Advancements and challenges in culturing patient-derived cancer cells for personalized therapeutics

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Patient-derived cancer cells (PDCCs) have emerged as a key strategy for advancing personalized cancer treatment. Unlike traditional cancer cell lines, PDCCs retain the genetic and phenotypic characteristics of the patient's original tumor and can more accurately reflect tumor biology. This review explores recent advances in methods for culturing PDCCs, highlighting the role of these models in drug discovery and high-throughput screening of personalized therapeutic options. By establishing living models directly from patient tumors, PDCCs can more faithfully recapitulate tumor heterogeneity and microenvironmental features than traditional cell lines. These cultures bridge laboratory research and clinical reality, allowing functional testing of patients' cancer cells. Despite the promise of PDCCs, their culture remains fraught with challenges, including the extremely low number of cancer cells that can be obtained, difficulty maintaining tumor heterogeneity, low culture initiation success rates, and ethical considerations for using patient tissues. In addition, controversy remains regarding the reproducibility of results between different laboratories and patient samples. By examining the field's current state, this review identifies gaps in the application of PDCCs, such as limited modeling capabilities for specific tumor types and the lack of comprehensive, scalable protocols for broad clinical use. This article discusses future directions, including integration with advanced microengineering and AI-driven analysis, which have the potential to overcome existing limitations and optimize PDCCs-based therapeutic strategies. PDCCs are expected to transform the future of cancer treatment as they ultimately provide more accurate drug testing and personalized medicine models.

KEYWORDS

patient-derived cancer cells (PDCCs), personalized medicine, cancer therapeutics, drug discovery, 3D cell culture, tumor heterogeneity, high-throughput screening, organoids

1 Introduction

Advancing cancer research and treatment requires model systems that accurately reflect human tumors. Traditionally, researchers have relied on established 2D cancer cell lines and animal models (e.g., mouse xenografts) to study tumor biology and test drugs (Kamb, 2005; Ledur et al., 2017). While these models play an important role, they often fail to capture the

full complexity of human cancer. For example, traditional 2D monolayer cell cultures lack the three-dimensional architecture, multicellular interactions, cellular diversity, and tumor microenvironment of real tumors (Seidel et al., 2015; Aboulkheyr Es et al., 2018). Animal models provide a richer microenvironment, but patient-derived tumor xenograft (PDX) generation is time-consuming and expensive, and the interaction between the immune system and tumorigenesis cannot be studied in PDX models due to their immunodeficient nature (Perez et al., 2025). As a result, many therapies that appear to be effective in 2D cancer cultures and current animal models do not translate into clinical success, and the FDA approval rate for oncology therapies is as low as 3%. These limitations highlight the need for more physiologically relevant *in vitro* cancer models.

The emergence of patient-derived cancer cells (PDCCs) fills this gap, allowing researchers to culture and study cancer cells obtained directly from patient tumor samples. Compared with immortalized cell lines, PDCCs better retain the genetic and phenotypic heterogeneity of the original tumor (Vlachogiannis et al., 2018). PDCCs can be obtained by surgical resection of solid parts (Dekkers et al., 2021), puncture, fine needle aspiration (Lai et al., 2020; Sachs et al., 2019) or liquid biopsy (Sachs et al., 2019; Kopper et al., 2019; Schutgens et al., 2019). PDCCs culture covers a range of techniques, from 2D cell monolayers (Ricci-Vitiani et al., 2007), 3D tumor spheroids (De Witt Hamer et al., 2008), organoids (van de Wetering et al., 2015) and advanced co-culture systems (Tsai et al., 2018). Researchers usually choose or combine these techniques according to specific research questions and clinical application requirements. Currently, PDCCs have been used to study a variety of cancers, including breast cancer, lung cancer, gastrointestinal cancer, gastroesophageal cancer, pancreatic cancer, ovarian cancer, prostate cancer, glioblastoma, liver cancer, colorectal cancer, retinoblastoma and bladder cancer (El Harane et al., 2023; Boj et al., 2015; van de Wetering et al., 2015; Broutier et al., 2017). Using PDCCs to establish *in vitro* models for personalized drug screening (Vlachogiannis et al., 2018), immunotherapy efficacy evaluation (Tsai et al., 2018), individualized vaccine design (Ott et al., 2017; Sahin et al., 2017) and real-time monitoring (Kumari et al., 2021; Shen et al., 2023; Shickh et al., 2022) can enable the formulation of precise treatment strategies and promote clinical translation (Schmid et al., 2018).

This review provides a comprehensive overview of PDCCs culture technologies and their application in personalized medicine, and discusses the challenges faced in technical and clinical translation. We outline future developments at the intersection of PDCCs culture and AI that are expected to improve model fidelity and clinical utility.

2 PDCCs culture techniques and development

The primary methods for culturing PDCCs include traditional 2D monolayer culture, 3D spheroid models, organoid culture, co-

culture systems including multiple cell types, and microfluidic chip-based culture (Figure 1). Each method has different operational complexity and tumor fidelity (Table 1).

2.1 2D cell monolayers and 3D tumor spheroids

The two-dimensional culture of PDCCs is the most straightforward approach, and it shares similarities with classical cancer cell line cultures, such as ease of manipulation and rapid cell proliferation, which have made them a workhorse in cancer research for decades (Kang et al., 2023) (Figure 2a). Large-scale drug sensitivity screening has traditionally relied on panels of two-dimensional cell lines from different patients or tumor types (Bresnahan et al., 2020; Qiu et al., 2019). However, PDCCs grown in a two-dimensional environment often fail to maintain the original tumour phenotype, with cells undergoing genetic and epigenetic drift, resulting in a loss of tumor-specific heterogeneity (Kamb, 2005; Ledur et al., 2017). In addition, cell-cell and cell-matrix interactions are absent or abnormal in 2D monolayers due to the absence of their native extracellular matrix (ECM) and tissue architecture (Kapałczyńska et al., 2018).
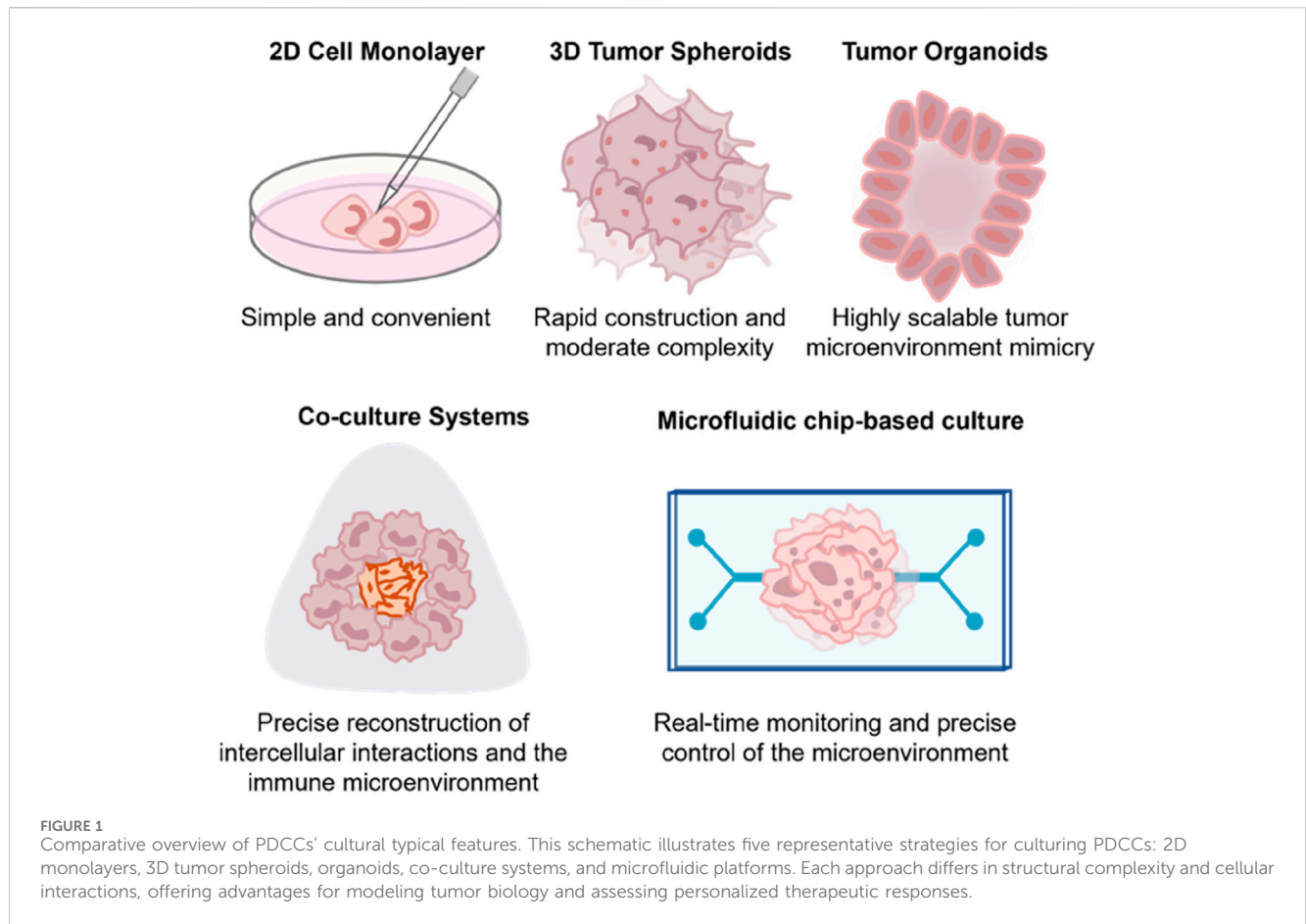
The shift from 2D flat layers to 3D spheroid cultures has significantly improved the physiological relevance of *in vitro* cancer models (Tung et al., 2011; Koledova, 2024; Hagemann et al., 2017). Due to cell-cell contacts and 3D structure, tumor spheroids can more closely resemble *in vivo* gene expression patterns, differentiation, and therapeutic response (Ricci-Vitiani et al., 2007; El Harane et al., 2023; Pampaloni et al., 2007; Ponti et al., 2005) patterns. And as spheroids grow, they develop nutrient and oxygen gradients, with a proliferative outer layer and a more quiescent or necrotic core (Han et al., 2021). This induces physiologically relevant features such as hypoxia, acidosis, and barriers to drug penetration (Groebe and Mueller-Klieser, 1996; Grimes et al., 2014; Thakuri et al., 2019; Costa et al., 2016). However, spheroids remain simplified models of tumors (Zhang et al., 2018) (Figure 2b). They are typically composed of a single cell type (usually the cancer cell itself) and lack the supporting stromal cells, immune infiltrates, and vascular structures found in real tumors (Faria et al., 2023). Nonetheless, tumor spheroids have laid the foundation for developing more complex 3D models such as organoids with more cellular complexity.

2.2 Patient-derived tumor organoids

Patient-derived tumor organoids are miniature tumors grown from a patient's cancer cells (usually from surgery or a biopsy sample) that reflect some characteristics of *in vivo* tissues and functions.

Organoids often contain multiple cell lineages present in a tumor (Dominijanni et al., 2020; Devarasetty et al., 2020). For example, patient-derived colorectal cancer organoids are composed primarily of malignant epithelial cells. Still, they may also contain cancer stem cells and embedded stromal cells from the original tissue (van de Wetering et al., 2015). Organoids often maintain tumor heterogeneity, retain key

FIGURE 1
Comparative overview of PDCCs' cultural typical features. This schematic illustrates five representative strategies for culturing PDCCs: 2D monolayers, 3D tumor spheroids, organoids, co-culture systems, and microfluidic platforms. Each approach differs in structural complexity and cellular interactions, offering advantages for modeling tumor biology and assessing personalized therapeutic responses.

mutations and gene expression patterns, and are genetically stable even after multiple passages (Roerink et al., 2018). They can also be expanded and cryopreserved to create living biobanks of patient tumors for research or drug testing (Sachs et al., 2018; Yan et al., 2018; Lee et al., 2018) (Figure 2c). Furthermore, because organoids can be generated relatively quickly (sometimes within 1–4 weeks) (Jacob et al., 2020a; Jacob et al., 2020b) (Figure 2d), there is growing interest in using them as real-time clinical avatars to guide treatment decisions for individual patients.

Despite their promise, organoids have limitations. Most organoids lack a complete tumor microenvironment (e.g., functional blood vessels, immune cells, and nerves), making it challenging to fully assess immune checkpoint responses and angiogenesis. In addition, organoid formation efficiencies vary between tumor types; for example, non-small cell lung cancer is difficult to construct due to the overgrowth of normal airway cells into lung cancer organoids (Dijkstra et al., 2020). Furthermore, their culture requires expertise and infrastructure (Gjorevski et al., 2016), and during culture, fast-growing clones may dominate, potentially reducing heterogeneity.

Researchers are working to standardize organoid culture media, develop synthetic matrices, and increase organoid derivation rates to overcome these issues. Overall, patient-derived organoids represent a high-fidelity tumor model that has rapidly become integral to cancer research and are at the forefront of personalized medicine efforts.

2.3 Co-culture system

Co-culture systems combine patient-derived tumor organoids or cells with other cell types to fully recreate the tumor microenvironment. The goal of co-culture models is to mimic the complex cell-cell interactions within a tumor, such as cancer cells and immune cells, fibroblasts, or endothelial cells.

Co-culture models allow researchers to directly observe the interaction between patient-derived organoids and immune or stromal and endothelial cells, realistically simulating tumor immune responses and microenvironments. For example, co-culturing organoids with patient-derived autologous T cells or CAR-T cells can produce tumour-killing activity, and PD-1/PD-L1 checkpoint blockade enhances this effect (Neal et al., 2018; Yu et al., 2021; Xie et al., 2020) (Figure 2e). At the same time, co-culture with cancer-associated fibroblasts and endothelial cells can reproduce tumor-stromal interactions and angiogenesis (Sharpe et al., 2024; Lim et al., 2022) (Figure 2f), making up for the lack of a complete microenvironment in single 3D organoids.

Co-culture systems can reproduce the tumor ecosystem more fully, such as immune evasion, immune cell activation, and stromal-tumor drug interactions, which are difficult to capture in a single organoid. For example, co-culture of organoids and immune cells can not only secrete cytokine profiles, but also show cell killing dynamics similar to the patient's actual tumor (Chakrabarti et al., 2021), providing a basis for developing personalized immunotherapy and combination therapies. However, co-culture

TABLE 1 Comparison of PDCC culture methods.

| Method	Advantages	Disadvantages	Complexity	Success rate	Tumor heterogeneity	Microenvironment mimicry	Drug screening Capability	Clinical translation potential	Ref
2D Monolayer Culture	Simple, cost-effective, easy to scale	Poor TME mimicry, lacks 3D architecture	Low	High	Low	Low	High throughput	Limited	Lenin et al. (2021), Gomez-Roman et al. (2017), Grillet et al. (2017), Kodack et al. (2017)
3D Tumor Spheroids	Mimics some 3D structure, easy to form	Limited heterogeneity, lack of stromal/immune cells	Moderate	Moderate	Moderate	Moderate	Good	Moderate	Miyoshi et al. (2018), Weiswald et al. (2009), Kondo et al. (2011)
Organoids	Retains patient-specific features, high fidelity	Variable success, Matrigel dependency, cost	High	Variable	High	High	Very Good	Good	Rajan et al. (2023), Kim et al. (2019), Gmeiner et al. (2020)
Co-culture Systems	Simulates immune/stromal interactions	Complex setup, risk of overgrowth by certain cell types	High	Moderate	High	Excellent	Very Good	Promising	Jenkins et al. (2018a), Chapman et al. (2010), Liu et al. (2017), Cattin et al. (2018), Courau et al. (2019)
Microfluidic Platforms	Precise control, real-time monitoring	High technical demand, low throughput, costly	High	Variable	High	Excellent	Excellent	Promising	Pinho et al. (2021), Dadgar et al. (2020)
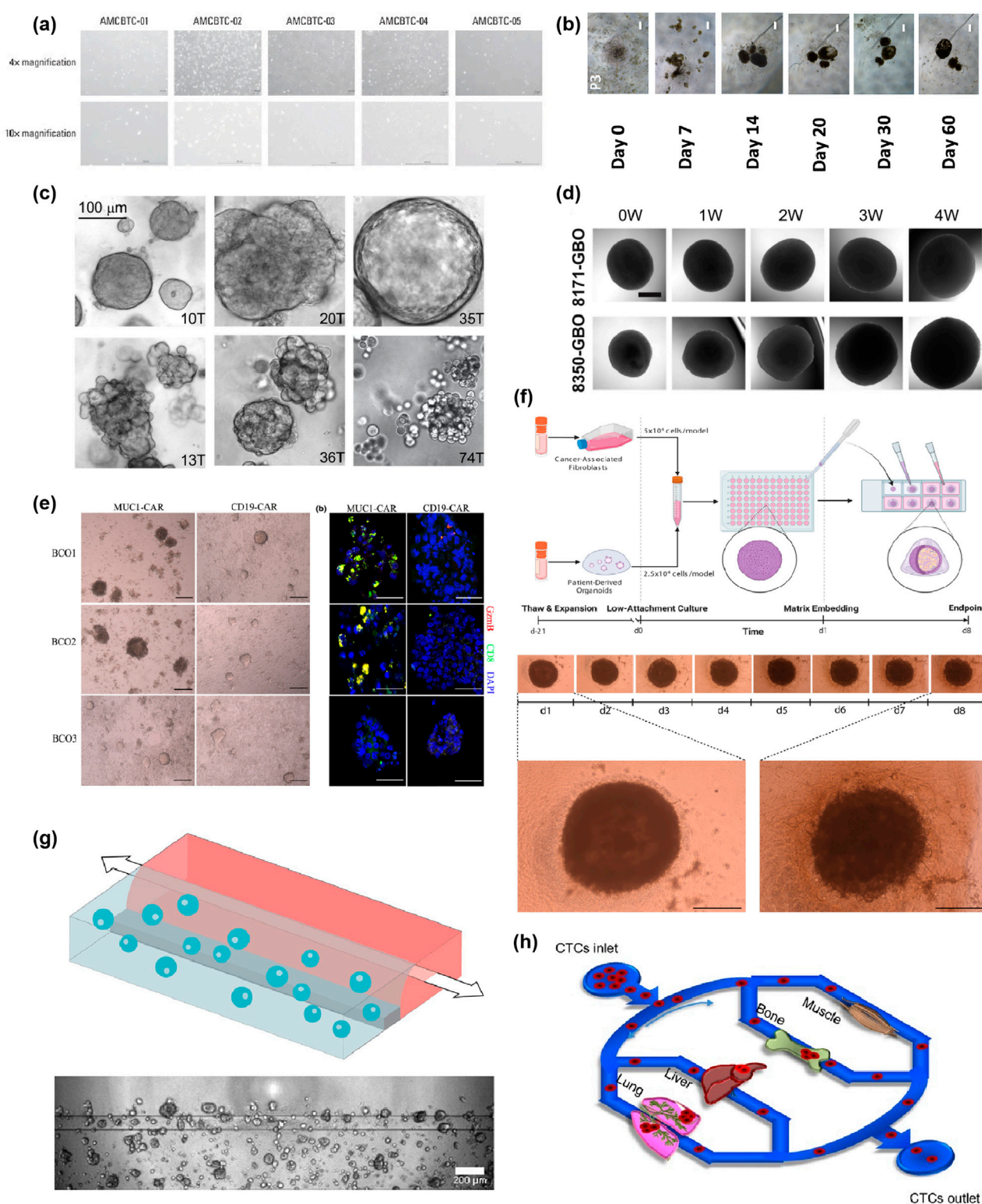
FIGURE 2
Spectrum and Evolution of PDCCs Culture Techniques. **(a)** Morphology of patient-derived cancer cell cultures when grown in 2D cell culture. The images are reproduced from reference (Kang et al., 2023) with permission from the Korean Cancer Association, copyright 2022. **(b)** Representative bright-field images of the tumour spheroids were shown and taken at ×40 total magnification. Scale bars correspond to 200 μm. The images are reproduced from the reference (Zhang et al., 2018) with permission from the Public Library of Science, copyright 2018. **(c)** Bright-field images depicting primary breast cancer organoid phenotypes. The top row shows cohesive organoids (left and middle: dense and solid; right: cystic and hollow), while the bottom row shows increasingly discohesive organoids (from left to right). Scale bar, 100 μm. The images are reproduced from reference (Sachs et al., 2018) with permission from Elsevier, copyright 2018. Shown in **(d)** are sample brightfield images of individual glioblastoma organoids over 4 weeks. Scale bar, 500 μm. The images are reproduced from reference (Jacob et al., 2020a) with permission from Elsevier, copyright 2020. **(e)** Images of bladder
(Continued)

also has challenges. The addition of multiple cell types increases complexity and variability. Immune cells are often short-lived and require specific activation. Obtaining and expanding autologous cells is difficult, and non-autologous cells may induce unreal immune responses (Wu et al., 2012). In addition, co-culture conditions (such as cell ratio, addition time, and culture format) and analysis methods still need to be further standardized.

In summary, co-culture PDCC models represent a significant advance in recreating the tumor microenvironment *in vitro*. By including multiple cellular players, they provide a more comprehensive understanding of tumor biology.

2.4 Microfluidic chip-based culture

Microfluidics has revolutionized *in vitro* modeling, including patient-derived cancer culture. Through small fluidic channels and chambers, these platforms dynamically culture 2D cells, spheroids, or organoids under tightly controlled conditions while delivering culture media, drugs, or immune cells, creating an engineered microphysiological system that recreates tissue-level structure and function (Khoo et al., 2018; Deng et al., 2021; Deng et al., 2023; Li et al., 2025; Zhang J. et al., 2025; Fu et al., 2022).

Microfluidics technology makes *in vitro* culture closer to the *in vivo* environment (Fu et al., 2023). The chip platform can precisely control flow rate, shear stress, nutrient and oxygen supply, and chemical gradients. Continuous perfusion simulates blood flow, forms nutrient gradients and waste removal similar to capillaries, and thus affects cancer cell behavior and drug sensitivity (Jung et al., 2019; Shirure et al., 2018). In addition, these systems allow for partitioned co-culture, integrating tumor organoids with endothelial, immune and other cells, and reproducing *in vivo* processes such as immune cell infiltration and tumor invasion of blood vessels (Aung et al., 2020; Geyer et al., 2023; Haque et al., 2022) (Figure 2g). Microfluidics platforms also support high-throughput parallel experiments, real-time imaging and sensor monitoring, providing rich data for drug screening and personalized treatment decisions (Schuster et al., 2020). The application range of PDCC chips continues to expand, including high-throughput tumor spheroid generation, tumor slice culture and multi-organ interaction simulation. It is expected to build a

more biomimetic system by combining bioprinting and microfabrication technology (Skardal et al., 2010; Aleman and Skardal, 2019). The organ homing preference of cancer cells was studied in a four-organ panel, and it was demonstrated that perfused breast circulating tumour cells invaded the lung, bone, and liver, but not muscle, which is consistent with animal studies (Kong et al., 2016) (Figure 2h).

Against this backdrop, microfluidic PDCC workflows have demonstrated end-to-end feasibility from scarce patient samples to functional readouts. Chip-based workflows can directly amplify circulating tumor cells (CTCs) from liquid biopsies and enable short-term, parallel drug testing within approximately 48–72 h under controlled perfusion, demonstrating a minimal tissue approach for rapid drug profiling for precise decision-making (Khoo et al., 2018). For example, Khoo et al. reported the development of a 3D microfluidic tumour model of bladder cancer, demonstrating that the incorporation of clinically relevant microenvironmental biofilm factors modulated tumour growth and treatment response, allowing for on-chip evaluation of combination therapies at specific flow rates (Deng et al., 2021). Similarly, recent studies have combined a microfluidic PDCC platform with a deep learning classifier to automate image-based cell viability and phenotype readouts, enabling rapid and reproducible analysis of patient cells directly from liquid biopsies (Hua et al., 2023; Li et al., 2022).

Overall, microfluidics improves the accuracy and complexity of patient-derived cancer models through engineered environments, complementing the biological fidelity of organoids to build tumor chip models that more realistically reproduce the tumor microenvironment and human tumor behavior. These advanced platforms are expected to improve the predictive power of drug screening and discovery and accelerate the application of PDCCs in clinical workflows.

3 Personalized medicine based on PDCCs

The primary motivation for developing PDCC culture is that it can retain the unique characteristics of the patient's tumour (such as gene mutations and drug sensitivity), thus providing a basis for

personalised cancer treatment. As an *in vitro* test platform, PDCCs can be used for personalized drug screening and evaluating the effect of immunotherapy, building tumor immune models, designing personalized vaccines, and promoting clinical translational research.

3.1 Personalized drug screening

Personalized drug screening is one of the most impactful applications of PDCCs. By exposing expanded tumor organoids to different drugs (or drug combinations) in multi-well plates or microfluidic chips, cell viability, growth inhibition, and apoptosis can be measured to obtain a drug sensitivity profile of the patient's tumor, providing a basis for selecting the most likely effective treatment. Traditionally, high-throughput screening has been challenging to achieve due to the limited number and lifespan of primary cells. Still, organoid culture and improved 3D detection technology have made this process possible (Kamb, 2005; Ledur et al., 2017).

Studies on various cancers have demonstrated the potential of PDCC-based drug screening. For example, drug responses in colorectal cancer organoids correlate with patient clinical manifestations and have been shown to successfully predict the sensitivity of metastatic colorectal cancer to targeted therapies, which were subsequently treated and clinically proven to be effective [6] (Figure 3a). Pancreatic cancer organoids have shown high consistency in drug responses and actual patient outcomes when determining chemotherapy combinations (Ponz-Sarvise et al., 2019). PDCC cultures can not only serve as therapeutic diagnostic tools in the laboratory to guide personalized medication. Still, they can also be used to screen new drugs and explore resistance mechanisms by establishing organoid biobanks of multiple tumor subtypes (Figure 3b) (Yan et al., 2018).

Although personalized drug screening based on PDCCs shows great potential, its widespread application still faces challenges. It is challenging to obtain results quickly to guide clinical decisions. Although most organoids can be cultured within a few weeks, this may not be timely enough for invasive cases, and growth is currently being accelerated by improving the culture medium. Second, the effect of certain drugs in organoids may not accurately predict clinical responses due to the lack of microenvironmental factors such as liver metabolism or tumour-stroma interactions, which have also promoted the integration of co-culture or microfluidics technology (Ooft et al., 2019).

3.2 Immunotherapy effect evaluation

Immunotherapy has revolutionized cancer treatment, but only a minority of patients can benefit. By adding immune components to the PDCCs co-culture system, researchers could mimic the tumor immune microenvironment, providing a new method for personalized prediction and study of immunotherapy responses.

The co-culture system of tumor organoids and immune cells can reproduce the patient's anti-tumor immune response *in vitro*. By co-culturing patient-derived organoids with autologous T cells, NK cells, macrophages, *etc.* (Tsai et al., 2018; Chakrabarti et al., 2018;

Fang et al., 2022; Chan and Ewald, 2022), researchers can observe phenomena such as immune cell infiltration, immune synapse formation, tumor cell killing and cytokine release, and use them to test the effects of immunotherapies such as PD-1/PD-L1 checkpoint blockade (Larkin et al., 2015; Garon et al., 2015; Borghaei et al., 2015; Le et al., 2015; Le et al., 2017). The PDCCs culture model reveals the mechanisms of immune escape and resistance, and by adjusting the co-culture conditions (such as the introduction of dendritic cells, specific antigen stimulation), it is observed whether the immune attack is improved. For example, adding IL-2 to the co-culture of melanoma organoids and peripheral blood lymphocytes significantly enhanced T cell proliferation and tumor killing (Dijkstra et al., 2018) (Figure 3c).

Despite the promise of co-culture systems, they also have key limitations that warrant further exploration. For example, immune cell viability and function decline rapidly *in vitro*, limiting the assessment of long-term efficacy (Finnberg et al., 2017; Yuki et al., 2020). Microspheres derived from human tumor suspensions containing immune cells in custom microfluidic devices have demonstrated responses to immunotherapy but lack tumor immunospecificity (Deng et al., 2018; Jenkins et al., 2018a). The static nature of co-cultures fails to recapitulate the dynamic immune cell recruitment and chemotaxis observed *in vivo*, reducing the predictive accuracy of therapies that rely on trafficking signals (Jenkins et al., 2018b). Comprehensive assessment of all stromal and immune components is also impeded. Furthermore, excessive immune cell proliferation can create interfering imaging features, leading to false-positive results (Stüve et al., 2023).

Advanced immune organoid platforms have emerged to overcome these challenges. Air-liquid interface organoids retain the *in vivo* association between native tumor-infiltrating lymphocytes and tumor cells *in vitro*, enabling robust assessment of the efficacy of PD-1 blockade (Neal et al., 2018; Li et al., 2016; Elbadawy et al., 2018; Li et al., 2014; Katano et al., 2013). Furthermore, a study has established an *in vitro* immune assessment platform based on patient-derived colorectal cancer organoids. Combined with CAR-natural killer cells, this platform can quantitatively monitor effector cell recruitment and tumor lysis in real time, and assess the killing efficacy and specificity of antigens such as EPCAM, thereby enabling personalized immunotherapy screening and off-target risk verification. (Schnalzger et al., 2019). Similarly, immune-enhancing organoids incorporating macrophages or dendritic cells can mimic antigen presentation and myeloid suppression, providing new insights into combination immunotherapy (Jiang et al., 2023; Chen et al., 2021; Subtil et al., 2023).

Furthermore, the Glioblastoma-on-a-Chip system enables real-time monitoring of T cell and tumor-associated macrophage recruitment by different glioblastoma subtypes. It analyzes the spatial expression patterns of PD-1 immune checkpoint and immunosuppressive factors such as the and TGF-β, thereby optimizing personalized immunotherapy strategies (Cui et al., 2020). PDCCs' immune models also provide a patient-specific testbed for next-generation immunotherapies. By screening different chimeric antigen receptors in CAR-T cells co-cultured with PDCCs organoids, researchers can identify the most effective designs *in vitro* (Figure 3d) (Yu et al., 2021; Kumari et al., 2021; Zou et al., 2021).
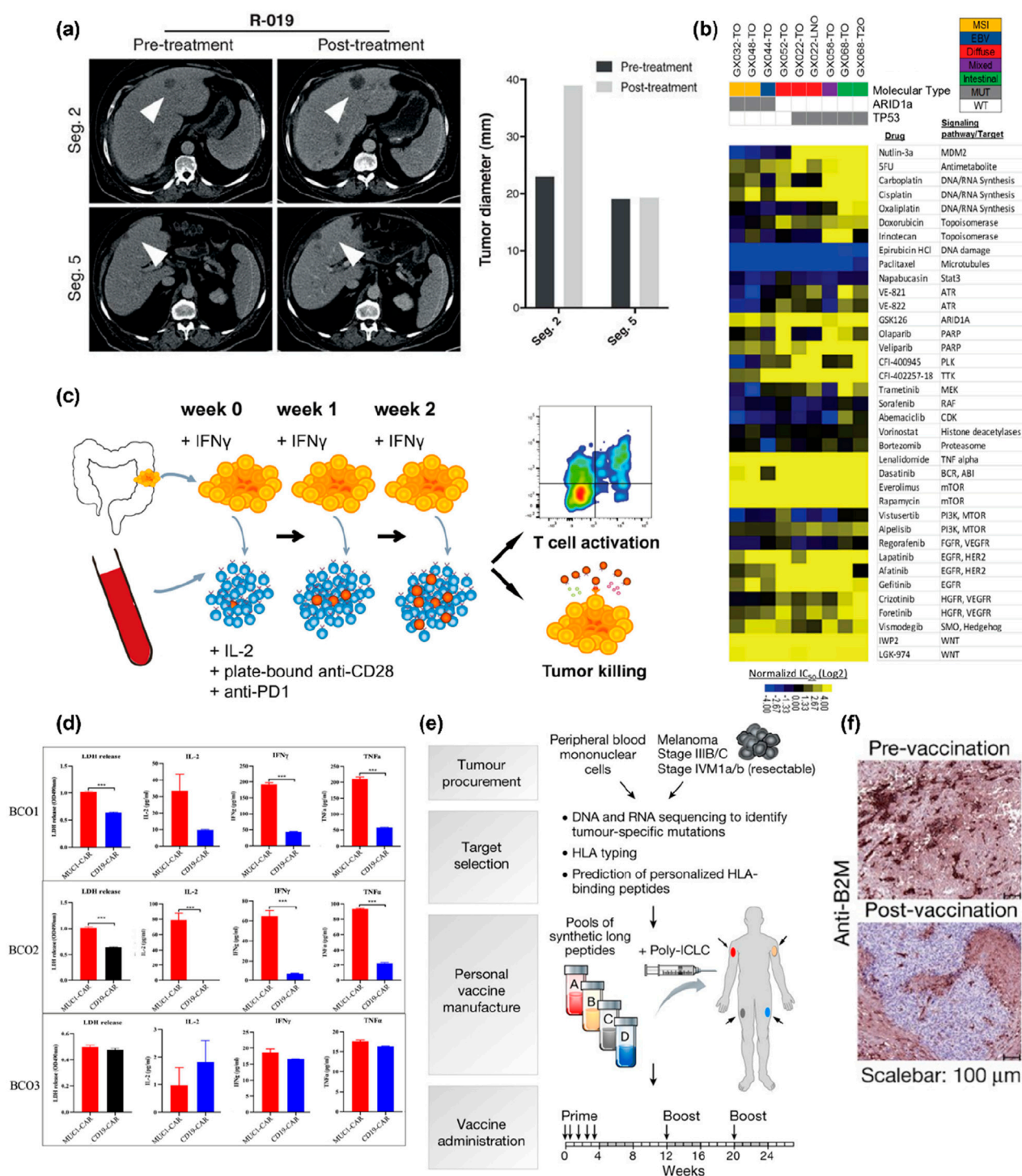
FIGURE 3
Applications of PDCC Cultures in Personalized Therapy. **(a)** Patient-derived organoids were established from a patient (R-019) with a mixed response to TAS-102. Whereas the segment two metastasis rapidly progressed, the segment five metastasis remained stable upon TAS-102 treatment (white arrowheads in the CT scan indicate metastases; bars represent pre- and posttreatment measurements of the indicated metastases). The images are reproduced from the reference (Vlachogiannis et al., 2018) with permission from AAAS, copyright 2018. **(b)** Large-scale drug screening in patient-derived organoid cultures. Heatmap of IC_{50} values for 37 compounds used to treat nine gastric cancer organoids derived from seven patients. The relative sensitivity against individual drugs is expressed as log2-transformed IC_{50} values and mean-centered against the therapeutic plasma steady-state concentration (Css). Therapeutic plasma concentrations of individual drugs (if available) are indicated on the x-axis of the dose-response graph. The images are reproduced from reference (Yan et al., 2018) with permission from Elsevier, copyright 2018. **(c)** Experimental workflow. Tumor organoids were established from dMMR CRC (resections or biopsies of primary tumors or metastases). They stimulated with IFNγ for 24 h before co-culture with peripheral blood lymphocytes (PBLs) from the same patient. PBLs were stimulated weekly with fresh tumor cells. After 2 weeks of co-culture, T cell effector functions and sensitivity of tumor organoids to T-cell-mediated killing were evaluated using flow cytometry. The images are reproduced from reference (Dijkstra et al., 2018) with permission from Elsevier, copyright 2018. **(d)** Quantification of lactate dehydrogenase release and cytokines products
(Continued)

FIGURE 3 (Continued)

(IL-2, IFN-γ and TNF-α) from Bladder cancer organoids after co-culture with either MUC1 CAR-T cells or CD19 CAR-T cells. Values represent the mean ± SEM (n = 3; unpaired parametric t-test; ***P < 0.001). The images are reproduced from reference (Yu et al., 2021) with permission from John Wiley and Sons, copyright 2021. **(e)** Generation of a personal, multi-peptide neoantigen vaccine for patients with high-risk melanoma. Somatic mutations were identified by whole-exome sequencing of melanoma and germline DNA, and their expression confirmed by tumour RNA-seq. Immunizing peptides were selected based on human leukocyte antigen binding predictions. Each patient received up to 20 long peptides in four pools. The images are reproduced from the reference (Ott et al., 2017) with permission from Springer Nature, copyright 2017. **(f)** B2M staining of melanoma cells in pre- and post-vaccination metastases. Whereas the pre-treatment tumour sample of P04 stained almost homogeneously for B2M, all tumour cells in the post-vaccine resectate were B2M-negative. The images are reproduced from the reference (Sahin et al., 2017) with permission from Springer Nature, copyright 2017. Human primary and metastatic pancreatic tumor organoid cultures (hT).

In summary, PDCCs culture technology reproduces the laboratory's interaction between tumors and the immune system, providing functional data for personalized immunotherapy research. With the help of new technologies such as microfluidic immuno-oncology chips, the culture of PDCCs is gradually becoming an important tool for predicting the efficacy of checkpoint inhibitors and customising cell therapy.

3.3 Personalized vaccine design

Personalized vaccine design is gradually becoming an important strategy for precision tumor immunotherapy. This method usually uses the patient's tumor cells as an antigen library, identifies tumor-specific neoantigens through high-throughput sequencing and bioinformatics, and then constructs a vaccine targeting the patient's specific mutations (Ott et al., 2017) (Figure 3e). *In vitro* models constructed using PDCCs, tumor spheroids or organoids can screen and verify the immunogenicity of candidate neoantigens in a short time while evaluating the effect of vaccines on activating T cell responses (Ott et al., 2017; Sahin et al., 2017) (Figure 3f). This personalized vaccine based on PDCCs can construct a personalized immune strategy for tumor-specific antigens and overcome the problem of tumor immune escape, providing patients with more precise and effective treatment options.

3.4 Real-time monitoring

In recent years, integrating microfluidic chips with machine learning has significantly improved the sensitivity and specificity of real-time patient-derived circulating tumor cells (CTCs) detection. For example, the fusion of microfluidics and deep learning in CTC detection enables automated classification and real-time monitoring, promising applications for the dynamic tracking of early-stage and metastatic cancers (Kumari et al., 2021; Shen et al., 2023; Shickh et al., 2022). Similarly, a dye-free, real-time imaging technique based on digital holographic phase microscopy and machine learning can distinguish cancer cells from blood cells at a rate of tens of cells per second, with an accuracy of 92.56% (Nissim et al., 2021). Furthermore, vapor nanobubbles-enhanced Cytophone technology has been used noninvasively to detect CTCs in melanoma patients. Accurate diagnosis was achieved in 27 of 28 patients, with a detection limit as low as 1 CTC per liter of blood, approximately 1,000-fold higher than existing detection methods (Galanzha et al., 2019).

However, the transition from research to clinical application still faces numerous obstacles. Inconsistent technical standards, high equipment costs, inadequate laboratory staff training, and incomplete regulatory policies have severely limited the adoption of liquid biopsy in routine clinical practice (Alix-Panabières and Pantel, 2021; Shickh et al., 2022; Lone et al., 2022; Martins et al., 2021; Mizutani et al., 2010). Furthermore, the inherent heterogeneity of CTCs and their extremely low concentration in blood present significant challenges for their capture and analysis (Vermesh et al., 2018; Lux et al., 2021; Chen X. et al., 2022; Kapeleris et al., 2022; Jiang W. et al., 2020; Andree et al., 2016). This leads to poor reproducibility, high false-negative/false-positive rates, and insufficient clinical confidence.

3.5 Clinical translation

The ultimate goal of PDCCs in personalized medicine is to inform and improve patient care directly. Although still in the experimental and early trial stages, significant progress has been made in the clinical translation of patient culture results.

One approach is combined clinical trials, whereby a patient receives standard care or an experimental treatment, and their tumour is studied in the laboratory in parallel using PDCC cultures. The laboratory test results are then compared with the patient's clinical response to adjust the treatment regimen. For example, in a metastatic gastrointestinal cancer trial, organoid tests established with PDCCs were used to screen alternative therapies (Vlachogiannis et al., 2018). In another case of glioblastoma, organoids from a patient's tumor predicted drug sensitivity, thereby guiding clinical decisions and observing tumor regression (Jacob et al., 2020a).

The main challenges facing clinical translation are scalability and turnover rate. These include ensuring that a high proportion of patient samples can quickly generate effective cultures (Centenera et al., 2018; Fu et al., 2021), especially since the success rate of some lung cancer organoids is low (Kim et al., 2019). And establishing an efficient process from hospital to laboratory to clinic. To in obtaining formal approval and widespread application. Multidisciplinary coordination and regulatory validation also need to be addressed to ensure the standardisation and predictive reliability of PDCCs testing.

Despite the challenges, PDCCs are gradually moving from the laboratory to the clinic and have played a role in trial design, such as screening out drug-sensitive patient subgroups, thereby providing information for biomarker-driven trial recruitment. PDCCs culture

technology is providing solid support for the realization of truly personalized cancer treatment.

4 Current challenges

Despite the powerful capabilities of PDCC cultures for therapeutic applications, critical technical challenges, clinical translation, and ethical issues need to be addressed to ensure their reliable and responsible use.

4.1 Challenges in technology and clinical transformation

Differences in organoid culture conditions (e.g., media composition, growth factors, and ECM types) exist between laboratories (Neal et al., 2018; Maru and Hippo, 2019; Huch et al., 2017; Tuveson and Clevers, 2019), leading to inconsistent drug response results for the same tumor type, complicating comparisons between studies. For clinical application, it is urgent to develop unified standard operating procedures, including consensus culture media, chemically well-defined matrices, and unified assay readout standards (Gjorevski et al., 2016), and to verify reproducibility through inter-lab ring trials (Niepel et al., 2019).

Tumor heterogeneity is a significant challenge for PDCCs culture, as clonal selection may occur during *in vitro* culture, resulting in overgrowth of specific subclones and loss of representation of the original tumor (Broutier et al., 2017; Gao et al., 2014; Boretto et al., 2019). To address this issue, researchers are working to ensure that organoids retain the diversity of real tumors by optimizing culture media (van de Wetering et al., 2015; Gjorevski et al., 2016), validating key mutations (Farshadi et al., 2024) and utilizing single-cell sequencing (Wang et al., 2022). While maintaining heterogeneity is a necessary and challenging task, organoids currently perform quite well in this regard.

Not all patient samples can generate usable cultures, especially tumors with genomically unstable or extensive necrosis (e.g., the success rate for some lung cancer subtypes is less than 50%) (Dijkstra et al., 2020), which may lead to research biased towards those tumors that are easy to grow. To improve success and fairness, researchers are exploring improved culture techniques (such as air-liquid interface culture) (Esser et al., 2020), multiple sampling strategies, and *in vivo* support (such as short-term patient-derived xenograft passage) (Daniel et al., 2009; Rubio-Viqueira and Hidalgo, 2009; Simpson-Abelson et al., 2008) to expand the scope of application of PDCCs culture.

The time factor is critical for clinical applications because if organoid drug screening results take 2–3 months to be available, patients may have already switched to other treatments. Researchers are speeding up organoid expansion and testing to shorten this time by optimizing culture media (Georgakopoulos et al., 2020), automated processing (Jiang S. et al., 2020), and imaging readouts. *In vitro* tissue slice culture is faster (Centenera et al., 2013) (drug testing can be performed within a week), and the experience can provide a reference for improving PDCCs organoid co-culture methods.

4.2 Sample logistics and regulatory challenges

PDCCs are increasingly used in preclinical research and personalized medicine due to their ability to recapitulate patient-specific tumor phenotypes. However, the clinical translation of PDCC-based platforms is hampered by sample logistics and regulatory frameworks. From a logistical perspective, current tissue collection and processing workflows are fragmented across institutions. For instance, Yoko S. DeRose demonstrated that breast tumor specimens must be transported on ice immediately after surgical resection to minimize ischemic time and preserve tissue viability, which is essential for successfully establishing patient-derived models (DeRose et al., 2013). Else Driehuis further reported that placing freshly resected tumor tissue in ice-cold culture medium supplemented with Rho kinase inhibitor significantly enhances cell viability and organoid formation efficiency, with organoid generation remaining feasible after up to 72 h of cold storage at 4 °C (Driehuis et al., 2020). In addition, Michael Gock and colleagues showed that transport delays negatively affect model establishment. However, Matrigel can significantly improve tumour engraftment rates (Ding et al., 2022) during delayed cooling conditions.

From a regulatory standpoint, ensuring cross-laboratory consistency and reproducibility is essential to generating reliable, clinically actionable results. In a colorectal cancer drug screening study, the pilot study of only eight patients was expanded to a registrational clinical trial of 250 patients (NCT05189171) to verify its sensitivity and reproducibility in predicting drug responses. This move aims to meet the key regulatory requirements for data consistency and traceability of results in *in vitro* diagnostic models for clinical applications (Ding et al., 2022). In the application of patient-derived 3D culture systems as disease-specific drug sensitivity models, it is emphasized that quality control and cross-laboratory consistency must be met before entering clinical translation. For example, by systematically evaluating the reproducibility indicators of IC_{50} and maximum inhibition rate, the stability and consistency of the organoid drug sensitivity testing platform in different batches are verified to meet the reproducibility standards in the test performance regulatory guidelines (Boehnke et al., 2016).

4.3 Ethical challenges

Regarding ethics, PDCC culture involves informed consent, privacy protection, ownership, and commercialization issues. Patients are required to understand the use of their tumor samples, storage period, and sensitive genetic information that may be generated (Mollaki, 2021). There is an obligation to protect patient privacy, and whole genome sequencing of organoids may reveal sensitive information about germline mutations (Mollaki, 2021; Hendriks et al., 2020; Driehuis and Clevers, 2017). In addition, due to the high cost of personalized PDCC organoid testing, measures must be taken to ensure this technology is accessible to patients at all levels. The regulatory framework is still unclear (Munsie et al., 2017), and guidelines are urgently needed to ensure strict quality control and

validation of PDCCs in clinical applications and to clarify their status in companion diagnostics.

5 Future development potential

The field of PDCC culture is rapidly evolving. Several exciting developments are expected to enhance PDCC culture further and expand its use in research and personalized medicine.

5.1 Combining advanced micro-engineering

The combination of PDCC culture with microfluidics and bioengineering methods is expected to be further deepened. Future tumour chip models will integrate multiple tissue types and achieve synergistic interactions between tumours, the immune system and normal tissues through microfluidics (Aleman and Skardal, 2019), 3D bioprinting (Chen et al., 2022b; van Pel et al., 2018) and sensors (Field 154, 155) (Figure 4a). The ultimate goal is to build personalized microphysiological systems to simulate "clinical trials on a chip" to predict tumor response, normal tissue toxicity and pharmacokinetics, formulating comprehensive personalized treatment plans.

5.2 AI Across Domains

As data generated from PDCCs' culture experiments becomes increasingly complex, artificial intelligence will become a key tool for extracting actionable insights (Kong et al., 2020; Hua et al., 2024). Organoid pharmacogenomic data and network-based computational methods have successfully derived reliable drug biomarkers to treat human tumors (Kong et al., 2020). A novel deep neural network can effectively detect organoids and dynamically track them throughout the culture process (Bian et al., 2021). In addition, large databases of patient-derived culture data could be used to train predictive models (Bian et al., 2021).

Tumor spheroids, a popular preclinical model, lack reliable image segmentation. A fully convolutional network, including U-Net and HRNet, automatically segments treated and untreated multicellular tumour spheroids, achieving a Jaccard index of approximately 90% and segmentation error comparable to interobserver variability (Streller et al., 2025). This work enables more reproducible and high-throughput quantification of treatment responses in 3D tumor models, complementing software tools for high-throughput image analysis of spheroids. Using matched colorectal tumor–organoid transcriptomes, Zhang et al. developed a network-based biomarker selection strategy to predict patient-specific chemotherapy responses, achieving superior performance compared to conventional gene prioritization approaches (Zhang W. et al., 2025) (Figure 4b). Furthermore, by combining longitudinal imaging of patient-derived tumor organoids with AI-powered segmentation and mathematical modeling, the study achieved high-resolution tracking of individual organoid growth trajectories, laying the foundation for further modeling efforts aimed at predicting treatment response kinetics and the duration

of drug resistance (Gunnarsson et al., 2024) (Figure 4c). Fillioux et al. developed a deep learning framework that integrates segmentation (SAM), feature extraction (DINOv2), and attention-based multiple-instance learning to analyze time-lapse videos of patient-derived organoids, enabling accurate, non-invasive prediction of chemotherapeutic efficacy over time (Fillioux et al., 2023). While these methods allow efficient and precise detection, classification, and measurement of PDCC culture platforms, they often require programming skills to create specialised code to train the networks and process the images (Spiller et al., 2021). Deep learning-based analyses can be very effective when processing large datasets, but extracting specific information requires additional data processing. Furthermore, overlapping organoids formed by PDCCs have contacts not present in individual organoids, making segmentation difficult using deep learning image processing tools (Park et al., 2023).

5.3 Enhancing the integration of the tumor microenvironment

Future PDCC cultures aim to fill gaps in TME mimicry by recreating a more realistic tumor microenvironment through vascularization strategies (such as co-culture with endothelial cells to form perfused capillaries (Silvestri et al., 2020)) and integrating a more complex immune system. Some studies have also explored microbiome-tumor co-cultures to evaluate the impact of the gut microbiota on cancer progression and immunotherapy response (El-Derby et al., 2024). Although there are challenges in maintaining these additional components without disrupting the core tumor culture, incremental progress brings us closer to building a "tumor ecosystem in a dish."

5.4 Automation, standardization, and data accessibility

Automation and scale-up are essential to making the clinic's PDCC culture model widely used. The next-generation of culture platforms will use robotic systems to automatically process each step from tissue dissociation to organoid generation and drug testing (Zhang et al., 2017) to achieve high-throughput processing, improve consistency and reduce costs. At the same time, a central facility or "living biobank" (Sachs et al., 2018) linked to the hospital will be established to send the patient's tumor to the hospital, conduct organoid or drug testing, and quickly report to the clinician.

The lack of standardised protocols for establishing PDCC culture platforms can lead to batch-to-batch variability and an overall lack of quality control across and within institutions (Brancato et al., 2020). Furthermore, establishing PDCC culture platforms is technically challenging and requires highly trained personnel to handle and prepare patient-derived cells (Kondo and Inoue, 2019). Therefore, standard operating procedures must be adopted to govern each step, including sample handling, culture conditions, quality control measures, and result interpretation, to ensure consistent and reproducible laboratory results (Xiang et al., 2024). This is crucial for providing reliable and comparable results
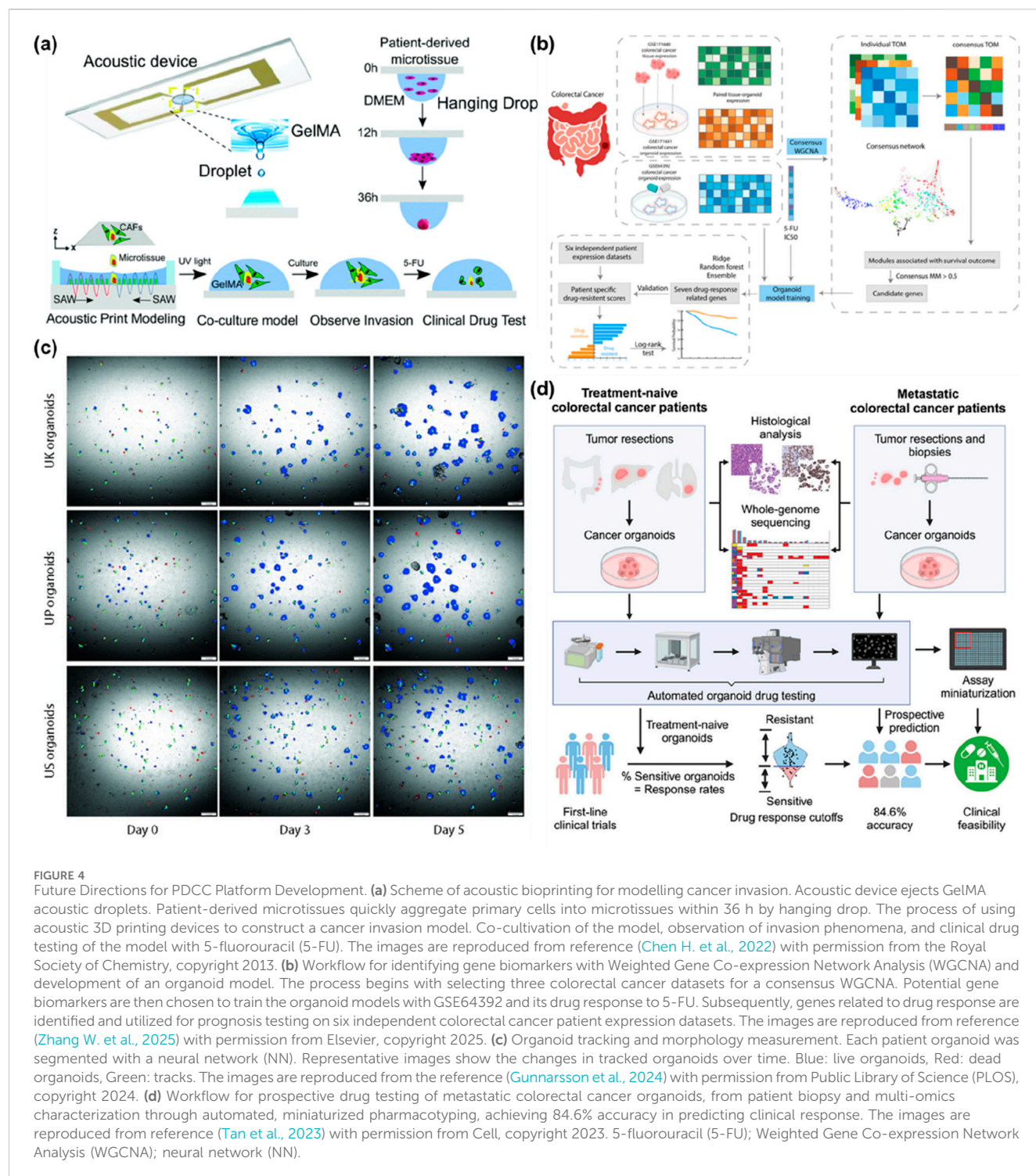
FIGURE 4
Future Directions for PDCC Platform Development. **(a)** Scheme of acoustic bioprinting for modelling cancer invasion. Acoustic device ejects GelMA acoustic droplets. Patient-derived microtissues quickly aggregate primary cells into microtissues within 36 h by hanging drop. The process of using acoustic 3D printing devices to construct a cancer invasion model. Co-cultivation of the model, observation of invasion phenomena, and clinical drug testing of the model with 5-fluorouracil (5-FU). The images are reproduced from reference (Chen H. et al., 2022) with permission from the Royal Society of Chemistry, copyright 2013. **(b)** Workflow for identifying gene biomarkers with Weighted Gene Co-expression Network Analysis (WGCNA) and development of an organoid model. The process begins with selecting three colorectal cancer datasets for a consensus WGCNA. Potential gene biomarkers are then chosen to train the organoid models with GSE64392 and its drug response to 5-FU. Subsequently, genes related to drug response are identified and utilized for prognosis testing on six independent colorectal cancer patient expression datasets. The images are reproduced from reference (Zhang W. et al., 2025) with permission from Elsevier, copyright 2025. **(c)** Organoid tracking and morphology measurement. Each patient organoid was segmented with a neural network (NN). Representative images show the changes in tracked organoids over time. Blue: live organoids, Red: dead organoids, Green: tracks. The images are reproduced from the reference (Gunnarsson et al., 2024) with permission from Public Library of Science (PLOS), copyright 2024. **(d)** Workflow for prospective drug testing of metastatic colorectal cancer organoids, from patient biopsy and multi-omics characterization through automated, miniaturized pharmacotyping, achieving 84.6% accuracy in predicting clinical response. The images are reproduced from reference (Tan et al., 2023) with permission from Cell, copyright 2023. 5-fluorouracil (5-FU); Weighted Gene Co-expression Network Analysis (WGCNA); neural network (NN).

across institutions and developing clinical implementation guidelines. Tao Tan described a unified framework for predictive testing based on patient-derived colorectal cancer organoids, encompassing standard chemotherapy, biologics, and targeted therapy regimens (Tan et al., 2023) (Figure 4d). Interlaboratory variability and the lack of standardized protocols limit reproducibility and scalability. To overcome these obstacles, future work should focus on developing standardized culture methods and shared biobanks (Heydari et al., 2025). Training programs and collaborative platforms can also help disseminate technical expertise. The CancerModels.Org platform embodies a global open-access framework for patient-derived cancer models that standardizes, harmonizes, and integrates clinical, genomic, and functional datasets according to FAIR principles, significantly enhancing accessibility and data sharing across the research community (Perova et al., 2025).

5.5 Clinical implementation and personalized treatment algorithms

In the next 5–10 years, PDCCs data may be integrated with information such as genomic sequencing into clinical decision-making to support tumor boards, and oncologists will also use artificial intelligence to support decision-making based on these *in vitro* functional data (Kong et al., 2020). Personalized treatment plans based on PDCC will guide first-line treatment and provide a basis for maintenance strategies and next-line selection. At the same time, regular biopsies to generate organoids can achieve real-time monitoring, detect drug-resistant mutations early, and adjust treatment.

6 Conclusion

PDCC models, encompassing 2D monolayers, 3D spheroids, organoids, co-culture systems, and microfluidic platforms, have collectively transformed our ability to recapitulate patient-specific tumor biology and drug responses. We discuss in detail the key applications of PDCC models in personalized drug screening, immunotherapy evaluation, individualized vaccine design, real-time monitoring, and clinical translational research. While advances in advanced microengineering, AI-driven analytical techniques, and enhanced integration of the tumor microenvironment continue to refine these platforms, widespread challenges remain in clinical translation, standardized sample logistics, quality management, and navigating the evolving regulatory landscape. By overcoming these obstacles through multidisciplinary collaboration and robust validation studies, PDCCs technology has the potential to become an indispensable tool in precision oncology, enabling truly personalized treatment strategies and accelerating the translation of laboratory discoveries into patient benefit.

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YF: Writing – original draft. BK: Writing – review and editing. CL: Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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