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Advancing tumor organoid 3D culture with functionalized hydrogels: Applications in precision medicine and technological challenges

Guangshun Zeng[1†], Jiayi Niu[1†], Jinqi Wang[1], Lulin Qiao[2], Na Tang[3], Mingzhu Zhang[4*], and Fushan Hou[4*]

[1]Shanxi Medical University, Taiyuan, Shanxi, China

[2]Third Hospital of Shanxi Medical University, Taiyuan, Shanxi, China

[3]Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, China

[4]Department of Foot and Ankle Surgery, Beijing Tongren Hospital of Capital Medical University, Beijing, China

***Corresponding authors:** Fushan Hou (houfushan@sxmu.edu.cn); Mingzhu Zhang (mingzhuzhang@mail.ccmu.edu.cn)

†These authors contributed equally to this work

Citation: Zeng G, Niu J, Wang J, *et al*. Advancing tumor organoid 3D culture with functionalized hydrogels: Applications in precision medicine and technological challenges. *Organoid Res.* 2026;2(3):026050007. doi: 10.36922/OR026050007

Received: January 29, 2026

Revised: March 18, 2026

Accepted: March 18, 2026

Published online: June 5, 2026

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Abstract

Traditional tumor models are limited by short *in vitro* survival, pronounced interspecies differences, and inadequate recapitulation of tumor heterogeneity. This review systematically classifies hydrogels into matrix-supporting, signal-regulating, and stimulus-responsive categories based on functional characteristics, and summarizes the fabrication strategies of natural, synthetic, and composite hydrogels. Tumor organoids preserve the genotype and phenotype of primary tumors; their integration with hydrogels provides complementary advantages, enabling the reconstruction of biomimetic tumor microenvironments, long-term *in vitro* culture, and personalized tumor modeling. Hydrogel-tumor organoid systems therefore demonstrate substantial value in tumor model development, high-throughput drug screening, and the investigation of drug resistance mechanisms. However, several challenges remain, including insufficient matching of mechanical properties, batch-to-batch variability of hydrogels, stringent organoid culture requirements, and the lack of standardized protocols—among which the standardization of natural hydrogels represents a major barrier to clinical translation. Finally, this review highlights emerging technological directions, including intelligent hydrogels and 3D bioprinting, outlines trends in interdisciplinary integration, and discusses future clinical application prospects. Addressing issues related to standardization and cost will be critical for fully unlocking the potential of hydrogel–organoid platforms in precision oncology.

Keywords: Hydrogels; Tumor organoids; Tumor models; Precision medicine

1. Introduction

Traditional tumor research models play a pivotal role in the field of tumor biology. However, each model type has its inherent limitations. Taking *in vitro* models as an example, precisely prepared tumor slices and patient-derived explants can preserve the original tissue architecture, extracellular matrix (ECM) components, and multicellular heterogeneity of tumors. These models enable direct testing of therapeutic agents in a physiologically relevant environment and facilitate investigations of cell–cell and cell–matrix interactions. Nevertheless, they are constrained by short survival periods and low experimental throughput. In addition, logistical challenges associated with the

acquisition and processing of fresh human tumor tissues further limit their widespread application.[1]

In vivo models remain indispensable for investigating systemic tumor biological characteristics. Among them, xenograft models—including cell line-derived xenografts (CDXs) and patient-derived xenografts (PDXs)—are widely used for drug efficacy evaluation and the simulation of human tumor biology. PDX models can retain the histopathological features and genetic background of donor tumors, thereby enabling the investigation of patient-specific therapeutic responses. However, reliance on immunodeficient hosts restricts their utility for studying tumor–immune interactions. Moreover, the selective engraftment of aggressive tumor subclones during transplantation may introduce experimental bias.[2] Notably, the complexity of the human organ system differs substantially from that of rabbit and rodent models. This fundamental interspecies disparity often results in the failure to translate therapeutic successes from animal models to human clinical applications, underscoring the translational limitations of conventional *in vivo* models.[3]

Syngeneic models involve transplanting mouse-derived tumor cells into immunocompetent mice, enabling the investigation of tumor immune dynamics and the evaluation of immunotherapy efficacy. However, their translational relevance to human disease is limited by interspecies differences. Genetically engineered mouse models (GEMMs) allow spontaneous tumor initiation, progression, and metastasis within an intact microenvironment. Although GEMMs provide valuable insights into tumor biology, they are associated with high costs and extended timeframes; moreover, they are typically restricted to specific genetic alterations and cannot fully capture the heterogeneity observed in human tumors.[4]

Tumor organoids, as an emerging *in vitro* model, effectively address several limitations of traditional models. By recapitulating the spatial architecture of native tissues, organoids provide a robust platform for modeling tumor drug resistance, conducting drug screening, and investigating the tumor microenvironment (TME), thereby serving as a critical bridge between conventional in vitro and in vivo models[5] (Figure 1).

2. Methods

2.1. Literature search methodology

To ensure the systematic approach and comprehensiveness of this review, a structured literature search was conducted across major biomedical databases, including PubMed/MEDLINE, Web of Science, Scopus, Embase, and CNKI. The search strategy employed the following terms: ("hydrogels" OR "hydrogel") AND ("tumor" OR "cancer") AND ("organoids" OR "3D culture" OR "*ex vivo* model")

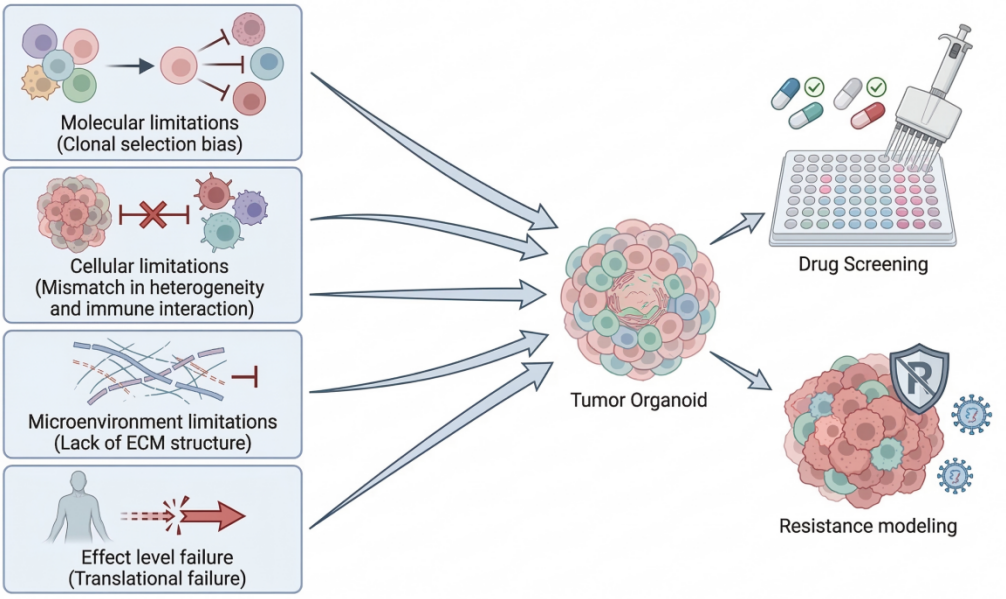

Figure 1. Tumor organoids overcome the limitations of traditional models to facilitate drug screening and resistance modeling. Image created by the authors. The left panels summarize the inherent limitations of conventional tumor models at the molecular, cellular, microenvironmental (*e.g.*, lack of extracellular matrix [ECM]), and translational levels. By recapitulating the spatial architecture and heterogeneity of native tissues, tumor organoids (center) serve as a bridging platform to overcome these barriers, enabling robust downstream applications such as drug screening and resistance modeling (right).

AND ("drug delivery" OR "tumor microenvironment" OR "tissue engineering"). The search covered publications from January 1, 2011, to February 28, 2025. Inclusion criteria prioritized studies published in high-impact journals (IF > 5), research articles from the past six years (2019–2025), and high-quality, cutting-edge studies focusing on three-dimensional (3D) tumor models. Article screening was conducted independently by multiple researchers, with discrepancies resolved through discussion and consensus. A total of 2,348 records were initially identified. Following title, abstract, and full-text screening, 85 high-quality articles were ultimately included in the review. This systematic search strategy ensures the comprehensiveness, representativeness, and scientific rigor of the literature synthesis, laying a solid foundation for subsequent analysis.

2.2. Figure generation and originality statement

All figures were generated exclusively using Adobe Illustrator 2023 (for vector graphics) and WPS Office 2025 (for supplementary data visualization). No figures were adapted, modified, or reproduced from previously published sources. All graphical elements, including diagrams, charts, and schematic illustrations, are original creations of the authors and are developed specifically for this manuscript.

3. Integration

3.1. Hydrogels

Hydrogels, as 3D polymeric network systems, combine high water content with structural stability and have emerged as core biomaterials for tumor organoid culture. Departing from the conventional classification based on chemical origin (natural or synthetic), this review adopts a function-oriented framework, categorizing hydrogels into three types: matrix-supporting, signal-regulating, and stimulus-responsive hydrogels (Figure 2).

3.1.1. Matrix-supporting hydrogels: Structural foundation for organoid survival

Matrix-supporting hydrogels are designed based on the principles of biocompatibility and ECM mimicry, providing a biomimetic microenvironment that supports the proliferation, differentiation, and structural integrity of tumor organoids. Representative materials include collagen, decellularized extracellular matrix (dECM), gelatin, and gelatin methacryloyl (GelMA).[6,7] Due to their structural homology with native ECM, collagen-based hydrogels can guide breast tumor organoids to form vascular-like structures and sustain stable culture for over 8 weeks.[7] dECM hydrogels retain tissue-specific bioactive components, thereby significantly enhancing the phenotypic fidelity of organoids.[6] Meanwhile, gelatin and GelMA enable precise regulation of porosity (10–100 μm) and mechanical stiffness (1–10 kPa) through technologies such as 3D bioprinting, allowing adaptation to the distinct mechanical requirements of different tumor types—for example, low stiffness for pancreatic cancer organoids and higher stiffness for breast cancer organoids.[6] The relatively low mechanical strength of natural polymer hydrogels can be improved through crosslinking strategies, such as glutaraldehyde-mediated covalent crosslinking or ionic crosslinking, while preserving their essential ECM-mimetic properties.[7]

3.1.2. Signal-regulating hydrogels: Precise modulators of organoid behavior

Signal-regulating hydrogels enable precise modulation of organoid behavior by targeting key cellular signaling pathways. Polycaprolactone (PCL) scaffolds, for instance, mimic the mechanical stiffness of tumor tissues (5–50 kPa). Their tunable stiffness promotes volumetric growth and morphological stability of liver cancer organoids through activation of the integrin-FAK-YAP signaling pathway.[8] Similarly, RGD-modified polyethylene glycol (PEG) hydrogels enhance cell–matrix interactions by facilitating integrin binding, which induces FAK phosphorylation, thereby strengthening cell–matrix adhesion and reducing anoikis.[9] In addition, gelatin-based hydrogels loaded with bone morphogenetic protein 4 (BMP4) enable sustained BMP4 release with a half-life of approximately 72 h, activating the BMP/Smad signaling pathway and promoting the differentiation of intestinal organoids toward an epithelial phenotype.[10] Furthermore, self-healing poly(2-hydroxyethyl methacrylate) (PHEMA)-based hydrogels maintain microenvironmental stability during long-term culture, thereby supporting sustained and reliable signal-mediated regulation of organoid development.[8]

3.1.3. Stimulus-responsive hydrogels: Intelligent platforms for dynamic regulation

As "smart" biomaterials, stimulus-responsive hydrogels enable spatiotemporally precise regulation of organoids by responding to endogenous cues in the TME (*e.g.*, pH 6.0–6.5 and MMP activity) or to external stimuli such as light (400–500 nm) and magnetic fields (0.1–0.5 T). pH-responsive alginate hydrogels trigger the targeted release of metronidazole within the acidic microenvironment of colorectal cancer organoids, thereby significantly reducing off-target toxicity.[11] Matrix metalloproteinase (MMP)–degradable gelatin hydrogels exhibit degradation kinetics synchronized with organoid growth, preventing premature scaffold collapse.[12] Photoresponsive PHEMA hydrogels enable light-controlled spatial patterning of growth regions,[8] while magnetic nanoparticle-modified gelatin-alginate composite hydrogels regulate cellular spatial distribution under magnetic fields to mimic tumor invasion processes.[13]

3.1.4. Functional synergy: Multidimensional regulation strategies of composite hydrogels

By integrating natural and synthetic polymers or functionalized nanoparticles, composite hydrogels achieve synergistic enhancement of the three aforementioned functions.[11,14] The gelatin-alginate composite system combines ECM-mimetic characteristics with pH-responsive properties, providing dual functions of structural support and targeted drug delivery for colorectal cancer organoids.[11] PCL-collagen composites integrate tunable mechanical signaling with high biocompatibility, thereby optimizing the microenvironment for breast tumor organoids.[7] In addition, RGD-BMP4 dual-functionalized gelatin-alginate composite hydrogels simultaneously regulate cell adhesion and differentiation pathways, enabling more precise modulation of organoid development.[9]

3.1.5. Physicochemical basis for hydrogel function implementation

The multifunctionality of hydrogels arises from their key physicochemical properties, including biodegradability, bioadhesiveness, and shear-thinning behavior.[15,16] Together, these properties establish a synergistic regulatory framework integrating mechanical strength, biocompatibility, and stimulus responsiveness, providing the physicochemical foundation for the three core functions (Figure 3).

3.1.6. Functional evolution: From drug carriers to organoid matrices

The application of hydrogels in tumor research has undergone a notable paradigm shift. Initially developed as targeted drug delivery carriers to reduce off-target toxicity,[17] hydrogels have progressively evolved into core matrices for organoid culture, with their functional focus expanding

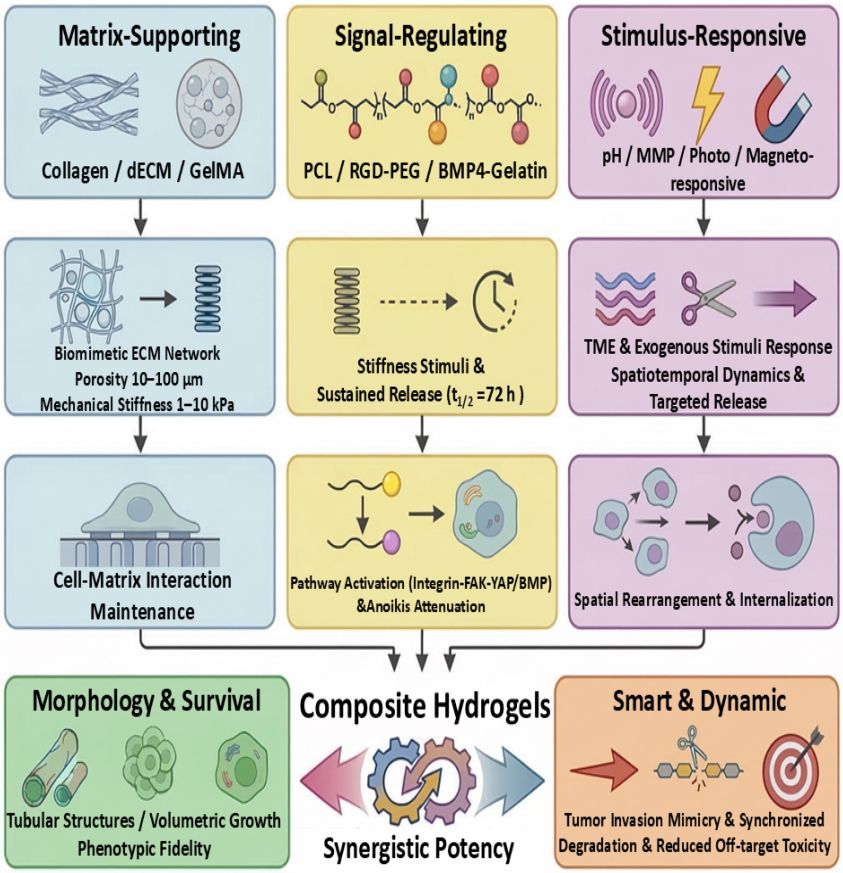

Figure 2. Function-oriented classification and synergistic applications of hydrogels in tumor organoid engineering. This schematic categorizes hydrogels into three distinct functional types: matrix-supporting (blue), signal-regulating (yellow), and stimulus-responsive (purple). Each column details the representative materials, mechanisms of action, and their respective biological outcomes. The bottom section highlights how composite hydrogels integrate these distinct functionalities to achieve synergistic potency, enhancing both phenotypic fidelity (morphology & survival) and dynamic regulation (smart & dynamic) in tumor organoids. Image created by the authors.
Abbreviations: BMP4: Bone morphogenetic protein 4; dECM: Decellularized extracellular matrix; GelMA: Gelatin methacryloyl; MMP: Matrix metalloproteinase; PCL: Polycaprolactone; PEG: Polyethylene glycol; TME: Tumor microenvironment.

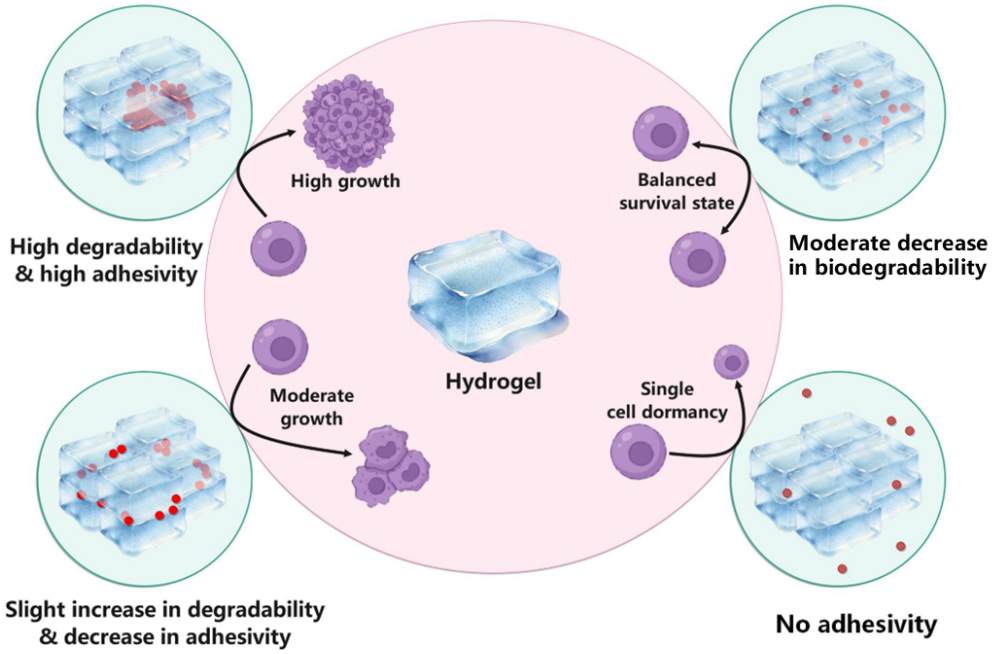

Figure 3. Physicochemical properties of hydrogels dictate the developmental fate of tumor organoids. This schematic illustrates how tuning the biodegradability and bioadhesiveness of the hydrogel matrix directly regulates cellular behavior. A microenvironment with high degradability and adhesivity promotes robust volumetric growth, whereas the complete absence of adhesivity induces single-cell dormancy. Intermediate modulation of these parameters results in either moderate growth or a balanced survival state, highlighting the fundamental role of material properties in customized organoid engineering. Image created by the authors.

from single-purpose drug delivery to comprehensive biological regulation. Compared with traditional ECM materials such as Matrigel, hydrogel-based scaffolds offer improved reproducibility and biosafety, making them increasingly preferred matrices for tumor organoid culture.[7]

Notably, the application of hydrogels in the tumor field exhibits a clear dynamic development trend. In the early stage, their primary application focused on targeted delivery and sustained release of antitumor drugs; through controlled release behavior, hydrogels reduced off-target toxicity and prolonged drug efficacy.[18] With advances in organoid technology, the application scope of hydrogels has been further expanded. At present, hydrogels have become the core scaffolding materials for tumor organoid construction, achieving a transition from "drug delivery carriers" to "tumor model construction matrices." Their integration with tumor organoids has established a research framework with enhanced clinical translational value.[7,19,20] Compared with animal—or tumor—derived ECM traditionally used in organoid culture, hydrogel-based scaffolds avoid issues such as poor preparation reproducibility and potential carcinogenic risks, making them preferred matrices for tumor organoid culture[21,22] (Figure 4).

3.2. Tumor organoids

Tumor organoids are 3D cellular structures cultured from isolated patient-derived tumor tissues, capable of retaining the complete genotypic and phenotypic characteristics of the original tumors. Among these, patient-derived organoids (PDOs) not only preserve the genetic and phenotypic features of tumor tissues but also exhibit chemoresistance patterns consistent with clinical observations, thereby providing a feasible platform for personalized therapeutic testing and the establishment of tumor biobanks. Accumulating evidence has demonstrated a significant correlation between drug responses observed in PDOs and patient clinical outcomes, which strongly supports their practical application in precision oncology.[23]

The main advantages of tumor organoids are as follows:

(i) *Preservation of tissue heterogeneity*. 3D hydrogel systems can mimic the TME by incorporating bioactive ECM components or their analogs. Such platforms facilitate the investigation of cell–cell and cell–matrix interactions, gene expression patterns, and tumor heterogeneity.[20]

(ii) *Simulation of chemoresistance*. Co-culture of cancer cells with stromal components, such as fibroblasts

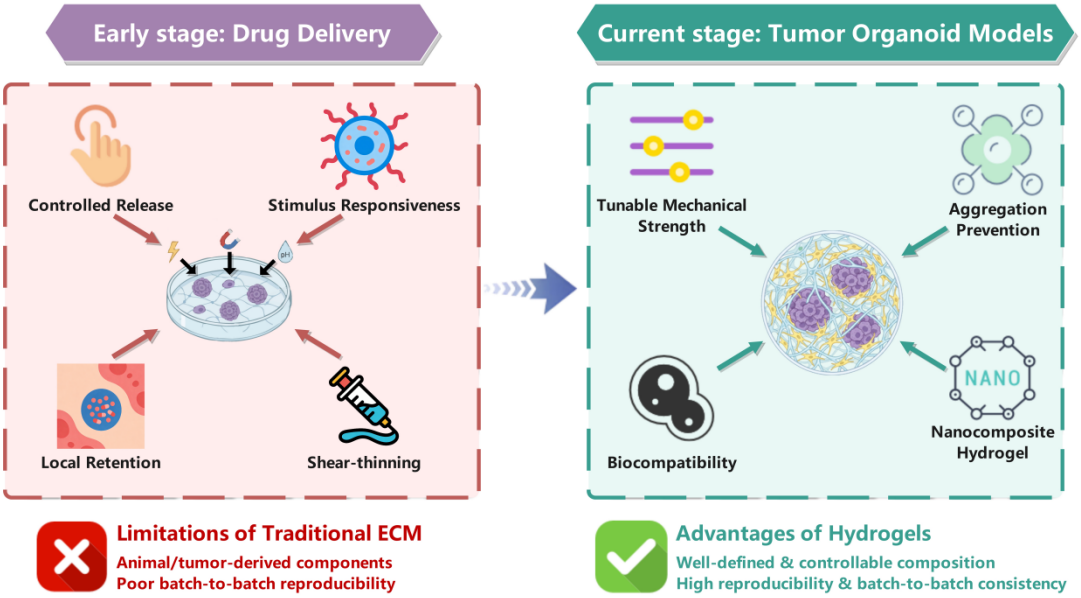

Figure 4. The paradigm shift of hydrogel applications in oncology: from drug delivery to tumor organoid modeling. The schematic illustrates the evolutionary trajectory of hydrogels in the tumor field. In early-stage applications (left), hydrogels primarily served as smart drug delivery carriers by virtue of their properties, such as controlled release and stimulus responsiveness. The current stage (right) highlights the transition of hydrogels into core scaffolding matrices for constructing tumor organoids. Compared to traditional animal- or tumor-derived ECM, which suffers from undefined components and poor reproducibility (bottom left), hydrogels offer significant advantages, including well-defined compositions, tunable mechanical strength, and high batch-to-batch consistency (bottom right). Image created by the authors.
Abbreviation: ECM: Extracellular matrix.

and macrophages, within hydrogel scaffolds enables effective modeling of tumor heterogeneity and chemoresistance. For example, alginate-encapsulated cryopreserved tumor cells combined with macrophages can create an immune-like microenvironment.[24]

(iii) *Support for long-term culture.* Advanced organoid models incorporate immune components, thereby enabling investigations into immunotherapy responses and microbiome interactions. PDOs also provide a robust platform for studying the long-term effects of hormone therapy in estrogen receptor-positive breast cancer.[25,26]

Crucially, the applicability of water-based hydrogel systems in tumor organoid culture differs fundamentally from that in non-tumor organoids due to the intrinsic heterogeneity of the TME. While Figure 5 illustrates a generalizable paradigm in which hydrogel mechanical properties (*e.g.*, stiffness and ECM composition) regulate organoid differentiation through signaling pathways such as YAP and Wnt in non-tumor systems,[20] this framework does not readily translate to tumor organoids. For instance, the same hydrogel stiffness (*e.g.*, 10 kPa) may activate Wnt signaling in colorectal cancer PDOs while suppressing YAP signaling in breast cancer PDOs,[10] reflecting the context-dependent nature of TME signaling. Consequently, this heterogeneity prevents the establishment of a standardized "material parameter → signaling pathway" mapping, rendering tumor organoid engineering inherently more complex than that of non-tumor systems.

This limitation also underlies a major challenge in standardization (Section 5.4). Tumor heterogeneity can lead to batch-to-batch variability in hydrogel responses; for example, collagen hydrogels derived from bovine sources may produce inconsistent Wnt activation in PDOs. Consequently, single-parameter hydrogel design is insufficient for reliable clinical translation.[27]

Similarly, while BMP4 regulates differentiation in non-tumor organoids (*e.g.*, promoting intestinal epithelial phenotypes through the BMP/Smad pathway in colon organoids), its role in tumor organoids is highly context-dependent. Within heterogeneous TMEs, BMP4 may simultaneously activate Wnt signaling in cancer stem cells while inhibiting it in stromal fibroblasts,[10] thereby preventing consistent pathway activation. This context dependency further highlights why tumor organoids

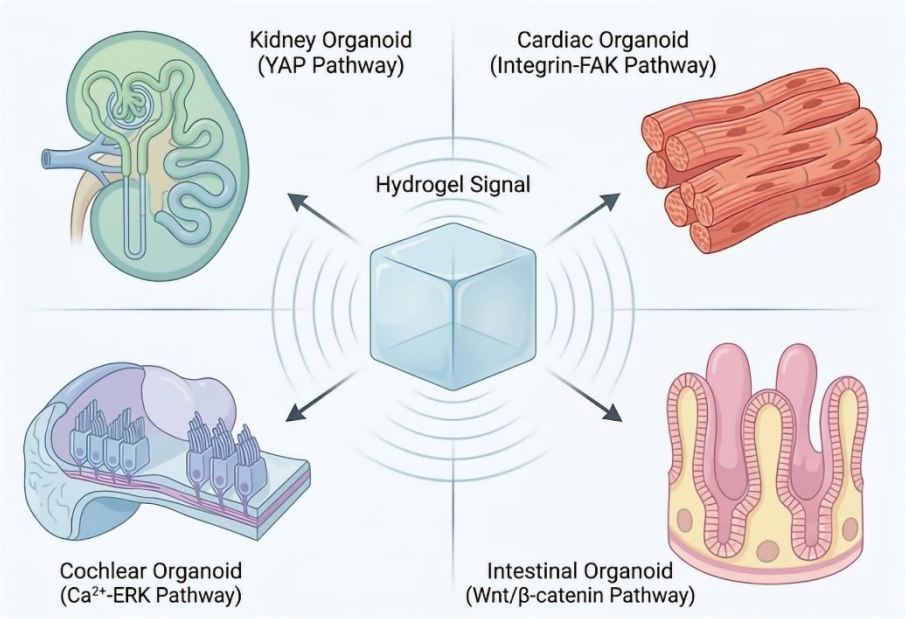

Figure 5. The standardized "parameter-to-pathway" regulatory paradigm in non-tumor organoids. This schematic illustrates the generalizable regulatory model in non-tumor organoid systems, where specific hydrogel physicochemical signals reliably direct organoid development via distinct signaling pathways (*e.g.*, YAP in kidney, integrin-FAK in cardiac, Ca^{2+}-ERK in cochlear, and Wnt/β-catenin in intestinal organoids). Unlike the highly heterogeneous tumor microenvironment, where signaling is extremely context-dependent, this straightforward "material parameter → signaling pathway" mapping provides a standardized framework for engineering conventional non-tumor models. Image created by the authors.

cannot adopt the simple "parameter → pathway" regulatory model used in non-tumor systems (Figure 5).

Current research has increasingly focused on integrating organoids with gene editing technologies. By engineering organoids to express specific genes or functional characteristics, their applicability in targeted cancer therapy can be substantially enhanced.[28] Meanwhile, the adoption of specialized culture strategies can further improve organoid maturation and model fidelity. Among these approaches, dynamic fluid culture and multilayer microfluidic culture are considered the most promising.[29,30]

Dynamic fluid culture systems employ microfluidic platforms to establish a controlled fluidic environment, in which culture media flow continuously or in a pulsatile manner to simulate physiological conditions, such as blood or lymphatic circulation. This dynamic environment helps maintain normal cellular physiological states and accelerates organoid maturation. For example, microfluidic devices can reconstruct a stable TME, promote interactions among tumor tissue fragments, and support the continuous migration of tumor-infiltrating lymphocytes. Consequently, lymphocyte-mediated tumor immune responses and infiltration processes can be effectively recapitulated, providing valuable reference for basic cancer research and the development of immunotherapies.[20]

Regarding multilayer microfluidic culture, the channel architecture of multilayer microfluidic systems allows different cell types or culture media to flow through separate channel layers, thereby enabling cell separation or controlled intercellular interactions. By precisely regulating fluid flow within each layer, the intrinsic multilayer structural features of *in vivo* organs can be faithfully reproduced. For instance, in the construction of PDO models of hepatocellular carcinoma, multilayer microfluidic chips can generate the physiological TME required for organoid growth. These platforms integrate a co-culture system comprising mesenchymal stem cells (MSCs) and peripheral blood mononuclear cells (PBMCs), ultimately establishing an MSC-PDO-PBMC composite model. In preliminary evaluations involving chemotherapeutic and molecular targeted agents, this model demonstrated considerable potential for applications in drug screening and fundamental cancer research.[30]

3.3. Background of integration of the two systems

The integration of hydrogels and tumor organoids arises from the need to leverage the complementary advantages of these two systems. Although traditional *in vitro* models can preserve tissue structure, they are limited by short survival durations and low experimental throughput.[1] In contrast, *in vivo* models are capable of simulating the

systemic physiological environment but are constrained by species differences and immune deficiencies. Moreover, the substantial disparity in organ complexity between humans and experimental animals results in low drug translational efficiency of anticancer drugs.[2,4] As 3D culture platforms, hydrogels provide a microenvironment that more closely resembles *in vivo* conditions while overcoming the inherent limitations of conventional models, thereby offering stable and controllable scaffold support for organoid growth.[31,32]

The application of hydrogels in tumor research has evolved progressively rather than emerging abruptly, exhibiting a clear trajectory of dynamic development. In the early stage, owing to their excellent biocompatibility and controlled drug-release capability, hydrogels were primarily utilized as carriers for targeted delivery and sustained release of antitumor agents. Through precise drug localization and prolonged therapeutic action, they enhanced treatment efficacy while reducing off-target toxicity.[18] With the rapid advancement of tumor organoid technology, the application scope of hydrogels has expanded substantially, and they have now become core scaffold materials for tumor organoid construction.[5,20] Recent advances in bioengineering platforms have further accelerated this development: 3D bioprinting enables precise spatial control of tumor architecture by integrating ECM and vascular components, thereby generating customizable microenvironments for tumor organoids.[33] Hydrogel-based dynamic scaffolds can mimic key biomechanical and biochemical features of the tumor matrix, including stiffness, degradability, and molecular gradients, providing more physiologically relevant culture conditions. In parallel, microfluidic platforms have enabled the integration of fluid flow, vascular-like channels, and compartmentalized co-culture systems, further refining tumor organoid models and offering robust technical support for the dual application of hydrogels in tumor research.[34,35]

The integration of hydrogels and tumor organoids has created new opportunities for tumor research.[5,20] This integrated model exhibits several notable advantages:

(i) Simulating TME complexity, including the composition, mechanical properties, and biochemical signaling of the ECM; [32,36]
(ii) Support for long-term culture and high-throughput screening, thereby overcoming the limitation of short survival times associated with traditional explant models;[1,20]
(iii) Facilitation of personalized tumor modeling, enabling the construction of patient-specific tumor models through the combination of PDOs and customized hydrogels;[37,38]
(iv) Provision of controllable experimental conditions, allowing systematic investigation of the effects of distinct microenvironmental factors on tumor cell behavior by modulating hydrogel composition and structural properties;[32,36]
(v) In addition, hydrogels can be loaded with signaling molecules such as BMP4 to precisely regulate the differentiation trajectories of organoids, thereby further enhancing the biological authenticity and practical practicality of the model.[10,38]

4. Fabrication technologies of hydrogels

The fabrication methods, functional characteristics, and application scenarios of various hydrogel types are systematically summarized in Table 1.

4.1. Fabrication of natural hydrogels

The fabrication of natural hydrogels primarily involves the extraction of polymers from natural sources, followed by appropriate chemical or physical treatments.[39] Fabrication methods for several commonly used natural hydrogels are summarized in the following.

Collagen hydrogels are typically prepared by extracting collagen from animal tissues such as cowhide and pigskin. Collagen is the principal ECM protein in human breast tissue and represents the most abundant protein in the animal kingdom. As a major structural component of the ECM, collagen can be easily and cost-effectively isolated from tissues including skin, tendons, and pericardia, which has led to its widespread application as a biomaterial.[40,41] Owing to its excellent biocompatibility, collagen provides an optimal microenvironment for cell adhesion and proliferation, making it particularly suitable for tissue regeneration and organoid culture.[42,43] The preparation of collagen hydrogels generally involves several steps. First, collagen is extracted from tissues through enzymatic or acid hydrolysis; subsequently, gelation is induced by adjusting the pH and temperature, which promotes the self-assembly and aggregation of collagen molecules into a 3D network.[40,44] In breast tumor models, type I collagen is extensively utilized because of its high abundance in breast tissue and its critical role in maintaining ECM structure.[20,40] Bioprinting using collagen-based bioink can further enhance tissue fidelity and biological relevance, enabling the construction of vascularized tumor organoids for drug screening applications.[7] In addition, Enrico *et al.*[45] reported a strategy for 3D vascular bioprinting based on collagen hydrogels, in which microchannels and cavities are generated through femtosecond laser irradiation. This approach enables the fabrication of millimeter-scale channels with diameters ranging from 20 to 60 μm, which remain stable for at least 8 days under physiological conditions. Importantly, this method allows the creation of 3D microchannels and cavities of arbitrary shapes and sizes while preserving cellular bioactivity within the hydrogel

matrix. Its key advantage lies in providing biologically relevant yet precisely controllable angiogenic structures, thereby facilitating the development of advanced 3D tissue models for investigating complex biological targets such as tumors and neural tissues.[45]

The fabrication of gelatin hydrogels is relatively straightforward, as gelatin is a hydrolysis product of collagen and is primarily composed of glycine, proline, and hydroxyproline. Gelatin exhibits excellent biocompatibility, hydrophilicity, and thermosensitivity, making it a widely utilized material for tissue engineering scaffolds. Upon heating, gelatin forms a viscous solution, which subsequently transitions into a hydrogel upon cooling. This property enables the fabrication of high-resolution scaffolds using 3D printing technology.[19,46] Gelatin can reduce the viscosity and shear stress of bioinks, thereby improving printability and minimizing cell damage during cell-laden bioprinting.[6,47]

The fabrication of alginate hydrogels is primarily achieved via ionic crosslinking.[48,49] Alginate, a natural polymer extracted from brown algae, is composed of β-D-mannuronic acid and α-L-guluronic acid. Renowned for its excellent biocompatibility, non-immunogenicity, and gelation properties, alginate has become a widely used material for medical biomaterials, antitumor drug delivery carriers, and tumor organoid culture scaffolds.[48,50] In tumor model construction and organoid culture, alginate can function as a surrogate for glycosaminoglycans, effectively immobilizing tumor-associated proteins and tumor cells.[11,48] Its favorable controlled-release characteristics also make it widely applied in the sustained delivery of antitumor drugs in early studies, thereby reducing drug-related toxicity and side effects.[48,51] Crosslinking agents such as calcium chloride are commonly employed to modulate the mechanical strength and structural integrity of alginate-based hydrogels.[11] During tumor organoid culture, cells require appropriately interconnected pores to facilitate effective communication. Alginate scaffolds can be fabricated using methods such as gas foaming, microfluidic foaming, electrospinning, leaching, and freeze-drying, thereby creating a suitable microenvironment for organoid growth. For instance, Fang *et al.*[50] achieved high-throughput generation of tumor organoids within alginate microbeads using microfluidic droplet technology. The organoids in these microspheres exhibited both tubular and solid-like structures that closely resembled the original tumors in terms of cellular phenotype and lineage, fully demonstrating the expanded application of hydrogels from drug delivery carriers to organoid scaffolds.

The fabrication of chitosan hydrogels typically involves deacetylation treatment. Composite chitosan hydrogels incorporating whitlockite nanoparticles have been shown to enhance blood coagulation through ion release, reducing bleeding more effectively than commercial products. Chitosan is a natural, biodegradable polymer with broad applications; however, its utility is limited by poor solubility.[52,53] Derivatives such as N-trimethyl chitosan, N-carboxymethyl chitosan, and N-carboxyethyl chitosan (CEC) have been developed to overcome this limitation.[13]

Several key factors influence the properties of natural hydrogels during fabrication. Polymer concentration directly affects the mechanical strength and porosity of the hydrogels. Crosslinking density can be tuned by adjusting the crosslinker concentration and reaction time. Ionic strength plays a crucial role in the characteristics of ionically crosslinked hydrogels, such as alginate.[11,54] Additionally, the decellularization method is decisive for maintaining the structural and biochemical integrity of decellularized ECM (dECM) hydrogels.[5,55]

It should be noted that natural hydrogels possess inherent limitations. For instance, while alginate hydrogels are widely used, they exhibit restricted mechanical properties, instability during degradation, and insufficient durability under application conditions. Currently, various strategies have been developed to mitigate these deficiencies.[49,50] Similarly, collagen hydrogels generally display relatively low mechanical strength without covalent crosslinking, which may pose challenges for culturing organoids that require stiffer matrices.[44]

4.2. Fabrication of synthetic hydrogels

The fabrication of synthetic hydrogels involves precise chemical synthesis and polymerization reactions, offering high tunability.[8,56] The main preparation methods are summarized as follows:

Free radical polymerization is one of the most commonly employed methods for hydrogel synthesis. Polymers such as polyethylene glycol (PEG) and polyacrylamide (PAM) can form hydrogel networks via free radical polymerization. The reaction is typically conducted in the presence of initiators (*e.g.*, ammonium persulfate) and catalysts (*e.g.*, tetramethylethylenediamine). The network structure, porosity, and mechanical properties of the resulting hydrogels can be precisely tuned by adjusting the monomer concentration, crosslinker dosage, and reaction conditions. PEG-based hydrogels are extensively used in tumor research due to their excellent biocompatibility and low immunogenicity.[6,47]

Photoinitiated polymerization enables spatially selective gelation. Gelatin methacrylamide (GelMA) is particularly favored for its adjustable mechanical and tunable physiochemical crosslinking properties.[6,47] Innovative strategies combining GelMA with bioactive components—such as calcium silicate ($CaSiO_3$) or methacrylate-based κ-carrageenan (CarMA)—via 3D printing have produced

scaffolds that support adipogenesis, angiogenesis, and mimic native tissue.[57]

Step-growth polymerization is suitable for preparing polyester-based hydrogels, such as poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL). These polymers can be synthesized via ring-opening polymerization or polycondensation reactions. The degradation rate and mechanical properties of the resulting hydrogels can be precisely controlled by adjusting monomer ratio, molecular weight, and end-group functionalization. PCL-based hydrogels are capable of replicating the mechanical properties of natural tissues, supporting volumetric regeneration, and can be fabricated using 3D printing techniques such as fused filament fabrication and selective laser sintering.[8]

Chemical crosslinking involves various types of covalent bond formation reactions. For instance, polyvinyl alcohol (PVA) hydrogels can form physical crosslinks through freeze-thaw cycles or covalent crosslinks via chemical crosslinkers, such as glutaraldehyde.[56,58]

The key parameters for the fabrication of synthetic hydrogels include the following: monomer purity affects the controllability of polymerization reactions and the homogeneity of the resulting products; reaction temperature and duration, which influence molecular weight and crosslinking density; the dosage of initiators and catalysts, which determine the polymerization rate and network structure; solvent selection, which impacts reaction kinetics and product properties; and post-treatment processes, including washing and drying steps, which affect the purity and performance of the resulting hydrogel.[14,56]

Compared with natural hydrogels, synthetic matrix hydrogels are produced via chemical synthesis, offering high tunability and precise control over their composition, mechanical properties, and degradation characteristics. Moreover, synthetic matrices can be chemically modified or functionalized to precisely regulate cellular behaviors and organoid development, thereby providing unique advantages for the construction of personalized organoid models.[59]

4.3. Construction of composite hydrogels

The construction strategies of composite hydrogels aim to combine the advantages of natural and synthetic polymers while overcoming the limitations of single-component materials.[14,60] The main construction methods are as follows:

The physical blending method is the simplest approach to preparing composite hydrogels. It involves mixing natural and synthetic polymers in solution, followed by inducing gelation through physical or chemical means. For example, studies on gelatin/alginate composite hydrogels fabricated via 3D printing have shown that increasing the alginate concentration enhances scaffold elasticity and reduces surface roughness without compromising biological properties. These findings highlight the excellent mechanical tunability and biocompatibility of alginate, reinforcing its applicability in *in vitro* tumor modeling.[54]

The chemical crosslinking method constructs composite networks by forming covalent bonds between different polymer chains. For instance, dopamine-modified hyaluronic acid and polydopamine-coated graphene oxide exhibit strong mucoadhesion and wound-healing capacity through quinone-mediated crosslinking.[61] Similarly, ECM-mimetic hydrogels composed of dopamine-modified gelatin and chondroitin sulfate methacrylate achieve tight adhesion via borate ester and thioether bonds.[62]

The layer-by-layer assembly method constructs composite hydrogels with gradient structures by alternately depositing polymer layers with distinct properties. It can generate regions with varying mechanical properties and biological functions, thereby better mimicking the heterogeneity of natural tissues. Layer-by-layer hydrogels are particularly suitable for supporting organoid growth in multilayer microfluidic culture systems, as they recapitulate the inherent multilayer structural characteristics of *in vivo* organs.[30]

Nanocomposite technology incorporates nanoparticles (*e.g.*, carbon nanotubes, graphene, bioceramics, *etc.*) into hydrogel matrices to enhance mechanical properties or impart special functions.[63,64] For instance, composites of alginate, gelatin, and cellulose nanocrystals (CNCs) demonstrate improved mechanical strength, enhanced cell viability, and slow degradability. PEG-based dynamic hydrogels with surface-immobilized RGD maintain cell adhesion, emphasizing the critical role of ligand presentation and molecular migration.[9]

The key considerations for the construction of composite hydrogels include compatibility, as the interaction between different polymers affects the homogeneity of the mixture;[39,60] component ratio, which requires optimization of the proportion of natural to synthetic polymers to achieve the desired performance; processing technology, taking into account the differences in processing conditions among various components;[54,65] functionalization strategy, which involves introducing specific bioactive groups via chemical modification, such as the incorporation of signaling molecules including BMP4.[10,38]

Table 1 summarizes fabrication methods, core functional characteristics, representative application scenarios in tumor organoid culture, and corresponding references for natural, synthetic, and composite hydrogels. The table systematically organizes the key information

Table 1. Fabrication methods, functional characteristics, application scenarios in tumor organoids, and representative references for different hydrogel types

Hydrogel type	Fabrication method	Core functional characteristics	Tumor organoid application scenarios	Ref.
Natural hydrogel (collagen)	Enzymatic extraction from animal tissues (*e.g.*, bovine hide, porcine skin) → pH/temperature-induced gelation	High biocompatibility, ECM-mimetic architecture, and strong cell adhesion support	Vascularization modeling and long-term stable culture of breast tumor organoids	7,39,44
Synthetic hydrogel (PCL)	Ring-opening polymerization → 3D printing (*e.g.*, fused deposition modeling or selective laser sintering)	Tunable mechanical stiffness (5–50 kPa), and controllable degradation rate (several months)	Morphological maintenance of liver cancer organoids and development of high-throughput drug screening platforms	8,59
Composite hydrogel (gelatin/alginate)	Physical blending of gelatin and alginate → calcium ion-mediated ionic crosslinking	Balanced mechanical strength and biocompatibility with injectable properties suitable for minimally invasive applications	High-throughput culture of colorectal cancer organoids and personalized drug sensitivity testing	11,48,61

Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.

discussed in **Section 3**, providing a comparative overview of the application characteristics and functional advantages of different hydrogel types in tumor organoid systems.

5. Specific applications and limitations of hydrogels and organoids

The application scenarios, core advantages, existing limitations, and optimization directions of hydrogel-tumor organoid models are systematically summarized in Table 2.

5.1. Applications in tumor model construction

Hydrogels provide an ideal 3D culture environment for tumor organoids, effectively recapitulating the complexity of the TME.[20,32] Three-dimensional hydrogel systems mimic the breast TME by incorporating bioactive ECM components or analogs, such as chitosan, alginate, hyaluronic acid, cellulose, collagen, gelatin, and silk fibroin.[7,19] These platforms facilitate detailed investigations of cell–cell and cell–matrix interactions, gene expression, and tumor heterogeneity.

In tumor model construction, the primary applications of hydrogels include:

(i) *Simulation of cell–cell interactions*. Co-culturing cancer cells with stromal components (*e.g.*, fibroblasts and macrophages) within hydrogel scaffolds enables the modeling of tumor heterogeneity and chemoresistance. For instance, alginate-encapsulated cryopreserved cells combined with macrophages can establish an immune-like microenvironment. Patient-derived cells or tissues cultured in PEG-heparin or decellularized scaffolds retain their original phenotype and drug resistance, advancing personalized tumor modeling for precision medicine and high-throughput drug screening.[24,31,66] Furthermore, microfluidic bioprinting platforms allow the study of tumor-normal cell interactions and migration, while multilayer microfluidic culture systems can construct more complex co-culture models through the integration of hydrogel scaffolds and different cellular layers. For example, the MSC-PDO-PBMC hepatocellular carcinoma model co-cultures MSCs, peripheral blood cells, and liver cancer organoids, more accurately simulating cellular within the *in vivo* TME.[29,30,34]

(ii) *Study of cell–matrix interactions*. Functionalized hydrogels, such as recombinant spider silk containing RGD motifs or elastin-like recombinant (ELR)-based gels incorporating MMP-degradable sequences, can enhance cell adhesion and modulate drug resistance. Although certain non-human ECM sources may limit clinical translation, these models remain highly valuable for investigating cell–matrix interactions.[7,19,20] Moreover, hydrogels can regulate the differentiation of organoid cells by delivering signaling molecules, such as BMP4, thereby providing a platform to explore the mechanism of signaling molecule-mediated cell–matrix interactions. For example, the role of BMP4 in promoting differentiation of intestinal epithelial cells in colorectal cancer organoids has been elucidated using BMP4-loaded hydrogel scaffolds.[10,38]

(iii) *Maintenance of tumor heterogeneity*. Three-dimensional hydrogel culture better recapitulates the TME than two-dimensional systems, thereby enhancing the relevance of drug testing. Patient-derived organoids retain the genotypes and phenotypes of the original tumors and exhibit clinically relevant chemoresistance, enabling personalized treatment evaluation and biobanking. Studies have demonstrated a strong correlation between PDO drug responses and patient prognoses, supporting applications in precision oncology.[5,37] Interindividual heterogeneity of tumor cells is a major contributor to treatment failure, and hydrogel-organoid integrated models can

effectively preserve this heterogeneity. For instance, gastric cancer organoids cultured in hydrogel scaffolds can differentiate into multiple subcellular subtypes, enabling the construction of activated models of the RAS and WNT signaling pathways to simulate the heterogeneous characteristics of tumors *in vivo*.[23,67]

(iv) *Reconstruction of the TME*. Advanced models integrate immune components, facilitating research on immunotherapy and microbiome interactions. PDOs also enable long-term studies on hormone therapy, such as for estrogen receptor-positive breast cancer.[25,26] By tuning the composition and architecture of hydrogels, changes in the microenvironment at different tumor development stages can be simulated, including increased matrix stiffness, initiation of angiogenesis, and infiltration of immune cells.[36,68] Dynamic fluid culture, combining microfluidic systems with hydrogels, can mimic physiological fluid environments such as blood and lymph flow, maintain normal cellular physiology, accelerate organoid maturation, and simulate lymphocyte-mediated tumor immunity and infiltration, providing an ideal model for studying the tumor immune microenvironment.[29,34] Furthermore, 3D vascular bioprinting using collagen hydrogels can construct microchannels that replicate *in vivo* vasculature, enhancing vascularization simulation and improving the physiological relevance of tumor models.[45]

5.2. Applications in drug R&D and screening

The hydrogel-tumor organoid model demonstrates significant potential in drug research and development (R & D) and screening, primarily in the following aspects:

(i) *Drug efficacy evaluation*. This model enables more accurate assessment of drug efficacy within an environment closely resembling *in vivo* conditions. Compared with conventional two-dimensional cell cultures, 3D hydrogel-based models better simulate drug penetration, distribution, and metabolism within tumor tissues.[5,37] For instance, when evaluating chemotherapeutic agents, this model can reveal differential effects of drugs on distinct tumor cell subsets, more faithfully reflecting clinical outcomes. Tumor organoids encapsulated in alginate hydrogel microbeads have been employed for high-throughput drug screening, with the internal tumor structures closely resembling the original tumors, thereby accurately capturing the inhibitory effects of drugs on tumor cells.[50]

(ii) *Personalized drug screening*. By combining patient-derived tumor organoids with customized hydrogels, patient-specific drug screening platforms can be established. Such platforms have shown strong correlations with clinical responses across multiple cancer types.[37] For example, in breast cancer research, analysis of PDO responses to various therapeutic agents allows prediction of patient sensitivity to specific treatment regimens, guiding individualized therapy. Similarly, the MSC-PDO-PBMC hepatocellular carcinoma model, constructed using multilayer microfluidic chips, has demonstrated promising potential in personalized drug screening for liver cancer, as validated in preliminary experiments involving chemotherapeutic and molecular targeted therapies.[30]

(iii) *Drug resistance research*. The hydrogel-tumor organoid model can more accurately recapitulate both intrinsic and acquired drug resistance in tumor cells. Through long-term culturing of tumor organoids under drug pressure, the mechanism and progression of drug resistance can be systematically investigated.[25,26,37] Organoids retain tumor heterogeneity, and the stable microenvironment provided by hydrogels simulate the physiological conditions under which drug resistance develops *in vivo*. For instance, gastric cancer organoid models in hydrogels have been employed to assess dynamic changes in drug sensitivity and to study the signaling pathways underlying drug resistance.[67]

(iv) *Screening of combination drug*. This model is particularly suitable for evaluating the effect of combination drug regimens. By testing multiple drugs simultaneously within the same hydrogel-organoid system, synergistic or antagonistic interactions between drugs can be more accurately predicted, providing valuable insights for the development of novel combination therapies.[69,70]

(v) *Drug delivery system evaluation*. Hydrogels can serve both as drug carriers and as platforms to evaluate other drug delivery systems.[18] For example, host-guest gelatin hydrogels combined with MgFe nano-hybrids enable sustained ion and drug release, promoting osteogenesis and bone tissue repair. The use of amphiphilic stabilizers and electrospinning techniques can optimize the dispersion of nanocarriers. Moreover, these systems can incorporate stimuli-responsive drug release mechanisms triggered by pH, ionic strength, temperature, or light, enabling precise, on-demand delivery.[71,72] Additionally, the controlled-release properties of hydrogels allow simulation of *in vivo* drug release kinetics, supporting the evaluation of long-term efficacy and targeting performance of drug delivery systems.[17,18]

5.3. Limitations

Despite the numerous advantages of the hydrogel-tumor organoid model, several important limitations remain.

Several hydrogel-related limitations are described as followed:

(i) *Insufficient mechanical property matching*. Although the mechanical properties of hydrogels are tunable, fully recapitulating the complex and spatially heterogeneous mechanical environment of tumor tissues *in vivo* remains challenging.[36,68] Some natural hydrogels exhibit inherently limited mechanical strength and unstable degradation, making it difficult to maintain the matrix stability required for long-term organoid culture.[44,49]

(ii) *Batch-to-batch variation*. Natural hydrogels, such as collagen and hyaluronic acid, display source-dependent variability, limiting their consistency in clinical applications. Variations in polymer synthesis, crosslinking density, or biofunctionalization between batches can significantly alter the properties.[73]

(iii) *Immunogenicity risk*. Although hydrogels are generally considered biocompatible, certain synthetic hydrogels may provoke immune responses. Residual crosslinkers, degradation by-products, or non-natural chemical modifications can induce inflammation, fibrosis, or immune rejection.[74,75]

(iv) *Long-term stability issues*. Many hydrogels undergo degradation or changes in performance during extended culture periods, compromising the stability of the culture environment. Natural hydrogels in particular may lose structural integrity within days to weeks due to enzymatic degradation.[75,76]

Tumor organoid-related limitations also warrant consideration, as outlined below:

(i) *Difficulty in obtaining biopsy samples*. High-quality tumor tissue samples are a prerequisite for establishing organoid models, yet clinical biopsy samples are often limited and may not fully represent tumor heterogeneity. Small biopsy samples may fail to capture the complete characteristics of the tumor.[25,26,77]

(ii) *Complex culture conditions*. Tumor organoid culture requires specialized medium formulations and precise culture conditions. Different tumor types may necessitate distinct culture protocols, and organoid differentiation relies on tightly regulated signaling molecules such as BMP4, with precise control over concentration and exposure time being challenging.[10,38] This complexity increases both the costs and technical difficulty of organoid culture.[25,26,78]

(iii) *Limited model representativeness*. Although organoids retain many tumor characteristics, they cannot fully replicate the complexity of tumors *in vivo*.[25,26,78] For example, organoids typically lack a complete vascular network, innervation, and a fully functional immune microenvironment. Even with the incorporation of immune and stromal cells via multilayer microfluidic culture, faithfully simulating the complex intercellular and organ-level interactions remains difficult.[30,34]

(iv) *Genetic differences and model efficiency issues*. Inter-patient genetic variability poses a challenge for developing effective personalized treatments. Moreover, organoid culture using engineered matrices is often less efficient than using traditional extracellular matrices, limiting their broader application in tumor organoid modeling.[77]

Challenges in technology integration are summarized as follows:

(i) *Difficulty in standardization*. Currently, there is a lack of unified standards for hydrogel preparation and organoid culture. Laboratory-specific variations in methods and conditions—including hydrogel component ratios, loading concentrations of signaling molecules, and parameter settings in microfluidic culture—cannot be easily addressed due to a lack of standardized guidelines, thereby affecting the comparability and reproducibility of results.[27,73]

(ii) *High cost*. The requirement for high-quality hydrogel materials, specialized culture reagents, and advanced equipment substantially increases research costs, limiting the widespread adoption and application of this technology.[79,80]

(iii) *Technical complexity*. The construction and maintenance of hydrogel-tumor organoid models demand multidisciplinary expertise, encompassing materials science, cell biology, tissue engineering, and microfluidic technology, placing high technical requirements on researchers. Furthermore, the integration of hydrogels with microfluidic systems and gene editing techniques further raises the technical difficulty.[30,34]

(iv) *Lack of long-term follow-up data*. Given the relative novelty of this technology, long-term clinical validation data are limited. In particular, the accuracy of these models in predicting treatment responses and patient prognosis requires further verification. Additionally, data regarding the long-term stability of organoid-hydrogel integrated models and the correlation between model outcomes and clinical efficacy remain scarce.[75,76]

5.4. Standardization challenges in natural hydrogels

Standardization remains a major barrier to the clinical translation of natural hydrogels, largely due to intrinsic batch-to-batch variability arising from biological sources and processing procedures. For instance, collagen hydrogels derived from bovine tissues often exhibit variations in molecular weight distributions and purity depending on tissue origin and extraction methods, which directly influence mechanical properties and gelation kinetics. Similarly, dECM hydrogels display considerable compositional heterogeneity as a result of differences in decellularization protocols and storage conditions.

Current standardization efforts focus on the establishment of reference materials, harmonized preparation protocols, and clearly defined critical quality attributes (CQAs). Guidelines such as ISO 10993-1 provide a foundational framework for the biological evaluation of biomaterials, although dedicated standards for hydrogel systems remain limited. Modern characterization strategies increasingly integrate rheological analyses (*e.g.*, assessments of shear-thinning behavior and yield stress) and multi-parameter evaluations of mechanical, chemical, and biological properties to improve reproducibility. In addition, defining quality control specifications for CQAs, including endotoxin levels, gelation time, and molecular weight, helps ensure consistency in hydrogel manufacturing and performance.

It is noteworthy that key limitations remain. The absence of standardized reference hydrogels hampers method validation and inter-laboratory comparability. Regulatory frameworks such as EU Medical Device Regulation (EU 2017/745) classify many natural hydrogel-based products as class III medical devices, yet provide limited guidance on detailed standardization requirements, creating uncertainty for manufacturers. Furthermore, the inherently complex and multi-component composition of natural hydrogels complicates the development of comprehensive and universally applicable protocols.

Future progress will depend on collaborative efforts among industry, academia, and regulatory bodies (*e.g.*, International Society for Biomaterials) to establish consensus standards. Emerging analytical approaches, such as multi-attribute testing (MAT) and AI-driven prediction of hydrogel performance based on raw material characteristics, offer promising strategies for addressing material variability. Without these advances, achieving reproducibility and regulatory acceptance for natural hydrogels will remain challenging, ultimately limiting their clinical translation in regenerative medicine.[27]

Table 2 summarizes the typical application scenarios, core advantages, key limitations, and targeted optimization strategies of hydrogel-tumor organoid models, along with representative references. The table systematically integrates the main information discussed in Section 4, providing an overview of the practical value of these models and the directions for future improvement.

Table 2. Application scenarios, core advantages, existing limitations, optimization strategies, and representative references for hydrogel-tumor organoid models

Application scenarios	Core advantages	Existing limitations	Optimization directions	Ref.
Tumor model construction	Recapitulates TME, including ECM composition and cell-cell/cell-matrix interactions; preserves intratumoral heterogeneity; supports co-culture of tumor cells with stromal and immune components; enables simulation of stage-specific TME dynamics during tumor progression	Lacks of functional vascular networks and innervation; limited integration of immune components; use of non-human ECM sources restricts clinical translation; difficulty in reproducing organ-level intercellular interactions	Incorporates 3D-bioprinted vascularized scaffolds with immune/stromal co-culture; optimizes hydrogel composition to mimic stage-specific TME features; employs dECM hydrogels to improve clinical compatibility; develops multilayer microfluidic co-culture platforms	27,73
Drug screening	Enables accurate evaluation of drug efficacy and penetration; supports personalized and high-throughput drug screening; facilitates studies of intrinsic and acquired drug resistance; suitable for assessing combination therapies and drug delivery systems	Limited standardization of culture systems; inadequate simulation of *in vivo* drug metabolism; hydrogel batch variability affects reproducibility; lack of unified criteria for drug sensitivity evaluation	Establishes standardized drug sensitivity assessment frameworks; integrates microfluidic systems to simulate drug metabolism and penetration; standardizes hydrogel preparation and organoid culture protocols; develops multi-cell co-culture models to improve prediction accuracy	73,79,80
Precision medicine	PDOs demonstrate strong correlation with clinical treatment outcomes; support personalized therapy optimization; enable tumor biobank construction; provide platforms for studying immunotherapy and hormone therapy	Scarce long-term clinical translation data; difficulty in obtaining representative biopsy samples; relatively low culture efficiency with engineered matrices; insufficient long-term follow-up on predictive performance	Conducts multicenter clinical validation studies; optimizes tumor sample cryopreservation and culture protocols; establishes clinical translation guidelines for hydrogel-organoid models; integrates gene-editing technologies to enhance predictive predictability	27,79,80

Abbreviations: dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; PDOs: Patient-derived organoids; TME: Tumor microenvironment.

6. Future perspectives

6.1. Directions of technological innovation

The fabrication technologies of hydrogels and culture techniques of tumor organoids are evolving toward higher precision and intelligence, mainly in the following aspects:

(i) *Development of stimuli-responsive hydrogels*. Future hydrogels are expected to exhibit more precise environmental responsiveness. Cancer cells often display extracellular acidification and intracellular alkalization, with elevated intracellular pH promoting glycolysis, hypoxia adaptation, and proliferation. Exploiting such pH differences, pH-responsive hydrogels have been designed to achieve site-specific release of cytotoxic drugs within the TME, thereby minimizing off-target toxicity. The incorporation of ionizable moieties, such as amines, carboxylic acids, or imines, imparts pH responsiveness to hydrogels.[13] Moreover, hydrogels capable of responding to concentration changes of signaling molecules, such as BMP4, in the TME can be developed to dynamically regulate organoid differentiation, further enhancing the physiological relevance and authenticity of the model.[10,38]

(ii) *3D bioprinting technology*. 3D bioprinting technologies—including extrusion, inkjet, stereolithography, and microfluidics—enable the precise fabrication of complex hydrogel structures that closely mimic natural tissues. The injectability and shear-thinning properties of dynamic hydrogels facilitate high-resolution printing with stimuli-responsive crosslinking, allowing spatiotemporal control over scaffold formation.[81] When combined with 3D vascular bioprinting technologies based on collagen hydrogels, hydrogel scaffolds with dynamic vascular networks can be constructed, providing organoids with nutrient supply and metabolic environment that more closely resemble *in vivo* condition.[45]

(iii) *Self-folding hydrogel technology*. Self-folding hydrogels can form intricate 3D structures that support cell adhesion, migration, and tissue development. Their applications have been extended to micro-grippers for minimally invasive surgery and shape-memory drug delivery systems for controlled targeted release. Integration into surgical tools can further enhance flexibility and navigation capabilities.[82] Dynamic hydrogels enable spatiotemporal control over mechanical and biochemical cues, facilitating detailed studies on chemotaxis, haptotaxis, and mechanotransduction under physiologically relevant forces.[83]

(iv) *Intelligent hydrogel systems*. Future hydrogels are expected to integrate multiple functionalities, including real-time monitoring, adaptive response, and intelligent drug release.[72] For example, hydrogels embedded with sensors can monitor changes in the TME in real time, such as pH value, oxygen concentration, glucose levels, and automatically adjust drug release rates based on these readings.[17] Simultaneously, they can be combined with sustained-release systems for signaling molecules to precisely regulate the release kinetics of factors such as BMP4, thereby achieving accurate control over organoid differentiation.[10,38]

Several breakthroughs have been achieved in tumor organoid culture techniques, which are outlined as follows:

(i) *Improved culture success rate*. Optimization of medium formulations and development of new matrix materials can enhance the culture success rate of organoids across diverse tumor types.[29,30]

(ii) *Enhanced model complexity*. Future organoid models are expected to incorporate more cell types, including immune cells, vascular endothelial cells, and fibroblasts, to better replicate the TME. In combination with gene editing technologies, organoids can be engineered to express specific genes, thereby increasing their utility in targeted therapy research.[17,28]

(iii) *Establishment of standardized culture protocols*. The development of standardized culture procedures and quality control systems—including hydrogel preparation standards, organoid culture parameters, and signaling molecule loading concentrations—will ensure the comparability and reproducibility of results across laboratories.[27,73]

(iv) *Long-term culture technology*. Advanced technologies that support the stable, long-term culture of organoids—such as improved hydrogel degradation profiles and optimized culture environments—will provide more durable models for chronic disease research and drug screening.[75,76]

6.2. Trends of multidisciplinary integration

The integration of hydrogel-tumor organoid models with other cutting-edge technologies is opening new avenues in tumor research:

(i) *Integration with microfluidic technology*. Microfluidic platforms enable the incorporation of fluid flow, vascular channels, and compartmentalized co-culture, facilitating the study of invasion, intravasation, extravasation, and drug delivery. This integration allows precise control over delivery of nutrients, drugs, and signaling molecules, effectively simulating blood circulation and interstitial fluid flow *in vivo*.[34,35] Further optimization of multilayer microfluidic culture systems can better recapitulate the structural characteristics of *in vivo* organs. For example, refinement of the

MSC-PDO-PBMC co-culture model enhances its applicability in immunotherapy and targeted therapy research.[30]

(ii) *Integration with gene editing technology*. Combining gene editing tools such as CRISPR-Cas9 with hydrogel-organoid models enables precise modification of tumor cell genes, facilitating the study of the role of specific gene mutations in tumorigenesis and progression.[17] Moreover, the integration of organoid culture with gene editing allows the design of organoids expressing defined genes and functions, thereby enhancing their utility in targeted cancer therapy research.[17,28]

(iii) *Integration with nanotechnology*. Incorporating nanotechnology into hydrogels can impart novel functionalities.[28,64] For instance, the addition of magnetic nanoparticles can render hydrogels magnetically responsive, enabling controlled drug release or guidance of cell migration under an external magnetic field.[84,85]

6.3. Clinical translation prospects

The integrated technology of hydrogels and tumor organoids holds broad potential for clinical applications; however, several key challenges must be addressed.

The clinical diagnostic applications of hydrogels and tumor organoids include the following:

(i) *Personalized diagnosis*. Patient-derived tumor organoids can be employed to establish personalized diagnostic models, enabling clinicians to more accurately assess tumor malignancy and prognosis.[25,37] When combined with hydrogel-simulated TMEs, the invasiveness and metastatic potential of tumors can be evaluated with greater precision, providing a more comprehensive basis for diagnosis.[36]

(ii) *Drug sensitivity testing*. Evaluating the efficacy of different drugs using organoid models allows prediction of patient-specific treatment responses, thereby guiding individualized therapeutic decisions.[37] For instance, multilayer microfluidic organoid models have been applied for drug sensitivity testing in cancers such as liver cancer and gastric cancer, offering precise guidance for medication selection.[30]

Formulation of treatment regimens encompasses several considerations: (i) *Neoadjuvant therapy screening*. Prior to surgery, PDO models can be utilized to test various treatment regimens, facilitating the selection of the most effective neoadjuvant therapy;[25,26,37] (ii) *Drug resistance prediction*. Organoid models can anticipate potential drug resistance during treatment, allowing timely adjustments to therapeutic strategies;[25,26,37] (iii) *Combination therapy optimization*. These models enable rapid screening of optimal combination therapy regimens, improving treatment efficacy.[69,70]

Clinical translation of hydrogels and tumor organoids presents several challenges, which are outlined below alongside potential solutions:

(i) *Regulatory approval challenges*. Hydrogels often occupy a regulatory gray area, being classified as both medical devices and drug carriers. This can be potentially addressed through close collaboration with regulatory authorities to clarify product classification and establish unified technical standards and quality control systems.[27]

(ii) *Large-scale production issues*. Translating laboratory-scale models to clinical applications presents challenges including sterilization methods, shelf-life optimization, storage conditions, and scalable production standardization. The solutions include development of hydrogel-specific sterilization technologies, optimization of storage conditions using methods such as freeze-drying, and implementation of automated production workflows.[80]

(iii) *Cost control*. High-quality hydrogel materials and specialized culture conditions contribute to elevated costs. To control the costs and expenses, it is imperative to develop cost-effective hydrogel materials, optimize culture protocols to reduce reagent consumption,[79] and establish centralized organoid preparation centers to achieve economies of scale.[80]

(iv) *Technical training*. Clinicians and technicians require specialized training to effectively adopt this technology. Potential solutions include establishing standardized training systems, developing user-friendly kits and equipment, and providing ongoing technical support and remote guidance.[79]

(v) *Ethical and legal considerations*. These involve the use of patient-derived tissues and privacy protection. This challenge can be mitigated through rigorous ethical review mechanisms, robust informed consent procedures, and secure data management systems.[79]

Through continuous technological innovation and interdisciplinary collaboration, the integrated hydrogel-tumor organoid platform is expected to achieve clinical translation, providing more precise and effective diagnosis and treatment options for cancer patients. The successful application of this technology will drive the transformation of tumor medicine from a one-size-fits-all approach to personalized precision medicine, significantly improving patients' treatment outcomes and quality of life.

7. Conclusion

The integrated technology of hydrogels and tumor organoids represents a pivotal breakthrough in tumor biology research and precision medicine, effectively addressing the inherent limitations of traditional *in vitro* and *in vivo* models. By leveraging the 3D scaffold properties and functional

versatility of hydrogels, together with the tissue fidelity and heterogeneity retention of tumor organoids, this integrated system establishes a tumor research platform that closely recapitulates the *in vivo* physiological state. It plays a critical role in simulating the TME, enabling personalized drug screening, and investigating mechanisms of drug resistance. The distinct applications of natural, synthetic, and composite hydrogels further expand the functional scope and applicability of the model.

Nevertheless, several key challenges remain, including insufficient replication of the spatial heterogeneity of mechanical properties, lack of standardization in culture and fabrication processes, and limitations in large-scale production and cost control. Future progress will depend on the development of stimuli-responsive hydrogels, the integration of 3D bioprinting and gene editing technologies, and the establishment of standardized experimental protocols.

With continued interdisciplinary collaboration and improvement of clinical translation pathways, this integrated technology is expected to transform tumor diagnosis and treatment from an empirical paradigm to a precision-based model. It has the potential to provide patients with personalized diagnostic evaluation, drug screening, and therapeutic regimens, ultimately improving treatment outcomes and quality of life.

Acknowledgements

The authors thank Zibo Yimore Translation CO. LTD for English language editing.

Funding

This work was funded by the General Program of Natural Science Foundation of Shanxi Province, China (Grant No. 202203021211030) and the Shanxi Provincial Administration of Traditional Chinese Medicine Program (Grant No. 2023ZYYC2037). The authors gratefully acknowledge the support provided by these funding agencies.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization: Fushan Hou, Mingzhu Zhang
Visualization: Guangshun Zeng, Jiayi Niu, Jinqi Wang
Writing–original draft: Guangshun Zeng, Jiayi Niu, Jinqi Wang, Lulin Qiao
Writing–review & editing: Fushan Hou, Na Tang, Mingzhu Zhang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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