


## REVIEW ARTICLE

# Colorectal cancer organoids: Construction, applications, and hydrogel microsphere-based engineering strategies

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## Abstract

Colorectal cancer (CRC) is one of the most prevalent malignancies globally, characterized by high incidence and mortality rates. Its marked heterogeneity and complex tumor microenvironment (TME) pose considerable challenges to traditional preclinical models. Patient-derived organoids (PDOs) have emerged as pivotal tools for elucidating disease mechanisms, enabling personalized drug screening, and advancing precision medicine, as they faithfully preserve the histological structure, molecular features, and genetic heterogeneity of primary tumors in vitro. However, conventional matrix gel-based culture systems suffer from inherent limitations, including ill-defined composition, significant batch-to-batch variation, and a lack of precise control over mechanical properties, which impede their ability to faithfully recapitulate dynamic intercellular crosstalk and spatiotemporal TME heterogeneity. Recent advances in biomaterials and tissue engineering have provided new opportunities to innovate organoid technologies. This review systematically summarizes the establishment strategies, major applications, and core challenges of CRC organoids, highlighting the potential of modular and programmable biomaterials, particularly hydrogel microspheres, for developing novel construction systems. We propose that hydrogel microspheres, with well-defined chemical composition, tunable mechanical properties, and function as basic building blocks, can be integrated with modular or controllable assembly strategies to construct next-generation CRC organoid models with enhanced biomimetic properties, greater structural complexity, and improved reproducibility. In summary, this review outlines core challenges and future directions, emphasizing that deep integration of engineered culture systems with organoid technology is a critical approach to advancing CRC research toward improved biomimetic recapitulation and greater clinical translational relevance.

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## 1. Introduction

Colorectal cancer (CRC) is a malignancy of the digestive tract characterized by persistently high global incidence and mortality rates. It ranks among the most commonly diagnosed cancers worldwide, posing a substantial burden on public health systems.<sup>1,2</sup> Currently, the mainstay of clinical management for CRC involves surgical resection combined with chemoradiotherapy. Nevertheless, this conventional strategy presents considerable limitations. On the one hand, CRC displays pronounced tumor heterogeneity, with marked genetic and phenotypic diversity observed not only among patients but also within individual tumors. Such heterogeneity contributes to inconsistent treatment responses and divergent clinical outcomes.<sup>3</sup> On the other hand, the development of chemoresistance often leads to treatment failure, a challenge particularly pronounced in patients with advanced, recurrent, or metastatic disease, for whom effective treatment options remain severely limited.<sup>4</sup>

Conventional research models, including cell lines and animal xenograft models, are often inadequate for recapitulating the complex tumor microenvironment (TME) and genetic diversity of human cancers. This discrepancy has resulted in frequent failures of drugs that show promising efficacy in preclinical settings to translate into successful clinical outcomes.<sup>5</sup> Consequently, there is a pressing need to develop novel *in vitro* models capable of faithfully preserving the features of primary tumors and facilitating personalized research. Organoids have emerged as a promising three-dimensional (3D) culture system that retains the intrinsic architecture, genetic profile, and cell–cell interactions of the original tumor tissue. These models offer a powerful platform for studying CRC pathogenesis, enabling patient-specific drug screening, investigating drug resistance, and advancing precision oncology.<sup>6–8</sup>

Despite remarkable progress in organoid technology, current mainstream protocols remain heavily dependent on animal-derived hydrogel matrices. Such substrates possess inherent drawbacks, including poorly defined composition, significant batch-to-batch variability, and limited tunability of mechanical properties. Consequently, they not only fall short of capturing the heterogeneous

nature of the native human extracellular matrix (ECM), but also fail to faithfully model the intricate TME.<sup>9–11</sup> Hence, the development of novel biosynthetic scaffolds with well-defined chemistry and adjustable physicochemical properties represents a crucial step toward standardizing, engineering, and clinically translating organoid-based platforms.

In this context, hydrogels have emerged as ideal alternatives to animal-derived matrices, owing to their excellent water-retention capacity and physicochemical characteristics that closely mimic the native ECM.<sup>12,13</sup> Importantly, when fabricated into micrometer-scale spherical constructs, termed hydrogel microspheres, they can be engineered into highly tunable functional modules.<sup>14</sup> Through rational and precise modulation of their material composition, mechanical stiffness, degradation kinetics, surface functional ligands, and encapsulated bioactive cargoes, these microspheres can act as modular building blocks for assembly with tumor cells. This approach thus provides a promising avenue for multi-scale and multi-dimensional recapitulation and spatiotemporal regulation of the TME.<sup>15</sup>

This review provides a systematic overview of the current state, recent progress, and major limitations of CRC organoid research. Furthermore, it discusses the potential of hydrogel microsphere-based engineering strategies to develop CRC organoid models that more faithfully recapitulate the physiological TME.

## 2. Evolution and comparative analysis of CRC research models

Over the course of CRC research, experimental models have been instrumental in driving both fundamental discoveries and clinical advances. From conventional two-dimensional cell cultures to animal xenograft systems, and more recently, to the widely adopted organoid technology, each model has provided essential tools for investigating stage-specific biological questions. Conversely, their respective limitations have consistently spurred the development and emergence of newer, more refined modeling approaches (Table 1).

**Table 1. Systematic comparison of preclinical research models for CRC**

Comparison	2D cell	PDX	Organoid	Ref.
Construction cycle	Short (several days)	Long (several months to half a year)	Moderately short (several weeks)	16

(Cont'd...)

**Table 1. (Continued)**

Comparison	2D cell	PDX	Organoid	Ref.
Cost	Low (affordable via routine 2D culture with minimal reagents)	High (cost-intensive due to murine housing, engraftment maintenance, and long experimental cycles)	Moderate (balanced cost for 3D matrix reagents and co-culture components, if applicable)	17,18
Genetic fidelity	Low (susceptible to genetic drift during serial passaging)	High (retains the genomic characteristics of primary tumors during <i>in vivo</i> passaging)	High (recapitulates the genetic heterogeneity and genomic stability of patient-derived tumors <i>in vitro</i> )	19,20
TME simulation	Not achievable	Partially achievable (retains <i>in vivo</i> TME but lacks functional immune components)	Partially achievable (can be reconstituted with key microenvironmental components via co-culture strategies)	21,22
Throughput capacity	High (well-suited for high-throughput screening)	Extremely low (not amenable to high-throughput applications)	Moderately high (amenable to scalable culture and high-throughput screening)	16,18
Tumor heterogeneity	Not maintainable	Well preserved (retains intratumoral heterogeneity of the original tumor)	Well preserved (recapitulates intratumoral and intertumoral heterogeneity <i>in vitro</i> )	19,23
Application scope	Molecular mechanism exploration, gene function research, and preliminary drug screening	<i>In vivo</i> efficacy validation, biomarker discovery and validation, and metastasis mechanism investigation	Personalized drug sensitivity testing, high-throughput drug screening, disease modeling, and toxicity assessment	24,25
Gene editing feasibility	Highly feasible (amenable to efficient gene editing via routine protocols)	Poorly feasible (hindered by <i>in vivo</i> delivery barriers and long engraftment cycles)	Feasible (compatible with <i>ex vivo</i> gene editing prior to 3D culture)	26,27
Immune crosstalk capability	Not feasible (lacks native immune components for crosstalk)	Limited (only achievable in humanized mouse models to recapitulate human immune crosstalk)	Feasible (functional immune crosstalk can be recapitulated via immune-tumor organoid co-culture systems)	28
Ethical and species considerations	Minimal ethical concerns	Subject to animal ethics regulations; associated with interspecies discrepancies between human tumors and murine hosts	Low ethical risk; free of interspecies compatibility issues	29

Abbreviations: CRC: Colorectal cancer; PDX: Patient-derived xenograft; 3D: Three-dimensional; TME: Tumor microenvironment; 2D: Two-dimensional.

## 2.1. Two-dimensional cell culture models

Two-dimensional (2D) cell culture models represent the earliest *in vitro* systems widely employed for tumor research. These models are established by seeding cells isolated from tumor tissues into flat-bottomed culture dishes and culturing them in nutrient-rich medium until they form a

confluent monolayer.<sup>30</sup> This model offers notable technical advantages, including ease of operation, cost-effectiveness, rapid cell proliferation, compatibility with genetic manipulation, and applicability for high-throughput drug screening.<sup>26</sup> In the early stages of CRC research, 2D cell lines have contributed substantially to elucidating key molecular

mechanisms, such as oncogene functions and signaling pathway regulation.<sup>31,32</sup> For instance, Zhu *et al.*<sup>33</sup> utilized HCT116 and other cell lines to demonstrate the pivotal role of the *MyD88* gene in mediating CRC cell proliferation, invasion, and migration. However, the limitations of 2D models have become increasingly evident as research has advanced. A fundamental shortcoming is their inability to recapitulate the 3D architecture and physiological context of tumors *in vivo*. These systems fail to mimic the spatial organization of tumor tissues and cannot reproduce the dynamic cellular crosstalk among tumor cells, stromal components, and immune cells within the TME.<sup>21</sup> More critically, prolonged *in vitro* passaging leads to genetic drift, causing the cells to lose the heterogeneity of the original tumor and thereby reducing their predictive value for clinical drug response.<sup>19</sup>

## 2.2. Patient-derived xenograft models

To more closely mimic the tumor growth environment in living organisms, patient-derived xenograft (PDX) models have been developed and established as a key standard in preclinical efficacy evaluation. The PDX model involves directly implanting fresh patient tumor tissue into immunodeficient mice, allowing the tumor to grow and persist in an *in vivo* setting.<sup>27</sup> Its primary strength lies in the high degree to which it preserves the original tumor's histopathology, molecular profile, and intratumoral heterogeneity.<sup>34</sup> Consequently, this model holds irreplaceable value for assessing *in vivo* drug responses, validating biomarkers, and studying the dynamics of tumorigenesis and progression.<sup>24</sup> Nevertheless, the PDX model also presents notable limitations. Its establishment requires an extended timeframe, often spanning several months, with variable success rates.<sup>17</sup> Additionally, the high costs, technical complexity, and limited scalability restrict its use in high-throughput drug screening. More critically, because immunodeficient hosts are used, PDX models cannot recapitulate interactions between the human immune system and tumor cells, thus restricting their utility in immuno-oncology research.<sup>22</sup> Furthermore, inherent species differences between mice and humans may affect the accuracy of drug metabolism and toxicity assessments.<sup>35</sup>

## 2.3. Organoid models

Organoid technology has emerged as a powerful and advanced platform for CRC research, effectively overcoming limitations associated with earlier model systems. Colorectal cancer organoids are 3D, self-organizing structures derived from patient tumor cells, capable of recapitulating key architectural and functional features of the original tumor *in vitro*.<sup>36</sup> A major advantage of this system is its ability to preserve the genetic profile, histological architecture, and cellular heterogeneity of the primary tumor.<sup>20</sup>

When compared to PDX models, organoids require a significantly shorter culture period, typically lasting only a few weeks, while also demonstrating higher success rates, easier cryopreservation, and greater scalability. These characteristics render them especially suitable for establishing living biobanks and conducting high-throughput drug screening.<sup>16,29,37</sup> A key advantage of organoid models is their exclusive human origin, which eliminates species-specific discrepancies. Furthermore, they can be engineered into more complex TME models through co-culture with stromal components such as immune cells and fibroblasts.<sup>38,39</sup>

Collectively, these attributes establish organoids as a highly promising tool for personalized oncology. By assessing the sensitivity of patient-derived organoids to chemotherapy, targeted agents, and immunotherapies, this platform can generate predictive insights to guide clinical treatment decisions, thereby supporting the advancement of precision medicine in CRC.<sup>40</sup>

Notably, it is important to distinguish true 3D organoid models from direct tissue biopsy culture. Organoids are defined as 3D structures that self-assemble *in vitro* from dispersed tumor cells or stem cells, relying on defined matrix components and exogenous cues to recapitulate tissue architecture. Their physiological complexity largely depends on the incorporation of multiple cell types, including tumor, stromal, endothelial, and immune cells, as well as the composition of the 3D matrix. In contrast, tissue biopsy culture mainly involves embedding intact or fragmented tissue explants in a matrix to preserve the existing tissue architecture and cell viability *ex vivo*, without *de novo* reconstruction. Clarifying this conceptual distinction helps avoid ambiguity and ensures accurate interpretation of model-derived results in CRC research.<sup>41</sup>

# 3. Establishment strategies and protocols for CRC organoids

## 3.1. Sample sources and preprocessing

Recent years have witnessed substantial progress in the establishment of CRC patient-derived organoids (PDOs). The successful establishment of these models relies on diverse tissue sources and systematic preprocessing protocols. Primary sample types currently include surgically resected specimens, colonoscopic biopsies, and cryopreserved tissues, each with distinct advantages and applications.

Surgically resected specimens typically provide a large amount of well-preserved tissue, facilitating the maintenance of tumor spatial heterogeneity and tumor-stroma interactions. Consequently, the success rate for generating organoids from these specimens is relatively



high, approximately 81.5% to 90%.<sup>18,42</sup> The standard processing workflow involves tissue cleaning, mechanical mincing, and enzymatic digestion (commonly using collagenase or trypsin) to obtain single cells or small clusters, which are then embedded into a 3D matrix for culture.<sup>23,43</sup> However, the invasive nature of surgical resection, which usually occurs after early diagnosis, limits the utility of these specimens for studies requiring dynamic monitoring or repeated sampling.

In contrast, colonoscopic biopsy represents a valuable source for early diagnosis and longitudinal studies owing to its minimally invasive nature and procedural convenience. A single biopsy typically yields 1–3 mm<sup>3</sup> of tissue. While the sample volume is limited and often contains a low proportion of tumor cells, culture efficiency can be increased to approximately 80% by optimizing sampling strategies (such as collecting at least 3–5 fragments from multiple sites, including the tumor margin and center) in combination with refined digestion and purification protocols.<sup>44,45</sup> Biopsy-derived specimens are particularly valuable for patients who are not surgical candidates, for monitoring responses to neoadjuvant therapy, and for guiding adaptive treatment strategies. A key limitation of this approach, however, is the potential for sampling bias, which may not fully capture the spatial heterogeneity of the original tumor.<sup>46</sup>

Furthermore, the use of cryopreserved tissues facilitates biobanking and multi-center collaboration. Studies confirm that human gastrointestinal biopsy specimens can be slowly frozen in a DMSO-based cryoprotective medium and stored at –80°C or in liquid nitrogen. Thawed tissues remain viable for organoid establishment, yielding cultures that are highly consistent with those derived from fresh tissues in morphology, growth kinetics, and gene expression profiles.<sup>47</sup> This approach not only mitigates time-sensitive processing constraints but also enables retrospective studies, analysis of rare cases, and the development of personalized treatment strategies.

### 3.2. Construction method

The most widely used technique for generating CRC organoids is the Matrigel-embedding method, also known as 3D suspension culture. This approach employs ECM-mimetic matrices such as Matrigel as a scaffold, which provides essential cell adhesion sites and growth-promoting signals. The standard workflow involves tissue dissociation, mixing of the resulting single cells or cell clusters with Matrigel, seeding into culture plates for gelation, and subsequent maintenance in specialized culture media.<sup>41</sup> This system is operationally mature, scalable, and compatible with high-throughput screening applications. However, it predominantly simulates the epithelial compartment and typically lacks critical stromal and immune components,

including fibroblasts and lymphocytes, which constitute the native TME.<sup>48</sup>

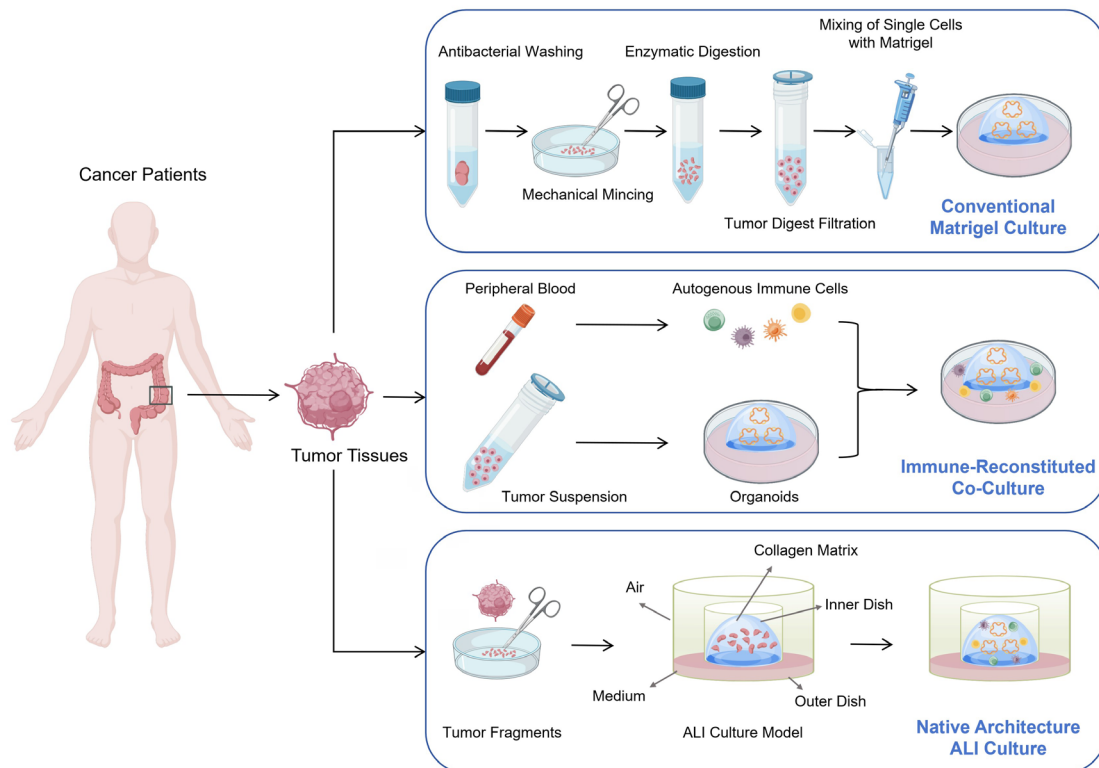
To address this limitation, the air-liquid interface (ALI) culture method has gained increasing attention. In this system, mechanically fragmented tumor tissue is embedded within a collagen gel and cultured on a Transwell insert.<sup>39</sup> This configuration enables efficient gas exchange at the apical surface while ensuring hydration and nutrient supply from the basal medium. As a result, the ALI method better preserves the original tumor stroma and endogenous immune cells (*e.g.*, T cells and macrophages), offering a robust platform for establishing highly physiologically relevant *in vitro* models of the tumor immune microenvironment<sup>49</sup> (Figure 1).

### 3.3. Cultivation system and key components

Regardless of the construction methodology used, culture system composition is critical for the successful establishment and long-term maintenance of organoids. The CRC organoid culture system originated from a systematic deconstruction of the signaling pathways present in the intestinal stem cell niche. By precisely modulating key growth factors and pathway inhibitors, this system successfully reconstitutes an *in vitro* organoid model capable of long-term self-renewal and multipotent differentiation.<sup>50</sup>

Advanced DMEM/F12 has been established as the universal basal medium for CRC organoid culture. It serves as the foundation for supplementing essential growth factors and small molecules to formulate a complete culture system.<sup>51</sup> The core of this system is a combination of three key factors that recapitulate the *in vivo* stem cell microenvironment: the Wnt agonist R-spondin-1, epidermal growth factor (EGF), and the bone morphogenetic protein (BMP) inhibitor Noggin.<sup>50</sup> These components act synergistically: Wnt pathway activation is essential for crypt stem cell proliferation and self-renewal, and its withdrawal leads to the loss of crypt structures and proliferation arrest.<sup>52,53</sup> EGF provides a core mitogenic signal for stem cell survival and expansion<sup>50</sup>, while Noggin, by inhibiting BMP signaling, relieves negative regulation on stemness and promotes crypt-like formation.<sup>54</sup> This “Wnt-EGF-Noggin” core represents the minimal requirement for sustaining long-term self-renewal and self-organization of CRC organoids.<sup>50</sup>

To specifically support the establishment and long-term expansion of human CRC organoids, and particularly to accommodate genomically unstable tumor cells, the core formulation is further optimized with specific small-molecule inhibitors.<sup>18</sup> Key additives include A83-01 (a TGF-β/activin/nodal pathway inhibitor) to block growth inhibition and differentiation, SB202190 (a p38 MAPK



**Figure 1.** Establishment workflow of colorectal cancer organoids. Created with BioGDP.com.  
Abbreviation: ALI: Air-liquid interface.

inhibitor) to mitigate cellular stress during digestion and early culture, and nicotinamide to improve metabolic adaptation and survival.<sup>51</sup> Additionally, supplements such as B27, N-acetylcysteine, and gastrin are routinely added. Together, these components create a stable, selective environment that promotes tumor cell proliferation while suppressing differentiation and apoptosis.<sup>18</sup>

A major advantage of this system is its ability to exploit the frequent Wnt pathway-activating mutations present in tumor cells. Under conditions of limited or absent exogenous Wnt ligands, tumor organoids, unlike their normal counterparts, can proliferate independently, enabling selective outgrowth of malignant cells.<sup>18</sup> Organoids cultured using this strategy faithfully retain the histological architecture, cellular heterogeneity, mutational landscape, and molecular subtypes of the primary tumor, providing a robust platform for building living biobanks and performing personalized drug testing and mechanistic studies.<sup>18,55</sup>

#### 4. Applications of CRC organoids

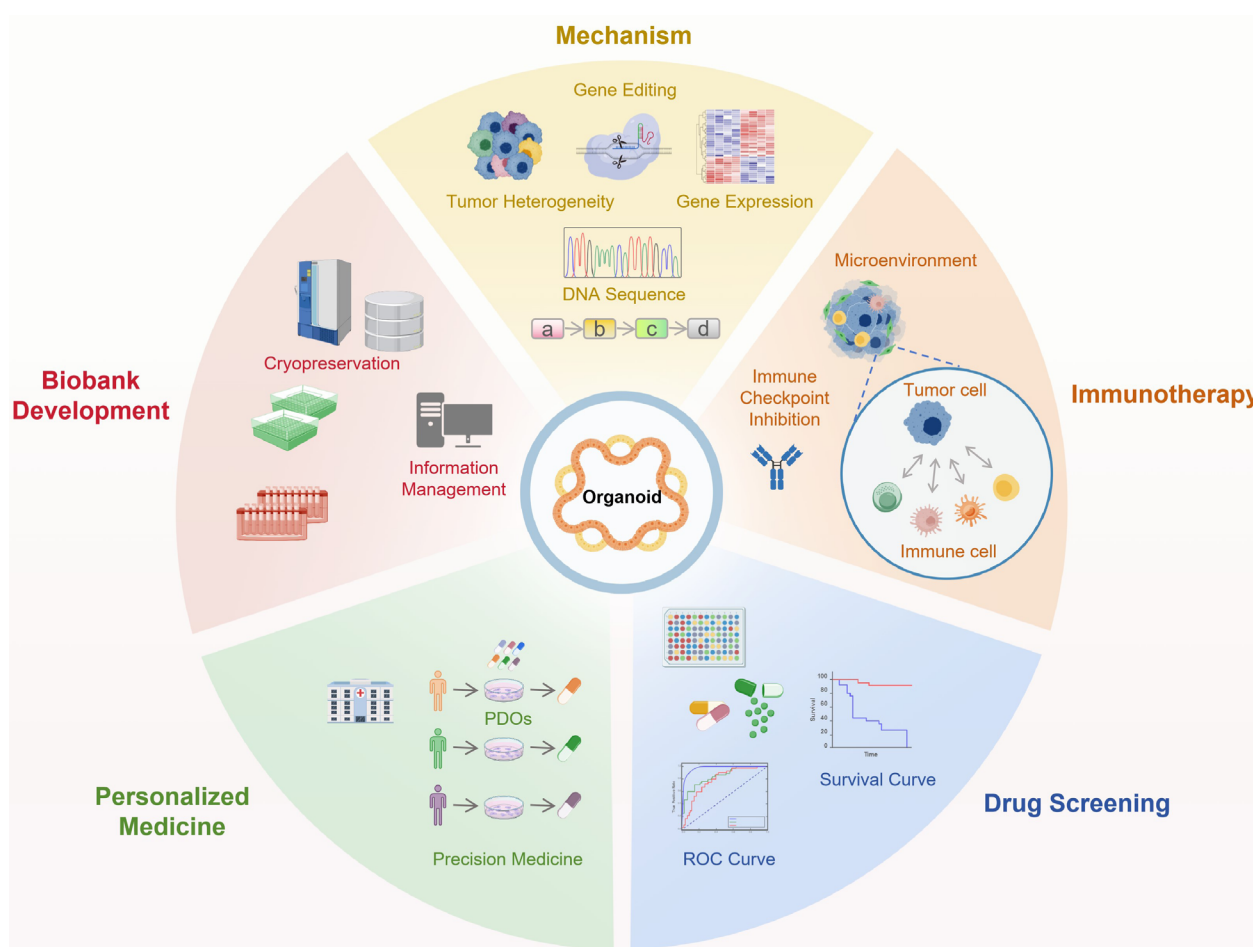
Colorectal cancer organoids, serving as highly physiologically relevant 3D *in vitro* models, hold significant promise for both basic research and clinical translation.

Their ability to preserve the genetic heterogeneity, histological architecture, and functional properties of primary tumors establishes them as a powerful platform for multifaceted investigations into CRC biology (Figure 2).

##### 4.1. Elucidating tumorigenesis and molecular mechanisms

Organoid models provide a unique system for modeling the carcinogenic process, including driver gene alterations and signaling pathway dysregulation in CRC. When combined with gene-editing technologies such as CRISPR-Cas9, sequential mutations in key driver genes (including APC, KRAS, TP53, and SMAD4) can be introduced into normal intestinal organoids. This approach enables the stepwise *in vitro* recapitulation of tumor progression from normal epithelium to adenoma and, ultimately, invasive carcinoma.<sup>56,57</sup> Studies utilizing these models have demonstrated that sustained activation of pathways such as Wnt/ $\beta$ -catenin, EGFR/MAPK, and p53 is critical for driving tumor initiation, maintaining stemness, and promoting metastasis.<sup>55,58</sup>

For instance, Xiong *et al.*<sup>59</sup> employed a chemically defined PDO system to capture a fetal-like plastic state in CRC, a phenotype associated with aggressive tumor



**Figure 2.** Multidimensional applications of colorectal cancer organoids. Created with BioGDP.com.  
Abbreviations: PDOs: Patient-derived organoids; ROC: Receiver operating characteristic.

behavior and poor prognosis. Their work identified the FGF2–AP-1 signaling axis as essential for maintaining this plastic state, offering a novel model for investigating the molecular mechanisms underlying tumor plasticity and therapy resistance. Furthermore, organoid models facilitate the functional study of cancer stem cells in processes such as self-renewal, differentiation, and drug resistance, providing critical insights into the mechanisms underlying tumor recurrence and metastasis.<sup>60</sup>

#### 4.2. Tumor microenvironment and immunotherapy

Conventional 3D organoids predominantly consist of epithelial cells and lack essential components of the native TME. To address this limitation, co-culture systems and ALI methods have been progressively integrated into organoid research. By co-culturing tumor organoids with cancer-associated fibroblasts (CAFs) and various immune cells, such as T cells and macrophages, complex *in vitro* models that incorporate both stromal and immune compartments have been successfully established.<sup>39,61</sup>

The ALI culture technique enables the maintenance of patient-derived tumor tissue fragments embedded in a collagen matrix for periods of up to 1–2 months. This approach fully preserves the endogenous immune cell populations (including T cells, B cells, and dendritic cells) and stromal cells, thereby generating a highly physiological model of the *in situ* immune microenvironment.<sup>49</sup> Building on this platform, immune-mechanistic studies and therapeutic evaluation can be further conducted. For instance, co-culturing patient-derived organoids with autologous peripheral blood lymphocytes or tumor-infiltrating lymphocytes enables the expansion and functional assessment of tumor-reactive T cells, offering predictive insights for personalized cellular immunotherapies such as CAR-T cell therapy.<sup>38,62</sup>

Moreover, microfluidic-based “mini-colon” models enable spatially compartmentalized co-culture of tumor cells, CAFs, tumor-infiltrating lymphocytes, and dendritic cells. This model dynamically simulates the processes of immune cell chemotaxis, infiltration, and tumor regression,

revealing the mechanism by which CAFs mediate immune escape through upregulation of PD-L1 expression in CRC cells.<sup>28</sup> Such models also demonstrate that PD-L1 blockade with agents such as atezolizumab can restore the activity of tumor-infiltrating lymphocytes and enhance cytotoxicity against microsatellite-unstable tumors, providing a reliable preclinical platform for *in vitro* evaluation of personalized immunotherapy strategies.<sup>28</sup>

### 4.3. Drug screening and novel drug discovery

Colorectal cancer organoids have become a pivotal platform for high-throughput drug screening and preclinical evaluation. Their capacity to faithfully recapitulate patient tumor heterogeneity and drug-response profiles enables broad applications in large-scale testing of chemotherapeutics, targeted agents, and novel compounds.<sup>18,23</sup> This model not only facilitates the identification of therapeutics against specific molecular subtypes but also supports the exploration of combination strategies to overcome drug resistance.<sup>55</sup>

A notable example is the work by Mao *et al.*,<sup>25</sup> who integrated computational drug prediction with organoid-based screening to systematically identify 34 marketed drugs with anti-CRC activity, such as fedratinib, trametinib, and bortezomib. Transcriptomic analysis further classified their mechanisms of action into five representative patterns, including differentiation induction, growth suppression, metabolic inhibition, immune activation, and cell-cycle arrest. The anti-tumor efficacy of these drugs was subsequently validated using patient-derived organoid xenograft (PDOX) models, offering a novel strategy for drug repurposing and mechanistic investigation. Recently, microfluidic chip-based multi-organ systems, for instance, integrated lung-liver platforms, have emerged as an advanced approach to extend organoid applications. By simulating inter-organ crosstalk and drug metabolism *in vitro*, these systems enable systematic assessment of both drug efficacy and organ-specific toxicity, thereby substantially improving the physiological relevance of preclinical models.<sup>63</sup>

### 4.4. Personalized precision medicine and clinical applications

The establishment of PDOs and subsequent organoid-based drug sensitivity testing represent a cornerstone for achieving personalized precision medicine in CRC.<sup>8,64-66</sup> (Figure 3). The potential of organoids in this field was demonstrated by a study employing machine learning for drug target screening, termed CANDiT. This research validated the specific cytotoxic effects of candidate drugs on the CDX2-low expression subgroup within PDO models and correlated genetic profiles with treatment responses.

The findings confirm that organoid models can effectively guide precision therapy for biomarker-defined populations, thereby facilitating biomarker-based patient stratification.<sup>67</sup>

By assessing organoid responses to a panel of standard chemotherapies, targeted agents, and immunotherapies, it becomes possible to predict the most effective treatment regimen for an individual patient *in vitro*. This strategy helps avoid the toxicity and financial cost associated with ineffective therapies.<sup>44,68</sup> Importantly, several clinical validation studies have demonstrated a high concordance between organoid-derived drug sensitivity profiles and actual patient outcomes, underscoring the significant utility of this platform, particularly in guiding treatment selection for advanced or therapy-resistant cases.<sup>45,69</sup>

### 4.5. Development of organoid biobanks

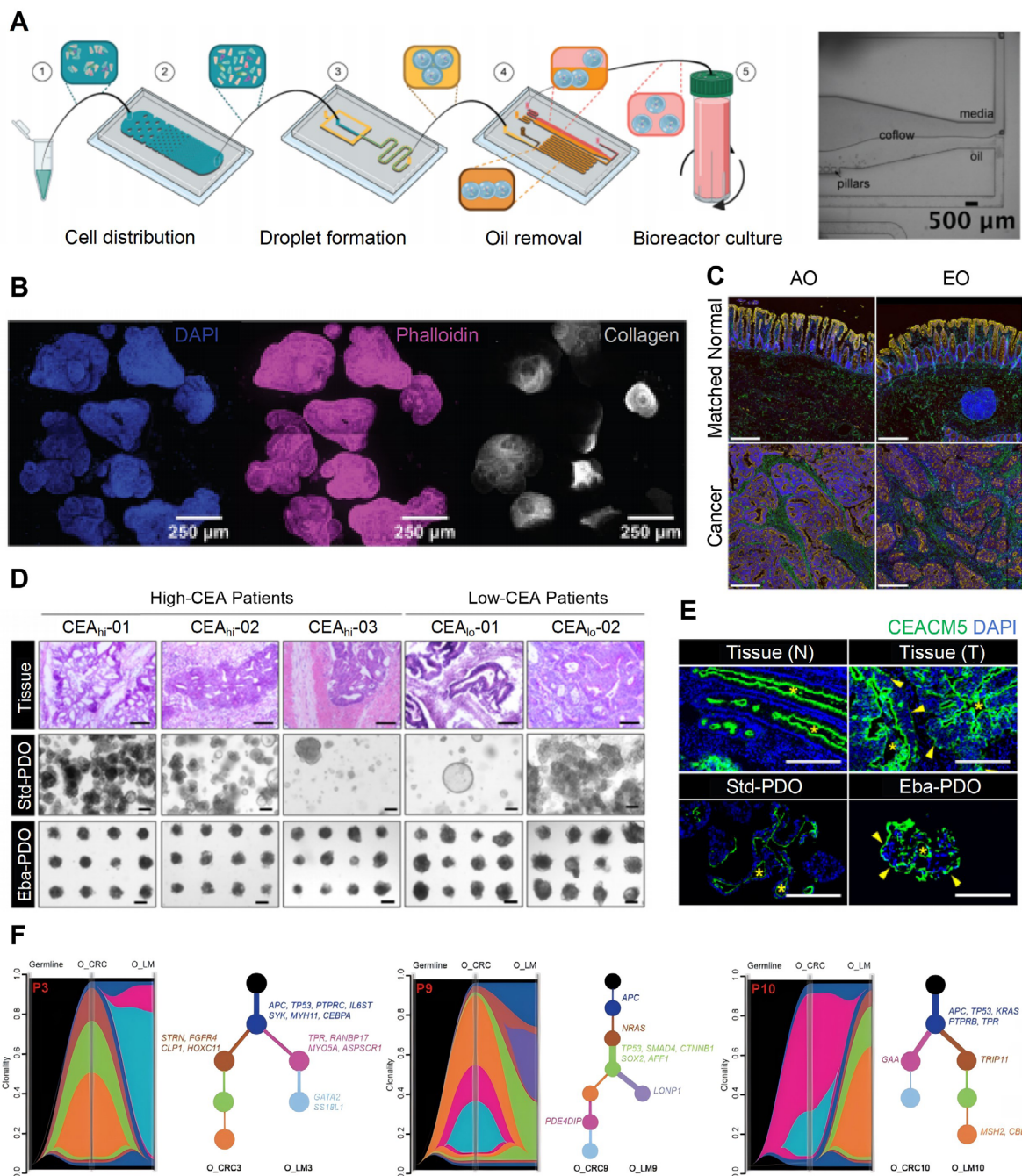
Large-scale, high-quality biobanks of CRC organoids constitute essential infrastructure for advancing translational research and personalized therapy. Globally, several research institutions have successfully established CRC organoid biobanks comprising dozens to hundreds of lines, thereby enabling the systematic integration of *in vitro* models with patient-specific tumor heterogeneity and clinicopathological data.<sup>18,45</sup>

Leading initiatives in the Netherlands, the United States, and China have developed comprehensive organoid biobank networks covering multiple cancer types, including colorectal, pancreatic, breast, glioma, and bladder cancers. This expansion reflects the growing maturation and standardization of organoid culture technologies.<sup>70</sup> Such biobanks systematically collect organoid samples across diverse molecular subtypes and clinical stages, along with annotated clinicopathological and genomic datasets. They not only permit the long-term preservation of patient-derived tumor models with high fidelity but also offer a stable, shared resource for investigating tumor heterogeneity, clonal evolution, and drug resistance mechanisms.<sup>71</sup>

For instance, an organoid library generated from patients with metastatic colorectal and gastroesophageal cancers demonstrated that even specimens with low tumor cellularity could be expanded while retaining the molecular and histopathological features of the original tumors.<sup>23</sup> This capability provides a solid foundation for retrospective analyses of tumor biology and for prospective patient stratification and biomarker discovery in clinical trials.

By implementing standardized protocols and rigorous quality control, organoid biobanks further support large-scale drug screening, disease mechanism studies, and multi-institutional collaborations, thereby substantially enhancing the efficiency and reproducibility of translational cancer research.<sup>72</sup>





**Figure 3.** Microsphere-based engineering for standardized, scalable, and functional organoid models in translational medicine. (A) Schematic of the integrated microfluidic platform featuring on-chip oil removal for automated organoid production. Reproduced with permission from Lavickova *et al.*<sup>64</sup> Copyright © 2026, Wiley-VCH GmbH. (B) Whole-mount immunofluorescence staining of colon organoids within hydrogel droplets. Reproduced with permission from Lavickova *et al.*<sup>64</sup> Copyright © 2026, Wiley-VCH GmbH. (C) Immunofluorescence staining of CRC tissues, depicting the SYTO13 nuclear marker in blue, the epithelial marker PanCK in yellow, and the stromal marker vimentin in green. Reproduced with permission from Huning *et al.*<sup>65</sup> Copyright © 2025, Wiley-VCH GmbH. (D) Brightfield images of Eba-PDOs derived from CRC patients with high or low serum CEACAM5 levels, illustrating patient-specific morphological heterogeneity. Reproduced with permission from Han *et al.*<sup>66</sup> Copyright © 2025, Wiley-VCH GmbH. (E) Immunofluorescence images comparing CEACAM5 expression and cellular polarity in standard PDOs versus Eba-PDOs, highlighting the disrupted apical-basolateral localization characteristic of CRC. Reproduced with permission from Han *et al.*<sup>66</sup> Copyright © 2025, Wiley-VCH GmbH. (F) Riverplot analysis depicting clonal evolution in paired primary CRC and liver metastasis organoids, capturing intratumor heterogeneity. Reproduced with permission from Mo *et al.*<sup>8</sup> Copyright © 2022, Wiley-VCH GmbH.

Abbreviations: CRC: Colorectal cancer; AO: Average-onset; EO: Early-onset; CEA<sub>hi</sub>: High-CEACAM5; CEA<sub>lo</sub>: Low-CEACAM5; Eba-PDO: Embedded bioprinting-enabled arrayed patient-derived organoid; Std-PDO: Standard patient-derived organoid.



## 5. Application of hydrogel microspheres in the construction of CRC organoids

Hydrogel microspheres, serving as tunable 3D culture platforms, are advancing CRC organoid research by enabling more customizable and physiologically relevant models. These systems overcome the inherent limitations of traditional matrices such as Matrigel, including batch-to-batch variability and poorly defined mechanical properties. As such, they provide a powerful tool for recapitulating the *in vivo* TME, deciphering mechanobiological signaling pathways, and establishing reproducible platforms for high-throughput drug screening (Figure 4).

### 5.1. Advantages of hydrogel culture systems

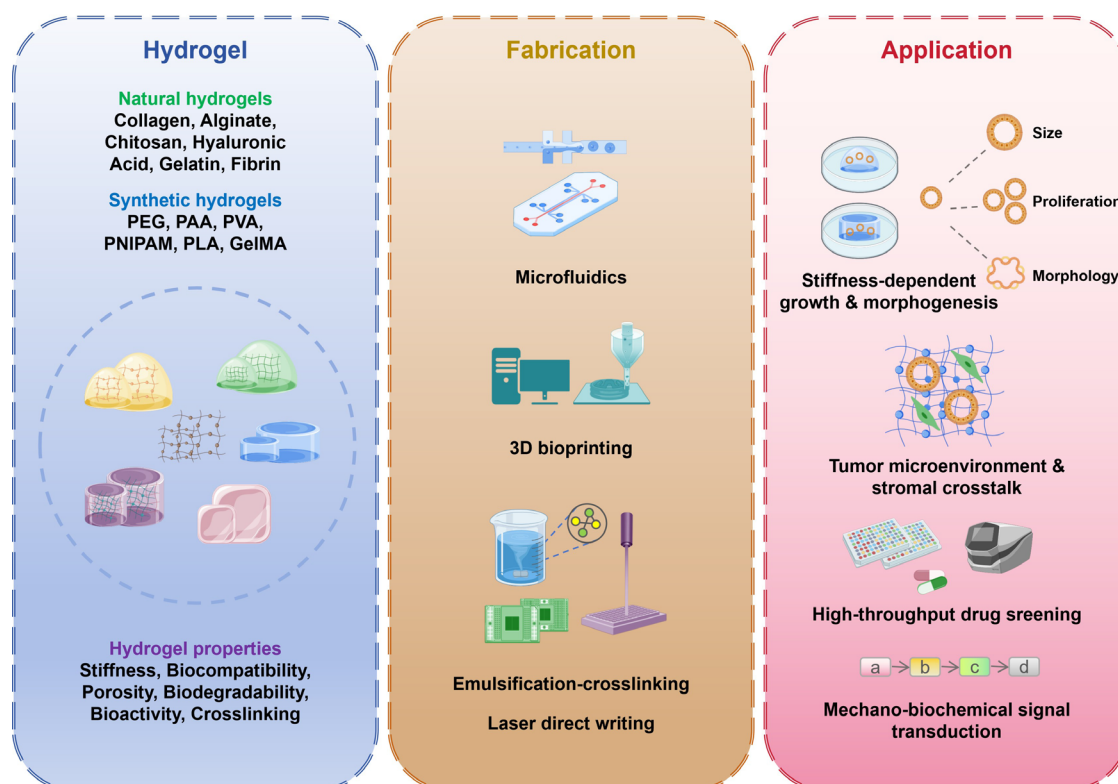
Despite its widespread use, Matrigel suffers from significant drawbacks, including an undefined composition and substantial batch-to-batch variation, which compromise experimental reproducibility and hinder mechanistic studies of ECM-mediated regulation.<sup>73</sup> In contrast, hydrogel-based systems offer several distinct advantages.

To begin with, hydrogels enable precise tuning of the mechanical microenvironment. Given that tissue development, fibrosis, and tumor progression are closely

linked to dynamic changes in ECM stiffness, the ability to modulate matrix mechanics is of critical importance.<sup>74</sup> Synthetic or semi-synthetic hydrogels, such as polyethylene glycol (PEG) and gelatin methacryloyl (GelMA), allow precise control over elastic modulus and viscoelasticity through adjustments in crosslinking density or polymer concentration. This capability facilitates systematic investigation into how substrate stiffness influences the growth, differentiation, and drug response of CRC organoids.<sup>75,76</sup> For example, He *et al.*<sup>77</sup> demonstrated that matrix stiffness regulates intestinal stem cell fate via mechanosensitive pathways such as YAP/TAZ.

Beyond that, hydrogel systems offer superior reproducibility and consistency. Chemically defined or standardized hydrogels exhibit stable physicochemical properties with minimal batch-to-batch variation, providing a reliable foundation for establishing standardized organoid models and reproducible drug-response assays.<sup>78-80</sup>

Most importantly, advanced hydrogel designs permit dynamic spatiotemporal control of mechanical cues. Photodegradable PEG hydrogels, for instance, allow non-invasive, localized reduction of matrix stiffness during culture, thereby mimicking *in vivo* tissue remodeling



**Figure 4.** Application of hydrogels in the establishment of colorectal cancer organoids. Created with BioGDP.com.

Abbreviations: GelMA: Gelatin methacryloyl; PAA: Polyacrylic acid; PEG: Polyethylene glycol; PLA: Polylactic acid; PNIPAM: Poly(N-isopropylacrylamide); PVA: Polyvinyl alcohol; 3D: Three-dimensional.

processes.<sup>81</sup> This capacity for dynamic mechanoregulation offers a valuable tool for studying CRC organoid behavior under time-varying microenvironmental conditions that closely resemble physiological dynamics.

## 5.2. Hydrogel microsphere-based platforms for organoid construction

An ideal hydrogel microsphere system for organoid culture should integrate several key design features. First, it must offer tunable mechanical properties, including stiffness and viscoelasticity adjustable via crosslinking density, in order to match the biomechanical cues of the target tissue and accurately recapitulate *in vivo* mechanosignaling.<sup>82</sup> Second, the material should exhibit excellent biocompatibility and intrinsic cell-supporting capacity, enhanced by the incorporation of integrin-binding motifs (such as the RGD sequence) or other adhesive signals to promote cell attachment, proliferation, and differentiation.<sup>83</sup> Structural stability with controllable degradation is another essential criterion, ensuring long-term maintenance of the 3D microenvironment while permitting localized remodeling by cell-secreted proteases such as matrix metalloproteinases (MMPs).<sup>84</sup> Finally, appropriate porosity and permeability are required to enable efficient diffusion of oxygen, nutrients, and metabolites, which is critical for supporting organoid viability, growth, and functional maturation.<sup>85</sup>

### 5.2.1. Microfluidic technology

Microfluidic technology represents a pivotal approach for fabricating hydrogel microspheres tailored to CRC organoid culture and research (Figure 5A). Its core strength lies in enabling precise microenvironmental control and high-throughput production. For standardized and scalable generation, droplet-based microfluidic devices, utilizing flow-focusing or co-flow geometries, allow precise regulation of oil- and aqueous-phase flow rates to produce hydrogel microspheres with highly uniform sizes suitable for organoid culture.<sup>64,86</sup> By optimizing the premixing of cells with the hydrogel precursor solution, such systems ensure consistent cell encapsulation. Integrated droplet collection and oil-removal modules further enable automated operation, substantially improving the throughput, homogeneity, and efficiency of organoid cultivation, thereby establishing a robust foundation for downstream applications such as drug screening.<sup>64</sup>

Beyond standardization, microfluidic platforms are instrumental in engineering complex physiological architectures, enabling the creation of intricate tissue-like structures. Through integration with techniques such as laser engraving in customized chip chambers, they permit precise patterning of collagen-based or Matrigel-based hydrogels to create biomimetic scaffolds with defined topological features.<sup>87</sup> These pre-designed structures

effectively guide the self-organization of colonic epithelial cells into “mini-colon” models that recapitulate key architectural hallmarks, including crypt-like domains and lumen formation, thereby providing more physiologically relevant systems for studying CRC pathogenesis.<sup>87</sup>

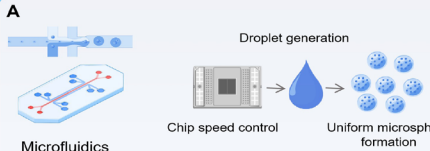

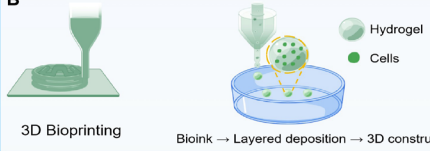

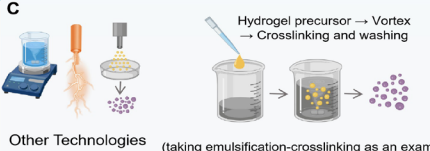

Furthermore, this technology plays a central role in advancing biomaterial applications, facilitating the development of next-generation matrices with tailored biochemical and mechanical properties. For example, when combined with advanced bioinks such as decellularized small-intestinal submucosal matrix, it enables the fabrication of functional microspheres that more closely recapitulate the composition and mechanical properties of the native ECM.<sup>86</sup> The synergy between material and platform not only enhances the physiological fidelity of organoid models but also retains operational simplicity and preparation controllability, offering an optimized solution for high-throughput drug screening.

### 5.2.2. 3D bioprinting

3D bioprinting technology represents a key strategy for fabricating hydrogel microspheres and more complex architectures to support CRC organoids (Figure 5B). This approach enables the layer-by-layer deposition of cell-laden bioinks, typically composed of hydrogels, via methods such as extrusion, inkjet, or photopolymerization. This process allows the construction of tumor organoid models with defined 3D organization.<sup>88</sup> Its primary advantage lies in the precise spatial control over cell and biomaterial distribution, which enables more faithful recapitulation of TME heterogeneity while ensuring consistent, reproducible organoid generation across batches.<sup>89</sup>

In practical applications, digital light processing (DLP) enables the rapid, large-scale production of uniformly sized, cell-loaded hydrogel microspheres, for example, those containing bone marrow-derived mesenchymal stem cells (BMSCs), in a single step. These microspheres can subsequently self-assemble into organoids under appropriate inductive conditions, achieving controlled and scalable production while maintaining high cell viability.<sup>90</sup> Extrusion-based bioprinting provides another widely used method to standardize tumor organoid culture. By depositing bioinks, which are mixtures of cells and matrix hydrogel, into 96-well plates to form uniform thin-layer 3D structures, this method significantly improves culture consistency and optimizes geometry. Such standardization facilitates downstream applications, including high-throughput live-cell imaging and single-organoid drug-response analysis.<sup>91</sup>

More advanced techniques, such as acoustic bioprinting, employ acoustic fields to precisely position droplets containing CRC organoids onto hydrogel scaffolds, thereby creating spatially organized TME models.<sup>92</sup> The technique

Technical Name	Core Principle	Key Advantages	Major Limitations	Typical Applications
<b>A</b> Microfluidics	 Droplet generation Chip speed control Uniform microsphere formation	<ul style="list-style-type: none"> <li>• Uniform particle size</li> <li>• High encapsulation efficiency</li> <li>• Controllable gradient regulation</li> <li>• Automated operation process</li> </ul>	<ul style="list-style-type: none"> <li>• High equipment cost</li> <li>• Complex scale-up process</li> <li>• Stringent operation requirements</li> </ul>	 <ul style="list-style-type: none"> <li>• Precise microenvironment simulation</li> <li>• Single organoid mechanism research</li> <li>• High-throughput drug screening</li> </ul>
<b>B</b> 3D Bioprinting	 Bioink → Layered deposition → 3D construct	<ul style="list-style-type: none"> <li>• Spatial controllability</li> <li>• Batch-to-batch consistency</li> <li>• Multicellular co-culture</li> <li>• Strong clinical translation potential</li> </ul>	<ul style="list-style-type: none"> <li>• High equipment dependence</li> <li>• Hard ink optimization</li> <li>• Low large-scale efficiency</li> </ul>	 <ul style="list-style-type: none"> <li>• Organoid biobank</li> <li>• Personalized graft development</li> <li>• Heterogeneity simulation</li> </ul>
<b>C</b> Other Technologies	 Hydrogel precursor → Vortex → Crosslinking and washing (taking emulsification-crosslinking as an example)	<ul style="list-style-type: none"> <li>• Simple operation</li> <li>• Low cost</li> <li>• Independent mechanical regulation</li> <li>• High viability after cryopreservation</li> </ul>	<ul style="list-style-type: none"> <li>• Poor size uniformity</li> <li>• Slight cell damage</li> <li>• Weak microenvironment regulation</li> </ul>	 <ul style="list-style-type: none"> <li>• High-throughput preliminary screening</li> <li>• Basic mechanism research</li> <li>• Large-scale production</li> </ul>

**Figure 5.** Comparison of hydrogel microsphere-based strategies for constructing colorectal cancer organoids. Created with BioGDP.com.

enables the controllable 3D assembly of organoids and stromal cells, thereby better mimicking *in vivo* tumor heterogeneity and invasive behavior. This enhancement may improve the clinical predictive value of subsequent drug testing.<sup>92</sup>

Furthermore, 3D bioprinting is increasingly integrated with other cutting-edge methodologies to build more sophisticated and functional models. For instance, embedded bioprinting systems utilize microgel particle suspensions as support baths, exploiting their shear-thinning and self-healing properties. This allows for the sequential printing of tissue contours and sacrificial vascular networks within reversible templates, a strategy that has successfully generated complex organ models featuring free-form vascular architectures, while also promoting the proliferation and directed differentiation of encapsulated stem-cell-derived organoids.<sup>93</sup> Another innovation, known as 4D bioprinting, achieves programmed temporal shape transformation by printing a biodegradable microgel support layer together with cells onto a gradient-crosslinked driving hydrogel, ultimately guiding the formation of specific 3D structures.<sup>94</sup> The spatiotemporal control offered by these integrated technologies opens new avenues for constructing morphologically programmable complex organoids in the future.

### 5.2.3. Additional fabrication technologies

Beyond microfluidics and 3D bioprinting, several other techniques are available for preparing hydrogel microspheres, each offering distinct features and advantages for CRC organoid research (Figure 5C).

Coaxial electrospinning, for instance, can be used to

fabricate core-shell microcapsules consisting of a Matrigel core encapsulated within an alginate shell.<sup>95</sup> This system not only supports organoid growth but also maintains high cell viability in stirred bioreactor cultures. Notably, organoids cultured in this format achieve post-cryopreservation recovery rates as high as 80%, a substantial improvement over the approximately 20% recovery typical of bulk Matrigel cultures, highlighting its value for scalable production and biobanking.<sup>95</sup>

Enzyme-catalyzed crosslinking offers another route. For example, gelatin-phenol (Gelatin-Ph) hydrogels enable independent tuning of mechanical strength through adjustment of hydrogen peroxide concentration without altering biochemical composition.<sup>80</sup> Similarly, thiol-norbornene click chemistry combined with photopolymerization allows precise control over stiffness, adhesive ligand density, and degradation kinetics of hyaluronic acid-based microspheres by modulating crosslinker concentration.<sup>80</sup>

Additionally, a dual-photoinitiator aqueous-oil emulsion technique enables the emulsification and photopolymerization of cell-containing PEG-fibrinogen precursor solutions, yielding PEG-fibrin hydrogel microspheres with highly uniform size and morphology.<sup>96</sup>

Another notable advancement is laser direct writing technology, which utilizes gentle laser pulses to precisely transfer and orderly array alginate microspheres encapsulating cells from a donor strip onto a recipient substrate. This process effectively maintains cellular viability and structural integrity, thus providing a novel strategy for fabricating spatially organized complex organoid constructs.<sup>97</sup>

### 5.3. Applications in organoid research for CRC

#### 5.3.1. Revealing stiffness-dependent growth and morphogenesis

Accumulating evidence has demonstrated that the growth of CRC organoids is highly sensitive to matrix stiffness. Using a gelatin-phenol hydrogel system, Ng *et al.*<sup>80</sup> showed that PDOs achieved maximal volume expansion in a moderately stiff matrix, whereas growth was suppressed in both excessively soft and excessively rigid environments. This finding suggests the existence of an optimal “mechanical niche” that favors tumor organoid proliferation. The hydrogel microsphere system offers an ideal platform for investigating this stiffness-dependent behavior, as it allows precise modulation of the local mechanical microenvironment and facilitates real-time observation of organoid growth and morphological dynamics.

In a complementary study, de Lau *et al.*<sup>98</sup> developed an organoid culture model based on a collagen I hydrogel system. By systematically tuning the physicochemical properties of the hydrogel, their work provides key insights into the growth mechanisms of organoids within a 3D matrix of defined composition and tunable mechanical properties.

#### 5.3.2. Simulating tumor microenvironment and matrix interactions

Hydrogel microspheres, featuring a unique 3D porous structure and tunable mechanical properties, represent an ideal carrier for constructing complex TME models that incorporate multiple cell types. Notably, Luo *et al.*<sup>99</sup> developed a hyaluronic acid-gelatin-based hydrogel system that supports the co-culture of CRC-PDOs with patient-matched CAFs. This system enables investigation of matrix stiffness-regulated effects on tumor cell invasion, epithelial-mesenchymal transition (EMT), and chemoresistance. Furthermore, Weng *et al.*<sup>100</sup> adopted a biomimetic design strategy to integrate RGD peptides, hyaluronic acid, and a stiffness-tunable network structure into their hydrogel system. This approach facilitated the construction of a 3D model capable of recapitulating key features of the *in vivo* TME, successfully reproducing ECM-mediated survival signals and enabling in-depth exploration of processes such as anoikis resistance and immune microenvironment regulation in CRC. At the material chemistry level, Gusarova *et al.*<sup>101</sup> developed a bioinspired buffering hydrogel that enables dynamic regulation of the organoid microenvironment pH via histidine grafting. This design mimics the acid-base equilibrium mechanism within tumors, offering a novel strategy for reconstructing *in vitro* tumor-stromal interactions that more closely approximate physiological conditions.

#### 5.3.3. Hydrogel-based platforms for drug screening and resistance studies

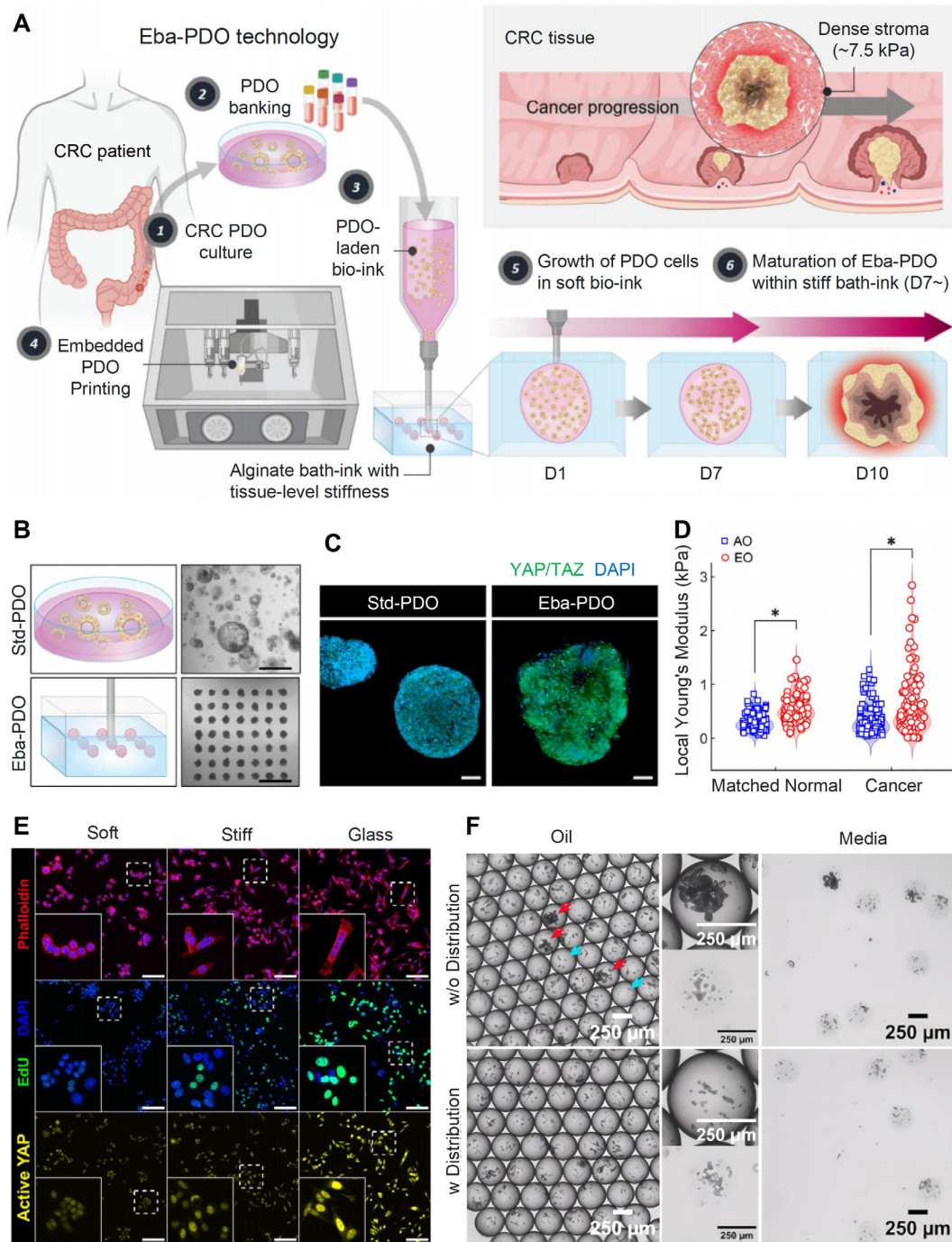
Hydrogel-based CRC organoid models hold considerable promise for drug screening owing to their tunable physicochemical properties and high reproducibility. By accurately recapitulating the mechanical attributes and cellular composition of the native tumor ECM, these systems substantially improve the predictive reliability of *in vitro* drug response assessments. Research indicates that organoids cultured in stiff hydrogels, which mimic the pathological fibrotic state, may demonstrate heightened resistance to chemotherapy, an observation that aligns with the treatment recalcitrance often seen in clinically dense, fibrotic tumors.<sup>99,102</sup> Wijnakker *et al.*<sup>79</sup> established a defined, animal-free 3D synthetic culture system by covalently conjugating invasin (INV; an integrin-activating protein derived from *Yersinia*) to a thermoresponsive polyisocyanate (PIC) hydrogel. The resulting functionalized PIC-INV hydrogel supports long-term expansion and serial passaging of human intestinal and airway organoids. Moreover, it enables liquid-perfusion assays for evaluating cystic fibrosis transmembrane conductance regulator (CFTR) function, highlighting its utility for testing drug-induced functional responses. The integration of automation has further advanced the standardization and throughput of drug screening. Using microfluidic droplet technology, Matrigel microspheres can be uniformly generated and robotically printed into single-well organoid arrays that faithfully retain patient-specific genetic features.<sup>103</sup> Screening a panel of 31 drugs across organoids derived from 21 patients using this platform achieved 81% accuracy in predicting clinical outcomes. The system also effectively assesses dose-response relationships for combination regimens such as FOLFOX, offering a standardized preclinical tool for guiding personalized precision therapy.<sup>103</sup>

#### 5.3.4. Mechanisms of mechanosensing and signal transduction

The programmable properties of hydrogel microspheres provide a distinct advantage for systematically investigating the conversion of mechanical cues into biochemical signals<sup>64-66</sup> (Figure 6). In CRC organoids, pathways such as YAP/TAZ, integrin-FAK, and Wnt/ $\beta$ -catenin have been identified as key mediators of mechanical force sensing and transduction.<sup>77,104,105</sup> By culturing organoids within hydrogels of defined stiffness and analyzing the activation status of these pathways, researchers can gain deeper insights into the molecular mechanisms through which the mechanical microenvironment promotes malignant progression.

Weng *et al.*<sup>100</sup> cultured tumor cells in GHP4a hydrogels with varying stiffness and observed that increased matrix





**Figure 6.** Engineering strategies to recapitulate the biophysical and biochemical cues of the tumor microenvironment. (A) Schematic of embedded 3D bioprinting for Eba-PDOs within a stiffness-tunable alginate bath. Reproduced with permission from Han *et al.*<sup>66</sup> Copyright © 2025, Wiley-VCH GmbH. (B) Brightfield images comparing the uniformity of Std-PDOs and Eba-PDOs. Reproduced with permission from Han *et al.*<sup>66</sup> Copyright © 2025, Wiley-VCH GmbH. (C) Immunofluorescence images showing YAP/TAZ upregulation in Eba-PDOs. Reproduced with permission from Han *et al.*<sup>66</sup> Copyright © 2025, Wiley-VCH GmbH. (D) Violin plot showing local Young's modulus of AO and EO CRC tissues, indicating elevated stiffness in EO CRC. Reproduced with permission from Huning *et al.*<sup>65</sup> Copyright © 2025, Wiley-VCH GmbH. (E) Immunofluorescence images of active YAP and EdU in CRC cells cultured on substrates of graded stiffness, demonstrating stiffness-dependent YAP-mediated proliferation. Reproduced with permission from Huning *et al.*<sup>65</sup> Copyright © 2025, Wiley-VCH GmbH. (F) Brightfield image of uniform cell-laden hydrogel droplets generated via flow-focusing microfluidics. Reproduced with permission from Lavickova *et al.*<sup>64</sup> Copyright © 2026, Wiley-VCH GmbH. Abbreviations: AO: Average-onset; CRC: Colorectal cancer; Eba-PDO: Embedded bioprinting-enabled arrayed patient-derived organoids; EO: early-onset; Std-PDO: Standard patient-derived organoids.



stiffness suppresses apoptosis by activating the PI3K/Akt signaling pathway and upregulating anti-apoptotic protein expression. This study reveals a molecular link between mechanochemical coupling and anoikis resistance in tumor cells. Furthermore, organoids cultured in collagen I hydrogels have been shown to maintain stable, physiologically relevant transcriptional profiles. This hydrogel-based culture system thereby enables the unbiased monitoring of mechanochemical signaling coupling within a 3D microenvironment.<sup>98</sup>

## 6. Challenges and outlook

### 6.1. Current challenges

Despite the rapid advancement of organoid technology, it still confronts multiple challenges, including a lack of standardization, prominent technical bottlenecks, and inadequate clinical translation.

Regarding standardization and reproducibility, the core issue lies in the absence of standardized construction protocols. Significant variations exist across laboratories in critical aspects such as digestive enzyme selection, medium formulation, and cell seeding density. Furthermore, commercial matrices such as Matrigel inherently exhibit substantial batch-to-batch variability and ill-defined composition,<sup>73,106</sup> factors that have significantly undermined the reliability and comparability of experimental results. In terms of quality control, a systematic evaluation framework encompassing morphology, functional validation, genetic stability, and mechanical properties has yet to be established, impeding the comparison of findings across different studies.<sup>107-110</sup>

Technical bottlenecks also hamper the further advancement and application of organoid models. On one hand, existing models have substantial limitations in recapitulating the authentic TME, often lacking key components such as functional immune cells and vascular networks.<sup>21,48</sup> This restricts their application value in research fields including immunotherapy and metastasis mechanism studies. On the other hand, genetic drift and phenotypic instability arising from long-term culture also impair the model's reliability,<sup>109,110</sup> representing a critical technical hurdle that requires urgent breakthroughs. Moreover, the high construction costs and limited throughput of current technologies further hinder their large-scale application in areas such as drug screening.

At the clinical translation stage, organoid technology still faces numerous practical obstacles. The protracted construction cycle and inconsistent success rates make it difficult to meet the demands of rapid clinical decision-making. Although preliminary studies have shown that

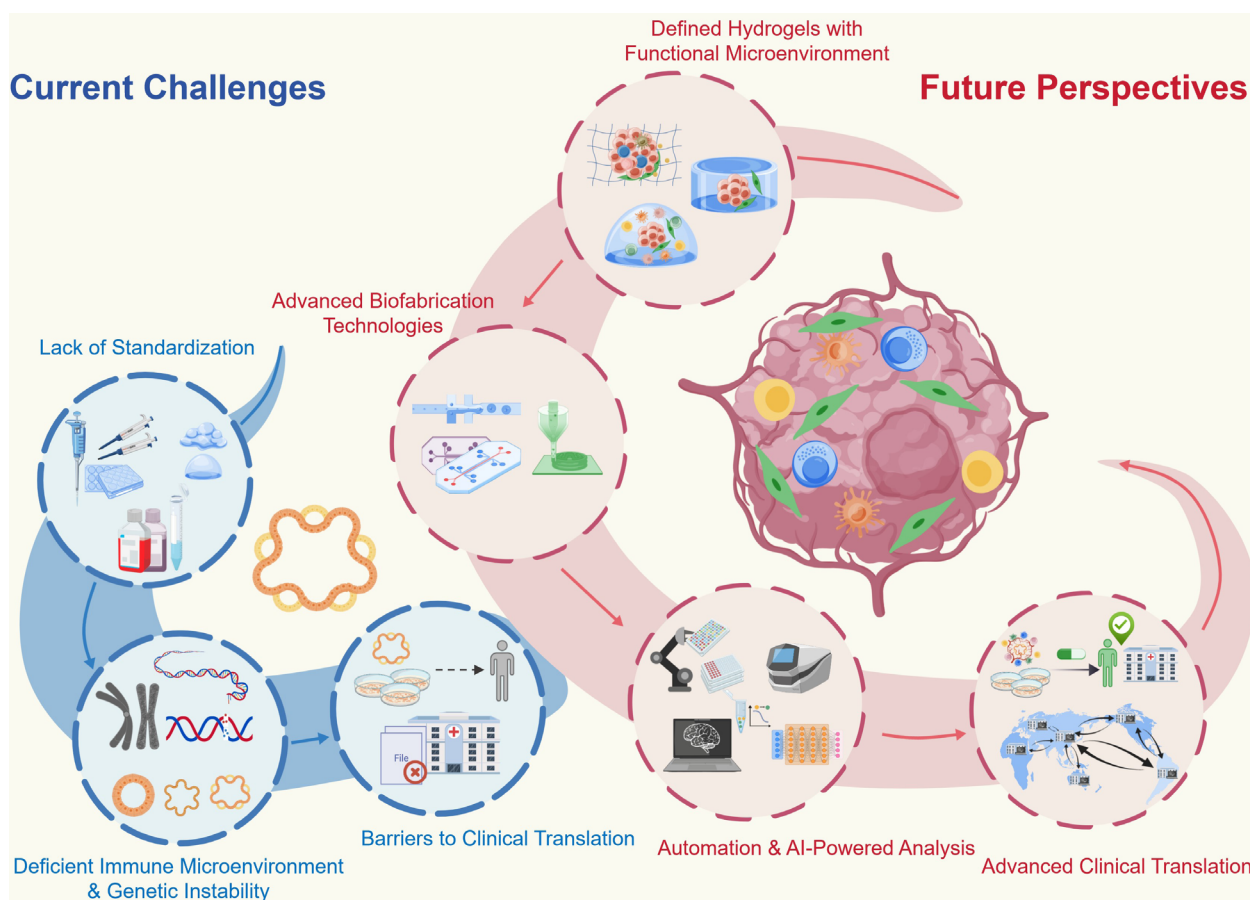
organoid drug sensitivity results correlate to some extent with patient outcomes,<sup>111,112</sup> their predictive value still requires further validation through large-scale prospective clinical trials. Further complexities arise from the underdeveloped ethical and regulatory frameworks. Clear regulations are lacking regarding intellectual property rights for biological materials, the scope of informed consent, data ownership and sharing, as well as clinical translation pathways. These issues constitute a significant institutional barrier to technology transfer.

### 6.2. Future directions and prospects

To address current limitations, organoid technology is advancing toward more sophisticated models, intelligent platforms, and systematic translation. A key research focus lies in developing complex co-culture systems that incorporate immune cells, stromal components, and other microenvironmental factors to better recapitulate tumor-immune interactions. The integration of 3D bioprinting offers novel approaches for engineering multicellular tissue architectures with spatial precision. Concurrently, the design of novel hydrogel materials with well-defined chemistry and tunable physical properties is expected to significantly improve experimental controllability and reproducibility. Furthermore, the convergence of organ-on-a-chip platforms with organoid technology holds promise in addressing vascularization challenges by enabling the simulation of *in vivo* perfusion, thereby facilitating the establishment of more physiologically dynamic culture systems.

The incorporation of automation and artificial intelligence (AI) is poised to drive organoid platforms toward high-throughput and standardized workflows. By integrating automated liquid handling, 3D bioprinting, and AI-assisted image analysis, efficient organoid fabrication and intelligent phenotypic evaluation can be achieved. Organ-on-a-chip systems equipped with real-time sensors will further enable dynamic monitoring of drug responses. Establishing standardized protocols and data-sharing frameworks will also facilitate multi-center collaborations and accelerate model optimization.

Clinical translation of organoid technology is progressing toward deeper integration. Two parallel pillars are critical to accelerating this process: validation through prospective clinical trials to demonstrate the predictive utility of organoids as biomarkers, and the development of standardized, shareable platforms to enable scalable multi-center studies. In the long term, by leveraging its potential in disease modeling, early screening, and personalized therapy, organoid technology is expected to transition from bench to bedside through interdisciplinary collaboration and industrial partnership (Figure 7).



**Figure 7.** Challenges and future perspectives of organoid technology from bench to bedside. Created with BioGDP.com.

## 7. Conclusion

The CRC organoid model has emerged as a transformative *in vitro* research platform that bridges conventional 2D cell cultures and complex *in vivo* animal models. It retains the genetic profile, histological architecture, and heterogeneity of the patient's original tumor, making it an ideal system for investigating disease mechanisms, studying dynamic TME interactions, and enabling efficient drug discovery and personalized treatment prediction.

The advent of engineered culture systems, including hydrogel microspheres, has introduced novel strategies for the controlled, standardized fabrication and functional modulation of organoids. While challenges persist in standardization, model complexity, and clinical translation, continuous technological innovation and deeper interdisciplinary integration are driving CRC organoids toward greater physiological relevance, operational intelligence, and broader accessibility.

Looking forward, this platform is poised to serve not only as a cornerstone for fundamental research but also as a critical translational link between laboratory discovery

and clinical practice. Ultimately, it is expected to propel CRC diagnosis and therapy into a new era of precision and personalization, offering transformative potential for improving patient outcomes.

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## Conflict of interest

The authors declare that they have no competing interests.

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## Ethics approval and consent to participate

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## Availability of data

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