

## REVIEW ARTICLE

# Top 10 organoid research breakthroughs of 2025

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## Abstract

Organoid technology has rapidly matured into a versatile three-dimensional in vitro platform capable of recapitulating key structural and functional features of native tissues. The field is increasingly driven by multidisciplinary integration, with research efforts shifting beyond simple cellular self-organization toward the reconstruction of complex physiological functions. Recent advances in bioengineering have provided organoids with more precisely controlled physical and chemical microenvironments. Approaches such as microfluidic platforms, synthetic biomaterials, and three-dimensional bioprinting enable the in vitro reconstruction of tissue-specific architectures. In addition, progress in vascularization strategies has alleviated long-standing challenges associated with nutrient delivery and metabolic waste removal in larger organoids. The development of assembloid systems further allows the modeling of inter-organ communication and complex physiological axes, expanding the scope of organoid-based studies beyond single-tissue contexts. Together, these technological innovations have substantially enhanced the utility of organoids in disease modeling, drug screening, and regenerative medicine. With continuous improvements in culture systems and the advancement of high-dimensional data analysis, organoids are increasingly serving as a critical bridge between fundamental research and clinical translation. In this review, we summarize the key developments in 2025 and highlight ten representative studies that exemplify recent practical breakthroughs, with the aim of providing useful insights and references for researchers working in this rapidly evolving area.

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## 1. Introduction

Organoid technology represents a significant advance in biomedical research, enabling stem cells to self-organize into three-dimensional (3D) tissue-like structures *in vitro*. Compared with conventional two-dimensional cultures, organoids more faithfully recapitulate the cellular composition, spatial architecture, and functional characteristics of native human tissues. Initially emerging from studies of tissue self-assembly, organoids have progressively evolved into controllable and versatile *in vitro* modeling platforms. Following the successful establishment of intestinal organoids in 2009, the approach has rapidly expanded to a wide range of organs, including the brain, liver, lung, and oral tissues. This progression reflects a broader shift in *in vitro* modeling—from simple cell aggregation toward increasingly sophisticated systems capable of reproducing complex organ-level features.

Despite their considerable promise in basic and translational research, organoids still face several technical limitations that hinder broader practical applications. First, most systems lack functional vascularization, resulting in insufficient nutrient delivery, metabolic waste accumulation, and subsequent necrosis, which restricts tissue size and long-term maintenance. Second, the absence of interactions with the nervous and immune systems limits their ability to model diseases involving complex multicellular or systemic responses. Finally, variability in culture conditions and batch-to-batch reproducibility further complicates standardization, posing challenges for large-scale screening and clinical translation.

Looking back at 2025, a series of disruptive technological breakthroughs have emerged in the field of organoid research. This review surveys organoid-related studies published in leading international journals, such as *Science* and *Cell*, in 2025 and highlights 10 particularly influential papers from multiple perspectives, including functional integration, clinical translational potential, bioengineering innovation, and integration with artificial intelligence (AI). These studies not only offer effective strategies to improve scaffold design, vascularization, and organoid maturation, but also propel organoid research to new heights of “organ-level reconstruction” and “digital assembly.” In conclusion, this article aims to outline the evolution of organoid research in the future by tracing the development of these studies, exploring their underlying scientific logic and philosophical implications (Figure 1).

## 2. Breakthroughs

### 2.1. Neurovascular organoids support post-stroke brain repair

Ischemic stroke, in its subacute phase, leads to liquefactive necrosis of brain tissue, forming a localized stroke cavity.<sup>1,2</sup> This not only disrupts neural circuits but also creates a

formidable physical barrier to tissue repair. Although the academic community has agreed that revascularization is key to initiating neurogenesis and functional recovery,<sup>3</sup> regenerative interventions within this confined intracranial space consistently face significant challenges: traditional tissue-engineered scaffolds struggle to strike a balance between providing physical support and preventing secondary damage.<sup>4</sup> On the one hand, many hydrophilic hydrogels absorb water and swell after implantation, leading to increased intracranial pressure, brain tissue compression, and even brain herniation.<sup>5</sup> On the other hand, if the matrix degrades too rapidly, the regenerative space collapses prematurely, failing to provide a stable microenvironment for the long-term development and integration of vascular organoids.<sup>6</sup>

To address the challenges, Xiao *et al.*<sup>7</sup> proposed a new type of non-expansive biodegradable matrix (NEBM) and also put forward the concept of “Regeneration in Constant Space”, which overcomes the mechanical limitations of intracranial implants. The study adopted a biomimetic assembly method, not a single material, combining the hydrophobic  $\beta$ -sheet structure of clinical-grade silk fibroin with an enzyme-crosslinked hyaluronic acid network. This unique design confers good volumetric stability, allowing the matrix to degrade *in vivo* while preserving its overall architecture and leaving space for vascular ingrowth, thereby reducing the risk of brain tissue compression.

Another important innovation of Xiao *et al.*<sup>7</sup> was the precise regulation of organoid developmental fate. The storage modulus of the optimal HS-75 formulation identified in the study is about 280 Pa, which matches the stiffness of native brain tissue. Single-cell sequencing and mechanical analyses show that the stiffness of this specific matrix activates the integrin–FAK–YAP mechanotransduction signaling pathway and upregulates artery-related genes (such as *DLL4*, *CXCR4*) in endothelial cells. Compared with Matrigel, the vascular organoids cultured from NEBM have higher vascular density, larger vessel diameter, and more pronounced arterial features. *In vivo* experiments in a mouse photothrombotic stroke model demonstrated that NEBM-embedded vascular organoids promoted angiogenesis, established functional anastomoses with host vessels, induced endogenous neurogenesis, and permitted axonal infiltration into the lesion area. This dual “vascular–neural” repair strategy resulted in significant motor recovery in mice. The work not only provides a clinical-grade culture medium that can replace Matrigel, but also offers a paradigm shift in addressing the challenges of biomechanical and biological compatibility in post-stroke brain tissue repair (Figure 2).

### 2.2. Human airway submucosal gland organoids model region-specific pathophysiology

In respiratory organoid research, investigators have often



**Figure 1.** Overview of the top 10 organoid research breakthroughs in 2025. Created with BioRender, Long Bai (2026). <https://BioRender.com/vqiq0xe>. Abbreviation: AI: Artificial intelligence.

focused on the surface airway epithelium (SAE) while overlooking submucosal glands (SMGs).<sup>8,9</sup> The SMGs are important parts of the respiratory tract, secreting mucus and contributing to airway hydration and host defense.<sup>10</sup> In diseases such as chronic obstructive pulmonary disease and asthma, SMGs become hypertrophic and hypersecretory, contributing to airway obstruction and disease exacerbations.<sup>11,12</sup> However, the SMGs are embedded in the cartilaginous airways and have a complex structure, and effective *in vitro* models remain lacking to simulate their functions, which has become a key “missing link” in respiratory tract research.

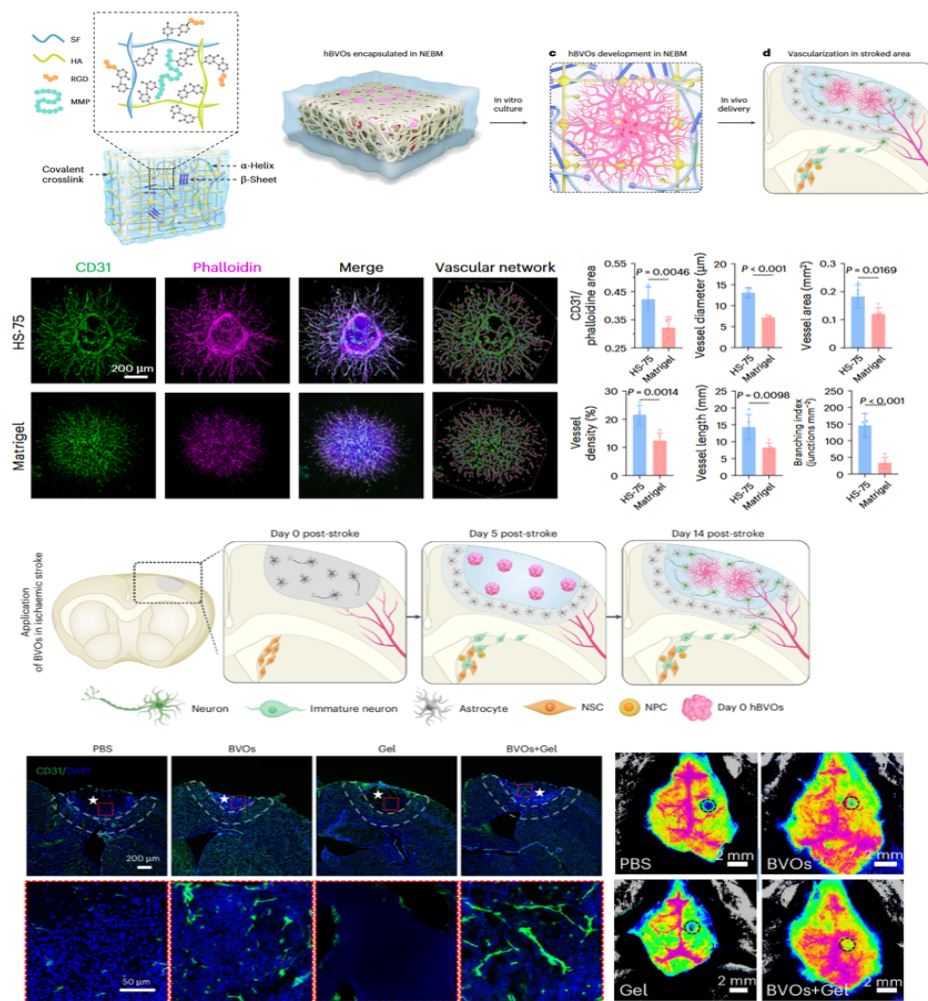
In 2025, Lin *et al.*<sup>13</sup> made a breakthrough, constructing a long-term expandable human bronchial SMG organoid model (Figure 3). They used a two-step enzymatic digestion method to separate the SAE sheet and release acinar clusters from between muscle fibers, and isolated and cultured these two regions *in vitro*. To verify the reliability of the model, they conducted an in-depth analysis using single-cell transcriptome sequencing (scRNA-seq). SMG organoids contained *MUC5B*<sup>+</sup> mucus-secreting cells and myoepithelial cells expressing  $\alpha$ -smooth muscle actin. Lin *et al.*<sup>13</sup> identified ANPEP/CD13 as a marker of SMG secretory cells and CD200 as a marker enriched in myoepithelial cells. The experimental results showed that SMG cells exhibit lineage plasticity and can differentiate into ciliated cells under specific airway conditions, implying that they may serve as reserve stem cells during airway injury repair.

The study further used this organoid system to investigate

differential glandular responses to inflammatory stimuli. For example, interleukin (IL)-1 $\beta$  and tumor necrosis factor alpha specifically upregulate major histocompatibility complex class II molecules in SMG mucin cells, which indicates that the gland may be involved in antigen presentation in the epithelial immune microenvironment. IL-13 transforms the mucin cell phenotype from *MUC5B* to *MUC5AC* through the transcription factor SPDEF, simulating the composite changes under the type 2 immune response. It is worth noting that the study found that HCoV-229E infection was not random and primarily targeted CD13<sup>+</sup> SMG secretory cells. Viral infection caused severe endoplasmic reticulum stress and an unfolded protein response, providing a new cellular mechanism for understanding how viral infection induces acute exacerbation of chronic obstructive pulmonary disease. Lin *et al.*<sup>13</sup> have opened a new era of full-thickness simulation in respiratory organoid research. Combining SMG and SAE organoids may enable more comprehensive modeling of respiratory physiology and pathology. This progress not only provides a more accurate drug screening platform for respiratory diseases but also lays a solid foundation for the development of future therapeutic strategies targeting mucus hypersecretion and virus-induced injury.

### 2.3. Vascularized heart organoids model early cardiac development

The early stages of organ vascularization in humans have long been an uncharted territory in developmental biology,



**Figure 2.** Stroke repair strategy based on NEBM-supported vascular organoids. The schematic diagram illustrates the “Regeneration in Constant Space” model that NEBM supports for the *in vitro* development and *in vivo* delivery of BVOs. The study showed that NEBM significantly improved BVO vascular maturity compared to Matrigel and enabled angiogenesis and restoration of blood perfusion in the infarct core area after transplantation. Image reprinted with permission from Xiao *et al.*<sup>7</sup> Copyright © 2025, The Author(s), under exclusive licence to Springer Nature Limited.

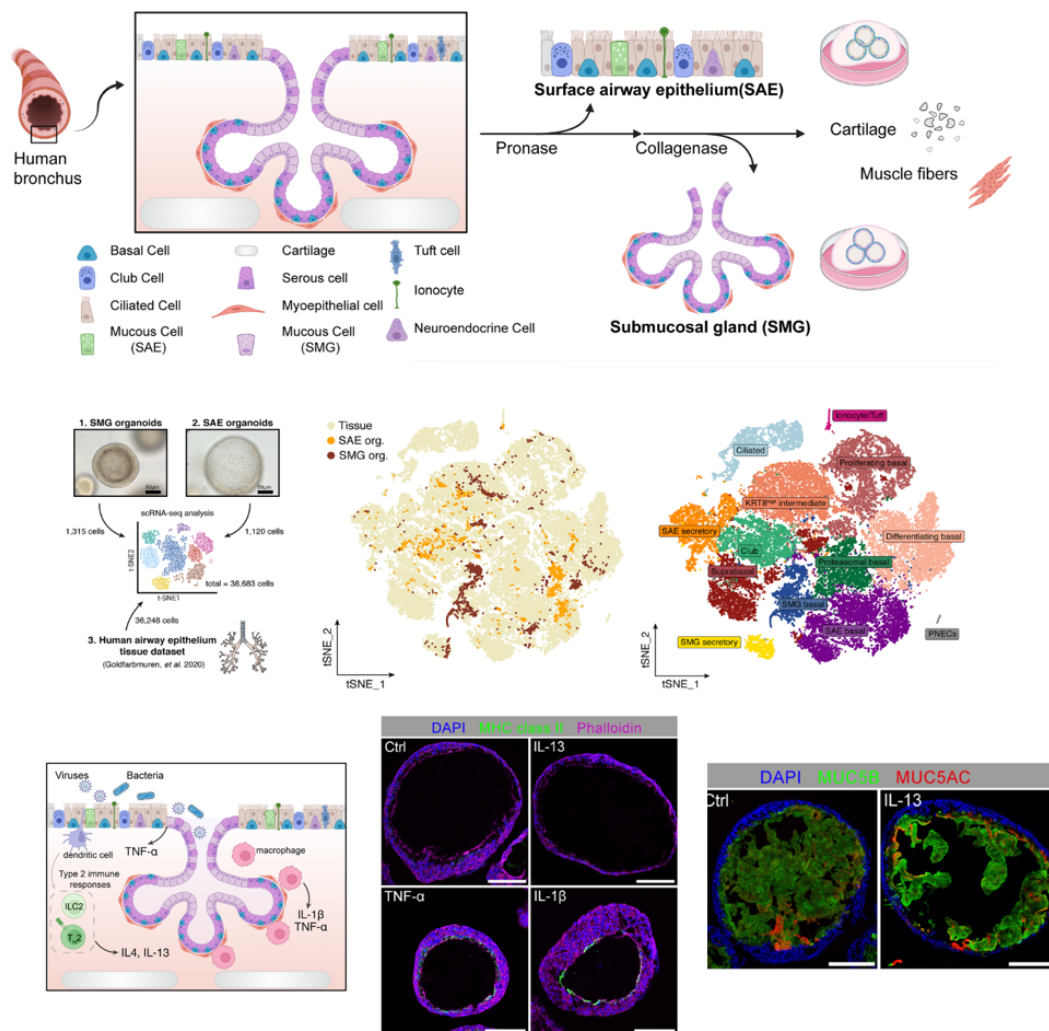
Abbreviations: BVOs: Blood vessel organoids; HA: Hyaluronic acid; hBVOs: Human blood vessel organoids; MMP: Matrix metalloproteinase; NEBM: Non-expansive biodegradable matrix; NPC: Neural progenitor cell; NSC: Neural stem cell; PBS: Phosphate-buffered saline; RGD: Arginine-glycine-aspartic acid; SF: Silk fibroin.

largely due to ethical and technical challenges. While organoid technologies derived from human pluripotent stem cells (hPSCs) have made significant progress in mimicking organ-specific cell differentiation, constructing functional, self-assembling vascular networks remains a major challenge.<sup>14–16</sup> Previous attempts at vascularization, such as classic tissue engineering co-culture,<sup>17</sup> forced overexpression of transcription factors (e.g., ETV2),<sup>18</sup> or 3D bioprinting,<sup>19,20</sup> while achieving some degree of integration of vascular cells, often fail to generate robust, hierarchical vascular networks that truly form lumina through self-organization. Furthermore, in existing cardiac organoid models, the co-differentiation efficiency of cardiomyocytes and vascular cells is usually unstable,<sup>21</sup> and existing models

do not faithfully reproduce the spatiotemporal features of *in vivo* development.<sup>22</sup> To overcome these limitations, Abilez *et al.*<sup>23</sup> developed an *in vitro* model based on micropatterning hPSCs, successfully simulating the vascularization process of the heart and liver corresponding to the first three weeks of human development (Carnegie stages 9 and 10). The study not only fills a gap in early vascular development models but also achieves simultaneous co-differentiation of complex vascular networks and organ parenchymal cells through a universal vascularization induction strategy.

In the study, Abilez *et al.*<sup>23</sup> first constructed a triple fluorescent reporter cell line (hESC-3R) to overcome the screening challenge. This cell line can track the





**Figure 3.** Modeling human bronchial SMG physiology and inflammation. Procedure for isolating SAE and SMG compartments from the human bronchus for separate organoid culture. Integrated Single-cell transcriptome sequencing atlas showing that SMG organoids accurately recapitulate native tissue heterogeneity, specifically capturing *MUC5B*<sup>+</sup> mucous cells and myoepithelial cells. Confocal images of SMG organoids capturing cytokine-specific responses: interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) induce major histocompatibility complex class II expression (green), while IL-13 triggers mucous metaplasia via MUC5B-to-MUC5AC (red) switching. Image reprinted with permission from Lin *et al.*<sup>13</sup> Copyright © 2025 The Author(s). Published by Elsevier Inc.

differentiation trajectories of cardiomyocytes (TNNT2-GFP), endothelial cells (CDH5-mOrange), and smooth muscle cells (TAGLN-CFP) in real time. Using this tool, the researchers tested 34 differentiation conditions and ultimately identified a “vascularization cocktail” containing vascular endothelial growth factor, fibroblast growth factor 2, a selective inhibitor of transforming growth factor-β type I receptor signaling, angiopoietin-1/2, platelet-derived growth factor-BB, and transforming growth factor-β1. Using micropatterned gastruloids, the researchers successfully induced the cardiac vascularized organoids (cVOs). The cVOs established in the study have more physiologically relevant structural and functional features compared with previous models. Structurally, cVOs form a spatially

ordered concentric circle arrangement, with endothelial cells forming a vascular network with clear branches (4–40 μm in diameter) and hierarchical organization. Microsphere experiments confirmed true lumen formation. scRNA-seq showed that the cellular composition of cVOs was highly similar to that of the human embryonic heart at 6.5 weeks post-conception, containing multiple cell types, including endocardium, myocardium, epicardium, and nerve cells. Furthermore, this study revealed the crucial roles of the Notch and bone morphogenetic protein (BMP) signaling pathways in early vascularization and used this model to discover that the opioid fentanyl abnormally increases angiogenesis, demonstrating the model’s potential for drug teratogenicity screening. Notably, the researchers

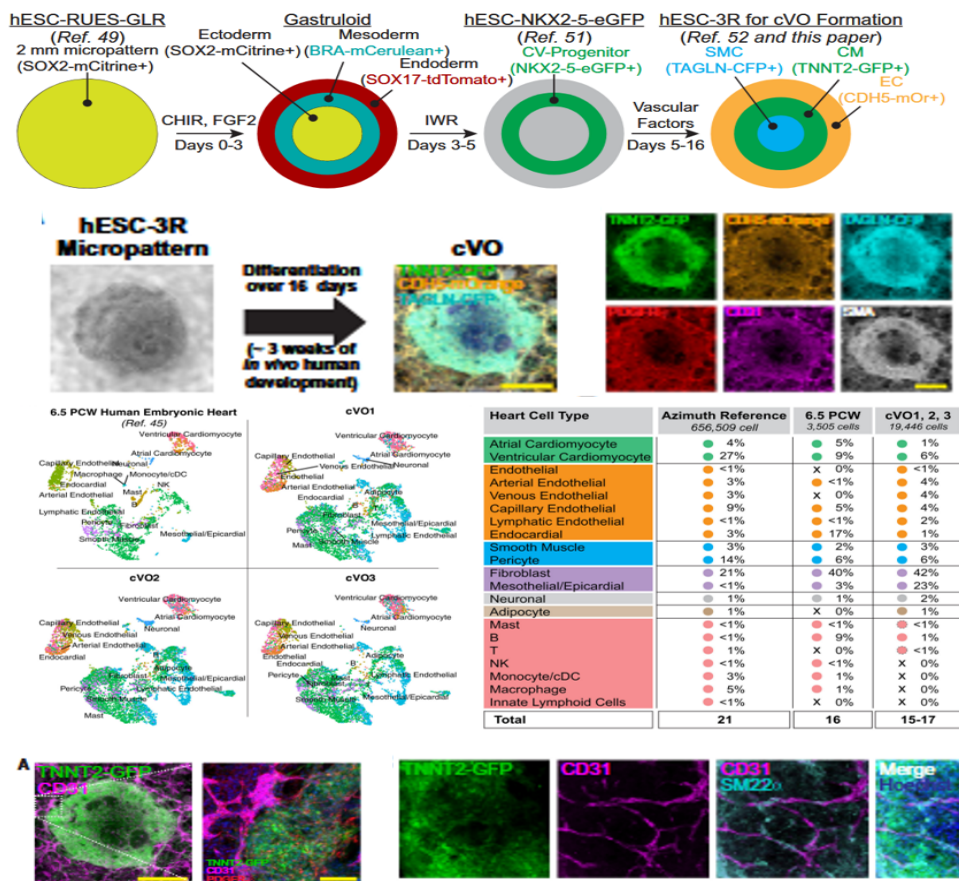
demonstrated the universality of this angiogenesis induction strategy; the same combination of factors can also induce the generation of hepatic vascularized organoids with functional vascular networks, providing a universal paradigm for vascularization modeling of multi-organ systems (Figure 4).

#### 2.4. Kidney assembloids achieve functional maturity

While kidney organoids derived from hPSCs offer hope for kidney disease research, constructing kidney tissue with complete anatomical structure and physiological function remains a significant technical challenge.<sup>24,25</sup> The complexity of the kidney stems from the precise spatiotemporal signaling regulation of interactions between different progenitor cell populations, such as nephron progenitor cells and ureteral bud progenitor cells. Most existing

induced pluripotent stem cell-based models generate either nephron or collecting duct lineages rather than an integrated system containing both.<sup>26-29</sup> As a result, these models remain arrested at an early developmental stage, fail to establish clear spatial patterning, and do not reproduce key physiological functions such as glomerular filtration and urine concentration. This developmental stagnation restricts the application of organoids in simulating late-onset nephropathy and regenerative medicine.<sup>30</sup>

To address these challenges, Huang *et al.*<sup>31</sup> established a platform for a kidney progenitor assembloid (KPA) platform. In the study, the culture system (v3 medium) was optimized for co-culture and self-assembly of human induced pluripotent nephron progenitor cells (iNPCs) and human induced pluripotent ureteric bud cells. Mechanistic studies showed that retinoic acid signaling is crucial in



**Figure 4.** Construction and characterization of cVOs. Micropatterning induced hPSCs to form spatially ordered gastrula-like structures, which then developed into cVOs containing concentrically arranged cardiac and vascular cells. Single-cell sequencing and imaging confirmed that their cellular composition mimicked the early human embryonic heart and possessed a complex branching vascular network tightly integrated with the myocardium. Image reprinted with permission from Abilez *et al.*<sup>23</sup> Copyright © 2025 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science.

Abbreviations: CHIR: CHIR99021, a glycogen synthase kinase 3 (GSK3) inhibitor and WNT activator; cVOs: Cardiac vascular organoids; FGF2: Fibroblast growth factor 2; hESC: Human embryonic stem cell; hPSCs: Human pluripotent stem cells; IWR: Inhibitor of Wnt response; PCW: Post-conception week.

early pattern formation: the agonist TTNPB induces *WNT4* expression, thereby enabling iNPCs to form polarized renal vesicles around the central ureteric bud, then develop into segmented nephrons and fuse with the collecting duct system. This process successfully reproduced the unique self-assembly mechanism and spatial arrangement of kidney development *in vitro* (Figure 5).

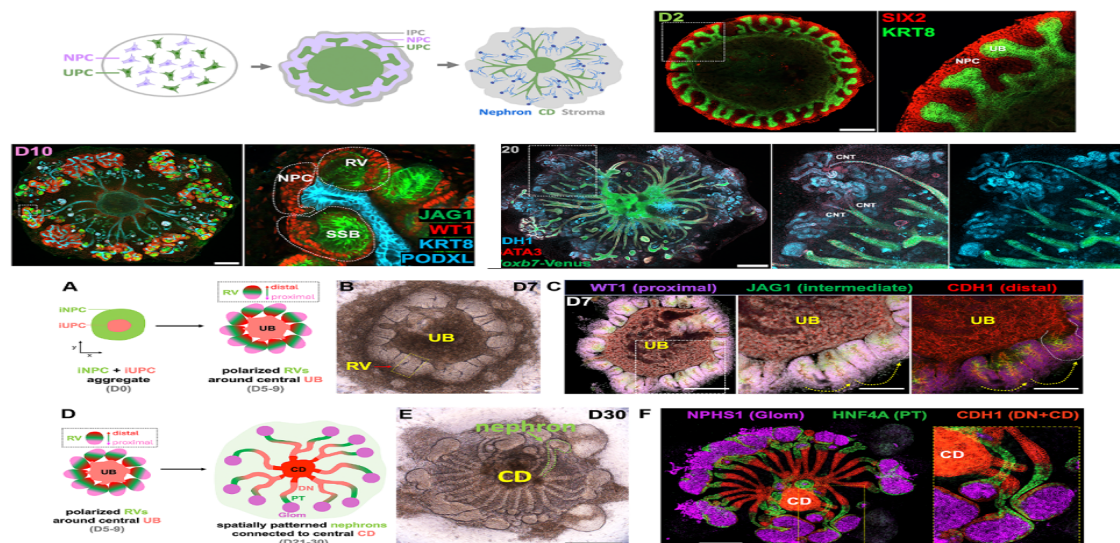
Functionally, KPAs exhibit a high level of maturity. *In vitro* experiments confirmed that human KPAs possess active renal tubular reabsorption (e.g., albumin uptake) and secretory functions, as well as the ability to secrete erythropoietin in response to hypoxia. More importantly, after transplantation into immunodeficient mice, human KPAs achieved extensive vascularization, forming a human-mouse chimeric vascular network. *In vivo* imaging directly observed the process of fluorescent dye entering the glomerulus and being reabsorbed by the tubules, confirming their filtration function. Single-cell multi-omics analysis further showed that *in vivo*-grown human KPAs were more mature at the transcriptomic level than the kidneys of 17-week-old human fetuses and expressed a variety of organic anion/cation transporters specific to adult kidneys. Furthermore, this study successfully constructed a high-fidelity *in vivo* model of autosomal dominant polycystic kidney disease using *PKD2* gene-knockout KPAs. This model not only replicated the formation of large cysts and ciliary polarity defects but also reproduced

microenvironmental pathological features such as fibrosis and macrophage infiltration, which are difficult to simulate *in vitro* models, providing a powerful tool for elucidating the mechanisms of complex kidney diseases.

## 2.5. Vascularized lung organoids mimic alveolar development

Previous strategies for constructing vascularized organoids often employed an “assembly” method, physically mixing independently differentiated endothelial cells with epithelial cells.<sup>32,33</sup> However, this non-*in situ* construction disrupts the complex spatiotemporal interactions of early embryonic development, leading to a disordered vascular network structure and a lack of organ specificity in endothelial cells, making it difficult to form a gas–blood barrier that matches lung tissue function.<sup>34,35</sup> A more developmentally faithful mesodermal–endodermal co-differentiation strategy was proposed by Miao *et al.*<sup>36</sup> This approach involves simultaneously inducing the development of two germ layers within a single embryoid and utilizing endogenous intercellular interactions to drive vascularization.

The core breakthrough of the study lies in revealing the crucial role of the BMP signaling pathway in determining early germ layer fate. The research team found that the timing of fine-tuning the BMP4 signaling pathway can balance the ratio of endoderm to mesoderm: brief activation of the BMP signaling pathway on days 0–1 of differentiation tends



**Figure 5.** Self-assembly and spatial development of KPAs. The illustration shows how mouse and human progenitor cells self-assemble to form polarized renal capsules surrounding the central ureteral bud. As development progresses, these capsules form segmented nephrons and eventually physically fuse with the collecting duct system, reproducing the complex spatial patterns and structural connections of the kidney. Image reprinted with permission from Huang *et al.*<sup>31</sup> Copyright © 2025 Elsevier Inc.

Abbreviations: CD: Collecting duct; CNT: Connecting tubule; DN: Distal nephron; Glom: Glomerulus; iNPC: Induced nephron progenitor cells; IPC: Interstitial progenitor cells; iUPC: Induced ureteric progenitor cells; KPA: Kidney progenitor assembloids; NPC: Nephron progenitor cells; PT: Proximal tubule; RV: Renal vesicle; SSB: S-shaped body; UB: Ureteric bud; UPC: Ureteric progenitor cells.

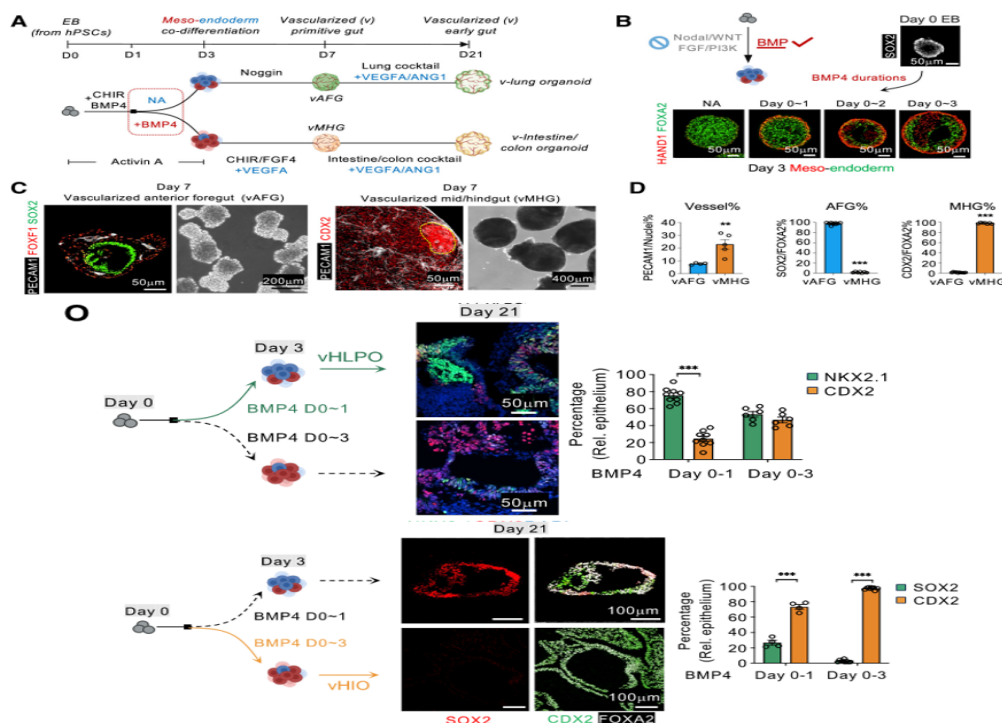


to induce lung (foregut) fate, while sustained stimulation until day 3 promotes intestinal (hindgut) development. This strategy successfully constructed vascularized lung progenitor organoids with organ-specific characteristics. Single-cell sequencing and functional experiments confirmed that these endothelial cells specifically express lung markers (such as *HPGD* and *FENDRR*) and have high transendothelial electrical resistance, contrasting sharply with the highly permeable, *IGFBP7*-expressing endothelial cells in intestinal organoids, thereby more faithfully recapitulating the heterogeneity of vascular beds in different organs *in vivo*.

Next, Miao *et al.*<sup>36</sup> transplanted the vascularized organoids into immunodeficient mice to functionally integrate human blood vessels with the host's circulatory system (Figure 6). Notably, the presence of blood vessels and interstitium significantly promoted epithelial maturation and diversification, inducing aerocytes and respiratory tract secretory cells, which are rarely seen in traditional organoids. Using a bioengineered scaffold, the researchers

also constructed a biomimetic sac-like structure *in vitro* in which endothelial cells tightly encapsulated alveolar epithelium, and observed gas-blood barrier units similar to those *in vivo* at the ultrastructural level. Furthermore, using this model, researchers successfully reproduced alveolar capillary dysplasia caused by *FOXF1* gene mutations, revealing that interstitial and vascular defects can arise secondary to epithelial developmental arrest and confirming the platform's unique advantages in elucidating non-autonomous pathogenic mechanisms.

Meanwhile, the logic of multi-germ layer co-development has also been validated in gastric organoid research.<sup>37</sup> Through a multi-germ layer co-differentiation strategy, Li *et al.*<sup>37</sup> successfully established gastroids, which are the first human gastric organoid model that mimics the self-organized fundic-antral bipolar patterning of early human stomach development. Furthermore, their study further revealed that neural-derived Wnt signaling plays a critical driving role in this regional specification



**Figure 6.** Mesodermal–endoderm co-differentiation for constructing vascularized organoids. Schematic diagram of differentiation process; BMP signaling time course determines the early germ layer ratio and subsequent foregut and hindgut fate; Immunofluorescence staining and quantitative analysis of vascularized foregut and midgut organoids on day 7, confirming the coordinated development of blood vessels, stroma, and epithelial cells. Image reprinted with permission from Miao *et al.*<sup>36</sup> Copyright © 2025 Elsevier Inc.

Abbreviations: AFG: Anterior foregut; ANG1: Angiopoietin 1; BMP: Bone morphogenetic protein; CHIR: CHIR99021, a glycogen synthase kinase 3 (GSK3) inhibitor and WNT activator; EB: Embryoid body; FGF: Fibroblast growth factor; hPSCs: Human pluripotent stem cells; MHG: Mid/hindgut; PI3K: Phosphoinositide 3-kinase; WNT: Wingless/Integrated signaling pathway; vAFG: Vascularized anterior foregut; vHIO: Vascularized human intestinal organoid; vHLPO: Vascularized human lung progenitor organoid; vMHG: Vascularized mid/hindgut.



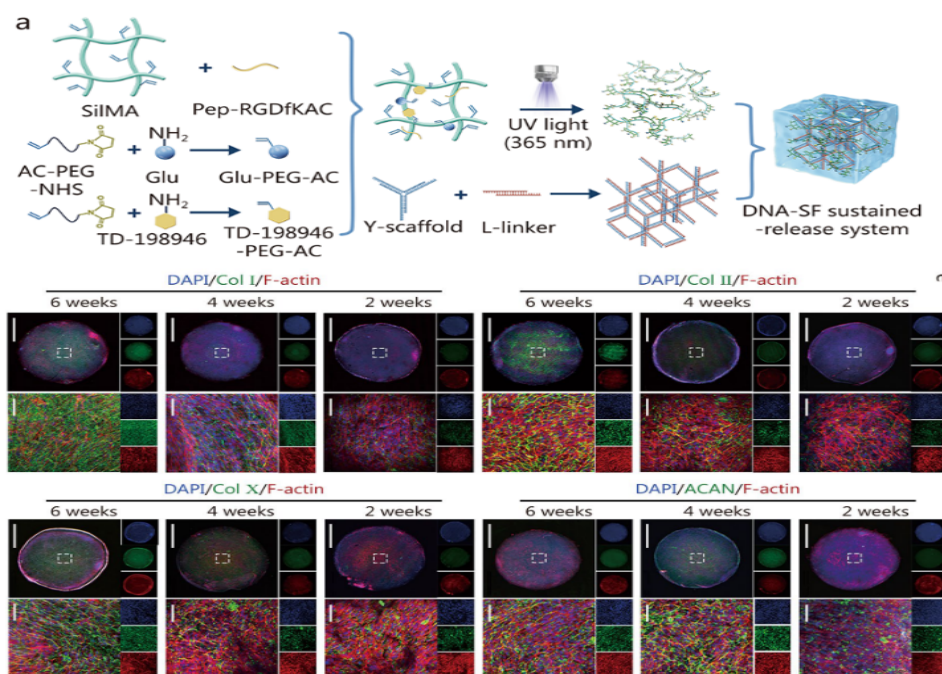
## 2.6. Bone and cartilage organoids accelerate skeletal repair

Articular cartilage, lacking blood vessels, lymphatic vessels, and nerve innervation, has extremely limited self-repair capabilities after injury.<sup>38,39</sup> Existing clinical treatment strategies (such as microfracture surgery or autologous chondrocyte transplantation) often fail to regenerate high-quality hyaline cartilage, instead producing fibrocartilage with poor mechanical properties, and graft integration with host tissue is often poor.<sup>40,41</sup> Although cartilage organoids offer new hope for tissue repair, clinical translation remains limited by two major challenges: maintenance of the hyaline cartilage phenotype after transplantation and the short *in vivo* half-life and rapid degradation of key growth factors such as transforming growth factor- $\beta$ 3 (TGF- $\beta$ 3), which reduce induction efficiency. These limitations remain major bottlenecks hindering their clinical translation (Figure 7).<sup>42</sup>

Shen *et al.*<sup>43</sup> recently achieved a breakthrough, proposing a new strategy to enhance cartilage regeneration by constructing an “organoid-hydrogel” synergistic microenvironment. They first addressed the short *in vivo* half-life and rapid degradation of growth factors, developing a smart sustained-release system based on DNA aptamers. By specifically anchoring TGF- $\beta$ 3 to the

DNA sequence, this system avoided the “burst release” of the drug, achieving continuous and precise delivery for up to 28 days. This long-lasting biochemical stimulation continuously activated the Smad2/3 signaling pathway, effectively maintaining the hyaline cartilage phenotype of the organoid and significantly inhibiting the tendency for fibrosis or hypertrophy, providing a stable molecular support for cartilage regeneration.

Building on the optimized biochemical microenvironment, the study further addressed the “mechanical fit” problem of the scaffold through materials engineering. To compensate for the insufficient mechanical strength of pure DNA hydrogels, researchers introduced silk fibroin, which has excellent mechanical properties, to construct a DNA-silk fibroin dual-network composite hydrogel. This composite design not only retains the good porosity and shear-thinning properties of the hydrogel, making it easy to inject into minimally invasive procedures, but also significantly improves the energy storage modulus of the scaffold, giving it sufficient mechanical strength to withstand the load-bearing environment of the joint. This “hard-soft” physical microenvironment provides the necessary mechanical support for the growth and maturation of cartilage organoids, mimicking the



**Figure 7.** Construction and regeneration of cartilage organoids based on DNA-silk fibroin (SF) hydrogel. The schematic diagram illustrates the use of DNA aptamers to provide long-term sustained release of transforming growth factor- $\beta$ 3 and the introduction of SF to enhance mechanical properties, creating a suitable microenvironment for cartilage organoid differentiation and maturation. Experiments in a rabbit knee joint defect model showed that, 12 weeks after implantation, this system effectively promoted the regeneration and repair of smooth, high-quality hyaline cartilage. Image reprinted from Shen *et al.*<sup>43</sup> Copyright © The Author(s) 2025.

physical properties of the native cartilage extracellular matrix. This strategy demonstrated remarkable repair potential in a rabbit knee joint cartilage defect model. The implanted complex successfully induced the regeneration of high-quality hyaline cartilage with a smooth surface, predominantly composed of type II collagen, and exhibited close integration with the host tissue, providing a novel and highly valuable translational approach for the clinical treatment of articular cartilage injuries.

## 2.7. Synthetic immune organoids sustain human antibody responses

Research on human adaptive immunity, particularly the exploration of immune response mechanisms in patients with non-Hodgkin B-cell lymphoma, has long been limited by the lack of *in vitro* models capable of accurately replicating the *in vivo* microenvironment.<sup>41,44</sup> Patients receiving immunotherapies such as anti-CD20/CD3 bispecific antibodies often face clinical challenges such as impaired antibody production and poor vaccine responses.<sup>45</sup> Traditional two-dimensional cell cultures or animal models struggle to accurately replicate the complex germinal center responses in human lymph nodes and patient-specific immune deficiencies.<sup>46,47</sup> Addressing this gap, Zhong *et al.*<sup>48</sup> developed a synthetic immune organoid, providing a powerful tool for elucidating the differences in B-cell responses between healthy individuals and lymphoma patients (Figure 8).

The core innovation of this research lies in the use of polyethylene glycol-maleimide (PEG-MAL) hydrogels to construct an engineered immune microenvironment. Integrin ligands (such as VCAM-1, ICAM-1) and key immune signaling molecules (such as CD40L) are integrated into the gel to simulate the mechanical stiffness and biochemical signals of lymphoid tissues. This system enables long-term three-dimensional co-culture of B cells and T cells from human tonsils or lymph nodes. It can induce B cell proliferation to form germinal center-like structures and drive them to differentiate into plasma cells, producing specific IgG antibodies against influenza virus or SARS-CoV-2, and fully recapitulating key features of humoral immunity *in vitro*. Using this platform, the team explored the immune suppression mechanism in patients with non-Hodgkin lymphoma under immunotherapy. Comprehensive experiments showed that the organoids from healthy donors had robust antigen-specific antibody responses, while the organoids from non-Hodgkin lymphoma patients (even in the remission period) showed markedly reduced B cell proliferation and antibody secretion defects. Mechanistic analyses showed that this functional impairment was associated with epigenetic suppression of B cells and impaired T-cell helper function. Of greatest clinical relevance, the study used this organoid

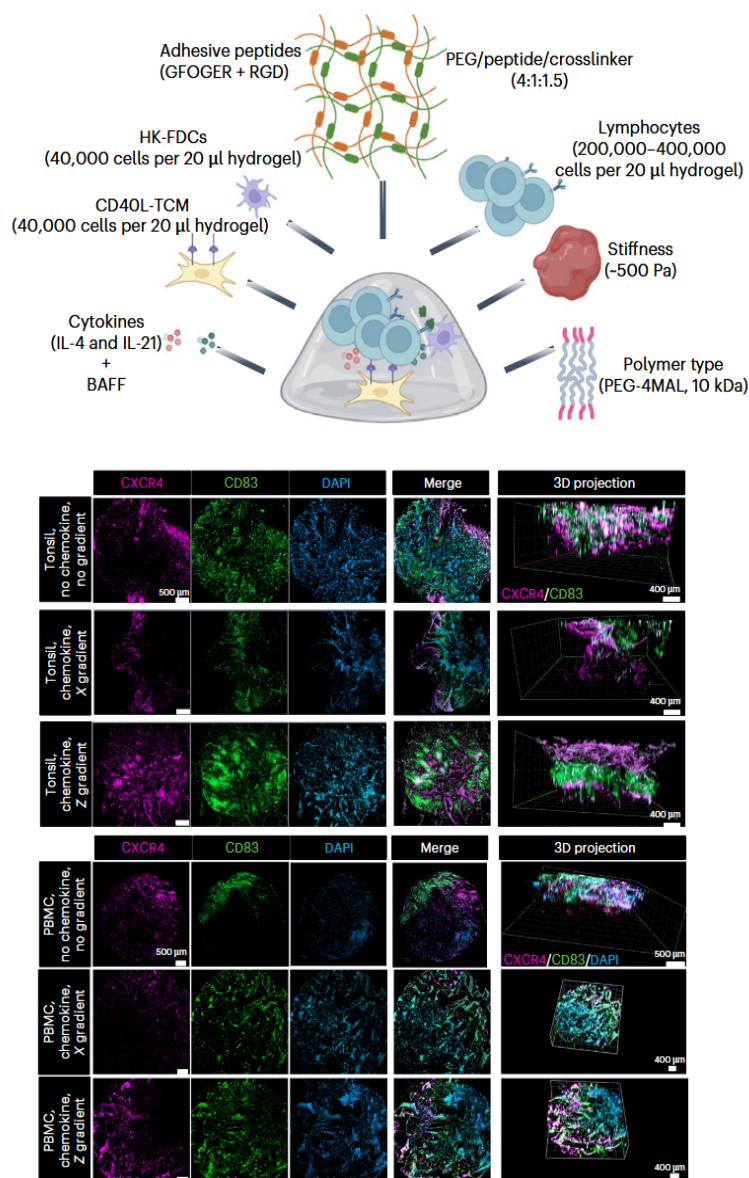
platform to screen epigenetic regulatory drugs (such as EZH2 inhibitors). Results showed that these drugs could partially restore germinal center responses and plasma cell differentiation capacity in patients' B cells. This achievement strongly demonstrates the practical application potential of organoids as a personalized medicine tool in assessing immune function and guiding precision treatment.

## 2.8. Integrin-activated matrices promote organoid growth

The widespread application of organoid technology has long been limited by its reliance on animal-derived matrices (such as Matrigel).<sup>49,50</sup> Although Matrigel is rich in extracellular matrix signals and can effectively maintain the survival and proliferation of stem cells, its unclear composition, poor batch stability, and potential risk of pathogen contamination have become major bottlenecks restricting the standardized production and clinical translation of organoids.<sup>51,52</sup> Although chemically defined synthetic hydrogels (such as PEG and polyisocyanate [PIC]) are considered ideal alternatives, they often lack the necessary cell-matrix adhesion signals, failing to effectively activate integrin-mediated survival pathways, leading to a high susceptibility to anoikis during culture in epithelial organoids.<sup>53,54</sup> Therefore, reconstructing crucial cell adhesion signals in a fully synthetic microenvironment has become a core challenge in achieving matrix-free organoid culture. With the aim of resolving this issue, Wijnakker J., *et al.*<sup>55</sup> had previously successfully constructed a fully defined, animal-free 3D culture system by covalently binding the functional domains of the Yersinia invasin protein to a PIC synthetic hydrogel. This demonstrated that artificially activated integrin signaling can replace the natural matrix to support the long-term expansion of organoids<sup>55</sup> (Figure 9).

In 2025, de Lau *et al.*<sup>56</sup> further proposed a simpler and more universal integrin activation strategy. The sophisticated molecular engineering strategy involves directly regulating receptor conformation to replace complex ligand modifications. Based on the integrin  $\beta 1$  activation antibody TS2/16, the research team constructed a single-chain variable region fragment (scFv-TS2/16). Unlike traditional extracellular matrix proteins (such as laminin) that rely on physical binding to recruit receptors, this scFv molecule can bind and stabilize integrin  $\beta 1$  in a high-affinity active conformation. This artificially induced conformational change mimics the natural matrix-binding state of cells, thereby activating key downstream survival and proliferation signaling pathways such as FAK and YAP in the absence of actual extracellular matrix ligands, providing an effective survival signal for organoids.

This strategy revolutionized the culture efficiency of synthetic hydrogels. Experimental results showed that

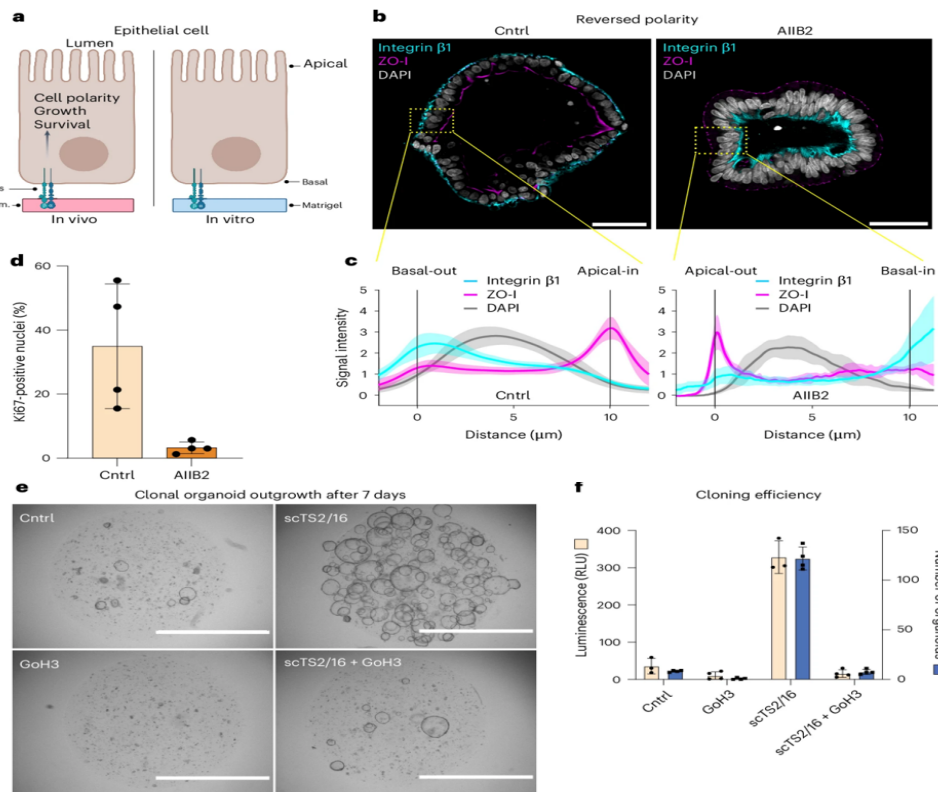


**Figure 8.** Construction of synthetic immune organoids and simulation of germinal center polarization. Schematic diagram of the construction process of tonsil immune organoids. Using the CXCL12 chemokine gradient in the microfluidic chip, B cells were successfully induced to form a spatial polarization distribution similar to the dark and bright areas of the germinal center in organoids derived from tonsils and PBMCs. Image reprinted with permission from Zhong *et al.*<sup>48</sup> Copyright © 2024, The Author(s), under exclusive licence to Springer Nature Limited. Abbreviations: BAFF: B-cell activating factor; HK-FDC: Human kidney fibroblastic dendritic cells; TCM: Tonsil-conditioned medium; PEG: Polyethylene glycol; RGD: Arginine-glycine-aspartic acid.

adding only trace amounts of scFv-TS2/16 to synthetic PIC hydrogels, completely free of bioactive ligands, significantly rescued human small intestinal organoids, preventing apoptosis and enabling them to self-assemble into healthy vesicle structures. Their growth rate and morphological characteristics were highly consistent with those under Matrigel or culture conditions containing expensive recombinant laminin. Furthermore, transcriptome analysis

confirmed that this antibody fragment treatment not only maintained the stem cell characteristics and differentiation potential of the organoids but also successfully eliminated dependence on animal-derived components. This study successfully constructed a chemically defined and xeno-free organoid culture system through a universal “outside-in” signal activation mechanism, removing a key regulatory hurdle for cell expansion and transplantation in





**Figure 9.** Integrin  $\beta 1$  regulates the polarity maintenance and clonal growth of intestinal organoids. Experiments have shown that blocking  $\beta 1$  with the antibody AIIB2 leads to inward basal-side orientation of organoids and a decrease in Ki67 levels, significantly inhibiting cell proliferation; conversely, activating  $\beta 1$  signaling with the activating single-chain antibody scTS2/16 enhances the colony-forming ability of single cells in Matrigel. Image reprinted with permission from de Lau *et al.*<sup>56</sup> Copyright © 2025, The Author(s).

regenerative medicine.

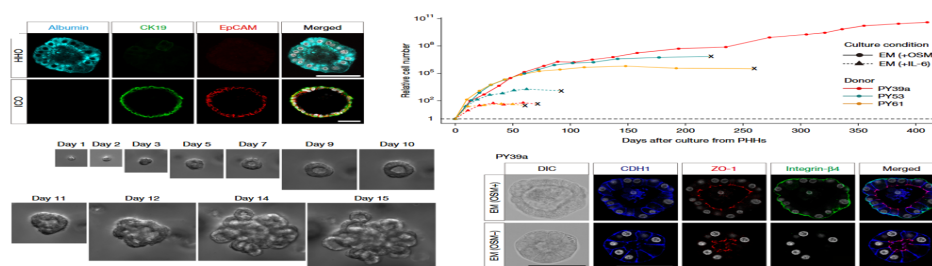
## 2.9. Adult human hepatocyte organoids exhibit metabolic function

The liver is the core organ of human metabolism, responsible for key functions such as gluconeogenesis, the urea cycle, bile acid synthesis, and drug metabolism.<sup>57</sup> Severe metabolic dysfunction can lead to life-threatening liver failure. However, constructing *in vitro* models that faithfully reproduce the complex metabolic functions of primary human hepatocytes has long been a significant challenge.<sup>58</sup> Primary human hepatocytes rapidly lose their proliferative ability after being cultured *in vitro*. Existing expansion strategies (such as using small molecule inhibitors) are often accompanied by serious side effects. During the process of proliferation, hepatocytes undergo ductular metaplasia, lose their original characteristics, and differentiate into the biliary lineage.<sup>59</sup> Although the need to balance proliferation with functional maintenance had long limited hepatocyte applications, Igarashi *et al.*<sup>60</sup> developed a human hepatocyte organoid (HHO) culture system that overcomes this bottleneck (Figure 10).

By analyzing pathways associated with hepatocyte self-

renewal, the researchers found that synergistic activation of the Wnt and STAT3 signaling pathways was critical for hepatocyte self-renewal and organoid expansion. The study showed that oncostatin M, as an activator of STAT3, plays a dual role: significantly promoting hepatocyte proliferation while effectively antagonizing duct metaplasia induced by the YAP and TGF $\beta$  pathways, thereby maintaining hepatocyte identity. This system supports organoid derivation from single adult hepatocytes sorted by flow cytometry and enables long-term culture for more than six months, achieving a million-fold expansion.

In terms of functional maturity, HHOs showed strong metabolic capacity, demonstrating excellent metabolic capacity. Through specific differentiation induction (removal of stem cell factors and addition of hormones), differentiated HHOs (dHHOs) can form hepatic cord structures with bile canaliculi networks and exhibit albumin secretion rates (50–150  $\mu\text{g/day/million cells}$ ) and drug-metabolizing enzyme (CYP450) activities comparable to those of *in vivo* hepatocytes. More importantly, this study successfully reproduced the liver's metabolic zonation *in vitro*, with dHHOs performing gluconeogenesis and urea cycling functions unique to periportal hepatocytes.



**Figure 10.** Establishment and expansion of HHOs. Immunofluorescence showed that HHOs expressed the hepatocyte marker albumin, distinguishing them from bile duct organoids that expressed CK19. OSM supported the long-term exponential expansion of HHOs for more than 300 days. HHOs could be established from a single primary hepatocyte. OSM induced HHOs to maintain epithelial-basal polarity characterized by integrin  $\beta 4$ . Image reprinted with permission from Igarashi *et al.*<sup>60</sup> Copyright © 2025, The Author(s), under exclusive licence to Springer Nature Limited. Abbreviations: EM: Expansion medium; HHOs: Human hepatocyte organoids; IL-6: Interleukin-6; OSM: Oncostatin M; PHH: Primary human hepatocytes.

Furthermore, this system established an efficient gene-editing workflow, successfully constructing disease models of glycogen storage disease (*G6PC* knockout) and urea cycle disorder (*OTC* knockout), demonstrating its significant potential in simulating hereditary metabolic liver diseases.

## 2.10. Artificial intelligence platform enables automated 3D organoid analysis

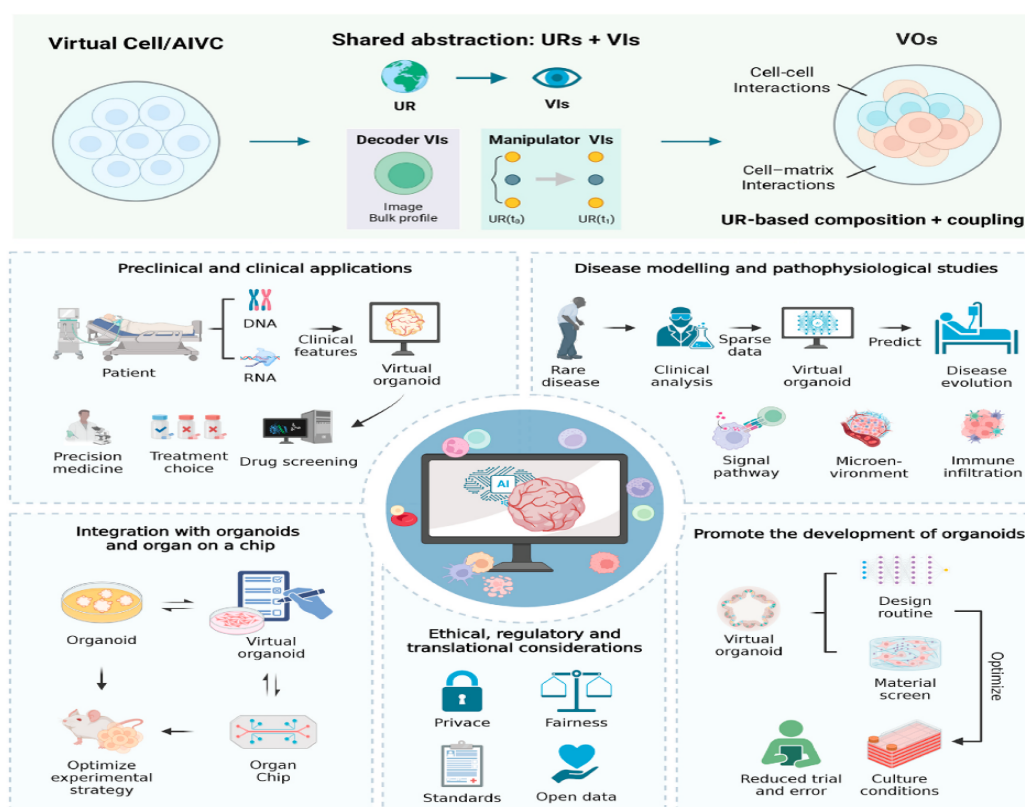
While organoid technology has shown great potential in replicating the anatomical structure and function of human organs, its standardization and clinical translation still face significant challenges.<sup>61,62</sup> The construction of physical organoids has long been limited by batch-to-batch variability caused by animal-derived scaffolds such as Matrigel, and its high dependence on manual operation leads to low experimental reproducibility.<sup>63,64</sup> Existing organoid analysis methods largely rely on destructive endpoint detection, making it difficult to achieve continuous, non-destructive monitoring of organoid states.<sup>65</sup> Traditional AI applications in organoids research have largely been limited to image analysis and data mining and have not generated longitudinal digital models that capture organoid evolution over time.<sup>66</sup>

Bai and Su<sup>67</sup> proposed the concept of AI virtual organoids (AIVOs), also termed silicon-based organoids, shifting the discussion from purely physical organoids toward digital twin frameworks. The study constructed a system framework with a data layer, a model layer, and an interaction layer. The model relies on integrating multimodal longitudinal data of single-cell genomics, spatial transcriptomics, and high-content imaging, using “virtual cells” as the smallest unit. Using the pancreatic cancer model ODFormer as a representative case, the AIVO framework leverages a pretraining dataset comprising 30,000 pan-cancer bulk transcriptomes and one million single-cell profiles. The system was subsequently fine-tuned using 14,000 drug-response assays derived from

183 patient-derived organoids. In predicting standardized drug responses, ODFormer achieved a Pearson correlation coefficient exceeding 0.9, validating the model’s high degree of quantitative precision. Moreover, by combining deep learning with biophysical modules such as finite element analysis and reaction–diffusion equations, the framework reconstructs cell–matrix interactions and tissue microenvironments *in silico*. This approach has transformed AIVOs from static simulations into organoid digital twins that can grow, respond, and evolve. Within this framework, a virtual organoid operates as a persistent digital object characterized by unique identifiers, versioning, and distinct temporal states. By employing a closed-loop workflow of measurement, update, and intervention, the system ensures real-time or near-real-time synchronization with physical entities, including solid organoids and clinical processes. The application value of this platform lies in achieving a “dry-wet two-way closed loop” between *in silico* modeling and wet-lab experimentation. AIVOs use modules such as “virtual stem cells” to first conduct high-throughput virtual experiments on the computer, including drug screening, dosage design, and drug resistance prediction. The prediction results can guide wet experiment design, reduce trial-and-error costs, and rely less on biological samples. For example, in tumor research, this platform can simulate tumor clonal evolution and microenvironmental selection pressure, thereby predicting patient-specific treatment effects. This strategy of integrating multi-scale data fusion and mechanism models provides an automated analysis tool with translatability, repeatability, and causal reasoning ability for drug discovery and precision medicine (Figure 11).

## 3. Conclusion

In 2025, organoid technology achieved significant progress, with the research focus shifting from simple *in vitro* simulations to the construction of systems with



**Figure 11.** Application of virtual organoids. Image reprinted from Bai and Su *et al.*<sup>67</sup> Copyright ©2025 The Authors.

actual physiological functions. Reviewing the top ten breakthrough studies selected this year, the advancements are reflected in three areas: biological construction, engineering material optimization, and AI integration.

In terms of biological construction, researchers successfully achieved internal vascularization in cardiac and hepatic organoids through co-differentiation techniques, significantly improving nutrient supply and tissue survival quality. Meanwhile, the metabolic functions of adult hepatocytes were maintained for longer periods during *in vitro* expansion, and spinal cord organoids were accurately constructed in segments based on anatomical positions. These achievements have brought *in vitro* models closer to actual human tissues in both structural complexity and functional authenticity. In the field of engineering materials, to solve safety and stability challenges in clinical translation, researchers introduced single-chain antibody activation technology, non-swelling matrices, and sustained-release systems. These innovations not only eliminated potential immune risks from animal-derived components but also ensured the physical stability of grafts in complex physiological environments. These material advancements provide a solid technical foundation for organoids to move from laboratory research toward clinical transplantation.

In digital applications, AI platforms have achieved precise prediction of experimental results and effective optimization of experimental conditions by performing analysis of large multimodal datasets combined with digital twin technology. In summary, this interdisciplinary path—fusing biological precision, material safety, and intelligent prediction—is accelerating the practical implementation of organoid technology in personalized precision medicine and human tissue repair.

Although organoids are developing rapidly, several technical bottlenecks remain before they can be fully put into practical use. First, most existing organoids are developmentally close to the fetal stage and lack coordination with the nervous, endocrine, and immune systems. This makes it difficult for them to simulate the long-term progression of chronic diseases in adults. Second, due to the lack of perfusion systems that mimic blood circulation, the interior of large-scale organoids is prone to necrosis caused by hypoxia. Although current synthetic materials are safer than natural matrices, they are often too simple to replicate the complex, dynamically changing signals of a native microenvironment during growth. Additionally, while AI has improved drug screening efficiency, its operational logic is often not transparent enough, and it is prone to errors when high-quality data is lacking.



Looking ahead, the focus of the field will shift toward system integration and standardized production. By connecting multiple organ systems through technologies such as microfluidics and performing real-time monitoring, organoids will transform from isolated tissue blocks into miniature physiological models with systemic feedback capabilities. With the improvement of digital simulation technology and evaluation systems, the research and development model will shift from traditional empirical trial-and-error to logical precision prediction. We believe that by combining clinical-grade materials, real-time feedback systems, and AI-driven design, organoids will truly move beyond the laboratory to become key tools for human injury repair and personalized healthcare.

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## Conflict of interest

Jiacan Su is an Editor-in-Chief of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

## Author contributions

*Conceptualization:* Long Bai, Jiacan Su

*Visualization:* All authors

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*Writing-review & editing:* Yuling Han, Han Lin, Wei Zhang, Junhong Luo, Lin Lin, Yifei Miao, Chenjie Xu

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

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## Availability of data

Not applicable.

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