of doxorubicin (DOX) and methotrexate (MTX) on MCF7 cell line had been analyzed. Aside from this, many dimensions of 3D culture have been successfully explored.

5.3 | Anticancer drugs screening

The 3D biomimetic models can meet many of the spatial requirements of drug testing. Cancer cell density, ECM, CA cells and the overall 3D multilayered cellular structure of the tumor affect every detail of tumor aggressiveness. ECM can affect cellular responses to drugs by changing amplified drug efficacy, drug action mechanisms, or by boosting cellular affinity for drug resistance [91]. Conducting in vivo experiments using 2D models to define drug inhibitory concentrations is a threatening issue, as the administration of low drug doses not only produces irrelevant results but also promotes tumor progression [86]. In contrast to monolayer culture, 3D models adhere to the concept of multilayer cell culture. Nonetheless, drug testing is still frequently performed on monolayers of cells (using 2D culture systems), which contradicts the natural compact heterogeneous multicellularity of in vivo systems. This is simply idealism. Apart from the experimental instances already discussed, there are other related studies. Arora and colleagues [92] used a tumoroid model to show that there is a significant difference in the IC_{50} of the anticancer drugs paclitaxel and doxorubicin (when performing cytotoxicity experiments on breast cancer and liver cancer cells, 3D models were tested simultaneously with 2D monolayer cultures). Cancer stem cells are not only the primary cause of tumorigenesis, but also promote tumor growth: (a) Invasion, (b) progression, (c) drug resistance, and (d) recurrence [93]. However, this is revealed when both tumor cells the TME are replicated. For example, many studies have found that chemosensitivity to cisplatin is due to exosomal secretion of the drug [94]. The mystery of how cisplatin promotes tumor growth will only be solved when studying other TME cellular components (e.g., CSCs and slow-cycling cancerinoma [SCC] cells) [86]. Cancer physiology differs from one microenvironment to another, and therefore from one site to another. Because the physiology and progression of two different cancers affecting nearby organs are not similar, simple treatments become ineffective when preclinical studies of these two cancers do not use separate diagnostic models [62]. Rectal cancer is one cause for concern, accounting for half of all deaths in the United States each year. In general, there are three treatments for rectal cancer: neoadjuvant chemoradiation therapy(CRT), cancer resection and additional treatment with 5-Fluorouracil (5-FU); however, clinical responses vary from patient to patient. Due to tumor heterogeneity among patients, some patients retain in complete response after CRT. This heterogeneity obscures the need for precise research models in step-standard analysis and treatment. An in-depth discussion of rectal cancer is partly beyond the scope of this review (Figure 5).

5.4 | Preclinical and clinical implications of precision and personalized medicine

Cancer cells have a variety of cellular components, each with unique compositions. TME heterogeneity with cancer types is one such issue. According to GLOBOCAN 2020, the 5-year prevalence of pancreatic cancer is only 10% [95] and therefore, the progression rate of pancreatic cancer accounts for 94% of the total estimated incidence in 2020. Half of them die within 6 months. In vivo

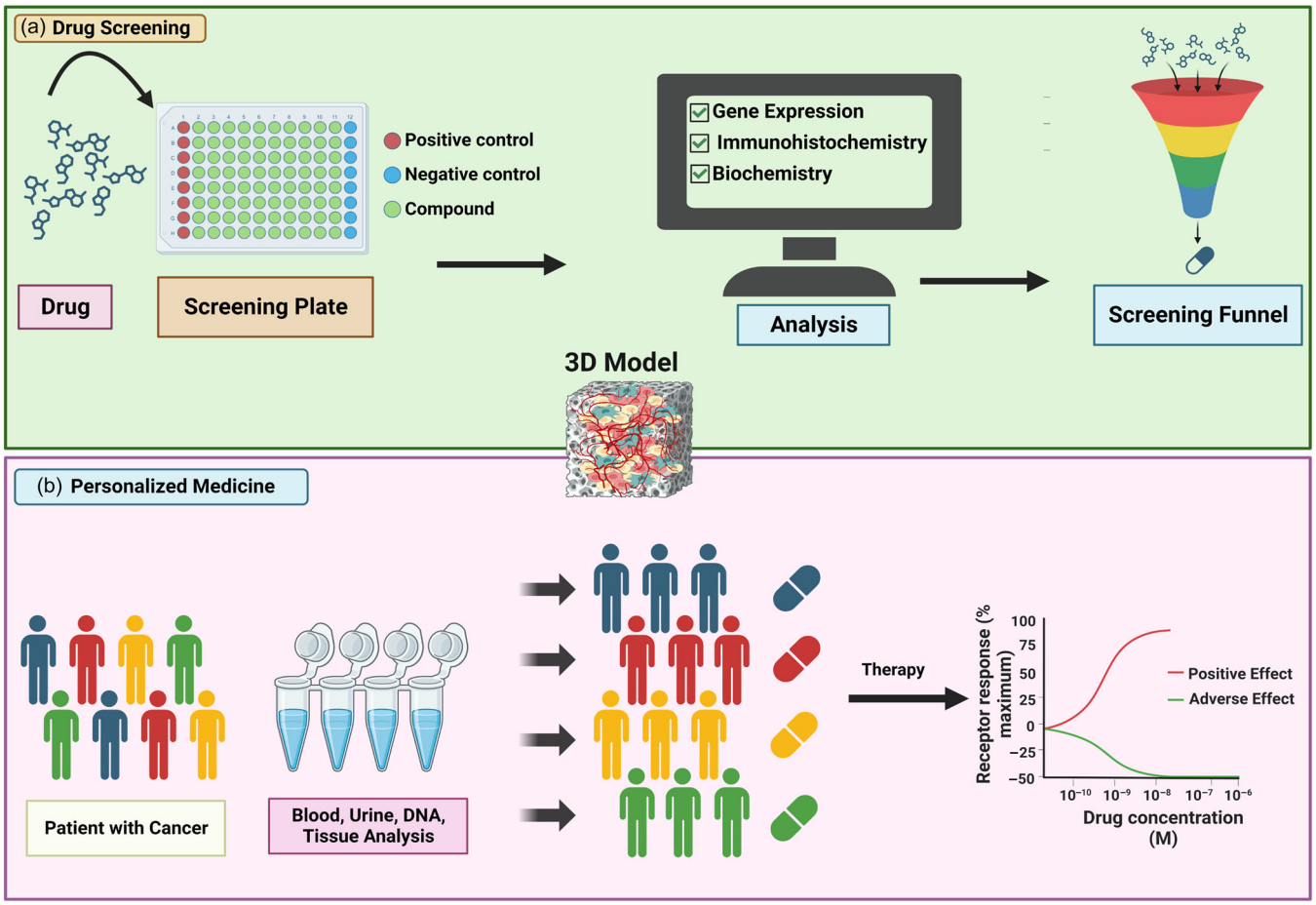

FIGURE 5 Depicting the significance of 3D cancer models. (a) Drug screening. (b) Personalized medicine. *Created with BioRender.com.

xenograft models are highly relevant as they are biological systems in their own types of cancer, but pancreatic cancer cannot receive precise treatments/medication due to relatively long turnaround time. CA cell composition and ECM density differ from cancer to cancer, necessitating the use of adaptive model systems. 2D models and most solid tumor studies are limited to a single cancer-related mechanism. Many studies, such as molecular signaling of metastasis, angiogenesis and others such as nutrient uptake and cancer-stroma interactions, require biological TME mimetics but have received little attention. Both cancer cells and other CA cells are responsible for drug sensitivity or resistance, and cell-dell interactions are important. As a result, 79% funding related to drug research is squandered because the drugs fail to perform as well as the rest of the drugs in clinical trials. In vitro models are used in studies to obtain minimal physiological relevant data. The concept of the 3D models is also rooted in preclinical [96, 97] and clinical phase studies [98]. If the metabolism and progression of known cancers are so devastating, imagine what new types of cancers or different variants of known cancers would look like. For example, if 20%–30% of cases are excluded, all newly diagnosed patients with ovarian cancer experienced recurrence after first-line treatment [99]. Ineffectiveness of research and new treatments is due to population-based tumor heterogeneity. If the patient has not recovered, treatment should be carried out according to the order of the disease. For a long time, there has been a strong desire for personalized medicine. Research highlights that this system could at least help eliminate ineffective treatments for different groups of people who may have even the same type of cancer [100]. Generalized treatments cause patients to develop chemosensitivity, with extremely low success rates for second chemotherapy after the first fails. Many studies of molecular signaling and cancer cell heterogeneity have benefited from the use of organoid models. Furthermore, one of the models developed by Lee and colleagues [101] examines the evolution of bladder cancer tumors. When organoid cell lines were independently prepared from 16 patients and mutational analysis was performed on organoids in mouse-derived xenografts, a high genotype similarity was observed. The

mutation profiles of the organoids in xenografts were also found to be similar to that of the parental tumors. The model can help analyze tumor progression. As basal and luminal subtypes change, phenotypic markers also change. The reason for this interconversion between basal and luminal subtypes may be a reflection of cellular plasticity observed during bladder cancer progression [102]. Increases in basal markers may also be due to early differentiation of tumors into a squamous cell phenotype [103]. Since these types of histopathology are also seen in the progression of bladder urothelial carcinoma and other bladder cancers [104], this model can serve as a model for studies of cancer evolution. The development of organoid models has not only become cost-effective, but has also achieved high success rates (even as high as 80%) [98] and has been used in clinical studies of personalized medicine for colon cancer. Some cancers (e.g., lung cancer) are extremely difficult to genetically analyze. One reason is that 2D culture systems are inefficient at growing them, but Yokota et al. [98] recently developed lung tumoroids. After extensive research by three groups of scientists, three lung tumoroids were successfully developed using a 3D biomimetic AO (airway organoid) model system, in which unforeseen DNA restricted the formation of tumoroids [105–107]. Nonetheless, the previously undetected BRAFV600E mutation was observed in Non-Small Lung Cancer-derived tumoroids, providing information for effective testing of targeted drugs. In this study, 41 patients volunteered to establish in vitro tumoroids to analyze the efficacy of targeted drugs against tumor-specific mutations, which may provide exemplary results for clinical research focusing on personalized treatment and may eliminate the possibility of cancer recurrence due to drug resistance.

6 | CONCLUSION

This review describes different 3D models that recapitulate different aspects of the in vivo microenvironment and can be used to achieve targeted clinically meaningful results. Specifically, 3D culture systems should aim to reduce the inevitable limitations of drug screening that still exist in the early experimental stages, save money and time, and improve the success rate of final late-stage clinical testing. Notably, it has been established that the histological characteristics of cancer cells in vivo are profoundly conserved in 3D model systems, whereas 2D culture systems exhibit a high degree of variability [13]. Additionally, cultured samples in both 3D and in vivo systems exhibit multicellular characteristics of growing cancers, which are completely different from monolayer cell cultures (due to isolated growing cells) [13]. Furthermore, a host of new experiments demonstrated that 3D culture is more realistic in terms of cancer cell-stroma interactions, physiological and morphological similarities, in vitro mechanophysiological recapitulation and a variety of other factors ranging from cancer invasion to metastasis. Talking about stromal cells, concepts like TME CA macrophages TAMs promote metastasis by inducing epithelial mesenchymal transition in cancer cells [30], while different types of other stromal cells largely influence the tumor histological pathologies discussed in this review. Concisely, these facts enumerate a explicit role of CAAs in tumor metastasis, thereby demonstrating their necessity. Although there have been a series of studies specifically targeting CA cells and their defined role in cancer progression [108, 109], they have still been ignored in analytical studies such as drug screening and drug discovery, and, since cancer is not just the physiology that needs to be studied, but the distorted physiology that needs to be treated/cured, all of these factors need to be addressed. Experiments employing 3D hydrogel platforms can examine changes in cell proliferation and cell cycle checkpoints [110], as well as alterations in morphology and gene expression due to the use of Engel breth-Holm Swarm gels [111]. It is conclusively elaborated here that the TME recapitulation (in vitro) provides explicit in vivo-like results for studies based on the morphology, biochemistry, mechanics and physiochemical organization of tumors, as well as for studies aimed at discovering new drugs. The collective advantages provided by 3D models have inspired their sophisticated applications in drug research and cancer research. 3D models hold an extremely bright future for 3D cancer research. This could lead to more biomimetic 3D models being used to accurately model the interactions between cancer and stromal cells. Furthermore, an ECM that is physiologically relevant in terms of composition and stiffness would allow the establishment of a barrier between the cancer mass and the tissue-engineered surrounding stroma. As the use of 3D modeling as a preclinical tool continues to increase, the number of animal studies may be reevaluated and lead to more ethical approach to research.

AUTHOR CONTRIBUTIONS

Karthikey Sharma: Conceptualization (equal); formal analysis (equal); investigation (equal); software (equal); validation (equal); writing—original draft (equal). **Sreenath Dey**: Conceptualization (equal); formal analysis (equal); software (equal); validation (supporting); writing—review & editing (equal). **Rounik Karmakar**: Formal analysis (supporting); software (equal). **Aravind Kumar Rengan**: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); resources

(equal); supervision (equal); writing—review & editing (equal).

ACKNOWLEDGMENTS

The authors S. D. would like to thank SERB–CRG, K. S. would like to thank CSIR and R. K. would like to thank DBT for their fellowship. All the authors would like to thank the Ministry of Education, Government of India and the Indian Institute of Technology, Hyderabad. Schematics were prepared using Biorender.com.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data sharing is not applicable as no new data was generated. The article is entirely theoretical research.

ETHICS STATEMENT

Not applicable.

INFORMED CONSENT

Not applicable.

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How to cite this article: Sharma K, Dey S, Karmakar R, Rengan AK. A comprehensive review of 3D cancer models for drug screening and translational research. Cancer Innov. 2024;3:e102. https://doi.org/10.1002/cai2.102