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Review

Engineering the Organoid Microenvironment: The Application of Hydrogel Matrics in Organoid Culture

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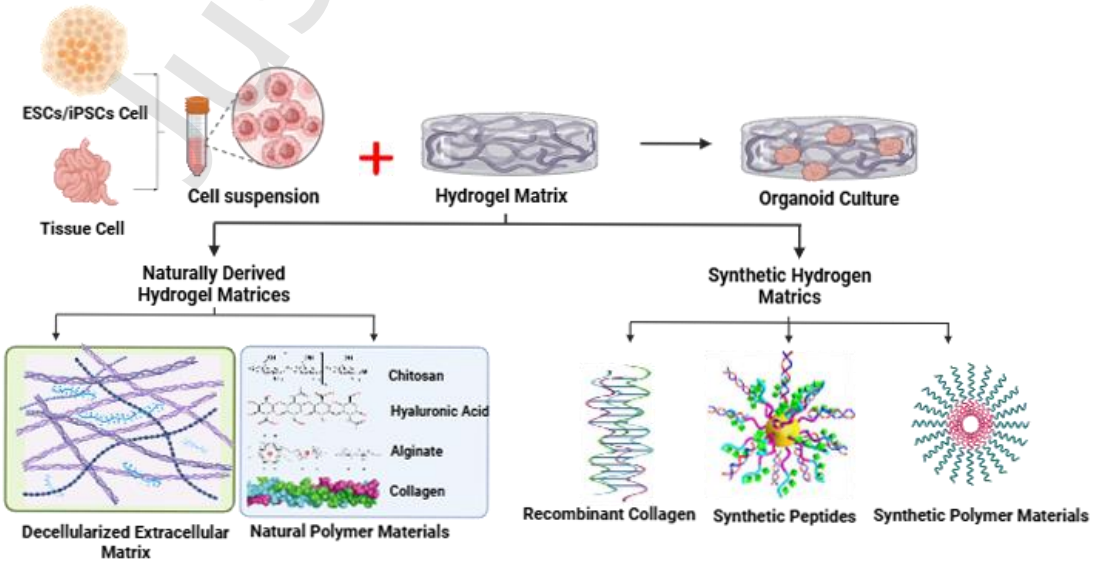
Abstract:

Organoids, which recapitulate the structure and function of human organs, have been widely used in various fields including disease modeling, drug discovery, personalized medicine, and regenerative therapy, demonstrating significant potential for future biomedical applications. Successful organoid culture depends on a matrix with the dual capability of mechanical support and mimicry of the *in vivo* microenvironment, both essential for cellular adhesion, proliferation, and differentiation. Owing to their tunable stiffness and viscoelasticity, which enable adaptation to the culture of different organoids, hydrogels have become a key material in the development of organoid culture matrices. This review summarizes current advances in hydrogels systems, encompassing both naturally derived and synthetic hydrogels used in organoid culture, with emphasis on their composition and physicochemical properties, with the aim of assisting researchers in selecting suitable hydrogels for their studies. We further explore how hydrogels composition governs gelation behavior and, ultimately, influences organoid growth and functionality, providing insights for the future development of "all-purpose" hydrogel matrices.

Keywords: Naturally Derived Hydrogel Matrices, Synthetic Hydrogel Matrices, Organoid Culture, Hydrogel Characteristics



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1 Introduction

Among the various *in vitro* microreaction systems currently available, organoids have emerged as one of the most representative models due to their three-dimensional structure, cellular heterogeneity, and partial recapitulation of organ-specific physiological functions. These attributes enable organoids to closely mimic the native tissue microenvironment and *in vivo* biological processes[1]. To date, organoid models representing more than twenty normal and pathological human organs have been successfully established, including brain[2], intestinal[3], gastric[4, 5], pancreatic[6], cardiac[7], hepatic[8], renal[9], and lung organoids[10]. These models have been widely applied in diverse research areas such as functional tissue induction, disease model construction, drug discovery and screening, personalized companion diagnosis, and regenerative medicine[11, 12].

Achieving high physiological fidelity in *in vitro* models necessitates precise engineering of the culture matrix. Hydrogels transcend the role of passive physical supports to act as sophisticated microenvironments. By strictly controlling physicochemical properties—such as biochemical ligands, viscoelasticity, and degradability—these matrices synergize with exogenous signals to orchestrate organoid morphogenesis. This active guidance of self-organization and maturation is critical for enhancing the reproducibility and standardization required for the clinical translation of organoid technologies. Efforts to develop suitable matrices can be traced back to the 1950s, when modified agar-based substrates were first employed for embryonic organ cultivation[13], and subsequently adapted for tumor tissue culture[14]. Despite its widespread use, agarose exhibited several intrinsic drawbacks, including low mechanical robustness, minimal bioactivity, inconsistency between batches, and limited functional versatility. These shortcomings impeded its effectiveness in supporting organoid culture and have prompted the development of alternative matrix systems with enhanced performance. A major breakthrough occurred in 1977, when a basement membrane extract containing extracellular matrix (ECM) was extracted from

Engelbreth-Holm-Swarm (EHS) mouse sarcoma. This material, later commercialized as Matrigel, provided a biologically active scaffold that revolutionized three-dimensional culture systems and established the foundation for modern organoid research. In 2009, Hans Clevers and his colleagues demonstrated that single mouse LGR5+ intestinal stem cells could self-organizing into intestinal organoids with crypt-villus structures in Matrigel *in vitro*, which marked the beginning of a new era for the early application of matrix gels in organoid culture.

With the rapid advancement of organoid technologies, the drawbacks of Matrigel, including compositional instability, high production costs, and potential biosafety issues, have become more apparent[12]. To address these challenges, researchers developed a broad spectrum of alternative matrix systems with enhanced reproducibility, tunable mechanical properties, and defined biochemical functions. Early efforts focused on biologically derived scaffolds such as collagen[13] and decellularized extracellular matrices[3], which provided improved biocompatibility and native-like cues for cell organization. Subsequent advances introduced fully chemically defined hydrogel systems, exemplified by the 2017 development of a polyethylene glycol (PEG)-based matrix incorporating arginine-glycine-aspartic acid (RGD) peptides and heparan sulfate to regulate cell adhesion and growth factor interactions[15]. Building upon these developments, Ganguly et al. further integrated matrix engineering with biofabrication approaches by employing bioprinting to deposit Gelatin Methacryloyl (GelMA)-carboxymethyl cellulose (CMC) composite hydrogels containing embedded cells in precisely predesigned structure. These engineered scaffolds, designed for *in vitro* culture, provided a significant step forward in creating customizable, functional alternatives to Matrigel for stem cell applications[16]. Collectively, these innovations highlight the evolution of Matrigel alternatives toward controllable, reproducible, and physiologically relevant culture systems for organoid modeling.

In general, hydrogel matrices for organoid culture can be broadly classified into two major categories: naturally derived and synthetic systems. Naturally derived hydrogels include decellularized extracellular matrix (dECM)-based formulations and

those composed of natural polymers, whereas synthetic matrices encompass hydrogels formed from synthetic polymers, synthetic peptides, or recombinant collagen. To date, more than ten types of hydrogel matrices have been commercialized for organoid applications, the majority originating from basement membrane extracts of mouse tumors and thus classified within the dECM group. Based on distinct physicochemical properties, biological activities, and application preferences, each matrix offers specific advantages and limitations depending on the organoid model and experimental objectives.

This article delineates the evolutionary trajectory of organoid culture matrices, moving from stochastic, ill-defined scaffolds toward engineered systems characterized by precise composition, tunability, and translational utility. By offering a systematic classification of hydrogels according to their material categories, this review seeks to guide researchers in navigating the complex landscape of matrix selection for enhanced experimental outcomes. Additionally, the relationship between the physicochemical properties and culture performance of hydrogel matrices is summarized, offering insights that may inform the rational design and future development of versatile, broadly applicable hydrogel systems.

2 Naturally Derived Hydrogel Matrices

2.1 Decellularized Extracellular Matrix

ECM is a complex and dynamic network of macromolecular fibers surrounding cells, mainly composed of fibrous proteins, glycoproteins, proteoglycans, and glycosaminoglycans. A typical derivative of ECM is the dECM, which is prepared by removing cellular components from natural ECM while retaining its intrinsic three-dimensional structural framework and bioactive substances. The dECM serves as a structural scaffold but also as a biofunctional microenvironment capable of transmitting biochemical and biomechanical cues, thereby modulating key cellular processes such as adhesion, migration, proliferation, and differentiation. Through these regulatory functions, dECM plays an essential role in orchestrating cell fate determination and

organ development *in vivo*[17, 18].Compared with other hydrogel sources, dECM-based hydrogels has two advantages[19]. Firstly, dECM retains natural biochemical signaling components, such as growth factors, polysaccharides, bioactive cryptic peptides, and natural proteins, which are essential for cell growth, differentiation, and functional maturation[15, 20], minimizing the need for chemical modification[12]. Secondly, owing to their biocompatibility and clinical safety, several dECM-derived materials have received Food and Drug Administration (FDA) approval and are already employed in tissue repair and regenerative medicine applications[21], underscoring their translational potential in both research and clinical contexts.

2.1.1 Matrigel

Matrigel is a soluble basement membrane matrix derived from the EHS mouse sarcoma. Its composition is predominantly composed of structural proteins and growth factors, notably laminin at approximately 60%, type IV collagen at around 30%, along with nidogen and other associated components. Together, these constituents assemble into a three-dimensional network that closely resembles the natural basement membrane, thereby offering stable mechanical support for cellular self-organization[22].

Building upon this compositional profile, Matrigel demonstrates remarkable biomimetic properties. It simulates the *in vivo* microenvironment to foster cell adhesion and differentiation, while its endogenous growth factors and adhesion motifs promote stem cell proliferation and directed differentiation[23]. These characteristics enable Matrigel to support the culture of various organoid types[3, 24-30] . Combined with its stringent quality control, these attributes have established Matrigel as the "gold standard" for organoid culture matrices[31].

Since the advent of Matrigel, a variety of commercial basement membrane matrix products have become available, such as Thermo Fisher Geltrex™[32], R&D Systems Cultrex® BME[33], TheWell Bioscience Vivogel™[34], BD Matrigel® Matrix [35], and Bio-Chain Matrigel® Matrix Organoid Culture Scaffold Gel [36]. Most of these products are extracts of the basement membranes of mouse-derived tumors, and therefore possess

159 similar culture properties to Matrigel. (**Table 1**)

160 **Table 1 Component Information and Applications of Commercially Available Matrix Gels**

Name of Matrix Gel	Company	Component	Applications
Corning® Matrigel® Matrix	Corning	Mouse-Derived Tumor Basement Membrane Extract	Small intestine organoids[3], stomach organoids[24], lung organoids[25], kidney organoids[26], liver organoids[27], brain organoids[28], pancreas organoids[29], breast organoids[30]
Geltrex™ Hydrogel	Thermo Fisher	Mouse-Derived Tumor Basement Membrane Extract	Lung cancer organoids[37], colorectal cancer organoids[38], lung organoids[39], intestinal organoids[40], human induced pluripotent stem cells (hiPSC)[41]
Cultrex® BME	R&D Systems	Mouse-Derived Tumor Basement Membrane Extract	Intestinal organoids[42], liver organoids[43], pancreatic organoids[44], breast organoids[45]
BD Matrigel® Matrix	Becton, Dickinson and Company	Mouse-Derived Tumor Basement Membrane Extract	Intestinal organoids[24], mammary organoids[24], pancreatic organoids[24], liver organoids[24], kidney organoids[24]
Mogengel™ Matrix Organoid Culture	Bio-Chain	Mouse-Derived Tumor Basement Membrane Extract	Colorectal cancer organoids[25]
Matrigel® Matrix Organoid Culture Scaffold Gel	Proteintech Group	Mouse-Derived Tumor Basement Membrane Extract mainly including laminin, type IV collagen, nidogen, and heparan sulfate proteoglycan A	Intestinal organoid[46]

Liwo Biotechnology™ Scaffold Gel	Liwo Biotechnology	Extracellular matrix derived from porcine small intestinal submucosal cells, mainly including laminin, type IV collagen, nidogen, heparan sulfate proteoglycan, etc.	Tumor organoids[47]
Vitrogel™ ORGANOID	TheWell Bioscience	Synthetic Hydrogel	Gastric cancer organoids[48], placental organoids[49], intestinal organoids[50]

Nevertheless, Matrigel presents substantial limitations for applications in organoid culture. Primarily, it is derived from mouse tumor tissue and inherently contains xenogeneic proteins, tumor-derived growth factors, and various enzymatic components. These heterologous components are prone to triggering an immune response in the human body during subsequent transplantation therapy research or clinical translation of human-derived organoids, posing a severe immunogenic risk[17]. Secondly, the physicochemical and mechanical properties of Matrigel, such as elastic modulus, pore structure, and stress relaxation properties, are hard to be precisely controlled or customized to meet the distinct biophysical requirements of organoids derived from different tissue types. The inability to tune these parameters limits the capacity to systematically modulate the cell-matrix interactions, morphogen gradients, and mechanotransduction cues, which are essential for optimizing organoid growth, differentiation, and structural maturation under tissue-specific culture conditions. Thirdly, Matrigel exhibits inherent compositional heterogeneity and instability, resulting in significant spatial variability in its mechanical properties, with the elastic modulus varying considerably across different regions of the gel. This variability, coupled with fluctuations in performance between batches, complicates the

maintenance of consistency across different product lots in organoid culture applications. Additionally, Matrigel faces production bottlenecks and high costs, stemming from limited raw material availability and the complexity of its preparation. These challenges fundamentally constrain its ability to meet the growing demands of large-scale and high-throughput organoid culture[12].

2.1.2 Other Animal-Derived Decellularized Extracellular Matrices

In the pursuit of more reproducible substitutes, other animal-derived dECM have gained attention due to their compositional similarity to Matrigel and their demonstrated efficacy in organoid culture. Among these, porcine tissue-derived dECM has emerged as the most extensively studied dECM type except murine-derived counterparts, attributed to its structural similarity to human tissue-derived dECM, easy accessibility, favorable biocompatibility, and minimal ethical concerns. María et al. developed a hydrogel for endometrial organoid culture by blending a 50:50 mixture of porcine endometrial dECM extract and 1% (w/v) PuraMatrix (PM) peptide solution[51]. PM consists of 16-residue peptides with a repeating $(RADA)_4$ sequence, enabling self-assembly into nanofibrous hydrogels under physiological conditions. The resulting hybrid hydrogel exhibited a honeycomb-like structure, where PM fibers infiltrated the type I collagen scaffold, leading to higher storage and elastic moduli compared to other composite formulations and Matrigel. The porcine kidney dECM hydrogel developed by Garreta et al. exhibits thermal-triggered gelation, with a gelation temperature of 25°C[52]. This allowed the hydrogel to form and maintain a stable three-dimensional structure at physiological temperature, which was higher than its gelation threshold. Following gelation, both the storage modulus (G') and loss modulus (G") were inclined to stabilization, indicating that the hydrogel maintains mechanical stability. These characteristics highlighted its favorable thermal responsiveness and consistent mechanical integrity post-gelation. Moreover, the dECM hydrogel could support the differentiation of human pluripotent stem cells (hPSCs) into kidney organoids, which exhibited differentiation characteristics and the formation of endogenous vascular components.

Beyond porcine-derived materials, dECM hydrogels from other mammalian sources have also shown promise in organoid culture. For instance, Kakabadze et al. found that the human placental dECM scaffold could support the survival of liver tissue fragments while maintaining functions such as albumin synthesis and bile transport[53]. Furthermore, transplantation of this scaffold restored liver function in sheep with acute liver failure. The natural vascular network and biocompatibility of placental dECM provided a physiological-level perfusion environment for organoids, while its preserved components such as collagen and laminin promoted cell adhesion and functional maintenance.

Despite these advances, dECM hydrogels sourced from non-murine mammals have not yet reached large-scale commercial availability. Moreover, although these matrix gels have expanded the available sources of materials for organoid culture, they still fail to completely address the issues of Matrigel, such as non-tunable composition, batch-to-batch instability and immunogenicity.

2.2 Natural Polymer Materials

Natural polymer materials refer to high molecular compounds extracted from natural sources, typically characterized by excellent biocompatibility, biodegradability, and renewability. The extracellular matrix primarily consists of protein-based materials, such as collagen[54-56], and polysaccharide-based materials, including hyaluronic acid and sodium alginate[57]. Compared with dECM, these materials retain high biocompatibility and possess tunable properties due to their well-defined chemical compositions, which can be modified through chemical and physical processes[58]. Furthermore, natural polymers offer a clear advantage over decellularized matrices by mitigating issues related to uneven composition and instability across batches. This is primarily due to their well-defined and consistent chemical structure, which allows for better control over material properties.

2.2.1 Single-Component Natural Biomaterial Hydrogels

2.2.1.1 Collagen

Collagen, the most abundant protein in animals, forms the structural framework and core component of the extracellular matrix. It provides essential support for organoid adhesion and growth, while also promoting cell proliferation and differentiation[12]. Its inherent thermosensitivity enables versatile manipulation in organoid culture, making collagen and collagen-based composite materials crucial components of hydrogel matrices for organoid applications. As a result, these materials have garnered considerable attention from researchers in the field.

The collagen types most widely studied are Types I, II, III, and IV, which can be classified into two groups based on their protein characteristics: Types I, II, and III are fibrillar proteins, whereas Type IV is a membrane-forming protein. Among them, Type I collagen accounts for approximately 90% of the total collagen in the human body and is widely present in tissues such as the skin, bones, and tendons. It is composed of two α1 (I) chains and one α2 (I) chain, which assemble into a triple-helical structure that provides strength and stability to tissues. Type II collagen is mainly distributed in cartilage and forms a triple-helical structure with three α1 (II) chains. It can bind to proteoglycans to provide compressive strength to cartilage, maintain cartilage function, and reduce joint friction. Type III collagen has a distribution similar to that of Type I but is softer and more elastic; consisting of three α1 (III) chains, it plays a prominent role in tissue repair. Type IV collagen is a major structural component of the basement membrane, featuring a heterotrimeric helical chain structure formed by six α-type peptide chains. As a characteristic component of the basement membrane, Type IV collagen is an important constituent of Matrigel, accounting for approximately 30% of its composition.

As a main component of the ECM, collagen, particularly Type I collagen, is one of the core materials in the exploration of Matrigel-free systems, as evidenced by its prevalence in the research literature[13, 59-61]. For instance, Irene et al. employed type I collagen as a complete substitute for Matrigel to constitute the core of the culture matrix for A549(human lung adenocarcinoma cells), normal fibroblasts (NFs), and cancer-associated fibroblasts (CAFs)[59]. They observed that within the culture system

constructed with type I collagen hydrogel, CAFs significantly enhanced the proliferation and invasion of A549 cells, and this promotive effect was markedly stronger than that exerted by NFs—with 1.5~2-fold increase in organoid area and a 2~3-fold increase in invasive cell protrusions. Jee et al. mixed Type I collagen extracted from bovine tendons with Ham's F-12 medium (II) and $NaHCO_3$ at a ratio of 8:1:1, successfully culturing small intestinal, gastric, and colonic organoids[13]. Additionally, after co-transplanting small intestinal organoids with this matrix into an EDTA-induced colitis mouse model, the organoids were able to successfully colonize the damaged tissue. Compared to organoids cultured in Matrigel, those grown in this collagen matrix exhibited a smaller perimeter and fewer buds, yet demonstrated higher stability and better preservation of stem cell properties. The reduction in bud formation was linked to precise regulation of the stem cell "self-renewal – differentiation" balance. Specifically, collagen, particularly type I collagen, binds to integrin β1 on the cell surface, activating downstream proliferative signaling pathways. The reduction in bud formation was linked to precise regulation of the stem cell "self-renewal – differentiation" balance. Specifically, collagen, particularly type I collagen, binds to integrin β1 on the cell surface, activating downstream proliferative signaling pathways. This leads to upregulated stable expression of stemness markers such as LGR5 and SOX9, inhibits excessive differentiation of stem cells into functional bud structures, and enhances the long-term passaging stability of organoids[62]. The smaller perimeter reflects a more compact arrangement of the epithelial layer, which results from the mechanical stiffness of the collagen matrix being closer to that of the ECM in vivo tissues. This property promotes correct localization of epithelial polarity markers such as E-cadherin and ZO-1 by regulating cytoskeletal organization, thereby avoiding the loosening of the epithelial layer caused by the compositional heterogeneity of Matrigel[63]. Furthermore, the collagen matrix formed a stable three-dimensional network through physical encapsulation or chemical conjugation, which reduced disordered diffusion of signaling molecules. On one hand, this network suppressed

excessive cell proliferation via the integrin–FAK–YAP/TAZ mechanotransduction pathway; on the other hand, it helped prevent oxidative stress-induced damage to stem cell properties by modulating the balance between reactive oxygen species (ROS) production and clearance. Ultimately, this led to the functional phenotype of fewer buds – high stability – high stemness[64].

However, a crucial bottleneck restricting the widespread application of collagen hydrogels is the challenge of its preparation. Collagen preparation methods mainly include traditional extraction, chemical synthesis, and genetic engineering. While traditional extraction methods are cost-effective and relatively simple to execute, they may leave residual pathogenic components in the product, posing a risk of heterologous or allogeneic rejection. Furthermore, residual acid, alkali, and salt reagents from the extraction process may induce cytotoxicity. Although collagen preparation via chemical synthesis or genetic engineering eliminates the risks of rejection and pathogenicity, these methods are hindered by high costs and technological complexity. Moreover, obtaining collagen with full-length sequences and maintaining its native three-dimensional structure remain challenging with these approaches65.

2.2.1.2 Alginate

Alginate is a cost-effective natural biomaterial derived from algae. Calcium ions can coordinate with the oxygen atoms in two adjacent guluronic acid (G) residues on two chains of alginate, forming the "egg-box model" and further generating a hydrogel through lattice stacking[66]. These hydrogels have been extensively utilized for various *in vitro* applications, encompassing the culture of mesenchymal stem cells, chondrocytes, and tissues such as ovarian primary follicles [67, 68, 69, 70]. Unmodified alginate does not have cell adhesion characteristics, and its hydrophilicity inhibits protein adhesion, making it a minimal support matrix that can only provide mechanical support for human intestinal organoids (HIOs) in a 3D environment[71]. Capeling et al. examined the mechanical properties of hydrogel prepared with different concentrations of sodium alginate solution (0.5% to 4% w/v), and the results showed that with the increase of sodium alginate concentration, the G' and G" of the hydrogel also

increased[71]. The G' and G" of the hydrogel derived from 1% (w/v) sodium alginate ranged between 2~5 kPa, which fell within the typical mechanical range of Matrigel (1~10 kPa) and simulated the viscoelasticity of native lung tissue ECM[71]. In contrast, the G' of the 2% (w/v) sodium alginate hydrogel was 5~8 kPa, slightly exceeding that of Matrigel and aligning with the G' (4~7 kPa) reported for PEG hydrogels optimized for human intestinal organoid culture[15]. This matching of mechanical moduli allowed cells to perceive a physiologically relevant mechanical microenvironment, which in turn regulated cytoskeletal organization and mechanotransduction pathways to support organoid differentiation[25]. Despite lacking inherent cell-adhesive properties, the alginate matrix—when mechanically tuned —enables human lung organoids (HLOs) to acquire the following differentiation characteristics: key lung differentiation markers, including the epithelial marker NKX2-1 and the airway marker SCGB1A1, are expressed at significantly higher levels than in Matrigel cultures. Moreover, the proportions of multiciliated cells and mucus-secreting goblet cells more closely approximate those found in native lung tissue[25].

2.2.1.3 Hyaluronic Acid

Hyaluronic acid (HA), a major glycosaminoglycan in the ECM, possesses a linearly repeating glycan chain structure and a large number of hydrophilic groups in its molecular architecture. These structural features endow it with excellent biocompatibility, tissue-mimetic viscoelasticity, and facile chemical tunability, thereby positioning it as one of the core scaffold material for organoid culture[72]. As a key regulator of cell signaling via interactions with Cluster of Differentiation 44 (CD44) and Receptor for Hyaluronan-Mediated Motility(RHAMM) receptors, HA is prone to rapid enzymatic degradation, exhibits weak mechanical integrity, and limited cell adhesion. These limitations are typically addressed through strategic functionalization, which remains a central focus in biomaterials research[73]. Common modification strategies introduce reactive moieties, such as acrylate, aldehyde, ketone, to enable controlled crosslinking, yielding hydrogels with customizable mechanical properties matching specific tissue microenvironments[74]. These modification strategies allow for

precise tuning of hydrogel properties. For example, acrylate-modified hyaluronic acid (AHA), when crosslinked with peptides containing matrix metalloproteinase (MMP)-cleavable sequences, forms a gel whose mechanical strength could be easily adjusted by varying the crosslinker concentration, while also imparting MMP-mediated enzymatic degradability[75].

Functionalized HA hydrogels have demonstrated efficacy across various organoid culture systems. Owing to their defined composition, they often exhibit superior compositional consistency and batch-to-batch reproducibility compared to Matrigel[10]. The adhesion peptide sequence RGD can be functionalized onto the surface of hydrogels to regulate cell adhesion[76]. Collectively, these studies establish strategically modified HA hydrogels as a versatile, defined alternative to Matrigel, with applications spanning oncology, regenerative medicine, and personalized drug testing[73-77].These studies collectively demonstrate that strategically modified HA hydrogels offer a versatile, defined alternative to Matrigel, with applications spanning multiple organ systems.

Natural polymer materials, such as collagen, hyaluronic acid, and sodium alginate are recognized for their high biocompatibility, widespread availability, and ease of acquisition. Furthermore, each material system provides its own distinct advantages: for instance, natural or bio-functionalized matrices often incorporate essential bioactive cues such as cell adhesion motifs, while synthetic or engineered systems tend to offer highly tunable physical properties including mechanical stiffness [59, 78]. However, these natural polymer materials have certain limitations when used as a single-component scaffold material for organoid culture. For example, pure collagen hydrogel scaffolds often have poor mechanical properties[79]; unmodified sodium alginate hydrogel do not have cell adhesion characteristics and inhibit protein adsorption[25]; hyaluronic acid hydrogel scaffolding materials also have fewer cell adhesion sites and insufficient toughness[80]. Consequently, researchers are increasingly focusing on composite natural polymer materials that integrate the complementary advantages of multiple natural components.

2.2.2 Composite Natural Polymer Materials

Composite natural polymer hydrogels stand as the optimal scaffolds in current organoid culture, with their core advantage lying in the integration of the inherent biocompatibility and biodegradability of natural polymers, while achieving precise regulation of key physicochemical properties such as cell adhesiveness, stiffness, viscoelasticity, and porosity[81]. Typically constructed using collagen, alginate, hyaluronic acid, and other core components via crosslinking or blending processes, these materials could tailor exclusive microenvironments to meet the growth requirements of different organoids. Crucially, these composites mitigate the limitations of single-component materials, such as the insufficient mechanical strength of pure collagen, while simultaneously overcoming the critical drawbacks of Matrigel, namely its significant batch-to-batch variability and ill-defined composition. Owing to these attributes, they have facilitated the development of diverse organoid models, positioning them as critical tools for sophisticated disease modeling and efficient high-throughput drug screening[82, 83].

Targeted investigations have focused on optimizing material performance and organoid adaptability. For example, Curvello et al. prepared carboxylated nanofibers (TONC) from 2,2,6,6-Tetramethylpiperidin-1-Oxyl(TEMPO)-oxidized nanocellulose. These were crosslinking with collagen to form collagen - nanocellulose (Col-NC) hydrogels through concentration and pH adjustment, which were further functionalized with RGD peptides to enhance cell adhesion. This hydrogel exhibits a sol-gel transition at 37°C and possesses G' and G" comparable to those of Matrigel. Increasing the TONC content yielded a high-stiffness matrix suitable for 3D tumor organoid culture and concurrently shortened the gelation time by 40% relative to pure collagen hydrogels. These findings confirmed the dual role of nanocellulose in enhancing mechanical properties and accelerating gelation kinetics[79]. In a similar approach, the team led by Ort developed a printable, stiffness-tunable collagen-alginate microgel system. After mixing diluted collagen with sodium alginate and a dextran cell suspension, followed by automated dispensing and crosslinking with calcium chloride, the system

successfully maintained the viability of HS-5 human bone marrow fibroblasts and MDA-MB-231 human breast adenocarcinoma cells for up to 9 days and 5 days, respectively. This provided an ideal model for investigating the regulatory mechanisms of matrix stiffness on cell morphology, contractility, and drug sensitivity[84].

In the field of 3D bioprinting, Shi et al. used silk fibroin as a embedding medium to stabilize bioinks composed of low-concentration type I collagen and poly(N-isopropylacrylamide)-modified thermoresponsive hyaluronic acid (CH) [78]. The grafting of poly(N-isopropylacrylamide) conferred thermoresponsive properties to the HA, thereby extending its retention time on the collagen matrix. This constructed microenvironment supported the growth of various tumor cell types and successfully recapitulate *in vitro* tumor angiogenesis.

To address the specific matrix requirements for hepatic organoid culture, Roudaut et al. enhanced cell adhesion and recognition capabilities by grafting RGD sequences and galactosamine groups onto HA. This modified HA was subsequently mixed with equal proportions of type I and type IV collagen. Following pH adjustment, the mixture was crosslinked with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and purified to yield a hyaluronic acid-collagen scaffold. This scaffold exhibited both high mechanical stability and an elastic modulus that closely matched that of the native liver ECM. Hepatic organoids cultured in this scaffold can maintain Cytochrome P450 enzymes activity and protein secretion capacity for 21 days, verifying its functional applicability[85].

Overall, these studies collectively demonstrate that composite natural polymer hydrogels surmount the inherent contradictions of single-component materials by leveraging the complementary performance advantages of their constituents (**Table 2**). This compositional synergy allows for high tunability—whether regulating mechanical properties with nanocellulose or optimizing cell-matrix interactions via biofunctionalized hyaluronic acid—enabling matrices to be tailored for specific applications. Such platforms can faithfully mimic physiological ECM characteristics while also ensuring experimental reproducibility. They thus emerge as ideal, defined

437 alternatives that circumvent the critical drawbacks of Matrigel, poised to continuously
438 drive progress in organoid research and translational medicine.

439 **Table 2 Categories, Components, and Applications of Natural Polymer Biomaterials for**
440 **Organoid Culture**

Category of Natural Polymer Materials Matrix Gels	Components of Matrix Gels	Key Functional Features & Mechanistic Advantages for Organoid Culture	Applications
collagen and its derivatives	Type I Collagen	Native ECM core component (90% of human ECM collagen); binds integrins (α2β1) on cells; supports tumor cell proliferation, maintains intestinal stemness, and promotes renal tubular differentiation	Patient-Derived Tumor Organoids (PDTOs)[86], Intestinal Organoid[87], Renal Organoids[61], Mammary Organoids[60]
	Type I Collagen + Oxidized Gum Arabic	Oxidized gum Arabic forms covalent crosslinks with collagen; enhances mechanical strength and reduces enzymatic degradation	Pancreatic Organoids[88]
alginate and its derivatives	Alginate	Ca^{2+}-responsive crosslinking; adjustable stiffness supports pulmonary multiciliated/goblet cell differentiation and hepatic organoid viability	Pulmonary Organoids[25], Mammary Cancer Organoids[89], Hepatic Organoids[90]
	Alginate + Gelatin - 3D Printing	Gelatin supplements RGD motifs (collagen-derived) to compensate alginate's poor cell adhesion; 3D printing enables customized matrix structure, matching liver lobule microenvironment	Hepatic Organoids[91]
	Alginate + Fibronectin	Fibronectin binds integrins (α5β1) on hepatic tumor cells; enhances cell-matrix interaction, simulating tumor stromal microenvironment	Hepatic Tumor Organoids[92]

		and promoting tumor organoid proliferation	
HA and its derivatives	Methacrylated Hyaluronic Acid (Me-HA) Hydrogel	Photocrosslinkable (UV/LAP) for precise gelation; viscoelasticity matches neural/spinal cord tissue, reducing mechanical damage to neural progenitors	Neural Organoids[93], Spinal Cord Organoids[94]
	HA	Native glycosaminoglycan; interacts with cell surface CD44 receptors; regulates biliary epithelial cell proliferation and ductal structure formation	Biliary Duct Organoids[95]
composite natural polymeric biomaterials	Hyaluronic Acid + Alginate Double Network	Double network (HA's viscoelasticity + alginate's rigidity) enhances mechanical stability; HA binds CD44 on cancer cells to regulate migration, simulating HCC microenvironment	Hepatocellular Carcinoma Organoids[96]
	Hyaluronic Acid Methacrylate (HAMA) + Pancreatic Extracellular Matrix - 3D Printing	Pancreatic ECM provides tissue-specific signals (e.g., laminin, growth factors); HAMA ensures 3D printability; synergistically promotes islet organoid insulin secretion	Pancreatic Islet Organoids[91]
	HA+ Matrigel - Mixed Hydrogel	HA reduces Matrigel's xenogenic protein content (lower immunogenicity); Matrigel provides biomimetic basement membrane, balancing safety and pulmonary organoid maturation efficiency	Pulmonary Organoids[83]
	Carboxymethyl Cellulose (CMC) - Sodium Alginate (NaA) Composite Hydrogel	CMC enhances hydrogel viscosity to prevent tumor cell aggregation; NaA enables stiffness adjustment (matching patient-specific tumor ECM), supporting personalized drug testing	Patient-Derived Tumor Organoids (PDTOs)[97]

Methacrylated Hyaluronic Acid (Me-HA) - dECM Composite Hydrogel - 3D Printing	dECM retains native bioactive components (e.g., collagen, growth factors); Me-HA ensures printability; supports stem cell adhesion and maintains multipotency for organoid co-culture	Bone Marrow Mesenchymal Stem Cells[98]

3. Synthetic Hydrogel Matrices

Synthetic hydrogel matrices are chemically engineered, three-dimensional polymer networks characterized by their precisely defined compositions. In the context of organoid culture, these synthetic matrices are primarily classified into two major categories: synthetic polymeric biomaterials and synthetic polypeptides. Commercialized products derived from both classes have already confirmed their feasibility for xeno-free organoid culture. A prominent example is the commercially available VitroGel ORGANOID, developed by TheWell Bioscience. This material is produced through a fully synthetic manufacturing process, which inherently excludes all animal- or human-derived components and thereby significantly reduces immunogenic risks associated with biological source variability[48-50]. Furthermore, it offers core advantages including a well-defined composition, tunable biofunctional moieties, and customizable mechanical strength and degradability. These features enable it to meet the culture requirements of diverse organoid types while maintaining high batch-to-batch stability. These properties collectively position synthetic matrices as a promising alternative to traditional animal-derived matrices like Matrigel, though performance gaps remain, as discussed below.

3.1 Synthetic Polymer Materials

Synthetic polymeric biomaterials refer to a class of biomaterials artificially prepared via chemical synthesis, with their core structure consisting of macromolecular chains composed of repeating structural units linked by covalent bonds. In terms of design, these materials allow precise regulation of molecular composition and degree

of polymerization, offer tunable biodegradability or long-term stability, and can be customized on demand to achieve physicochemical functions such as mechanical strength, hydrophilicity/hydrophobicity, and pore size. Thus, they are widely used in the biomedical field. Two primary subclasses dominate this category: PEG and its derivatives, and polyisocyanopeptides (PICs).

PEG is recognized as an ideal ECM alternative due to its excellent biocompatibility, low cost, and biological inertness. PEG hydrogels are typically formed by the chemical crosslinking of PEG macromonomers into a three-dimensional network structure[99]. Common derivatives include multi-arm PEG and functionalized PEG variants, for instance acrylated and thiolated PEG[100], which form hydrogels through diverse crosslinking reactions—such as photo-crosslinking[101], click chemistry crosslinking[99]—to provide structural support for organoid growth. The key advantage of PEG hydrogels lies in their highly tunable mechanical strength[102], properties that directly influence organoid formation and function. To date, PEG-based hydrogels have been applied to the culture of neural[86, 103], hepatic[86], pancreatic[104, 105], intestinal[86], renal [86], mammary[86], placental[106], and tumor organoids[86, 101, 107], highlighting their versatility.

The second major subclass, PICs, is distinguished by its thermoresponsiveness—a property critical for simplified organoid handling. PICs are synthesized via nickel(II)-catalyzed polymerization of di-, tri-, or tetraethylene glycol-modified isocyanic acid-(D)-alanine-(L)-alanine monomers. Their polyisocyanide main chain is stabilized by hydrogen bonds, forming a helical structure, while alanine side chains enable rapid transformation into a transparent hydrogel upon heating[108]. The lower critical solution temperature (LCST) of PICs correlates directly with ethylene glycol side chain length, shorter chains exhibit a lower LCST. Notably, above the transition temperature, strong hydrogels can be reversibly formed even at extremely low polymer concentrations (0.1 wt%) [109]. A study by Wang et al. highlighted the utility of PIC hydrogels in organoid maturation[110]. They found that these hydrogels, when supplemented with sodium butyrate and valproic acid, promoted the maturation of liver intrahepatic biliary epithelial cell organoids into functional biliary epithelial cells. The core mechanism lies

in the fact that, as a chemically defined material, PIC hydrogels have suitable mechanical properties that can assist in the polarity remodeling of organoids. Furthermore, they work in synergy with sodium butyrate and valproic acid to regulate the expression of genes related to biliary differentiation, collectively promoting the functional maturation of organoids.

Collectively, synthetic polymeric materials offer significant operational convenience for researchers: mechanical properties can be regulated via grafting or modifying groups to alter crosslinking methods, and thermoresponsiveness can be readily achieved by incorporating thermo-responsive functional groups or adjusting copolymer composition. However, the performance of synthetic matrices in organoid culture often lags behind that of Matrigel, primarily due to their inherent limitations in bioactivity and biocompatibility, indicating a clear need for improvement in these properties.

3.2 Synthetic Peptides

Self-assembling peptide hydrogels are formed when peptides spontaneously assemble into one-dimensional supramolecular structures via non-covalent interactions; these structures then self-organize into a 3D network that encapsulates water molecules[111]. Two primary commercially available products are employed in organoid culture systems: PeptiGels® manufactured by Manchester Biogel[112-114], and PM peptide analogs such as RADA16-1, supplied by Shanghai Biotech Bioscience & Technology[51, 115]. Olayanju et al. demonstrated that the stiffness (1~14 kPa) and charge of PeptiGels® synergistically regulate liver organoid development[112]. A moderate stiffness (10 kPa) combined with a surface charge yielded the highest organoid formation rate, morphologies most closely resembling Matrigel, and peak epithelial marker (CK18) expression. Conversely, excessive rigidity (14 kPa) or a neutral charge impaired organoid stability and reduced RNA content. This confirms that liver organoids require balanced mechanochemical properties—a finding consistent with organoid-specific matrix demands[116, 117]. Complementing this, Xu et al. showed that RADA16-I peptide hydrogels can support airway organoid formation, allowing cells to

form lung-like cavities, maintain proliferation, and express tissue-specific markers[115]. By virtue of its defined composition—a key advantage over Matrigel's undefined nature[87]—the hydrogel enabled adenovirus infection assays while also highlighting the potential of peptide hydrogels in translational disease modeling.

Together, these studies highlight the unique value of self-assembling peptide hydrogels. Their precise tunability, which is governed by established composition-mechanical correlations[78, 79, 84, 85], is a key advantage of both basic and translational research. However, these systems still exhibit a performance gap compared to Matrigel, notably in terms of lower RNA content and reduced culture stability, indicating the need for further optimization. Enhancing cell-matrix interactions, for instances, by integrating RGD motifs[85] or nanocellulose[79], is a key strategy. This positions them as strong contenders against other Matrigel alternatives, such as dECM or synthetic matrices, for organoid culture.

Table 3 Categories, Components and Applications of Different Synthetic Matrix Gels

Category of Synthetic Matrix Gels	Components of Matrix Gels	Key Functional Features & Mechanistic Advantages for Organoid Culture	Applications
PEG and its derivatives	PEG hydrogel	Biologically inert, low immunogenicity; 3D network provides stable mechanical support, avoiding neural cell cytotoxicity and maintaining pancreatic cancer organoid phenotype	Neural organoids[103], Pancreatic cancer organoids[107]
	Thiol-ene modified PEG hydrogel	Thiol-ene click crosslinking enables mild gelation (no harsh reagents); tunable stiffness matches neural tissue softness, supporting neural progenitor self-renewal	Neural organoids[86]
	PEG-RGD	RGD motif binds integrins (e.g., αvβ3) on organoid cells; enhances cell-matrix adhesion, promoting hepatic albumin secretion, intestinal crypt formation, and placental trophoblast differentiation	Hepatic organoids[104], Intestinal organoids[86], Renal organoids[86], Breast cancer organoids[101], Placental organoids[106]

	PEG-LMN	Laminin-mimetic sequence mimics basement membrane component; interacts with cell surface laminin receptors, supporting epithelial organoid polarization	Pancreatic organoids[86], Intestinal organoids[86], Renal organoids[86], Breast cancer organoids[86]
	PEG-MMP	MMP-degradable peptide links enable cell-driven matrix remodeling; allows intestinal stem cell migration for crypt-villus formation and pancreatic organoid expansion	Pancreatic organoids[86], Intestinal organoids[86], Renal organoids[86], Breast cancer organoids[86]
	PEG-Heparin	Heparin binds growth factors via electrostatic interaction; sustains local growth factor concentration, promoting mammary epithelial organoid branching	Mammary organoids[86]
	Mixed PEG hydrogel	Combines stiffness tunability of PEG derivatives and growth factor retention; matches liver ECM mechanical properties, maintaining hepatocyte P450 enzyme activity	Hepatic organoids[105]
PICs and their derivatives	PIC	Helical structure stabilized by hydrogen bonds; thermoresponsive (sol-gel at ~37 ℃, liquefies at 25 ℃); simplifies organoid passaging without enzymatic digestion	Hepatic organoids[118], Mammary organoids[118], Prostate organoids[118]
	PIC-RGD	RGD peptide enhances mammary epithelial cell adhesion; thermoresponsiveness balances culture stability (37 ℃) and easy collection (25 ℃), optimizing mammary organoid yield	Mammary organoids[119]
	PIC-LMN	Laminin-mimetic motif promotes hepatic biliary epithelial cell attachment; thermoresponsive property supports long-term culture of intrahepatic biliary organoids	Hepatic organoids[120]

Hydrogel materials constructed from self-assembling peptides and their derivatives	FEFEFKFK (FEK) self-assembling peptide hydrogel	Self-assembles into nanofibrous network (mimics ECM fiber structure); hydrophobic interactions between FEK peptides enhance mechanical stability, supporting hepatocyte aggregation	Hepatic organoids[121]
	FEFEFKFK-RGD	RGD peptide improves cell adhesion; nanofibrous structure matches skin/pancreatic/cardiac tissue microarchitecture, promoting skin keratinocyte differentiation, pancreatic islet formation, and cardiac cell contractility	Skin organoids[122], Pancreatic organoids[122], Cardiac organoids[123]
	PeptiGel®	Tunable stiffness and surface charge; adjusts to mammary epithelial branching and renal tubular formation via mechanosensing	Mammary organoids[114], Renal organoids[113]
	RADA16-I	Self-assembles into ~10 nm nanofibers (mimics native ECM); high water content ensures nutrient diffusion, supporting airway multiciliated cell differentiation and renal tubular viability	Mammary organoids[123], Airway organoids[115], Hepatic organoids[123], Renal organoids[124]
	RADA16-I-IKVAV/RGD	IKVAV motif promotes neural progenitor differentiation; RGD enhances adhesion; synergistically supports neurite outgrowth and cortical layer formation	Neural organoids[125]
	RADA16-I-RGD	RGD peptide enhances intestinal LGR5+ stem cell adhesion; nanofibrous network facilitates stem cell self-organization into crypt-villus structures	Intestinal organoids[126]
	HYDROSAP	Definable amino acid sequence; supports pancreatic islet-like structure maturation and neural organoid electrical activity via consistent microenvironment	Pancreatic organoids[127], Neural organoids[127]
	FAQ	Customized peptide sequence with neural cell-binding motifs; matches neural tissue viscoelasticity, reducing neural progenitor apoptosis	Neural organoids[127]

PuraMatrix	Self-assembles into porous network; allows cardiac cell migration and nutrient transport, supporting cardiac organoid contractile function development	Cardiac organoids[128]

In summary, synthetic matrices leverage their engineered nature to provide precisely defined and tailorable systems **(Table 3)** [48-50],. This directly addresses the principal drawbacks of Matrigel, namely its undefined composition, batch instability, and intrinsic immunogenicity. Nonetheless, as earlier highlighted, the broader application of most synthetic matrices is hindered by two pivotal constraints: limited biocompatibility and suboptimal culture outcomes, which collectively impede a full transition from Matrigel.

Notably, recombinant collagen-based hydrogels represent a distinct subclass of synthetic matrices that bridge the gap between animal-derived and conventional synthetic systems. Although artificially synthesized—thus ensuring definable composition and batch consistency—their inherent biological similarity to the native ECM confers superior biocompatibility. This characteristic, which sets them apart from other polymeric or peptide-based matrices, facilitates stronger cell-matrix interactions and reduces cytotoxicity. Consequently, they are positioned as a promising intermediate solution, offering the tunability of synthetic matrices without sacrificing the high biocompatibility of traditional animal extracts.

3.3 Recombinant Collagen

3.3.1 Classification and Organoid Culture Applications of Recombinant Collagen

As mentioned above, recombinant collagen (rCols) refers to a class of collagen produced via genetic engineering techniques, designed to mimic the structure and function of natural human collagen. Collagen materials can be categorized into three types according to their compositional and structural similarity to native human collagen: The first type is recombinant human collagen (rhCol), which is generated by

recombinant DNA expression. rhCol reproduces the full-length sequence and triple-helical structure of specific human collagen types and displays physicochemical and biological properties comparable to native collagen. The second type is recombinant humanized collagen (RHC), which is derived from sequences encoded by human collagen genes. This category includes full-length proteins, partial amino acid sequence fragments, or combinations of functional domains, all of which are sequence-identical to their natural human collagen counterparts. The third type is human-like collagen (HLC), which is encoded by specifically designed or modified gene sequences. These sequences produce full-length proteins, fragments, or combinations of functional domains. A defining characteristic of HLC is that its resulting gene and amino acid sequences possess low homology with those of natural human collagen[129]. Among the three types of recombinant collagen, rhCol and RHC closely mimic the structure of natural human collagen. Consequently, they exhibit high affinity, superior biocompatibility, and reduced allergenicity. Furthermore, their production via genetic engineering techniques effectively mitigates the risks associated with pathogen transmission.

Leveraging these aforementioned advantages, rCols hydrogels have been utilized in the culture of organoids derived from various organs. In the culture of neural organoids, rhCol III scaffolds modified with integrin-binding peptides (GFOGER/IKVAV) effectively supported the adhesion, proliferation, and differentiation of human neural stem/progenitor cells, and promoted the formation of a double-layered structure that mimicked the embryonic cerebral cortex[130]. Additionally, the rhCol III scaffolds contributed to softer and more regenerative healing processes by mimicking key characteristics of scarless fetal wound healing[131].

However, the molecular structure of rhCols usually contains only a single collagen chain. This structural characteristic makes it difficult for them to stably maintain hydroxylation and glycosylation modifications in *in vitro* culture environments. Furthermore, when rhCols used alone, they are often insufficient for organoid culture primarily because they lack spontaneous gelation capabilities. This deficiency stems

from their lack of the requisite oxidase systems necessary to promote the self-assembly of collagen molecules into fibrous structures[12]. Therefore, achieving efficient gelation of rCols through multi-pathway regulation is a key technological breakthrough for promoting the successful application of this type of biomaterial in the field of organoid culture.

3.3.2 Gel-Formation Methods of Recombinant Collagen

To overcome the inherent lack of spontaneous gelation in rhCols, strategies are required to induce their crosslinking and assembly into stable hydrogels. Currently, four primary approaches are utilized for this purpose: chemical crosslinking, enzymatic crosslinking, encapsulation gelation, and self-assembly gelation. Each approach offers a unique balance of advantages, such as enhanced tunability or high biocompatibility, and inherent limitations, including harsh reaction conditions or high cost. The optimal choice of method is therefore dictated by the specific organoid application and the requisite final matrix properties.

3.3.2.1 Chemical Crosslinking Method

Chemical crosslinking is the most widely used strategy for preparing rhCol hydrogels. This technique utilizes crosslinking agents to form covalent bonds between molecules or within the same molecule, thereby constructing a three-dimensional network structure[132-135]. A major advantage of chemical crosslinking lies in its well-understood reaction mechanisms, the variety of available crosslinking agents, and the broad range of compatible substrates. By adjusting the materials and reaction conditions, the properties of the resulting hydrogel, such as the degree of crosslinking, can be precisely controlled. This makes the method particularly suitable for fabricating complex network structures, especially composite hydrogels based on recombinant proteins[132]. However, chemical crosslinking often requires harsh reaction conditions, exhibits slow kinetics and low efficiency, and typically forms strong, irreversible covalent bonds, which limits its utility for synthesizing reversible hydrogels.

Among the direct chemical crosslinking strategies, the zero-length carbodiimide

method, primarily using EDC and N-hydroxysuccinimide (NHS), is the most established and widely employed. In this approach, EDC initially converts carboxyl groups into active O-acyl urea intermediates, which are then stabilized by NHS to form NHS esters; these esters subsequently react with amine groups (-NH_2) to form stable amide bonds, thereby achieving molecular crosslinking[136].

While the widely-used EDC/NHS system is effective, it relies on synthetic reagents that raise concerns over potential cytotoxicity and the need for rigorous post-reaction purification to remove byproducts. This has driven the exploration of natural alternatives. Genipin, a natural crosslinking agent derived from gardenia, offers high biocompatibility and low toxicity. Its crosslinking mechanism is pH-dependent. Under acidic or neutral conditions, polymer amino groups attack the C-3 carbon of genipin to form heterocyclic amines, leading to network formation. Conversely, under alkaline conditions, genipin hydrolyzes an aldehyde intermediate, which then forms crosslinks with amino groups via a Schiff base reaction[137].

Moving beyond the stable, irreversible bonds formed by crosslinkers like EDC or Genipin, Schiff base crosslinking offers a mechanism for forming dynamic and stimuli-responsive hydrogels, which result from the condensation between amino groups and carbonyl-containing compounds such as aldehydes or ketones. While these bonds can construct a stable three-dimensional network when multifunctional reactants are used[138, 139], their inherent reversibility is key to advanced material properties. This approach is often leveraged in dual-crosslinking systems to create sophisticated, tunable biomaterials.

Beyond dynamic linkers, other synthetic agents are employed to achieve high crosslinking efficiency and superior mechanical properties. Tetrakis (hydroxymethyl) phosphonium chloride (THPC), for example, is noted as a highly reactive crosslinking agent. Wang et al. utilized THPC as a crosslinking agent to prepare a recombinant collagen hydrogel for aging skin rejuvenation[140]. They reported that THPC demonstrated higher efficiency than traditional EDC/NHS, enabling complete hydrogel crosslinking even at low concentrations. Mechanistically, the four active hydroxyl

groups of THPC are proposed to form robust crosslinks with the trimeric collagen. This resulted in a hydrogel with superior mechanical strength, injectability, stability, and durability, showing promise for anti-skin aging applications.

In stark contrast to the direct chemical mixing methods described above, photocrosslinking offers unparalleled spatiotemporal control over gelation. To achieve this, the rCol polymer must first be functionalized, most commonly through methacrylation (MA). Upon exposure to ultraviolet (UV) light, these introduced methacrylate groups undergo a radical-mediated polymerization, driving the formation of a robust three-dimensional network with enhanced mechanical strength[141]. Methacrylated polymers generally exhibit excellent biocompatibility, support cell adhesion and proliferation, and are widely used in tissue engineering, 3D bioprinting, and drug delivery.

3.3.2.2 Enzymatic Crosslinking Method

Enzymatic crosslinking represents a highly specific and biocompatible method for hydrogel fabrication. It utilizes enzymes as biological catalysts to promote the formation of covalent bonds between protein molecules, effectively improving the structure and function of the resulting biomaterial[142]. While a wide array of enzymes can modify proteins, the crosslinking of rCol hydrogels predominantly relies on three main enzyme systems.

The first and most widely characterized enzyme for rCol crosslinking is transglutaminase (TG). Its popularity stems from its high specificity and ability to function under mild, physiological conditions. Mechanistically, TG catalyzes an acyl transfer reaction that forms highly stable isopeptide bonds between the γ-carboxamide group of glutamine residues and the ϵ-amino group of lysine residues, making it highly effective for crosslinking collagen[143-147]. The applications of TG-crosslinked rCol hydrogels are extensive, particularly in skin tissue engineering and wound healing.

In contrast to the transamidation mechanism of TG, oxidative enzymes provide an alternative pathway for crosslinking. Tyrosinase, a copper-containing redox enzyme, is

a prominent example and exhibits a dual catalytic function[142, 148]. First, it catalyzes the oxidation of monophenols to ortho-diphenols. Second, it further oxidizes these ortho-diphenols into highly reactive ortho-quinones. These quinones subsequently function as in-situ crosslinking agents that spontaneously react with nucleophilic residues present in the protein, such as lysine, tyrosine, or cysteine, to generate a stable and crosslinked network[142]. While this reaction can utilize the native tyrosine residues within rCol, the crosslinking efficiency and hydrogel properties are often significantly enhanced by grafting additional phenolic substrates onto the polymer backbone. A sophisticated example of this strategy was demonstrated by Yuan et al[148]. They first functionalized HLC by grafting tyrosine onto its backbone to produce HLC-TA. In parallel, they functionalized HA by grafting epigallocatechin gallate (EGCG) onto it, resulting in the modified derivative termed HA-EGCG. Separately, they prepared mesoporous polydopamine (MPDA) nanoparticles loaded with deferoxamine (DFO), thereby creating deferoxamine-loaded MPDA nanoparticles, which were designated M@D. Finally, tyrosinase was used to catalyze the rapid crosslinking between the enhanced HLC-TA and HA-EGCG components, entrapping the M@D nanoparticles within the hydrogel. The prepared hydrogel possessed versatile functionalities: it not only promotes M2 macrophage polarization but also exhibits anti-inflammatory properties. This function was crucial for supporting the survival and maturation of cells in complex structural vascularized organoid culture systems.

A second major oxidative system utilized for rCol crosslinking is based on Horseradish Peroxidase (HRP). In contrast to tyrosinase, HRP's catalytic activity is critically dependent on a co-substrate, hydrogen peroxide (H_2O_2). The underlying mechanism has been well characterized. H_2O_2 initially activates HRP, which subsequently oxidizes phenolic compounds, such as dopamine or tyramine, to generate highly reactive phenol radicals[149]. These radicals rapidly couple with one another to form covalent bonds, crosslinking the polymer chains into a 3D network. Similar to the advanced strategies seen with tyrosinase, this method is exceptionally powerful when used in composite systems where one component is first functionalized to provide the

phenolic substrate. Similar to advanced strategies involving tyrosinase, this method is particularly effective in composite systems where one component is pre-functionalized to serve as the phenolic substrate. A major advantage of the HRP/H_2O_2 system lies in its high tunability: gelation time and matrix rigidity can be precisely controlled by modulating the concentrations of enzyme and H_2O_2[149]. For instance, softer matrices can be tailored for neural organoids, while stiffer matrices are suitable for intestinal organoids.

In summary, enzymatic crosslinking offers a compelling strategy for rCol hydrogel fabrication, characterized by significant advantages in biocompatibility. The primary benefits stem from its physiologically mild reaction conditions, which avoid the extreme temperatures or pH values that could compromise the viability of encapsulated cells or the structural integrity of biomacromolecules[142]. This inherent "cell-friendliness," combined with the high biocompatibility of the enzymes themselves, makes the resulting hydrogels exceptionally well-suited for sensitive biomedical applications, particularly organoid culture where cell viability and functional differentiation are paramount. Moreover, the high specificity of enzymes minimizes off-target reactions, enhancing the reliability and reproducibility of the gelation process. Despite these advantages, several practical and technical limitations persist. The high cost of purified enzymes can be prohibitive for large-scale production. The process is also operationally complex, demanding precise control over environmental conditions to maintain optimal enzyme activity. Compared to more robust chemical methods, the range of compatible substrates is often narrower. Critically, the enzymes themselves are susceptible to inactivation during the crosslinking process. This poses a significant risk of incomplete gelation and inconsistent hydrogel performance—factors that could severely undermine organoid growth and experimental consistency[142].

3.3.2.3 Encapsulation Gelation Method

The encapsulation gelation method offers a distinct strategy that bypasses the common limitations of direct chemical or enzymatic crosslinking. Its core value lies in constructing a protective network via the physical crosslinking of a complementary

polymer, for instance sodium alginate, rather than relying on chemical modification of the rCol itself[150, 151]. n this approach, the recombinant collagen along with other sensitive biomolecules, such as enzymes, proteins, or cells, are passively entrapped within the resulting hydrogel matrix. This mechanism is highly advantageous: by leveraging well-characterized physical gelation processes and eliminating the need for harsh chemical agents or expensive enzymes, it ensures high biocompatibility while reducing costs. This makes it an ideal solution for applications where preserving the full biological activity and stability of the encapsulated cargo is the paramount objective.

In terms of application, this method has been refined into two distinct protocols to improve scenario adaptability, targeting either therapeutic use or *in vitro* culture. Although this protocol primarily focuses on wound therapy, its core logic is to achieve in situ gelation through encapsulation via physically crosslinkable matrices, and this logic can be extended to organoid culture. This extension reduces organoid damage by minimizing handling steps and enables seamless integration with the matrix.

Overall, the encapsulation gelation method is often the preferred choice for applications that require the preservation of recombinant collagen or encapsulated cell viability, due to its simplicity, low cost, and favorable biocompatibility. However, this approach is inherently constrained by its dependence on physical crosslinking, typically achieved through sodium alginate-calcium ion systems, which presents two main limitations. First, the hydrogel's physical properties, such as mechanical strength, and its biological functions, including cell adhesion, are intrinsically coupled. Because both are governed by the same collagen-to-alginate ratio, enhancing mechanical performance often comes at the expense of biofunctional efficacy, necessitating a compromise. Second, achieving uniform encapsulation remains challenging. The distribution of recombinant collagen is highly sensitive to mixing parameters and gelation kinetics, frequently yielding a heterogeneous matrix. Such heterogeneity can undermine the reproducibility of organoid growth and experimental outcomes. Consequently, in organoid culture applications, this method imposes a trade-off: while it offers excellent biocompatibility, it generally requires relinquishing fine-tuned

control over the final hydrogel's physical properties.

3.3.2.4 Self-Assembly Gelation of Recombinant Human-Derived Collagen

Natural collagen self-assembles into large molecular fibers and complex networks under specific *in vitro* conditions, such as temperature, concentration, pH and incubation time[152], and its large molecular weight, high anisotropy, and strong self-assembly ability—superior to most globular proteins—are key to its function as an extracellular matrix component. Recombinant collagen, however, lacks this inherent capability despite its advantages in organoid culture and tissue engineering, a longstanding barrier to its use as an independent hydrogel matrix[152-157]. Recent advances in synthetic biology and protein engineering have addressed this limitation by customizing recombinant collagen sequences to create self-assembling hydrogel variants, opening new paths for collagen matrices with modular functionality, full synthetic origin, and batch stability.

Across these approaches, synthetic biology enables precise modification of recombinant collagen to introduce self-assembly-mediating motifs or amino acids. The resulting hydrogels share three key advantages: batch stability which is vital for reproducible organoid culture, non-antigenicity reducing *in vivo* immune responses, and modular integration of functional sequences such as cell adhesion motifs, growth factor-binding domains.

By overcoming the poor biocompatibility of conventional synthetic polymers and the variability of Matrigel, rCol has established itself as a pivotal material for organoid technology. Its principal limitation, however, remains its lack of spontaneous gelation. This challenge has spurred the development of diverse strategies, many with significant trade-offs. Chemical crosslinking is highly tunable but relies on harsh conditions[133-135, 141, 158-160]. Enzymatic crosslinking offers a mild, biocompatible alternative but is often cost-prohibitive[142-149, 161]. Encapsulation gelation is simple and inexpensive, yet suffers from poor tunability and uniformity[150, 151]. Self-assembly gelation has thus emerged as a compelling fourth strategy, valued for its high modularity, consistency, and

789 biomimetic nature[152-157] This approach, particularly when driven by synthetic biology,
790 represents a highly promising avenue for future advancements.

791 **3.4 Key Performance of Major Hydrogel Categories**

792 As the core microenvironmental scaffold for organoid culture, the chemical
793 composition of hydrogels directly defines their physicochemical characteristics. These
794 properties, in turn, modulate organoid formation, differentiation, and functional
795 maturation via cellular mechanotransduction, biochemical signaling, and related
796 pathways, collectively establishing a "composition – property – function" cascade
797 regulatory network. Below, we provide a concise comparison and summarize the
798 regulatory principles of key hydrogel properties—such as mechanical stiffness and
799 thermoresponsiveness—in organoid culture. (**Table 4**)

800 **Table 4 Key Performance of Major Hydrogel Categories**

Hydrogel Category	Formation Efficiency	Functionality	Transcriptional Fidelity	Typical Advantages	Core Limitations
Natural Origin - dECM (Matrigel as representative)	High	High (Multi-organ adaptability)	High (Physiologically relevant)	Excellent biocompatibility, strong biomimetic microenvironment construction ability	Undefined composition, significant batch-to-batch variation, immunogenicity risk
Natural Origin - Composite Natural Polymers	Medium-High	Medium-High (Customizable)	Medium-High	Controllable composition, tunable mechanical properties	Insufficient bioactivity of some combinations
Synthetic Origin - PEG-based	Medium	Medium (Ligand modification required)	Medium	Defined composition, batch-to-batch stability, low cost	Weak bioactivity, need for additional ligands
Synthetic Origin - Synthetic	Medium	Medium (Specific	Medium	Precise tunability, no immunogenicity	Narrow application range,

Peptide-based	organ adaptability)	insufficient long-term stability

The performance variation observed among hydrogel categories fundamentally stems from the trade-off between bioactivity and controllability. dECM possesses functional superiority owing to its inherent biomimetic composition, yet it suffers from limited controllability and variable safety. Composite natural polymers, by contrast, achieve a more favorable balance between bioactivity and tunability via synergistic interactions among components, making them widely adopted in current basic research. Synthetic hydrogels — such as PEG and engineered peptides — emphasize controllability as a primary strength, though they typically necessitate conjugation with bioactive ligands to compensate for their inherent lack of cellular recognition. Recombinant collagen represents a "natural-synthetic hybrid system," combining high transcriptional fidelity with low immunogenicity, and has demonstrated promising utility in specific organoid culture models—despite remaining challenges in gelation process optimization.

These performance characteristics offer key criteria for selecting organoid culture matrices: in applications where functional maturation is paramount, dECM or composite natural polymers are often preferred; whereas contexts that prioritize clinical translatability or batch-to-batch consistency may favor PEG-based hydrogels or recombinant collagen.

Underlying these functional distinctions are differences in hydrogel chemical composition and structural design. Mechanical properties—including stiffness and viscoelasticity—serve as critical regulatory intermediates, directly linking material composition to organoid phenotypic outcomes.

4. Chemical Composition and Properties of Hydrogels

As the core microenvironmental scaffold for organoid culture, the chemical composition of hydrogels fundamentally dictates their physicochemical properties.

These properties regulate organoid formation, differentiation, and functional maturation through mechanisms such as cellular mechanotransduction and biochemical signal mediation, collectively establishing a "composition – property – function" cascade regulatory network. Here, we systematically outline the principles by which key hydrogel properties influence organoid culture, elucidate the underlying mechanisms through which compositional modifications govern material properties, and provide theoretical foundations for the rational design of organoid culture matrices.

4.1 Regulatory Principles of Hydrogel Mechanical Properties in Organoid Culture

The mechanical performance of hydrogels is quantified by key parameters including G', which reflects elasticity, G", which indicates viscosity, and compressive modulus (K) , which measures rigidity[84, 116, 117, 162, 163]. These parameters collectively constitute "mechanical microenvironment signals," and their compatibility with organoid functions — mediated through molecular signal transduction — directly influences culture efficiency.

Organoid-specific mechanical requirements have been well documented. For intestinal organoids, Guo et al. demonstrated that matrix softening with G' (0.5~1 kPa) was crucial for driving crypt formation, as stiffer matrices restricts cytoskeletal rearrangement necessary for crypt budding. When G' > 2 kPa, the polarized distribution of stem cells was disrupted and crypt budding rates were significantly reduced[164]. In contrast, bone organoid differentiation required a matrix with a higher compressive modulus K (10~20 kPa). Under pulsatile pressure, this modulus activated the Piezo1 ion channel, upregulated osteogenic markers such as runt-related transcription factor 2 (Runx2) and osteocalcin (OCN), and enhanced the osteogenic differentiation efficiency of mesenchymal stem cells (MSCs) by regulating cytoskeletal remodeling, reducing oxidative stress, and maintaining calcium homeostasis[16]. When K < 5 kPa, MSCs tend to differentiate into adipocytes rather than osteoblasts. Cardiac organoid culture benefited from dynamic stiffness regulation. Gradually increasing G' from 1 kPa to 3

kPa enhanced cardiomyocyte contractile response to isoproterenol via the integrin–YAP/TAZ pathway, yielding a 30% increased in contractile force compared with static matrices and calcium transient kinetics more closely resembling those of adult cardiomyocytes[163]. Moreover, dynamic stiffness suppressed the expression of the profibrotic factor TGF-β1 and reduced phenotypic dedifferentiation. Matrix stiffness could also modulate cell-drug interactions and differentiation outcomes. In soft microgels with G' (0.3~0.8 kPa), myogenic differentiation factors effectively promoted myoblast fusion into myotubes; in stiffer matrices with G' (> 5 kPa), intercellular junction formation was inhibited and differentiation is impaired[84]. For tumor organoids, when matrix stiffness matched the native tumor extracellular matrix—for instance, with G' (2~4 kPa) for breast cancer organoids—the membrane localization of drug targets such as HER2 was enhanced, thereby improving chemosensitivity and the accuracy of drug response evaluation[101].

4.2 Mechanisms Underlying the Compositional Regulation of Hydrogel Mechanical Properties

As a core component of the natural ECM, collagen constitutes approximately 30% of total mammalian proteins. Its capacity to bind integrins and support cell adhesion has established collagen as a foundational scaffold for most organoid hydrogels[78, 79, 85]. To address the diverse mechanical requirements of different organoids, compositional modification has emerged as a key strategy for precise performance tuning, primarily through additive integration, functional modification, and regulation of crosslinking methods.

Additive Integration is widely used for the targeted optimization of hydrogel mechanical parameters. The incorporation of functional materials enables precise control of stiffness and gelation kinetics. For example, composite collagen-nanocellulose (Col-NC) hydrogels, formed by blending collagen with nanocellulose (50~100 nm in diameter), increase G' from approximately 1 kPa (as seen in pure

collagen) to (5~8 kPa)—matching the mechanical range of Matrigel. Concurrently, gelation time is shortened by about 40% to (15 ± 2 min), reducing organoid exposure to sol-state matrix damage and making this system suitable for intestinal organoid culture[79]. Likewise, supplementing collagen hydrogels with 0.5~2% (w/v) alginate—a polysaccharide capable of Ca^{2+}-responsive crosslinking—can tune G' from 0.54 ± 0.15 kPa to 2.3 ± 0.28 kPa, covering the stiffness requirements of intestinal organoids with G' (0.5~1 kPa)) and pancreatic organoids with G' (1.5~2 kPa)[84].

Functional Modification synergistically improves both mechanical performance and biocompatibility, thereby expanding the applicability of hydrogels. For instance, plant-derived nanocellulose hydrogels functionalized with RGD peptides can achieve a G' comparable to Matrigel with only 0.1% nanocellulose content under ionic crosslinking. Additionally, RGD peptides bind to cell-surface integrins (αvβ3), enhancing cell adhesion and self-organization in intestinal crypt organoids and yielding a transcriptomic correlation of 0.89 with Matrigel-cultured controls[165]. These results demonstrate how functional modification can concurrently optimize mechanical and biological properties.

Crosslinking Methods directly govern hydrogel mechanical strength and stability, thus determining their suitability for specific applications. Compared with physically crosslinked systems, chemically crosslinked biopolymer hydrogels—formed through irreversible covalent bonds—typically exhibit superior mechanical strength and stability both *in vitro* and *in vivo*. For example, UV-crosslinked glycidyl methacrylate-modified collagen (Col-GMA) combined with methacrylated hyaluronic acid (HA-MA) yields a Col/HA composite hydrogel with G' (350 Pa)—significantly higher than that of pure HA hydrogels (175 Pa)—which meets the mechanical demands for organoids used in bone-defect repair models[166]. These results demonstrate how functional modification can concurrently optimize mechanical and biological properties.

Crosslinking Methods directly govern hydrogel mechanical strength and stability, thus determining their suitability for specific applications. Compared with physically

crosslinked systems, chemically crosslinked biopolymer hydrogels—formed through irreversible covalent bonds—typically exhibit superior mechanical strength and stability both *in vitro* and *in vivo*. For example, UV-crosslinked Col-GMA combined with methacrylated HA-MA yields a Col/HA composite hydrogel with G' (350 Pa)—significantly higher than that of pure HA hydrogels with G' (175 Pa)—which meets the mechanical demands for organoids used in bone-defect repair models [167].

4.3 Thermoresponsive Hydrogel Materials

Thermoresponsive hydrogels, which are characterized by reversible sol-gel transitions at physiological temperatures (32~37°C) , have gained significant traction in organoid culture. This property enables them to simplify the passaging process by eliminating the need for enzymatic digestion and to enhance seeding uniformity. Their behavior is driven by either intrinsic material properties, such as collagen self-assembly, or through deliberate compositional modifications, with overall performance optimized by balancing thermoresponsiveness, mechanical strength, and biocompatibility.

Pure collagen hydrogels exhibit inherent thermoresponsiveness, transitioning from sol to gel at ~37°C via triple-helix formation and fibrillogenesis. However, their low mechanical strength with G' (0.5~1 kPa) limits use for organoids requiring structural support[79, 139]. To address this limitation, composite strategies have been developed. For instance, Col-NC hydrogels retain thermoresponsiveness, with a transition temperature of approximately 36°C , while simultaneously increasing G' to (~5 kPa). This enhanced mechanical performance can support liver organoid culture for durations of up to 21 days[79]. Similarly, collagen-chitosan hydrogels—prepared by adding β-glycerophosphate sodium (β-GP)—exhibit gelation at 37°C and K (8 ± 1 kPa), with Shen et al. showing that this composite maintains pancreatic organoid integrity during passaging[139].

Shi et al. grafted thermosensitive groups onto hyaluronic acid to prepare a thermally responsive hyaluronic acid-based polymer (HA-pNIPAM) [78]. By combining

this thermosensitive polymer with collagen, when the temperature exceeded the polymer's LCST, the hydration size of the polymer increased significantly, prolonging its retention time within the collagen hydrogel framework. Consequently, a stable hydrogel formed within the collagen network at 37 °C.

Compared to covalently crosslinked networks, physically crosslinked networks exhibit significantly lower bond energy, enabling the formation of reversible network structures, which consequently impart superior stimulus responsiveness and self-healing capabilities. For instance, 8-PEG-cytosine50 hydrogels can form physical crosslinked networks through triple hydrogen bonds between cytosine-cytosine pairs. These hydrogels can form gel-like structures at room temperature under physiological pH and liquefy at 65 °C, demonstrating thermoresponsive behavior[87]. Similarly, composite biomaterials formed by nickel(II)-catalyzed polymerization of isocyanide monomers modified with dimeric, trimeric, or tetrameric ethylene glycol and functionalized with (D)-alanine-(L)-alanine residues feature a polyisocyanide backbone stabilized by hydrogen bonds into a helical structure. The alanine side chains further participate in hydrogen bonding, enabling PICs to rapidly transition into transparent hydrogels upon heating and depolymerize upon cooling, thereby exhibiting thermoresponsive properties[108].

Collectively, thermoresponsive hydrogels bridge the gap between mechanical performance and operational convenience. Compositional modifications such as Col-NC, HA-pNIPAM, enable simultaneous optimization of transition temperature (34~37°C), stiffness (0.5~10 kPa), and gelation time (5~30 min), making them adaptable to intestinal, liver, neural, and pancreatic organoid cultures.

5. **Conclusion**

In conclusion, the advancement of organoid culture is inextricably linked to the sophistication of its hydrogel matrices. As this review has demonstrated, progress no longer lies in choosing between naturally derived or synthetic materials, but in their strategic synthesis. It is this very interplay—leveraging the biocompatibility of natural

polymers while harnessing the tunability of synthetic ones—that is collectively driving the field from an "empirical" model to a "precision-based" one.

Naturally derived hydrogel matrices are centered on dECM and natural polymeric materials. Among them, Matrigel, as a representative of dECM, has become the "gold standard" for organoid culture due to its ability to construct a biomimetic microenvironment. However, issues such as immunogenicity caused by xenogeneic proteins, batch instability resulting from component heterogeneity, and the inability to regulate mechanical properties on demand have limited its application in clinical translation. Although natural polymeric materials retain high biocompatibility, and their properties can be regulated through chemical/physical modifications to address the problem of uneven composition of dECM, single-component materials have obvious shortcomings, such as low mechanical strength from pure collagen, lack of cell adhesion sites from unmodified alginate, and insufficient toughness from hyaluronic acid. Therefore, composite natural polymeric hydrogels with "complementary advantages" have become the mainstream. They have demonstrated greater application potential than Matrigel in the culture of organoids such as tumor, liver, and intestinal organoids.

Synthetic hydrogel matrices are characterized by core advantages of well-defined components and controllable properties, mainly including synthetic polymeric material, synthetic peptide, and recombinant collagen. Although the first two categories can meet the culture requirements of different organoids through functional modification and have no immunogenicity risk, problems such as insufficient biocompatibility and lower organoid maturation efficiency compared to Matrigel still need to be solved. As a special subclass of synthetic matrices, recombinant collagen achieves structural similarity to natural human collagen through genetic engineering technology. It combines the batch stability of synthetic materials and the biocompatibility of natural materials, effectively avoiding the pathogenic risks and immunogenicity of natural collagen. It has shown higher organoid formation efficiency in the culture of neural and liver organoids; however, its core bottleneck lies in the lack of spontaneous gelation

ability. Currently, four major technical approaches—chemical crosslinking, enzymatic crosslinking, encapsulation gelation, and self-assembly gelation—can achieve gelation, but each has limitations: chemical crosslinking requires harsh conditions, enzymatic crosslinking has high costs, and encapsulation gelation has poor tunability. Although self-assembly gelation driven by synthetic biology is the optimal direction, further optimization is still needed in sequence design and large-scale production.

The performance of hydrogels is ultimately determined by their chemical composition and inherent properties. Mechanical properties, including stiffness, viscoelasticity, and porosity, must be tailored to match the physiological requirements of the specific organoid type. Simultaneously, thermoresponsiveness enables critical procedures such as subculturing passaging and cell seeding to be performed under suitable physical states of the material. Various properties of hydrogels can be achieved through component compounding, functional modification, and selection of crosslinking methods.

The future development of hydrogels for organoid culture needs to make breakthroughs in three main directions: first, optimizing the gelation efficiency and property tunability of recombinant collagen, especially promoting the industrialization of self-assembly gelation technology to reduce production costs; second, improving the biocompatibility of synthetic polymers and synthetic peptides, and narrowing the gap in organoid maturation efficiency between these materials and Matrigel by simulating the multi-component synergy of natural ECM; third, developing composite natural polymeric hydrogels, and optimizing the organoid culture performance of hydrogels through targeted modification and component combination. Beyond these established directions, several emerging trends are poised to redefine organoid culture and expand its translational impact. Dynamic hydrogels are engineered to possess stimuli-responsive mechanical and biochemical properties, enabling them to mimic the spatiotemporal remodeling processes characteristic of the native extracellular matrix. A key example is the progressive adjustment of matrix stiffness, which can be achieved through the incorporation of enzyme-cleavable linkers, such as sequences sensitive to

matrix metalloproteinases, or via photo-activation mechanisms triggered by factors secreted by the developing organoids. This dynamic adaptability provides stage-specific mechanical support critical for tissue maturation, for instance within intestinal crypts or hepatic lobules, thereby overcoming the fundamental limitations of static matrices in accurately recapitulating complex developmental dynamics. Multi-material and spatially patterned hydrogels leverage advanced fabrication techniques including 3D bioprinting, microfluidics, and microcontact printing to introduce region-specific heterogeneity. By incorporating distinct natural or synthetic components, cell-adhesive motifs such as RGD peptides, or precisely controlled growth-factor gradients within defined spatial domains, these engineered systems enable the reconstruction of complex tissue architectures. Examples include the intestinal crypt-villus axis, the hepatocyte-sinusoid interface in the liver, and heterotypic tumor-stroma microenvironments. Consequently, this approach significantly enhances the physiological relevance of organoids for research focused on tissue patterning mechanisms and disease progression pathways. Immunocompetent organoid models are enabled by immunomodulatory hydrogels that are functionalized with immune-active components, for instance encapsulated immune cells, cytokines, or checkpoint molecules. These engineered matrices regulate the bidirectional crosstalk between organoids and specific immune cell subsets, such as T cells or macrophages. Consequently, they offer robust and physiologically relevant experimental platforms for investigating fundamental immune responses, modeling autoimmune diseases, and evaluating the therapeutic efficacy of immunotherapies including PD-1 and PD-L1 inhibitors, with direct translational relevance to clinical applications. CRISPR-engineered matrices combine gene-editing technology with hydrogel carriers, either by modifying the genome of seed cells to alter their ECM-secretory profiles or by engineering hydrogel-encapsulated stromal cells to express specific growth factors or signaling molecules. This precise customization of the microenvironment according to organoid-specific genetic backgrounds substantially improves physiological fidelity and supports applications in personalized medicine. These breakthroughs will accelerate the translation of organoids from basic research to fields such as disease

modeling, drug screening, and regenerative medicine, and are expected to realize the clinical application goal of personalized organoid diagnosis and treatment.

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Author contribution statement

LI Yaxiao: Visualization, Writing-original draft. CHOU Wenhsuan: Writing-review & editing. ZHANG Weiwei: Data Curation, Visualization. WANG Yi: Conceptualization, Writing-review & editing. ZHANG Chong: Conceptualization, Writing-review & editing. All authors have approved the final manuscript.

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