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Review article

Artificial Intelligence Virtual Organoids (AIVOs)

Long Bai [a,b,d,e], Jiacan Su [a,b,c,d,*]

[a] Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China
[b] MedEng-X Institutes, Shanghai University, Shanghai, 200444, China
[c] Department of Orthopedics, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200092, China
[d] National Center for Translational Medicine (Shanghai) SHU Branch, Shanghai University, Shanghai, 200444, China
[e] Wenzhou Institute of Shanghai University, Wenzhou, Zhejiang, China

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ABSTRACT

Organoid platforms have reshaped in vitro human biology yet remain constrained by batch variability, sparse longitudinal readouts and barriers to scale. This review introduces Artificial Intelligence Virtual Organoids (AIVOs), also termed silicon organoids: organoid-scale digital twins instantiated in the computational space, with virtual cells-and, where appropriate, virtual organoids-serving as the minimal executable units. AIVOs fuse multimodal and longitudinal measurements into universal state representations and use virtual instruments constrained by biophysical priors to emulate assays and perturbations, while hybrid mechanistic modules (agent-based, continuum, finite-element) capture cell-cell, cell-matrix and transport dynamics. The article defines conceptual boundaries, formalizes a data-model-interaction architecture and construction strategies, and synthesizes evaluation and standardization practices. Applications span drug screening and dosing design, disease subtyping and resistance mapping, integration with organoid-on-chip systems and clinical decision support. Principal challenges include the acquisition and harmonization of high-quality longitudinal data, scalable computation and model reduction, interpretability and causal reasoning, and governance addressing privacy, safety and fairness. Virtual organoids ultimately provide a silicon-grounded, transparent and reproducible bridge between physical organoids and clinical practice, enabling high-throughput in silico experiments and active experiment design without added experimental burden and accelerating precise therapy, mechanism discovery and regulatory translation.

1. Introduction

Organoids are three-dimensional miniaturized tissue structures that arise from stem cells or progenitor cells under appropriate culture conditions [1]. They can reproduce key anatomical and functional features of native organs in vitro and are now widely used in developmental biology, disease modelling and drug discovery [2]. Compared with conventional two-dimensional cell lines and animal models, organoids better capture the cellular diversity, spatial organization and microenvironment of human tissues [3–6]. This makes them especially valuable for dissecting organ-specific disease mechanisms and for personalized drug-sensitivity testing based on patient-derived material [7].

At the same time, current organoid technologies face several fundamental limitations. Most organoids rely on animal-derived extracellular matrices such as Matrigel, which contain more than a thousand proteins with poorly defined composition and substantial batch-to-batch variability [8]. These factors lead to marked differences in organoid morphology, growth and function between experiments, reducing reproducibility and comparability across laboratories. In addition, organoid generation and maintenance depend heavily on manual operations. Small differences in cell source, culture medium composition and handling protocols can produce large technical variation, yet there is no widely accepted guideline for distinguishing technical from biological replicates or for standardizing quantitative phenotyping. Mainstream readouts, including microscopy and bulk or single-cell transcriptomics, are typically end-point measurements and do not provide continuous, non-invasive monitoring of organoid state. Emerging microsensors and multimodal monitoring platforms are still in early development and cannot yet deliver long-term, high-content measurements at scale. As organoids become more complex and more similar to

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* Corresponding author. Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China.
E-mail address: drsujiacan@163.com (J. Su).

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human organs, ethical review, production cost and scalability also become non-trivial constraints, limiting the use of organoid platforms in large-scale drug screening and routine clinical workflows [9].

The rapid development of digital-twin concepts and artificial intelligence (AI) [9] in the life sciences offers new ways to address these limitations. Early "virtual cell" research primarily employed mechanistic formalization methods such as ordinary differential equation/-partial differential equation systems and stochastic simulations to model specific cellular behaviors or functions within a simulation environment supporting model construction and computational experiments. With the rise of high-throughput multi-omics, high-content imaging and spatial profiling, a new class of AI-based models—artificial intelligence virtual cells (AIVCs)-has been proposed [10]. In this framework, deep learning is used to fuse heterogeneous knowledge, static structure and dynamic state into unified numerical embeddings, often referred to as universal representations (URs) [11]. Virtual instruments (VIs), implemented as neural networks, then decode these URs into human-interpretable outputs or manipulate them to approximate the effects of perturbations. In this way, AIVCs act as cell-level digital twins that can accept biological inputs and generate actionable predictions, enabling high-throughput in silico experiments on cell behavior.

Extending this idea from single cells to tissues, virtual organoids (VOs) have been introduced as organoid-scale digital models that use virtual cells as basic building blocks and combine machine-learning components with mechanistic descriptions [12]. In a VO, many virtual cells are connected through modeled cell–cell and cell–matrix interactions and are embedded in a digitally represented microenvironment. This allows tissue-level dynamics-such as growth, differentiation, patterning and response to drugs-to be simulated without relying exclusively on physical organoids. Importantly, VOs are designed to be coupled to experimental data streams: they can be updated and calibrated with omics, imaging and functional measurements from real organoids and patients, and in turn generate predictions that guide which experiments and interventions should be performed next. In this sense, virtual organoids are not just static simulations, but a class of digital twins that mediate a loop between computation and wet-lab or clinical practice. Several recent articles have discussed AI-enabled organoids and organoid-related digital twins from specific angles, for example focusing on imaging analysis, drug-response prediction or organoid-on-chip systems. However, the concept of "virtual organoids" as a coherent class of models, and their systematic construction from AI virtual cells, has not yet been comprehensively reviewed. Building on existing work on AIVCs and organ-level digital twins, this review proposes an integrated framework for VOs.

In recent years, the research on AI-enabled organs, digital medicine and single-cell modeling has developed rapidly, but the existing work mostly regards organ-like as data sources or in vitro verification platforms, rather than independent modeling objects with their own structure and dynamics. In response to this gap, we propose a conceptual framework of virtual organs with data layer-model layer-interaction layer as the main line, which is used for system organization of multimodal data, computational models and closed-loop coupling with physical organs and clinical decision-making. Under this framework, we identify virtual stem cells, virtual functional cells and virtual tumor cells as reusable virtual cell base elements, which are used to connect single-cell representation with organ-like scales and even the simulation of patient scales. It should be emphasized that the virtual organs (VO) discussed in this paper have relatively clear boundaries in the digital twin system in a broader sense. First of all, VO takes the specific biological system of organ-like-patient as the object, relying on virtual cell units such as AIVC and multimodal data to achieve sustainable and updated organ-level digital twins; while digital twins based on organ chips are usually centered on the platform behavior of microphysiological chips, focusing on drug replacement and toxicology. Function simulation, the two can be coupled, but they are not conceptually equivalent. Secondly, the traditional pure mechanism multi-scale model is usually composed of explicit differential equations and rule systems. Most of the parameters come from literature or limited experiments, and do not require long-term synchronization with specific types of organs or individuals; VO takes data constraints as the core, emphasizing the calibration and iterative update at the specific type of organ cohort or patient level. Thirdly, a large number of AI-enabled prediction models only output one-time prediction results at a given input, and do not constitute a digital twin in a sustainable maintenance state that supports closed-loop experimental design; in contrast, VO is clearly designed as a persistent virtual object with identifiers, version and timing states, which can the cycle of measurement-update-intervention is kept corresponding to solid organs and clinical processes. Based on these boundary definitions, this paper regards VO as the middle view level between virtual cells, physical organs and medical digital twins, and on this basis, the subsequent data layer, model layer and interaction layer framework are developed.

In this review, we first define the concept and scope of virtual organoids and clarify how they relate to physical organoids, traditional virtual cell models and broader digital-twin initiatives. We then summarize key data requirements and modelling strategies for constructing VOs, including multimodal data integration, the design of URs and virtual instruments VIs at the cell level, and the coupling of data-driven and mechanistic components such as biomechanical or diffusion models. We go on to review current analysis methods and application scenarios for VOs in drug screening, disease modelling and clinical decision support, and discuss major challenges linked to data quality, computational cost, interpretability, privacy, fairness and standardization. Finally, we outline future directions for research and translation. By providing a structured view of virtual organoids and their technological building blocks, we aim to offer a conceptual and methodological basis for further work at the interface of computational science, organoid biology and precision medicine.

2. Definition and evolution of virtual organoids

2.1. From AI-enabled organoids to virtual organoids

Organoids are three-dimensional in vitro structures that reproduce key architectural and functional features of native organs [13]. They are generated from stem cells or other precursor cell populations and are widely used to study organ development and behavior [14], as well as for drug screening and disease modeling. Because they can recapitulate essential aspects of tissue-specific microenvironments and organ-level functions, organoids have become powerful systems for dissecting disease mechanisms and for evaluating candidate therapies. However, organoid research is still at an early stage. Strategies for constructing, assessing and applying organoids are under active exploration, and current systems still show important limitations, including suboptimal bioactivity of scaffold materials, marked differences in cell composition and cell-type ratios compared with native tissues, and incomplete spatial organization [15].

These scientific and technical gaps have stimulated efforts to better control organoid self-organization, to standardize culture conditions and readouts, and to integrate organoids with advanced analytical tools. In this context, the concept of AI-enabled organoids has emerged [16]. Here, organoids remain physical three-dimensional cultures, but AI is systematically embedded along the experimental pipeline. Machine learning models are used to accelerate the screening of construction strategies [17], for example by predicting suitable hydrogel compositions and culture parameters from existing data. Deep learning methods extract multiscale features from bright-field and fluorescence images to quantify morphology, cell states and differentiation outcomes in a cost-effective and non-destructive manner. In addition, AI is applied to large-scale bulk, single-cell and spatial omics datasets generated from organoids to identify cell populations [18], reconstruct developmental trajectories and link molecular states to functional phenotypes. These

applications improve the efficiency and reproducibility of organoid experiments and extend their use in preclinical evaluation and personalized medicine.

However, in most AI-enabled organoid studies, AI algorithms still treat organoids primarily as data sources: models are trained on measurements derived from organoids and then used to make predictions, but they are not designed as persistent virtual counterparts that mirror organoid dynamics over time. In parallel, work on virtual cell models and AIVCs has shown that it is possible to build computational systems that simulate the behavior of individual cells under diverse perturbations [11]. Together, these trends point towards a next step: extending the digital representation from single cells to whole organoids, and coupling physical organoids with their computational counterparts in a digital-twin framework. On this basis, we introduce the concept of VOs, which generalize AI-enabled organoids into explicit, organoid-scale digital twins [19] built from virtual cells and linked to experimental systems (Fig. 1).

2.1.1. Virtual cells and AIVCs

Virtual cells are broadly defined as computational models that simulate the biological functions and interactions of a cell [20]. Existing virtual cell models often combine assumptions about underlying mechanisms with parameters fitted from observational data, and are implemented using explicit mathematical or computational formalisms such as differential equations, stochastic simulations and agent-based models. They have been used to study processes including gene regulation, signaling, metabolism and cytoskeleton-driven morphology, and demonstrate that cell behavior can be captured in silico with sufficient mechanistic detail and calibration.

Building on this foundation, the concept of an AIVC has been proposed as a next-generation, AI-driven virtual cell. An AIVC is envisioned as a multi-scale, multi-modal [21], large-neural-network–based model that can represent and simulate the behavior of molecules, cells and tissues across diverse states. It is structured around two core components: a universal multi-modal, multi-scale biological state representation, which transforms high-dimensional biological data into numerical embeddings-URs-that retain meaningful relationships and patterns [11]; and a set of VIs, implemented as neural networks that manipulate or decode these representations. Decoder VIs map URs to human-interpretable outputs, such as cell-type labels or synthetic images, whereas manipulator VIs transform one UR into another, for example by approximating the effect of a perturbation on cell state.

Through this architecture, an AIVC can capture biology at three physical scales: molecules and their structures, individual cells as spatial collections of interacting molecules, and multicellular arrangements that describe interactions between cells and their microenvironment. Each scale is represented by a distinct UR, and higher-level representations are built by aggregating and transforming lower-level ones [21], thereby linking molecular, cellular and tissue-level phenomena in a consistent way. In addition, VIs support virtual experimentation, such as simulating difficult or expensive assays, exploring combinations of perturbations that are impractical in the laboratory, and guiding data generation through active, lab-in-the-loop workflows.

Within the framework of virtual organoids, AIVCs serve as cell-level digital twins that can be instantiated for different cell types, states and patient contexts. Virtual organoids use these AIVC-based virtual cells as basic building blocks. By connecting many virtual cells through modeled cell–cell and cell–matrix interactions, and by embedding them in a digitally represented microenvironment, VOs form tissue- or organoid-scale virtual systems whose dynamics remain grounded in single-cell representations and responses.

2.1.2. Relationship between physical organoids and virtual organoids

Physical organoids are three-dimensional cell aggregates derived from stem cells or progenitor cells and grown in vitro [13]. They recapitulate key aspects of organ architecture, cell-type diversity and function, and have become important platforms for basic research, disease modeling and drug screening [7]. In contrast, VOs are data-driven and mechanism-informed digital models that represent organoid systems in silico. They emphasize cross-scale integration of multi-omics, imaging and clinical data, together with machine learning, generative AI and mechanistic simulations, to reconstruct organoid composition, spatial organization and functional responses in a computational form.

A defining feature of VOs is their role as organoid-scale digital twins. In a digital-twin setting, measurements from physical organoids or patients—such as single-cell and spatial profiles [22], high-content imaging or clinical markers—are continuously or iteratively integrated into the VO, updating its parameters and state. In the other direction, VO predictions are used to guide experimental design, including the selection of cell lines, culture conditions, perturbation schemes and readouts, and to prioritize drug combinations or genetic interventions for further testing. This bidirectional feedback establishes a dynamic link between the virtual model and the physical system, enabling near–real-time synchronization and iterative refinement. Supporting this synchronization requires robust VO-level assimilation, which necessitates explicit cross-scale coupling between cell-state URs and organoid phenotypes. Since organoid culture can partially erase native positional cues, emergent behaviors often become driven by cell–cell and cell–matrix interactions. Therefore, interaction modeling becomes a central determinant of VO validity rather than a secondary detail.

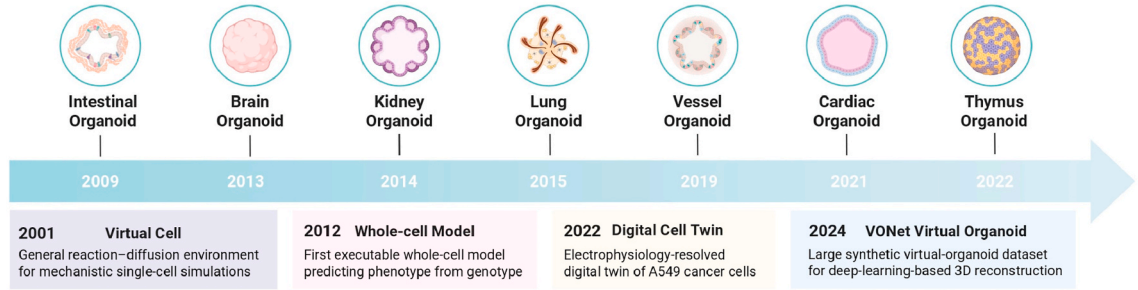
Crucially, a VO operates as an active system rather than a passive data repository. Central to this role are AI components that learn to encode organoid states from multimodal observations while performing state and parameter inference with quantified uncertainty. These systems further enable counterfactual perturbations through virtual interventions or surrogate models and simultaneously detect drift or out-of-distribution conditions during iterative updates. Without these core inference and prediction capabilities, the system effectively functions as a static database rather than a dynamic, AI-enabled digital twin.

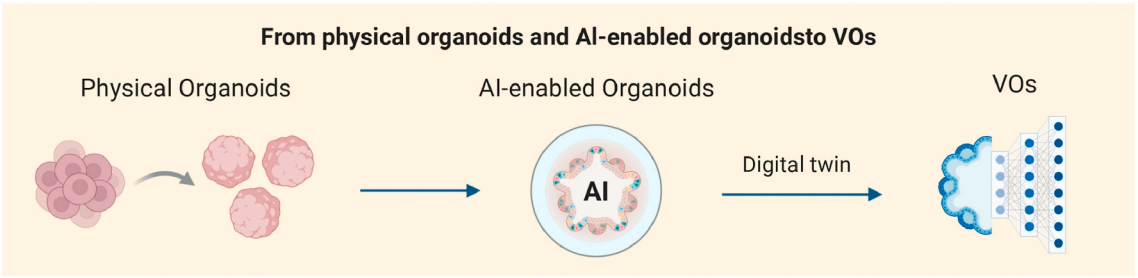
Thus, physical organoids and VOs are complementary rather than competing. Physical organoids provide the biological material and empirical data needed to train, calibrate and validate virtual models, while VOs extend the reach of organoid experiments by enabling high-throughput virtual screening, exploration of extreme or combinatorial perturbations, and systematic hypothesis generation. In particular, VOs can accelerate the identification of promising drug regimens, new disease subtypes and resistance biomarkers, and can help reduce reliance on large numbers of physical organoids or animal models for certain classes of questions. In summary, physical organoids act as the experimental substrate, whereas virtual organoids provide a computational counterpart that amplifies the power of organoid-based research and connects it to the broader digital-twin paradigm in biomedicine.

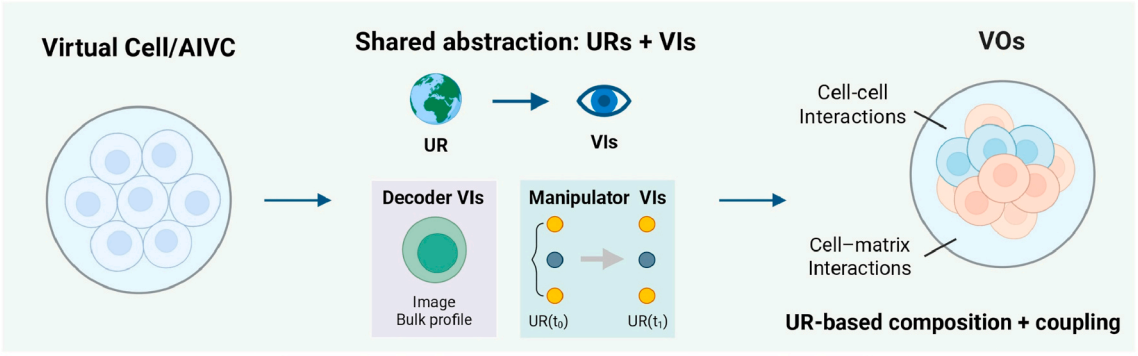
2.2. Components and layers in a virtual organoid

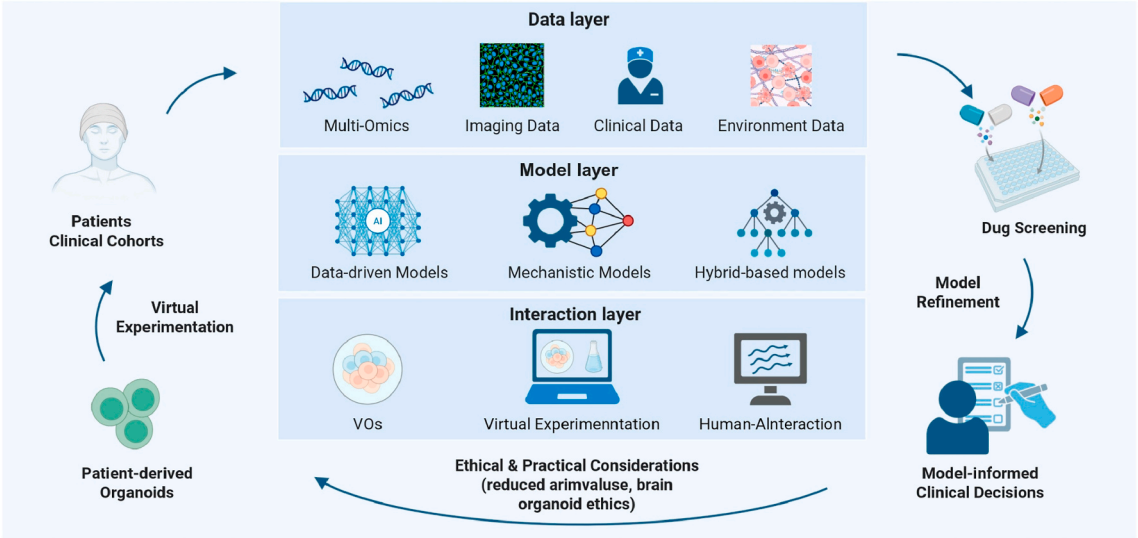
The construction of a virtual organoid can be organized into three interacting layers: a data layer, a model layer and an interaction layer. This layered view provides a practical blueprint for connecting virtual cells with organoid-scale phenotypes.

In VO, the most fundamental computational unit is a state-encoded virtual cell instance (or cell type-specific state distribution) coupled with shared microenvironment fields such as morphogen concentration, nutrient/oxygen diffusion, and mechanical stress/strain. This hybrid unit enables cross-scale integration: virtual cells/AIVCs provide cellular state UR and disturbance interfaces (VIs), while tissue-scale modules (e.g., reaction-diffusion PDEs or finite element/continuum mechanics) supply constraints and shared fields. To achieve interface docking for heterogeneous virtual cell models, VO coupling must rely on a set of shared anchoring observations (gene/protein/imaging features), ontologically typed state variables, and uncertainty perception adapters capable of mapping non-comparable latent spaces rather than assuming UR equivalence.









Benefits	Limitations
• High-throughput in silico screening and response prediction	• Insufficient, biased and noisy multi-modal data
• Multi-scale data integration and novel subtype discovery	• High computational cost and limited interpretability
• Reduced reliance on animal and sensitive organoid experiments	• Ethical, legal and privacy risks in patient-linked VOs

Fig. 1. Virtual organoids: evolution, construction and biomedical applications.

2.2.1. Data layer

The data layer collects and organizes the information needed to build a digital twin of the organoid system. In a typical setting, this layer integrates multi-omics profiles [23], including genomic variants, bulk and single-cell transcriptomes [24], epigenomic and chromatin accessibility maps, as well as proteomics [25] and metabolomics [26], with high-dimensional imaging data [27]derived from fluorescence and label-free microscopy, live-cell imaging and, when available, high-resolution or electron microscopy that captures organoid architecture. For patient-derived organoids that are linked to clinical cohorts, the data layer also encompasses clinical and phenotypic information such as patient demographics, treatment histories, response measurements and survival outcomes. In addition, it incorporates environmental and microenvironmental datas [28], including culture conditions, extracellular matrix composition, mechanical cues [29]and drug exposure schemes, which together define the external context in which organoids develop and respond. In line with the digital-twin literature, these heterogeneous datasets are not used in isolation; instead, they are integrated into multi-scale interaction maps, where methods from network science and complex systems are applied to connect genes, proteins, cells and microenvironmental factors into graphs or networks that summarize their relationships. Within the AIVC framework, such interactions are further encoded into URs that provide compact numerical descriptions of the multi-scale state of the system and serve as key inputs for virtual organoid models.

However, clinical and experimental alignment is a key bottleneck. Even if clinical records are available, mapping them to organoid measurements requires explicit metadata. This includes the source and sampling time of patients and organoids. The end points must be consistent. For example, clinical reactions need to correspond to molecular or functional surrogate indicators. We also need to correct for confounding factors, missing data, and batch effects. Without accurate alignment, clinical variables may become weakly supervised labels. This will exaggerate the apparent performance, but the model will not be generalized.

2.2.2. Model layer

The model layer contains the computational engines that translate raw or preprocessed data into predictions about organoid behavior, and is inherently heterogeneous in terms of modeling strategies. Classical machine learning models, including supervised, unsupervised and representation learning methods [30], are used to classify organoid samples, cluster cell states, infer latent factors and link molecular profiles to phenotypic outputs. Deep learning models, such as convolutional neural networks and Transformer architectures [31], are employed to handle complex data modalities like imaging and high-dimensional gene expression profiles, and to learn non-linear relationships both within and across modalities. Complementing these data-driven approaches, mechanistic numerical models—such as agent-based and multi-agent systems exemplified by frameworks like PhysiCell [32], together with continuum or finite-element models—are used to simulate cell–cell interactions, tissue growth, mechanical stress, diffusion of nutrients and drugs, and other physical processes at the tissue scale. Hybrid models sit at the interface of these categories by coupling data-driven and mechanistic components, for example by constraining neural networks with biophysical priors or by using mechanistic simulations to generate synthetic training data that regularize learning. Within this layered architecture, the AIVC framework plays a central role: by mapping multi-modal data into URs at molecular, cellular and multicellular scales, and by exposing VIs that act as virtual experiments, AIVCs provide a flexible interface between cell-level digital twins and organoid-level tasks. Generative AI tools can further augment sparsely sampled regions of the state space, for instance by synthesizing plausible gene expression patterns under untested perturbations or by predicting single-cell profiles from bulk or imaging data. In the context of virtual organoids, these capabilities enable in silico screening of perturbations and systematic exploration of developmental or disease trajectories under different assumptions.

However, it is very difficult to build a mixed VO model. Multimodal observations are rarely aligned in time and space. This makes parameter identification and supervised learning an ill posed problem. In addition, different AIVC frameworks use inconsistent URs to encode cell states. Therefore, coupling them to the VO model requires a clear interface design. This involves pattern switching and uncertainty propagation. Finally, adding the mechanical module will introduce simplified assumptions and boundary conditions. Unless constrained by validation data and sensitivity analysis, these assumptions may dominate the final prediction results.

2.2.3. Interaction layer

The interaction layer links the virtual organoid with its physical counterpart and, when relevant, with the patient, and is responsible for continuous data exchange, model updating and decision support in a digital-twin architecture. New measurements from physical organoids or from patients-such as updated imaging, genomic or transcriptomic profiling, and clinical response data-are incorporated into the virtual organoid to correct model drift, refine parameter estimates and adjust the internal state of the digital twin. In the opposite direction, predictions generated by the VO are used to guide experimental and clinical actions, for example by suggesting which perturbations or drug combinations are likely to be most informative, which organoid lines have a higher probability of responding to a given treatment, or which molecular readouts would be most valuable to collect in the next experimental round. Within the AIVC framework, this bidirectional interaction is mediated by VIs that act as computational counterparts of laboratory protocols. Applied to organoids, such VIs can simulate changes in drug dosing schedules, alterations in matrix stiffness or modifications in the composition of co-cultured immune cells, and the outcomes of these virtual interventions can then be compared with real measurements to iteratively improve the model. Through repeated cycles of prediction, measurement and update, the interaction layer drives the system towards real-time or near–real-time synchronization between the virtual organoid and the evolving biological system.

2.3. Benefits of virtual organoids

The current research on VO points out three main advantages. First, after calibration, they can achieve scalable prediction and counterfactual disturbance analysis. Secondly, VO can complete the cross-scale integration from molecular state to organoid phenotype. Finally, they can prioritize the experiments of uncertainty perception. This helps to reduce the workload of the wet laboratory. But these benefits are conditional. They rely on the adequacy of the data, a validated URs, and robust uncertainty estimates.

2.3.1. Efficient prediction and virtual experimentation

Virtual organoids allow high-throughput simulation of drug responses and developmental trajectories in silico, which can substantially reduce the cost and time of experimental screening. The ODFormer framework is a concrete example of this idea [33]. It is a virtual organoid for pancreatic cancer, designed to predict patient-specific therapeutic responses by simulating patient-derived organoids (PDOs) computationally. ODFormer uses two encoders pretrained on 30,000 pan-cancer bulk transcriptomes and one million pancreatic cancer single-cell profiles to learn tissue- and organoid-specific representations. It is then trained on a curated dataset of about 14,000 drug-response assays across 183 PDOs and 98 drugs, using a Transformer-augmented hybrid contrastive network. In benchmarking, ODFormer significantly outperforms existing methods and achieves a Pearson correlation coefficient greater than 0.9 in predicting standardized drug responses. Multi-cohort retrospective analyses show that ODFormer-guided treatment choices can improve clinical outcomes without requiring physical

PDO assays, demonstrating how a VO can serve as a practical surrogate for labor-intensive organoid experiments.

2.3.2. Multi-scale integration and discovery of new biology

By design, VOs integrate information across molecular, cellular and tissue scales. In the AIVC framework, URs aggregate diverse data types into a common space, while VIs enable controlled perturbations in that space. ODFormer illustrates how such models can reveal new biology: by analyzing predicted responders and non-responders, the study identifies novel pancreatic cancer subtypes with distinct therapeutic vulnerabilities and resistance biomarkers, which are further supported by independent datasets including TCGA-PDAC. This suggests that VOs can help uncover disease subtypes and markers that might be difficult to detect through conventional analysis of organoid or patient cohorts.

2.3.3. Ethical and practical advantages

Virtual organoids can, in principle, reduce reliance on animal models [34] and complex organoid experiments for certain tasks, especially for large-scale screening and exploration of extreme or combinatorial perturbations. In sensitive areas such as brain organoids, where concerns about emerging neural activity and consciousness have been raised, in silico experiments may provide an additional layer of ethical protection by allowing preliminary studies to be run in a virtual setting before moving to physical models. More generally, VOs offer a way to reuse existing data more fully and to prioritize the most informative experiments, thereby making better use of limited biological material.

2.4. Limitations and challenges

VOs hold immense potential, but we cannot automatically trust their reliability. To possess scientific value, these models require observability, interpretability, and calibrability, while also being validated under conditions of distributional bias. Current virtual organoid construction workflows are constrained by sparse measurement data and dataset biases, and model specification errors also pose a challenge. These issues often stem from the use of deep generative components or simplified physical assumptions.

2.4.1. Data sufficiency and quality

To build a high-fidelity digital twin of an organoid system, large volumes of high-quality, multi-modal, longitudinal data are needed. Current experimental technologies rarely capture all relevant molecular events simultaneously, and data from different modalities or time points may be missing or noisy. The AIVC framework explicitly notes that even when large datasets are available, they may be biased towards certain cell types, tissues or experimental conditions [11]. These constraints can limit the fidelity and generalizability of VOs, and highlight the need for careful experimental design and for models that can quantify and propagate.

2.4.2. Computational complexity and interpretability

AIVCs and VO models often rely on large, multi-modal neural networks [35] and on simulations of many interacting virtual cells. Training and running such models can be computationally demanding, especially when integrating high-resolution single-cell data, spatial information and realistic physical constraints. Moreover, although deep learning models can achieve high predictive accuracy, they may behave as black boxes, making it difficult to extract mechanistic insight. Developing approaches that balance complexity, efficiency and interpretability-such as hybrid models that embed mechanistic structure into neural networks-remains an active area of research.

2.4.3. Ethical, legal and privacy considerations

Virtual organoids that are linked to patient data, such as ODFormer, raise questions about data privacy, consent and model governance. As VOs become more tightly integrated into clinical workflows, it will be important to define how predictions are communicated to clinicians and patients, how responsibility is shared when decisions are informed by model outputs, and how to guard against misuse or unintended biases. These issues connect the technical development of VOs with broader discussions on AI ethics and regulation in medicine.

Taken together, these benefits and limitations indicate that virtual organoids are a promising yet still emerging concept. They extend the idea of virtual cells and AIVCs from the single-cell level to organoid-scale systems, and early work such as ODFormer shows that they can already provide actionable predictions in clinically relevant settings. At the same time, their success will depend on continued progress in data generation, modeling methodology, computational infrastructure and ethical frameworks.

2.4.4. Limits of mechanistic assumptions and simplified physics

Mechanistic modules provide interpretability and inductive bias, but their validity is bounded by assumptions such as simplified mechanics, boundary conditions, coarse-graining of ECM/cell behaviors, and parameter choices that are difficult to identify from sparse observables. A VO can therefore become “stable but wrong” if simplified mechanics or interaction rules are not sufficiently constrained by measurements. Hybrid VOs should explicitly state physical assumptions, perform sensitivity analyses, and validate predictions on withheld perturbation experiments rather than relying solely on retrospective fit.

3. Construction and modelling strategies

We next summarize the construction of this VO in four parts (Fig. 2). Data assembly integrates multimodal and longitudinal measurements-single-cell and spatial transcriptomics, proteomics, metabolomics, mass-spectrometry imaging, light and electron microscopy-together with clinical records and continuous organoid monitoring. Robust preprocessing addresses batch effects, noise, platform inconsistency and spatial misregistration, followed by standardized scaling to ensure cross-study comparability. The modelling layer couples self-supervised and contrastive representation learning with large-scale pretraining to derive transferable encodings; generative models (VAE, GAN) augment scarce cohorts and simulate untested perturbations; multi-model fusion combines graph neural networks and Transformers with biophysical components to capture signaling, cell-cell and cell-matrix interactions. Interpretability is strengthened through post-hoc attribution and physics-informed constraints that preserve mechanistic plausibility. Multi-scale simulation employs agent-based schemes, continuum formulations and finite-element analysis, which are unified in virtual experiment platforms for in silico intervention, design optimization and prioritization of wet-lab tests. Evaluation spans regression and classification metrics with explicit uncertainty and confidence scoring. FAIR-aligned data/model sharing, curated repositories and reusable model bases support reproducibility and translation. Governance emphasizes privacy, safety and fairness, aligning documentation and traceability with emerging regulatory expectations. Together these elements define a scalable, transparent and reproducible route to virtual organoids capable of bridging high-content experimentation with decision support in drug discovery and precision medicine.

3.1. Data acquisition and preprocessing

3.1.1. Multiomics and image data collection

The first step in constructing virtual organoids is to collect real data, especially multi omics data and imaging data [11]. These data provide a solid biological foundation for the construction of virtual organoids, and lay a foundation for the accuracy and authenticity of the model. The simulation of virtual organoids not only depends on genomics and transcriptomics data, but also needs to integrate multimodal information such as mass spectrometry, single cell sequencing, spatial transcriptomics and imaging data, so as to comprehensively reflect the

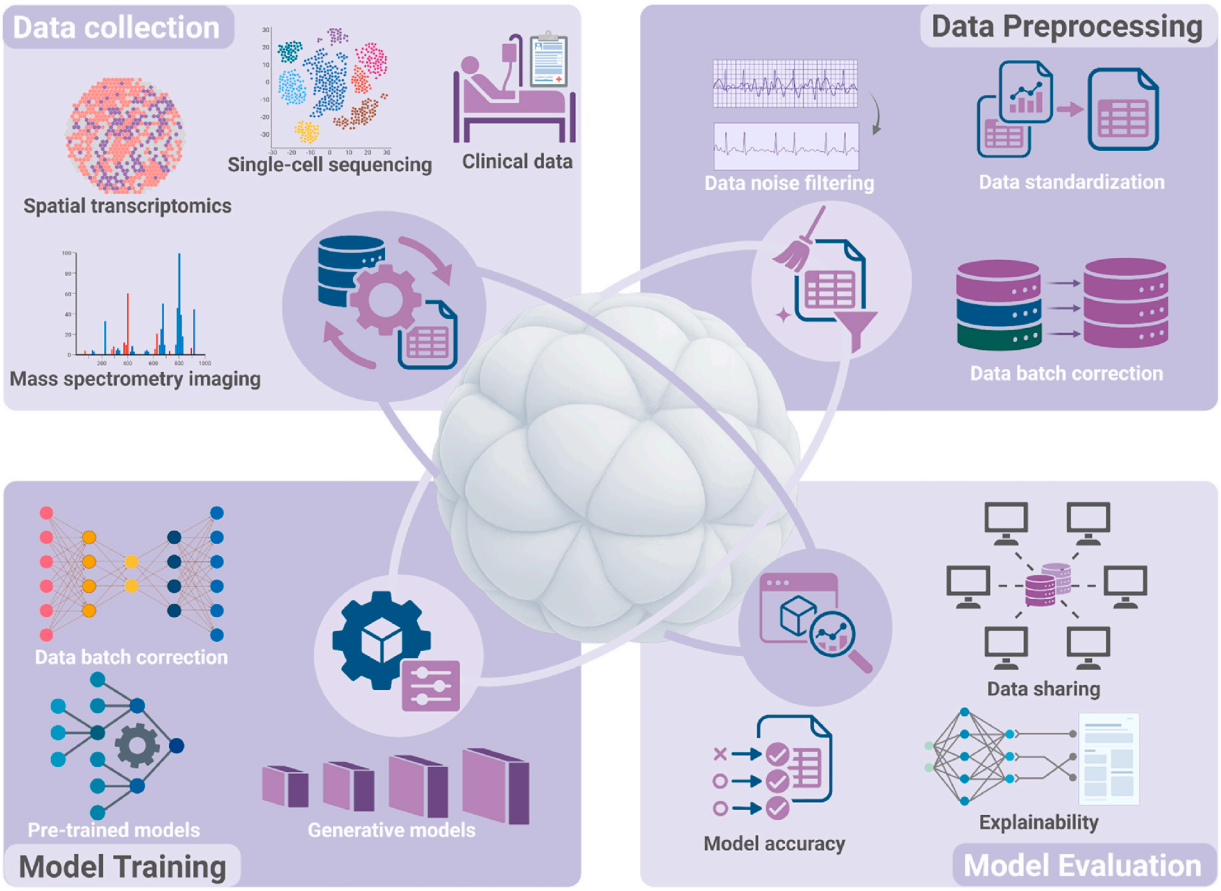


Fig. 2. The construction of virtual organoids.

complex biological processes of cells and tissues [36].

Single cell RNA sequencing (scRNA-seq) technology is an important tool for understanding cell heterogeneity, cell-cell interactions and cell distribution in tissues [37]. In the construction of virtual organs, scRNA-seq provides detailed data for each cell's functional state, transcriptional activity and its role in the organization. Through this technology, transcriptome data of different cell types in cell populations can be obtained, and then key gene expression patterns can be identified, revealing how cell communities in tissues regulate their growth, differentiation and function through gene expression [38].

Unlike AIVCs that prioritize single-cell states, the construction of VOs specifically requires spatial transcriptomics and mass spectrometry imaging. Since VOs are 3D multicellular entities, their functions emerge from complex spatial architecture and cell–matrix interactions. Integrating such spatially resolved data is essential to accurately reconstruct microenvironmental gradients for authentic tissue-level simulation.

By combining genomic analysis with the spatial structure of tissues, spatial transcriptomics enables us to obtain the transcriptional information of each cell in the space of tissue slices [39,40]. In the construction of virtual organoids, spatial transcriptomics can provide key spatial information for the model, and help virtual organoids better simulate the distribution and arrangement of cells in three-dimensional structure [41].

Mass spectrometry imaging (MSI) technology can accurately analyze and map the chemical composition and spatial distribution of extracellular matrix by scanning tissue sections [42]. Mass spectrometry imaging provides chemical information of cell microenvironment for the construction of virtual organs, such as the changes of extracellular matrix (ECM) components and the distribution of metabolites [43]. This information is essential for simulating the interaction between cells and matrix, the diffusion and metabolism of nutrients in organoids.

Light microscope and electron microscope (EM) are key tools for observing the ultrastructure of cells and tissues [44–46]. In the construction of virtual organoids, the integration of optical and electron microscopic imaging data plays an important role in the three-dimensional structure reconstruction and cell behavior simulation of organoids [47]. Furthermore, beyond hardware, advanced imaging technologies such as high-content live-cell imaging and fluorescence microscopy are essential. These technologies provide continuous, high-resolution data on cell migration, division, and subcellular ultrastructure, which are critical for reconstructing the realistic 3D geometry and morphological evolution of Virtual Organoids. Through these imaging techniques, researchers can obtain three-dimensional morphological data of organoids, and build a more realistic virtual organoid model based on these data.

3.1.2. Integration of clinical data and organoid monitoring data

Clinical data provide personalized biological background for virtual organoids, especially in the simulation of patient disease progression and drug response [41]. The key clinical data include genomic data, patients' phenotypic information and treatment history. Genome data, such as mutation information and transcriptome data, can reveal the genetic background of cells and help virtual organs simulate the genetic driving factors of diseases [48]. The clinical phenotype and treatment data provide information such as disease progression and treatment effect for the model, and can guide drug screening and personalized treatment optimization. The integration of clinical data can provide more realistic physiological and pathological states for virtual organoids, so as to enhance its application potential in personalized medicine.

The real-time monitoring technology of in vitro organoids provides continuous and dynamic high-dimensional data support for virtual organoids [49]. By means of on-line metabolic detection and unmarked

electrophysiological monitoring, the morphological remodeling, proliferation and differentiation, metabolic status and immediate response to drugs or stimuli of organoids can be continuously captured during the culture process, so as to realize the whole process tracking from structure to function [50,51]. These time series data can be fed back to the virtual organoid model in real time to correct parameters, update the state equation and optimize the prediction performance, so that the digital model is closer to the real evolution process of the real organoid [52]. On this basis, the monitoring results of organoids from different patients can also be linked with the clinical data to evaluate the sensitivity and tolerance of individuals to the treatment scheme, so as to provide more accurate and timely decision-making basis for individualized medication and course adjustment.

3.1.3. Data preprocessing and standardization

In the construction of virtual organoids, data collection and integration is the basis of building high-precision models. However, the collected multimodal data often have various problems, which will directly affect the accuracy and stability of the virtual organoid model if not handled. Common problems include batch effect of data, noise interference, data inconsistency between different technology platforms, and spatial coordinates mismatch [53,54]. These problems make multi omics data from different experimental platforms and technologies unable to be directly input into the model for training. Therefore, data standardization and preprocessing are the key steps to build a virtual organoid model.

Batch effect refers to the systematic deviation caused by experimental conditions or equipment differences, which is usually corrected by statistical methods such as combat and SVA to ensure the consistency of data from different batches. However, biological data is inherently high-dimensional, and simple statistical corrections are often insufficient to handle complex non-linear distortions. The core challenge in preprocessing is therefore to map these heterogeneous data onto a unified biological coordinate system. In this regard, artificial intelligence—particularly deep generative models and domain adaptation techniques—is playing an increasingly critical role. By learning disentangled representations, these AI-driven approaches can effectively separate biological signals from technical noise, promoting precise alignment across different platforms and ensuring that Virtual Organoids are built upon a robust, consistent ground truth.

Noise comes from sample pollution or instrument error [55]. Noise filtering methods include removing low expression genes and background noise correction to help improve the signal-to-noise ratio of data. Spatial coordinate calibration solves the problem of coordinate system inconsistency between different technology platforms. Common technologies include image registration algorithm and spatial transformation model to ensure the accuracy and consistency of spatial data. In addition, data standardization eliminates the scale differences between data from different platforms through Z-score or min max normalization methods [56], so that the data can be trained under a unified standard, and further improves the accuracy and stability of the virtual organoid model.

Through the pretreatment and standardization of the above series of data, the accuracy and stability of the virtual organ like model have been significantly enhanced. These preprocessed and standardized data can not only provide a more reliable biological basis for the construction of virtual organs, but also provide a more accurate prediction model for clinical treatment, drug screening and personalized medicine. With the continuous progress of data acquisition technology and the continuous improvement of preprocessing methods, the application of virtual organoids in precision medicine will continue to expand, providing more powerful support for disease treatment and new drug research and development.

3.2. Machine learning and deep learning framework

3.2.1. Representation learning and model pre-training

The construction of virtual organoids depends on high-quality data input, especially data from multi omics technology, such as single cell RNA sequencing, proteomics, etc. These data provide a wealth of cell characteristics information for virtual organs, such as gene expression patterns, protein interactions and network relationships between cells. In order to extract valuable biological information from these large-scale data, traditional supervised learning methods often rely on a large number of labeled data, while in the field of multi omics, the scarcity of labeled data makes supervised learning impractical. In this context, self supervised learning and comparative learning have become effective solutions [57]. They can learn general feature representation from a large number of data without manual annotation. The introduction of this technology provides great potential for the construction of virtual organ like model.

By designing a specific loss function, self supervised learning and comparative learning can promote the model to extract potentially useful features from unlabeled data [58]. For the construction of virtual organoids, these unsupervised learning methods can automatically extract the gene expression characteristics of cells from high-dimensional data such as single-cell transcriptome data and spatial transcriptome data, which can help us understand the roles and interactions of different cells in organoids. These features can be effectively transformed into the input of virtual organ like model, and provide strong data support for predicting cell behavior and tissue response.

On this basis, odformer and other virtual organ like models adopt a pre training strategy to generate a data-based representation by pre training on large-scale transcriptome data and single-cell data. Odformer automatically learns the feature representation of cells by inputting large-scale data into the pre training model, and provides powerful feature extraction ability for downstream tasks [33]. This pre training model can make full use of a large number of public data in the field of biology without clearly labeled data, so that the model can quickly adapt to and improve the prediction accuracy in the face of new data. For example, odformer uses single-cell RNA SEQ data to automatically learn the expression patterns of cells in organoids and map them to different tissue types and cell populations, so as to effectively predict drug reactions and therapeutic effects [33]. This method can greatly improve the personalization level of the virtual organ like model, so that it can adapt to the changes of different patients or different pathological states.

Through self-supervised learning and comparative learning, the model can learn valuable biological characteristics from large-scale multi omics data without manually labeled data, while the pre-training strategy can further improve the accuracy and personalization level of the model, providing a richer and more efficient data processing means for the construction of virtual organs.

3.2.2. Generative model

In the construction of virtual organoids, the scarcity and high dimensionality of data often lead to great challenges in model training. Especially in the face of limited samples, the traditional machine learning methods may not provide enough training data to learn effective feature representation. Therefore, the introduction of generative models such as VAE and GaN has become a very important technical means [59]. The generative model can enhance the existing data and generate new samples, so as to improve the diversity of the data set and the generalization ability of the model [60].

Variational auto encoder (VAE) and generative countermeasure network (GAN) are the most commonly used generative AI technologies in the scarce data environment [61]. In the construction of virtual organoids, VAE can be used to generate new cell expression data and expand the existing data set, so as to simulate more diverse biological

states without additional experimental costs [62]. The generation of confrontation network (GAN) generates more realistic data through the training process of confrontation (the game between generator and discriminator). Gan has achieved remarkable success in generating virtual cell images, simulating cell phenotypic changes, and cell response patterns. For example, in the process of drug screening, Gan can generate virtual cell images under different drug treatments, so as to predict the impact of drugs on organoids without actual cell culture and experiments [63]. This data enhanced and simulated generation ability makes Gan have unique advantages in the construction of virtual organs and virtual cells.

In the research of virtual organoids, the application of generative model enables scientists to predict the behavior of cells under different conditions without actual experiments, such as the reaction of cells to new drugs, the adaptability of cells under different environmental conditions, etc. Through these generative models, virtual organoids can not only reflect the response of actual cells, but also predict their behavior in the absence of conditions, which greatly improves the accuracy and reliability of prediction.

3.2.3. Multi model fusion

In the construction of virtual organoids, a single model is often unable to effectively simulate the complex interactions between cells and biological processes at the tissue level. Therefore, the introduction of multi model fusion technology plays an important role in capturing cell relationships, signaling pathways and cross scale spatial interactions [64]. Especially, deep learning models such as integrated map neural network (GNN) and transformers can better simulate the interaction between cells and the spatial structure within tissues [65]. In addition, the integration of biophysical models provides an important supplement, which can incorporate the physical behavior of cells into the virtual organ like model, and further improve the biological rationality and prediction accuracy of the model.

GNN can capture the relationship and interaction between cells through graph structure, which is very important for simulating the spatial distribution of cells in tissues and how they regulate the overall biological process through signal pathways and interactions [66]. Through GNN, the virtual organ like model can learn the layout of cells in tissues and predict the effect of cell-cell interaction on tissue behavior. Transformers, with its powerful self attention mechanism, can handle long-distance dependencies, and play an important role in simulating complex cell communication and signal transmission [67].

In addition to the deep learning model, the integration of biophysical models is also an indispensable part of the construction of virtual organoids. Cells are not only the product of the interaction between genes and proteins, their behavior is also affected by mechanical and physical constraints, such as cell mechanics, cell migration, proliferation and apoptosis [68]. These physical behaviors are crucial for the simulation of the structure, function and disease progression of organoids. For example, the mechanical force of cells can affect their arrangement in the three-dimensional structure, and then affect the morphological changes of tissues [69]. Therefore, the combination of biophysical model and deep learning model can not only enhance the biological realism of virtual organs, but also ensure more accurate prediction of cell behavior in dynamic environment.

3.2.4. Introduction of interpretable algorithm

Interpretability is the key factor to ensure the reliability and clinical availability of the model. Traditional deep learning models often face the “black box” problem, that is, the decision-making process of the model lacks transparency, and it is difficult to understand the biological mechanism behind its prediction [70]. To solve this problem, we can improve the transparency and interpretability of the model by introducing interpretable algorithms, such as lime (local interpretable model independent interpretation) and Shapley (Shapley value) [71]. These algorithms can help researchers understand how the model makes predictions, and reveal which features play a key role in the behavior of virtual organs, so as to avoid the black box problem.

In addition, by embedding biological networks, dynamic equations and physical constraints into the AI model, the interpretability of the model can be further enhanced. Adding physical constraints, such as cell mechanics model, can ensure that the behavior predicted by the model conforms to the actual biophysical laws.

3.3. Multi scale simulation and virtual experiment

3.3.1. Rule driven proxy model

Rule Driven agent models (ABM) is a computational model that simulates the behavior of complex systems. In this model, the system is composed of multiple “agents”, and each agent represents an independent entity in the system. In the construction of virtual organs, these agents usually represent a single cell, and each cell has its own behavior rules and decision-making mechanism [72]. The core idea of agent model is to simulate the dynamic evolution of cells in a specific environment by setting the behavior rules of each cell. Cells can interact with each other through physical contact, chemical signals, gene expression and other ways to form more complex biological phenomena.

In the construction of virtual organoids, rule driven agent models (such as physicell) are widely used to simulate basic biological processes such as cell proliferation, apoptosis and migration. Different from the traditional continuum model, the agent model can capture the heterogeneity and spatial distribution of cells in the organization by simulating the behavior of individual cells, and form the dynamic behavior at the organization level on this basis [73]. Each cell acts as an agent and makes calculations and decisions based on predefined rules. The advantage of agent model is its high flexibility and scalability. Biologists can adjust the interaction rules between cells according to actual needs, without relying on complex programming or mathematical derivation [74].

By introducing a rule driven proxy model, virtual organoids can simulate the dynamic relationship between cells and environment on multiple scales. In the tumor like organ model, the rules of cell behavior can describe the growth mode of tumor cells, the infiltration of immune cells, the evolution of tumor microenvironment and other key processes. This simulation not only provides intuitive biological interpretation, but also carries out virtual intervention to optimize drug screening and treatment strategies, which has significant clinical application prospects.

3.3.2. Continuum and finite element model

In the construction of virtual organs, finite element model (FEM) and continuum model are very important tools. They are widely used to simulate physical processes at the level of cells, tissues and organs, such as stress conduction, nutrient diffusion and matrix mechanics [75]. These physical processes play a key role in virtual organoids, because the growth, morphological changes and response to external stimuli of organoids are not only regulated by intracellular signals, but also affected by mechanical environment and physical constraints. For example, cell migration and proliferation are not only regulated by chemical signals, but also affected by physical factors such as matrix stiffness and stress gradient [76].

Finite element model is a numerical method, which is widely used to solve complex problems involving physical phenomena, especially in mechanical and structural analysis. In the modeling of virtual organs, FEM can effectively simulate the changes of cells and tissues in the stress environment, including the mechanical interactions between cells, the mechanical properties of matrix and how cells respond to external stimuli in these environments [77]. Combined with physical constraints, FEM can provide a more realistic simulation of cell behavior, making virtual organs not only biologically reasonable, but also physically in line with the actual physiological environment.

Coupled with the rule driven surrogate model, FEM and continuum

model can work together to simulate how cells respond to biological stimuli in different physical environments. Through this physical biological coupling model, virtual organoids can more accurately reflect the behavior of cells in the real environment, and improve the biological rationality and realism of the model. This integration of multiple physical field models provides a more accurate prediction tool for drug screening and disease research.

3.3.3. Combined simulation and virtual experiment platform

The virtual experiment platform can effectively carry out virtual intervention and experiment design optimization by integrating a variety of simulation technologies and data. In the research of virtual organs, this platform can combine biophysical simulation and generative model to accurately simulate the reaction of cells and tissues under different conditions, and optimize drug screening and treatment. For example, biophysical simulation can provide mechanical constraints to simulate the behavior of cells under different matrix stiffness, oxygen concentration and other conditions, while generative models such as Gan and VAE can generate virtual samples in the case of scarce data to further expand the experimental data space [78].

By combining these technologies, the virtual experiment platform can simulate the effects of drugs on virtual organs, predict the efficacy and side effects of different drug combinations, and reduce the trial and error costs in real experiments. In terms of experimental design, the platform can automatically design the experimental process, optimize the priority of drug screening, and provide more predictive experimental data support. In addition, virtual intervention enables scientists to adjust personalized treatment plans in a simulated environment, simulate the response of organoids under different treatment strategies, and provide decision-making basis for clinical treatment.

3.4. Model evaluation and standardization

To ensure that Virtual Organoids (VOs) can successfully transition from laboratory prototypes to clinically viable decision-support tools, a rigorous and hierarchical evaluation and standardization system must be established. This section proposes a three-tier framework extending from micro-technical validation to macro-societal governance: first, algorithmic performance evaluation, which emphasizes uncertainty quantification and confidence scoring alongside predictive accuracy; second, infrastructure standardization, ensuring reproducibility through FAIR (Findable, Accessible, Interoperable, Reusable) data and model sharing mechanisms; and finally, regulatory compliance, embedding model development within ethical and legal constraints. This hierarchical architecture aims to address core challenges in reliability and transparency, laying a solid foundation for the practical deployment of VOs.

3.4.1. Performance index

In the evaluation of virtual organ like model, using a variety of performance evaluation indicators is the key to ensure the effectiveness of the model. Common evaluation methods include Pearson correlation coefficient, mean square error (MSE), accuracy and AUC, which can quantify the prediction ability of the model in different tasks [79]. MSE is applicable to the regression problem, which measures the accuracy of the model in predicting the value [80]; The Pearson correlation coefficient is used to evaluate the linear relationship between continuous variables and help us understand the correlation between the model output and the real value [81].

However, in the face of complex and biologically uncertain virtual organoid data, relying only on traditional performance indicators may not be able to comprehensively evaluate the reliability of the model. At this time, the introduction of uncertainty evaluation and confidence score can significantly improve the interpretability and reliability of the model [82]. By quantifying the uncertainty of model prediction, uncertainty assessment can help identify which data points or prediction results have high risks, so as to guide subsequent experiments and interventions. The confidence score can provide a confidence level for each prediction result and further support clinical decision-making, especially in the face of data scarcity or uncertain experimental conditions [83]. In conclusion, the combination of a variety of performance evaluation indicators and uncertainty quantification methods can provide more comprehensive and reliable support for the accurate modeling of virtual organs, and improve its practicability and reliability in clinical application.

3.4.2. Data and model sharing

Data and model sharing is the core element to promote the development of virtual organ like technology, especially in biomedical research. The openness and reusability of data can accelerate scientific discovery and reduce resource waste. In order to ensure the openness and sustainability of virtual organoid data and models, it is essential to adopt the fair principle (findable, accessible, interoperable, reusable) [84]. The fair principle provides a standard framework for data management and sharing, ensuring that data can be widely found, accessed, interoperated and reused, so as to promote interdisciplinary cooperation, improve research efficiency, and promote the standardization and popularization of technology [85].

In the process of constructing virtual organoids, the establishment of data sets and model base is the key to realize the principle of fair. By collecting data from different sources and types, such as single-cell transcriptome data, tissue imaging data, genome data, etc., establishing a unified database and carrying out standardized processing [86], we can ensure that the data has good compatibility and operability between different platforms and research. At the same time, the model library is developed to collect and store the verified virtual organ like models, providing researchers with easy access and reuse tools. These libraries not only help to improve the reusability of data, but also accelerate the validation and optimization of models and promote cross agency cooperation.

The sharing of data and models not only promotes the standardization of virtual organ like technology, but also is crucial for clinical transformation. By sharing data and models in the virtual experimental platform, researchers can conduct virtual drug screening, gene editing simulation and other experiments on the shared platform, so as to improve the efficiency and accuracy of experimental design. The construction of the sharing platform enables the data and models to be continuously updated and iterated, and further promotes the clinical application of virtual organs. This open and reusable model library provides strong support for the cross domain application of virtual organ like, and promotes the rapid development of technology and clinical transformation.

3.4.3. Supervision and regulations

With the rapid development of virtual organ like technology and artificial intelligence (AI) in the medical field, the establishment of regulation and regulation is particularly important [87]. In order to ensure that the virtual organ like model is not only scientifically effective, but also can comply with ethical requirements in clinical practice, clear regulatory standards must be formulated. The regulatory standard of clinical decision support is the key, which can ensure the safety, accuracy and compliance of the virtual organ like model, especially in the field of patient health, and ensure the reliability and availability of the model.

From the ethical point of view, the construction and application of virtual organ like model need to follow the basic principles of medical ethics, including informed consent, privacy protection and maximizing the interests of patients. The application of virtual organ like model should ensure the rational use of patient data, protect patient privacy, and provide ethical decision support.

From the perspective of regulation compliance, the virtual organ like model must meet the legal and ethical requirements of various countries

and regions. For example, the EU AI regulations put forward strict requirements for the transparency, traceability and ethics of AI systems, which provides a standard framework for the development and application of virtual organs [88]. Especially in medical applications, the traceability and compliance of the model require that the model must be able to provide clear decision-making basis and logic, and ensure that it conforms to relevant legal and ethical standards in practical applications. Similarly, FDA has strict regulatory requirements for medical software and AI system to ensure the safety and effectiveness of the model [89]. These global standards have an important impact on the development and application of virtual organoids, promote the compliance development of virtual organoids technology, and ensure its global applicability.

In summary, multidimensional performance metrics, a standardized sharing ecosystem, and strict regulatory governance collectively form the cornerstone of trust for the clinical translation of Virtual Organoids. This integrated system demonstrates that a mature VO model must not only be a mathematically accurate predictor but also a scientifically reproducible public resource and an ethically responsible medical tool. By tightly coupling technical evaluation with standardization processes, we can overcome data silos and the "black box" trust crisis while providing traceable chains of evidence for regulatory bodies. This systematic construction, spanning from algorithms to norms, is the essential pathway for propelling Virtual Organoids out of the computational space and into widespread acceptance within precision medicine workflows.

4. Virtual cells informing the design of virtual organoids

In the framework of virtual organoids (VO), virtual cells (VC) are the smallest functional units [12]. It is not merely an average cell or a low-dimensional feature vector, but rather an executable cell state model learned under the constraints of multi-omics, spatio-temporal imaging, and perturbation experiments. The purpose of VC construction is to accept inputs, give predictions and support relevant decisions on the basis of multimodal and multiscale measurements, so that the single-cell basic model can be expanded to the level of organoids and even individuals [20]. For VO, VC not only reproduces the cell heterogeneity observed in the experiment, but also dynamically responds to virtual stimuli, which is the display between the upstream data layer and the downstream organ level simulation.

It has been proved in the field of organoids that the three-dimensional culture system derived from embryonic stem cells (ESC), adult stem cells (ASC) [90] and induced pluripotent stem cells (iPSC) [91] can reproduce human development, homeostasis and disease processes to a considerable extent, but it also exposes problems such as large batch differences, insufficient reproducibility, and limited readout indicators [92]. The purpose of VO's introduction of VC is not to replace these entity models, but to superimpose a layer of calculable and deducible digital copies on them, that is, using single-cell multi omics, lineage tracing and physical field measurement data to build virtual versions for stem cells, functional cells and tumor cells from different sources, and then build, perturb and screen them in the virtual space, and then reverse guide the experiments of solid organoids. In this section, VC is divided into three categories: virtual stem cells, virtual functional cells and virtual tumor cells, and their construction path, application scenarios and representative results in VO are discussed respectively.

4.1. Virtual stem cell

According to the cell source, stem cells can be roughly divided into ESC, ASC and iPSC. Corresponding to various types of virtual stem cells, its core goal is to reproduce the relevant characteristics in the digital space, including not only the multilineage differentiation potential and its probability distribution, but also the lineage tree structure, key regulators of fate decisions, and the response mode to microenvironment signals [93,94]. VO can take virtual stem cells as the lineage starting point, and simulate the whole life cycle process from normal development, tissue homeostasis to disease occurrence under a unified framework by introducing stem cell modules derived from embryos, adults or lesions (Fig. 3).

4.1.1. Virtual iPSC

iPSC has both the pluripotency like properties of embryonic stem cells and its individual specific genetic background, which makes it an ideal prototype for constructing virtual stem cells and lays the foundation for personalized disease modeling. The garmen framework proposed by Kaul et al. [95] embeds a large number of VC agents into a minimalist gene regulatory network containing a few key factors such as OCT4, SOX2, TBXT, SOX17, CDX2, and then couples it with BMP/WNT and other morphogenetic fields, so that a homogeneous human pluripotent stem cell population spontaneously differentiates into ectoderm, mesoderm, endoderm, and trophectoderm like concentric ring structures in a simulated environment. Its spatial pattern and response to signal and gene perturbation are highly consistent with the micropatterned human pluripotent stem cell model, systematically revealing the mechanism by which OCT4 and BMP/WNT cascade jointly shape the surrounding gastrulation pattern.

Complementary to this, Squidiff based on diffusion model represents virtual iPSCs as potential vectors of single-cell transcriptome, learns stimulus direction through conditional denoising, and reconstructs continuous intermediate states during differentiation of iPSCs into three germ layers and vascular lineages given only the initial pluripotent state and a small amount of terminal lineage data [96]. This model can not only predict the transcriptional changes of iPSCs under different inducers, gene editing and physical stimuli in a unified potential space, but also reproduce the lineage bifurcation of endothelium, pericytes and fibroblasts and the trajectory deviation after radiation exposure in iPSC derived vascular organoids, providing a computable virtual lineage map for understanding the cell fate dynamics in complex 3D organoids.

When extending to the downstream functional level, virtual iPSCs can also naturally connect to the digital twin work of iPSC derived cardiomyocytes (iPSC-CM). Yang et al. [97] provided a virtual iPSC cm prototype, which retrieved key parameters such as ion channels from experimental readings by aligning single-cell electrophysiological records with a parameterized cardiomyocyte model, which provided a calibrated cell level parameter set for the electrophysiological module of VO. This digital twin unit can not only reproduce the action potential waveform, conduction velocity and cell-cell heterogeneity [98], but also map drug exposure into quantifiable safety risk output, such as arrhythmogenic event probability [99] providing the cornerstone for the construction of VO.

The value of virtual iPSC goes beyond generating differentiated digital cells, as it can provide VO with a multi energy starting point that can be personalized, calibrated by data, and transmitted across scales. Upstream, a minimalist regulatory network and morphogenetic field can be used to quickly establish reproducible early lineages and spatial patterns in VO. Midstream, a generative model can be used to constrain differentiation trajectories within a unified latent space and parameterize disturbance responses. Downstream, digital twin units of specific functional cells can be embedded into VO as functional modules, achieving closed-loop verification from cell state to organ level readings. With the accumulation of patient derived iPSCs, multi omics of organoids, and longitudinal functional measurements, VO is expected to become a universal interface for connecting individual genetic backgrounds, lineage fates, and functional phenotypes, and a personalized virtual experimental platform that moves from structural simulation to predictability, comparability, and iteration.

4.1.2. Virtual ESC

In the blueprint of VO, the significance of virtual ESC is not only to

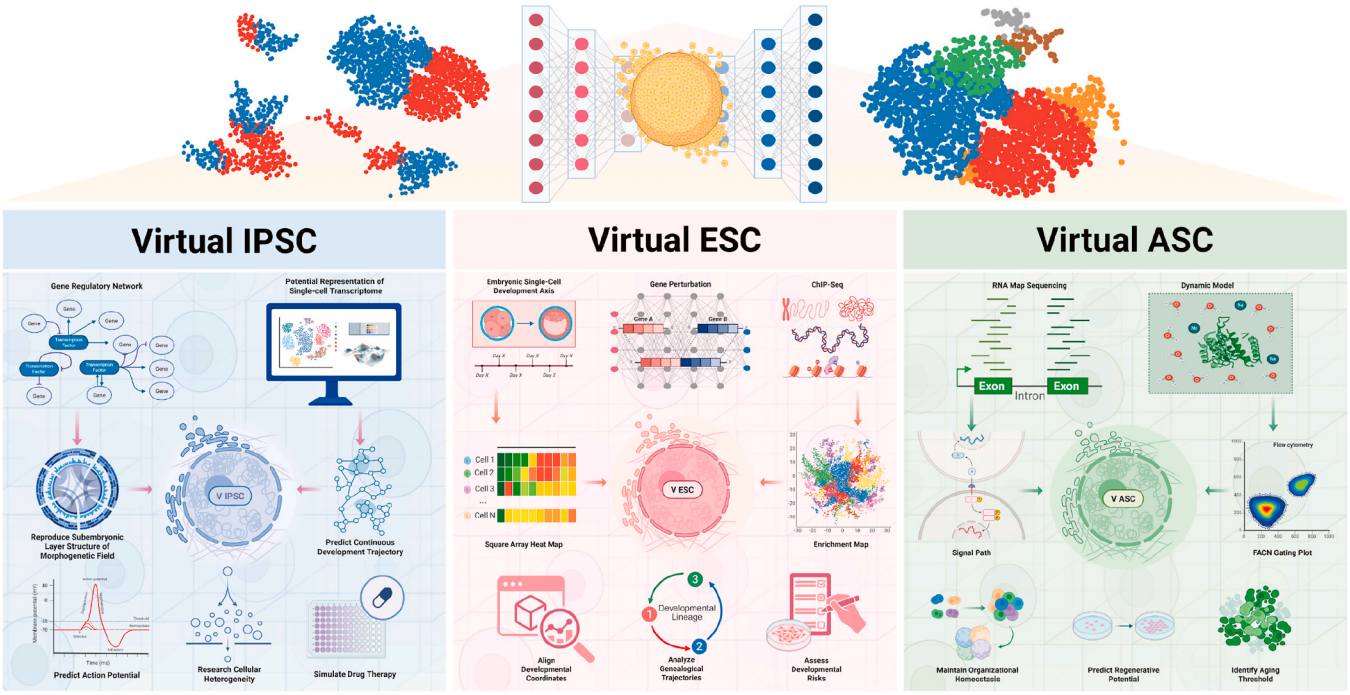


Fig. 3. Virtual stem cells as the developmental skeleton of virtual organoids.

create a digital embryo, but also to provide a calculable and calibrated starting layer for early human development. Zhao et al. [100] used multiple batches of single-cell RNA sequencing data to build a reference map of human embryos from zygotes to gastrula stage, and developed a tool to project various stem cell-derived embryo models onto a unified developmental trajectory to evaluate their molecular and lineage fidelity. Any ESC state generated in the model can be used as the developmental starting point of VO only if it can still fall within a reasonable germ layer and time interval when projected back to this reference map, which provides a yardstick for the development of VO.

Virtual ESC also needs to have clear examination criteria. Roohani et al. [101] selected human embryonic stem cell line H1 as the test platform of the first round of VC challenge, proposed to take the prediction of transcriptome effects after single gene perturbation as the core task, and emphasized open data, unified indicators and blind comparison across models, hoping to make the evaluation of VC a public benchmark similar to Turing test. This means that virtual ESC is not an arbitrarily designed black box, but to accept the direct comparison of different modeling frameworks on the same cell line and the same perturbation task. For VO developed on this basis, this offers quantitative criteria for embedding specific virtual ESCs into early developmental modules.

At the mechanism level, the chromatin transcription factor binding map of ESC provides rigid constraints for virtual ESC. Algorithms such as Macs have been widely used in ChIP-seq analysis of ESCs to systematically delineate the genome-wide enrichment patterns of core transcription factors and histone modifications [102]. These measurements can be transcribed a priori into the virtual ESC parameter space. No matter how high-dimensional the model is generated, the open or closed state near the key site, and the binding strength must be compatible with the known ESC map. The resulting virtual ESC not only aligns real embryos on the developmental trajectory, passes rigorous tests of perturbation predictions functionally, and abides by chromatin constraints mechanistically, but is more suitable as a computational agent for the early embryonic layer in VO, undertakes data from real embryo models upward, and provides a credible developmental starting point and traceable regulatory background for organ level virtual tissues downward.

In VO, the orientation of virtual ESC is more like a developmental ruler, which can anchor the initial state generated by the model on interpretable embryonic time coordinates, pull different modeling frameworks into the same evaluation system, and use chromatin transcription factor binding maps as prior constraints to avoid VO deviating from the biologically feasible domain in high-dimensional generation. Furthermore, this provides a starting layer specification that enables the subsequent lineage unfolding, spatial patterns, and functional differentiation of VO to be established on consistent developmental logic and verifiable molecular constraints, thereby advancing VO from being able to simulate to accurately and reproductively simulating such higher levels.

4.1.3. *Virtual ASC*

Virtual ASC emphasizes the long-term steady-state and regeneration ability in the specific tissue microenvironment, which is the key interface for embedding VC into VO. Taking hematopoietic stem cells as an example, Calvanese et al. [103] defined the HSC core signature of $RUNX^{+}HOXA9^{+}MLLT3^{+}MECOM^{+}HLF^{+}SPINK2^{+}$ through the single-cell Atlas of human hematopoietic tissues from early embryo to birth, and tracked its transfer and maturation stages from AGM area to fetal liver and then to different habitats before and after birth, providing a continuous coordinate axis of HSC ontogeny. For VO, this coordinate axis can be used to constrain the state space of virtual ASC. For example, virtual HSCs in bone marrow VO are no longer isolated discrete labels, but are anchored on a continuous lineage and maturation gradient from embryo to adult, so that they can naturally exhibit developmental history dependent functional differences when simulating radiotherapy, transplantation or chronic inflammation.

On the other hand, Mahadik et al. [104] constructed a dynamic model that explicitly included self-renewal, differentiation and feedback of cell secreted factors based on the in vitro HSC progenitor co culture data, and proved that exogenous cytokines alone were not enough to explain the lineage evolution. Instead, the feedback loop between adult stem cells and their microenvironment must be incorporated into the equation to accurately predict the expansion and decay of each subpopulation under different fluid exchange frequencies and culture conditions. This idea can naturally rise to the construction paradigm of

virtual ASC. In VO, adult stem cells are not just nodes of passive response, but dynamically remodel the cellular composition and functional output of the whole VO through the closed loop of VC – virtual matrix – virtual factors. In the future, when similar multi-scale dynamic models are extended to different adult stem cell systems such as intestine, skin and bone, it is expected to form a set of portable virtual ASC templates, which can not only predict the critical conditions of tissue regeneration, aging and failure in VO, but also provide an iterative in silico test site for personalized stem cell therapy and biological manufacturing.

From a more macro perspective, the unique value of virtual ASC lies in truly introducing organizational scale time into the model. It does not pursue the one-time generation of a specific terminal cell type, but instead focuses on slow variable processes such as steady-state maintenance, damage response, and repair regression, continuously outputting testable dynamic fingerprints such as lineage bias, clone update rate, etc. Therefore, virtual ASC compares the bifurcation paths of the system towards regeneration, compensation, or depletion under different initial conditions and intervention strategies, providing process controllers for the construction of VO. If in the future, it can form a closed loop with longitudinal sampling of organoids, such as time series single-cell omics, spatial transcription, etc., and feed back the key inflection points predicted by the model to experimental verification, virtual ASC is expected to become a core module in VO for evaluating regenerative therapy windows, optimizing culture processes, and even quantifying manufacturability.

In general, virtual iPSC focuses on individual specific multipotent starting points. Virtual ESC provides a time and lineage ruler aligned with human embryo development. Virtual ASC carries the process of tissue homeostasis and regeneration, and reshapes the long-term behavior of VO through feedback loop with the microenvironment. The key to future work is to build a unified computational representation framework to realize the continuous development trajectory integration from iPSC, ESC to ASC. By integrating various reference maps, disturbance response data and dynamic regulation model systems into a set of standardized virtual stem cell interaction interfaces, VC modules can achieve seamless compatibility and functional assembly in a unified VO platform. This technical path will ultimately promote VO to play a substantial role in the analysis of developmental mechanisms, the construction of disease models, and the computer-aided design and optimization of individualized stem cell therapy.

4.2. *Virtual functional cell*

Virtual functional cell is more like executive units in VO. Different types of virtual functional cells are embedded into a unified VO framework through parametric signal input-output relationship, metabolic flux, secretion spectrum and mechanical response, which jointly determine the overall performance of VO on multiple axes, such as immune defense, material metabolism, barrier homeostasis, information transmission and tissue remodeling (Fig. 4). The resulting virtual functional cell bank provides the basis for assembling, replacing and upgrading VO on demand for different organs and disease scenarios, so that VO not only has a growable structure, but also has multidimensional physiological functions that can be quantitatively characterized and intervened.

4.2.1. *Virtual immune cell*

Virtual immune cell are not simply digital clones of a certain immune cell, but attempt to reconstruct an immune cell population with developmental history, multi-level memory and environmental awareness in the computational space. Taking T cells as an example, Davenport et al. [105] showed that the diversity of peripheral T cell repertoire is not simply determined by antigen experience, but by superimposing T cells exported from different developmental batches in fetal, perinatal and adult stages. These cells with different developmental ages have stable differences in functional preferences and fate choices, which can be regarded as a source of diversity in the stealth layer.

In recent years, the research on virtual memory T cells (VM) has provided a clear biological grasp for the construction of virtual immune cells. White et al. [106] found that in mice that were not challenged by antigen, there naturally existed a group of VM $CD8^+$ T cells with CD44hi/CD122hi/CD49dlo, whose formation depended on IL-15-mediated steady-state proliferation and could play a bystander

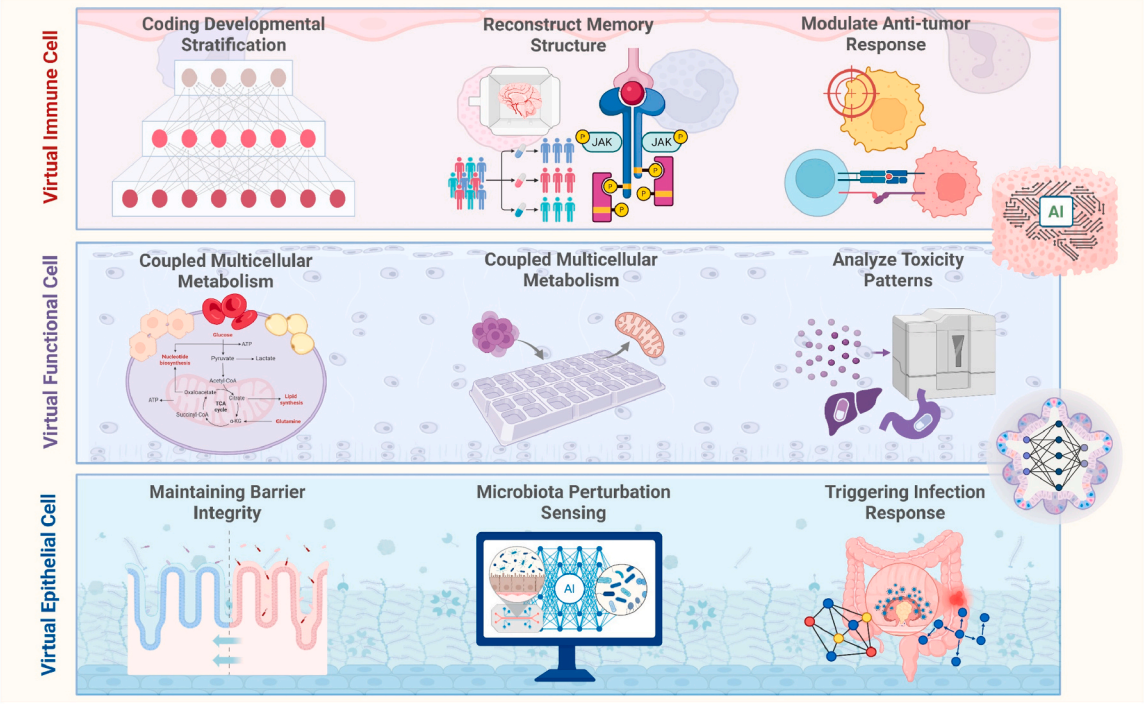


Fig. 4. Schematic diagram of multi-dimensional functional output of virtual organoids driven by virtual functional cells.

protective role under the stimulation of non-specific inflammatory factors. Lee et al. [107] further proved that such VM cells are close to true memory T cells in transcription factor expression and cell cycle status, but show a unique functional lineage after TCR triggering, which is considered as a bridge between innate immunity and adaptive immunity. The study of Borsa et al. [108] suggested that with age, the function of classical naive $CD8^+$ T cells that rely on asymmetric division to maintain diversity declines, while VM cells retain their metabolic characteristics and asymmetric division ability relatively, thus bearing a more important role in memory and response in older individuals. Tu et al. [109] gave practical directions from the perspective of tumor immunity. By treating mice with Alb-FLT3L, they could induce $CD44^{high}$ $CD8^+$ T cells with VM characteristics in vivo. These cells could significantly inhibit melanoma growth after adaptive transfer, showing the plasticity and application potential of VM cells in anti-tumor immunity. Looking ahead, embedding such developmentally layered and functionally plastic virtual immune cell populations into organ- and tumor-specific VO will make it possible to interrogate immune–tissue interactions, treatment responses and age- or context-dependent immune failure within a unified, mechanistically interpretable in silico framework.

Starting from the modeling practice of VO, the more important significance of virtual immune cells lies in transforming processes such as immune infiltration, antigen presentation, cytokine diffusion, and checkpoint regulation into parameterized and verifiable input-output relationships. Instead of pursuing the replication of the static phenotype of a certain type of T cell, it is better to treat the plasticity and population structure of immune cells as adjustable knobs of VO. Virtual immune cells are expected to become the most sensitive intervention evaluation layer in VO, used to quickly compare the effective window and side effect boundaries of immune regulation strategies, and provide an iterative computational testing ground for personalized treatment plans.

4.2.2. Virtual metabolic cell

The core task of virtual metabolic cell is not to reproduce the average phenotype of a cell, but to reconstruct how the multicellular metabolic network is cooperatively maintained or imbalanced in the computational space. Andreoni et al. [110] modularized the metabolic models of hepatocytes, endothelial cells and adipocytes, coupled them into a virtual "liver vessel fat axis" through medium flow and substrate exchange, and successfully reproduced the steady-state and disturbed process of glucose and lipid metabolism in the three culture systems, which can be seen as an example of early multi type virtual metabolic cells being assembled into prototype vo. The key to this idea is not how elaborate a certain cell model is, but to integrate the roles of different cells such as liver, endothelium and fat in carbon and lipid metabolism into a unified equation set through pluggable virtual metabolic cell modules, so as to lay a structural foundation for the subsequent construction of more complex virtual metabolic organoids.

At a finer scale, the virtual cell based assay developed by Worth et al. [111] continued to drill down the metabolic function into the intracellular compartment, established VC for HepaRG hepatocytes and icell cardiomyocytes, and explicitly added to the mitochondrial compartment to simulate the distribution of compounds in the culture pore, intracellular and mitochondrial, and then linked the changes of mitochondrial membrane potential with cytotoxicity. At the same time, Smith et al. [112] used the virtual mouse liver framework to embed the virtual hepatocytes with portal central vein gradient into the lobule like geometry. Through the virtual experiment of acetaminophen toxicity, they systematically compared different mechanism hypotheses, and finally gave a synthetic mechanism that can not only explain the drug clearance kinetics but also reproduce the spatial temporal pattern of necrosis around the central vein. Based on these constructs, in the future, in drug-induced liver injury, myocardial toxicity, metabolic syndrome and other scenarios, the virtual metabolic cell parameters can be replaced according to individual genotype and exposure history, and the in silico iteration of drug combination and dose regimen can be quickly completed in VO, and then the reverse direction can be used to guide solid organoids and animal experiments, so as to realize the real VO design path from system metabolism to whole organ response.

The most noteworthy aspect of virtual metabolic cells is not the study of a specific pathway, but whether they can translate metabolic activity into system level readings that can be compared in experiments, and explain why these readings exhibit spatial and temporal differences. It can ensure the closure of carbon flow, energy, and redox states in a multicellular network within VO, while also having the ability to convert small parameter deviations into detectable toxicity boundaries, metabolic imbalance inflection points, or tissue vulnerable areas. The research on virtual metabolic cells is expected to make VO a digital metabolic bridge connecting molecular mechanisms and overall physiological responses, thereby promoting the evolution of metabolic disease research, drug toxicity prediction, and personalized treatment strategies towards more accurate and predictable directions.

4.2.3. Virtual epithelial cell

Epithelial cells are regarded as a dynamic barrier system, which not only maintains the continuity of crypt villus or luminal tract surface, but also makes rapid and reversible responses to microbiota and pathogens. Peck et al. [113] based on SOX9 EGFP mice, performed transcriptome and miRNA sequencing on a variety of intestinal epithelial cell subsets and found that different cell types have highly specific miRNA profiles, while intestinal epithelial stem cells are the most sensitive to the presence of bacteria. Under sterile and normoxic conditions, proliferation related genes of stem cells and miRNA such as mir-375 undergo significant remodeling, which is directly related to epithelial renewal speed and barrier integrity. This suggests that virtual epithelial cells should not only carry the basic dynamics of "Division-Differentiation-Shedding" in the model, but also explicitly encode regulatory circuits such as microbiota miRNA stem cell axis, so as to reproduce the migration and imbalance of barrier homeostasis under different microbial states in VO.

At the tissue scale, the multi-scale colon crypt model constructed by Leeuwen et al. [114] can be regarded as an early virtual epithelial crypt. Each virtual epithelial cell is internally coupled with WNT signal and cell cycle equation. The adhesion and migration between cells are described by spring network and Voronoi segmentation. On the whole, it can spontaneously form bottom-up renewal flow and stable crypt structure in the computational space. The robustness of barrier renewal under different assumptions can be tested by in silico analysis such as virtual labeling index experiment. If this virtual crypt layer is directly embedded in the VO reconstructed by the geometry of real intestinal organoids, the long-term effects of nutrition, toxins and flora on barrier renewal can be simulated on the same platform, and then the combination of parameters under which the virtual epithelium will appear barrier rupture or repair failure can be discussed.

The construction of virtual epithelial cells is not only a structural simulation of physiological barriers, but also a systematic decoding of the dynamic interface behavior of living organisms. VO can achieve a transition from static structural similarity to dynamic functional similarity in its research, making it the module closest to the clinical symptom end, used to simulate barrier fate under infection, inflammation, and microbiota intervention, and provide verifiable quantitative basis for intervention timing and intensity.

Virtual immune cells undertake developmental stratification and memory networks, and are responsible for shaping an individualized immune landscape that can be reconstructed with age and treatment in VO. Virtual metabolic cells connect drug clearance, energy metabolism and organ level injury mode into a computable chain through the metabolic coupling of multi cells and multi compartments. Virtual epithelial cells assume the dual functions of barrier and infection response, and can evolve a variety of homeostatic and destabilizing situations under the disturbance of bacteria, toxins and inflammation. In

the future, if these three types of virtual functional cells can be seamlessly spliced with the aforementioned virtual stem cells and virtual tumor cells according to their lineage and spatial location, and information such as individual genotype, exposure history and treatment plan can be introduced, it is expected to build a multi-scale VO that is truly growable, responsive and intervenable, and provide an iterative in silico test platform for disease mechanism analysis and treatment strategy design.

4.3. Virtual tumor cell

The outstanding characteristics of tumor system are genetic heterogeneity, microenvironment dependence and continuous clonal evolution. For most solid tumors, this evolution mainly occurs on the malignant epithelial cell lineage [115]. Based on this perspective, the tumor module of VO is more suitable to start from the state set of epithelial origin, and then superimpose heritable physical differences and microenvironment fitness rules, so that clone differentiation, collective invasion, and treatment escape naturally emerge as evolutionary results in the model. Swat et al. [116] built a multicellular virtual tissue model based on CompuCell3d. Using nutrition, oxygen concentration gradient, and boundary conditions as external constraints, and heritable physical parameters such as cell adhesion and survival threshold as internal adjustable dimensions of virtual tumor cells. As a result, tumor cells spontaneously form spatial stratification in the computational space of a three-dimensional mass, where oxygen enriched surroundings are more likely to retain highly adherent cells, while hypoxic pressure promotes the gradual enrichment of invasive clones with reduced adhesion and higher integrin expression inside. This also explains epithelial evolution, where microenvironmental pressure not only selects tumor cells, but also continuously reshapes malignant epithelial phenotypes along a fitness landscape, resulting in spatially separated subclones that can be compared with spatially derived tumor microregions and subclones. For VO, the tumor cell module should take both physical heterogeneity and microenvironmental selection pressure as the core parameter set, in order to reconstruct the morphological evolution from primary lesion to focal invasion within VO and provide tissue scale phenotype readings that can be constrained and validated by data for subsequent treatment modules such as radiotherapy/immune intervention. Crucially, The progression of malignant epithelium is usually collective, not purely single-cell. Research has shown that collective patterns such as migration and invasion of epithelial tumor cells are deeply influenced by the microenvironment. Therefore, in addition to intracellular proliferation, virtual tumor cells targeting VO should also include collective rules that enable the model to reproduce queue level epithelial behavior commonly observed in spatial tumor tissues.

On the other hand, virtual tumor cells also need to connect gene protein networks and observable phenotypes at the level of single cells. The three-dimensional multi-scale agent-based brain tumor model proposed by Deisboeck group [117] provides a representative prototype for this. Each virtual glioma cell is embedded with an EGFR related gene protein interaction network, which is coupled with the simplified cell cycle module. At any time, the cell makes a choice between migration, proliferation, rest and death according to the network state, and finally forms a strip-shaped infiltration and marginal proliferation zone on the virtual volume of brain tissue. Complementary to this, Swanson et al. [118] used the reaction-diffusion equation to build a virtual glioma on the patient's real brain anatomic geometry. By treating tumor cells as a continuous density field with dual characteristics of diffusion and proliferation, they quantitatively characterized the infiltration front in gray matter and white matter under different migration velocities, and proposed that the basic diffusion coefficient and proliferation rate could be used as parameter pairs for individualized prognosis and surgical boundary assessment. From discrete virtual tumor cells carrying EGFR network to tumor cell density field with spatial continuity, realize the cross scale mapping from molecular regulation to infiltrative morphology, and lay the foundation for subsequent VO access to radiotherapy, chemotherapy and other intervention modules.

In the context of immunotherapy, virtual tumor cells assume the role of rivals coupled with virtual immune cells. The agent-based virtual tumor immune model built by Wang et al. [119] incorporates high antigenicity and low antigenicity virtual tumor cells, virtual CTLs with different killing mechanisms, such as fas/fasl and perforin/granzyme, and pd-1/pd-l1 immune checkpoint blockade strategies into a unified framework, and makes a parallel comparison with the corresponding ordinary differential equation model. The results showed that even if the overall cell number trajectory was similar, different spatial organization and antigenicity distribution of tumor cells could still form completely different survival clones and immune escape patterns in the virtual tumor, which directly affected the efficacy of immune checkpoint inhibitors. In VO, the tumor cell module must explicitly include the rules of antigenicity, checkpoint molecule expression and its evolution over time, and be coupled with the aforementioned virtual immune cell module, so as to truly investigate the therapeutic window under the interweaving of spatial heterogeneity, antigenicity spectrum and immune intervention in VO.

The role of virtual tumor cells in radiotherapy optimization is more reflected in the sensitive characterization of hypoxic structure. Schiavo et al. [120] proposed a virtual tumor model for radiotherapy. Starting from the fractal vascular tree, three-dimensional tumor microvascular network and coherent oxygen distribution map were generated at 10 μm resolution, which distinguished chronic hypoxia, acute hypoxia and anaemic hypoxia, and calculated the hypoxia fraction and oxygen enhancement ratio of virtual tumor cells in the corresponding voxel. On this basis, researchers can compare the efficiency of different segmentation schemes and different dose delivery strategies on the clearance of hypoxic clones in the complete in silico environment. If the vascular oxygen field derived from imaging or organoid imaging reconstruction is superimposed in VO geometry, and each virtual tumor cell is bound to its local PO_2 level and DNA damage repair ability, the optimal radiotherapy strategy in three-dimensional space of dose, time and oxygenation can be systematically scanned, which is suitable for individual chemoradiotherapy scheme rehearsal of bone, brain and other organs with complex blood supply.

In addition to behavior level simulation, virtual tumor cells also gradually extend to the virtual reading of morphological and molecular markers. The virtual Ki67/cytokeratin double staining method proposed by Røge et al. [121] uses image analysis algorithm to divide tumor cells and non-tumor cells at the pixel level by digitally fusing Ki67 and CK staining of adjacent sections, and automatically calculates the proliferation index. The result is highly consistent with the strict stereological manual counting. This kind of digital pathology method essentially endows each tumor cell with a virtual state and gives the spatial distribution, which can be used as an important basis for calibrating the proliferation parameters of virtual tumor cells. Similarly, the VC filter based on odep can continuously enrich circulating tumor cells by size using the photo induced electrophoresis effect on a microfluidic chip, while improving the purity while retaining the activity required for subsequent genetic detection [122]. These technologies provide experimental morphology and physical property distribution prediction for virtual tumor cells, so that the setting of tumor cell size, deformability and proliferation heterogeneity in VO no longer completely depends on theoretical assumptions.

At the molecular pathway scale, virtual tumor cells have also been used to carry the effects of drug action networks and transcriptional reprogramming. Manu et al. [123] studied the inhibition of CXCR4 and its downstream metastasis behavior by plumbagin, a natural product. On the one hand, they systematically verified the NF-κB-mediated transcriptional regulation pathway. On the other hand, they simulated the dynamic process of CXCR4 and other metastasis related gene expression downregulation caused by NF-κB inhibition on a virtual tumor pathway platform, and used the in vitro experimental results to

verify each other. This virtual tumor cell at the level of virtual functional proteome allows us to predict the comprehensive impact of multi-target intervention on the migration and invasion phenotype of tumor cells without relying on large-scale wet experiments. After embedding it into VO, it is possible to simultaneously track the coordinated changes of signal network status, cell behavior, and tissue level invasion mode within VO, providing a new computational perspective for the optimization of complex target combinations and drug delivery sequences.

More macroscopically, a systematic review of pairs of tumor digital twins by Shen et al. [124] shows that virtual tumors with real potential for clinical transformation often need to integrate imaging, multi omics, and clinical process data, and achieve dynamic prediction and bidirectional update of individual tumor dynamics through multi-scale models. At present, most digital twin prototypes still remain in coarse-grained tumor blocks at the organ or whole-body level, and it is difficult to naturally introduce heterogeneity at the experimental and cellular levels of organoids.

In the framework proposed in this subsection, taking virtual tumor cells with clear molecular networks, physical parameters and treatment response rules as the minimum unit, align real tumor organoids in geometry and microenvironment, construct mesoscale virtual tumor organoids, and then roll their statistical behavior to patient specific digital twinning, which is expected to become a missing link between solid organoid experiments and bedside decision support (Fig. 5). In other words, virtual tumor cells are not only the technical details of simulating tumor growth, but also the key interface of organically splicing VC, VO and tumor digital twin. It is expected to build a new paradigm of full chain tumor precise treatment with organoids as the experimental base, virtual tumor as the deduction engine, and digital twin as the clinical carrier.

5. Preclinical and clinical applications

Virtual organoids provide a computable framework that links patient-level molecular profiles to therapeutic decision-making, accelerates mechanism-driven discovery, and augments physical organoid systems. Trained on drug-sensitivity matrices and multi-omics, models forecast single-agent and combination responses, enable rapid in-silico screening and prioritization, and extend discovery to data-sparse indications. Multiscale simulations integrate signaling, cell–cell communication, and biophysical transport to recapitulate development and disease, including immune–stroma–vascular interactions and mechanics–epigenetics coupling. Coupling with organoids and organ-on-chip establishes a data–model–experiment loop for calibration, active experimental design, and automated high-throughput iteration. Ethical, regulatory, and translational dimensions are addressed through privacy preservation, bias mitigation, transparent performance bounds, standardized versioning, and alignment with emerging non-animal testing pathways. In bone research, virtual bone organoids unify material and mechanical determinants, predict scaffold and loading effects, guide experiment selection, and support individualized disease modeling and risk assessment, thereby compressing timelines while improving reproducibility and clinical relevance (Fig. 6).

5.1. Personalized medicine and drug discovery

The development of virtual organs can promote the research and development of precision medicine and drugs. Based on large-scale drug sensitivity data and transcriptome/mutation information training model, a nonlinear mapping between molecular characteristics and drug effects is established [125,126]. On this basis, the genetic mutations, transcriptomes and key clinical indicators of new patients are encoded into characteristic vector input models, and virtual organs can output different drugs and drugs. The expected reaction of the combination is used for the selection of auxiliary treatment plans. Taking pancreatic duct adenocarcinoma (PDAC) as an example, ODFormer inputs the multi-group characteristics (such as gene mutation, copy number variation, transcription expression spectrum) of patient-derived organoids (PDO), and fuses drugs at the same time. Sensitive spectrum and group/cohort information (such as systematic differences caused by different clinical stages, treatment plans or central sources), and learn the mapping relationship of "molecular state-drug response" under a unified framework. Based on this model, in the independent verification cohort, the reaction probability/sensitivity level of commonly used chemotherapy drugs and targeted drugs (such as gecitabine, FOLFIRINOX-related regimens or specific target inhibitors) can be predicted in the independent verification cohort, so as to provide a testable first for individualized drug use. Test the hypothesis. Furthermore, the predictive response model output by ODFormer can be used for treatment-related subtype stratification of patients: different subtypes

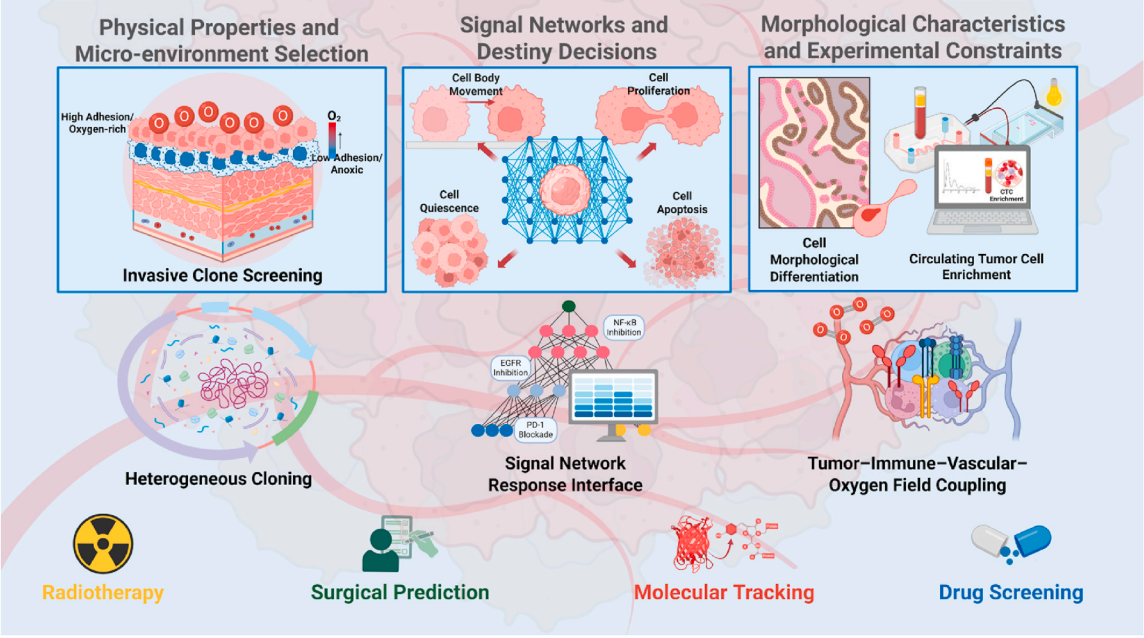


Fig. 5. Schematic diagram of the cross-scale role of virtual tumor cells in virtual organoids and tumor digital twinning.

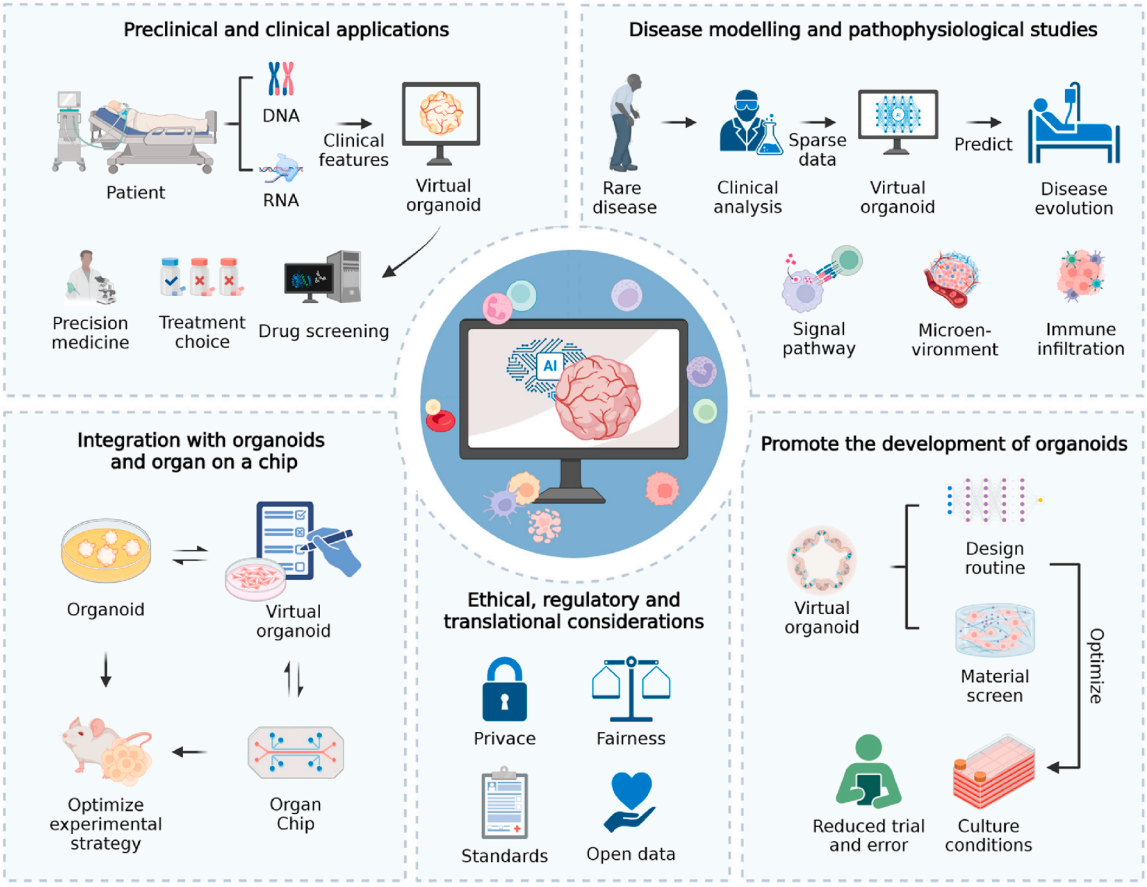


Fig. 6. Application of virtual organoids.

show systematic differences in the drug response spectrum and are related to clinical outcomes (such as progression-free survival, overall survival or risk of recurrence), thus supporting by response spectrum classification - according to The transformation path of type formulation strategy [127,128]. Retrospective analysis shows that the model predicts Highly consistent drug regimens are often accompanied by better survival outcomes, suggesting that the classification and drug recommendations based on virtual organs have practical clinical application potential. Studies on a variety of tumors such as pancreatic cancer, colorectal cancer and rectal cancer have confirmed that patient-based organs are closer to the primary focus than traditional two-dimensional cell lines in terms of tissue structure, molecular characteristics and drug response patterns, and can be used to build drug sensitivity spectrums related to real clinical efficacy, which is proposed for subsequent virtual organ models. Provide high-quality training data [129,130].

However, in the routine clinical process, the large-scale establishment and maintenance of organ-like platforms is expensive and the cycle is long, and it is difficult to carry out systematic screening of the whole drug combination for each patient. The advantage of virtual organs is that once the model training based on organ-multigroup-drug response is completed, the subsequent expansion of candidate drugs, increasing the dose gradient or designing complex joint schemes in the virtual space only needs to increase the amount of calculation, and is no longer limited by the culture time and sample number. Through virtual organs, the screening cycle can be compressed under the premise of maintaining the prediction accuracy, and the overall quality of the candidate for clinical trials can be improved.

In the early stage of drug research and development, virtual organs can also be used for mechanism exploration and lead compound optimization. Researchers can use trained models to conduct in vitro virtual screening of large-scale compound libraries, identify a group of compounds that are highly sensitive to virtual organs of specific molecular subtypes, and then combine methods such as structure-effect relationship analysis, network pharmacology and structural modeling to gradually narrow the scope of candidates. For rare diseases with limited sample size and difficulty in establishing stable organ-like or animal models, the strategy of virtual organ-like is particularly important, because under strict ethical and sample constraints, potential targets and drug combinations can still be systematically explored, providing a more focused direction for subsequent organ-like experiments and early clinical research.

5.2. *Disease modelling and pathophysiological studies*

Virtual organs can not only promote personalized medical care and drug research and development, but also be a multi-scale model platform for understanding the mechanism of development and disease. By integrating the cellular-level signal network, cell-cell interaction and tissue-scale physical processes into a unified framework, virtual organs can reproduce the critical stages of organs from development to disease progression in computers, helping researchers to put forward testable mechanism hypotheses without increasing the experimental burden [11,131].

In terms of development and disease mechanism, multi-scale virtual models have been used to simulate cell stratification, axon guidance and local loop formation in nervous system development, and to analyze the coupling relationship between intracellular signaling pathways, cell polarity and tissue geometry [132,133]. Similar ideas can be extended to the intestines, liver, bones and other organs. Virtual organs reveal the key regulatory nodes behind the abnormal development trajectory through comparative simulation in the state of health and disease, such

as specific genetic mutation, signal inhibition or stress exposure [134]. In some diseases where early phenotypes are difficult to directly observe in vitro, such as some neurodevelopmental disorders or early fibrosis, virtual organs can help judge when and which pathway cumulative changes are most likely to trigger irreversible structural remodeling through extraplating the time dimension.

For rare diseases and cell types that are difficult to culture, virtual cells build a generalizable cell-level basic model by integrating multimodal single cytomics and spatial glomics data, and then superimpose disease-specific disturbances on this basis, so as to build a dynamic model of rare cell groups in a virtual environment. For example, in some rare immunodeficiency or bone marrow diseases, real samples are very limited and the risk of acquisition is high. Virtual organs can further add tissue-level cells-cell interaction and cell migration on top of virtual cells, simulating the process of amplification, migration and conjugence of diseased cells in the microenvironment, and there are very few subsequent Digital in vitro experiments provide more targeted design.

Microenvironment and immune response simulation are another important application direction of virtual organs. The tumor microenvironment often involves immune cell infiltration, angiogenesis, metabolic reprogramming, and the diffusion and removal of drugs in three-dimensional tissues at the same time. It is difficult to fully describe in a single-scale model [135–137]. Virtual organs can couple proxy models with multi-physical field simulation to simulate the spatio-temporal distribution of chemotherapy drugs, immune checkpoint inhibitors or cell therapy in complex tissues, analyze the effects of different immune infiltration patterns, interstitial content and vascular density on efficacy, and predict potential drug resistance paths. This kind of simulation can be compared with the spatial transcriptome and multiple immunostaining results of real organ-like or tissue slices, thus building a more detailed bridge between the in vitro system and clinical pathology.

In addition, physical signals such as substrate topology and stiffness of virtual cells can drive systematic changes in cell morphology, nuclear shape and chromatin configuration. The virtual cell model constructed by Heydari and others can predict the morphological changes of cells and nuclei and the relevant rearrangement of intranuclear structures under different nanometer/micron scale topology conditions. By expanding this idea to virtual organs, mechanical environmental changes at the tissue level and epigenetic and transcription network reconstruction at the cellular level can be traced at the same time in a unified framework, thus helping to explain why the same genetic mutation produces significantly different disease phenotypes in different tissue backgrounds or different mechanical environments [138].

5.3. Integration with organoids and organ on a chip

Virtual organs and physical organs do not replace each other, but form a closed-loop system that is mutually calibrated and mutually strengthened. Solid organs provide high-fidelity experimental data, including organizational structure characterization, multi-group information and drug response curves; virtual organs are generalized and generalized on the basis of these data, and discrete experimental results are transformed into reusable predictive models and mechanism hypotheses [139–142]. Through this iteration of “data-model-experiment”, virtual organs can not only be used to explain existing experimental phenomena, but also reversely guide the design of a new round of organ experiments.

In terms of model verification and calibration, solid organs provide key benchmarks for virtual models. Researchers can systematically obtain data on the survival rate, differentiation status and spatial structure of organs under different drug, doses and culture conditions to train the initial virtual organ model, and then test the drug response, phenotypic transformation or long-term stability predicted by the model in independent organ-like experiments [143,144]. Through continuous comparison and Iteratively correct the model parameters to realize the evolution from empirical fitting to explainable mechanism models. Similar ideas can also be applied to emerging systems such as bone organs and liver organs to improve the prediction ability of complex phenotypes by introducing mechanical stimulation, trophic diffusion and cell-matric interaction into virtual models.

Organ chips provide a controlled bridge between virtual organs and in vivo physiology. The microfluidic control chip can accurately control the fluid shear force, concentration gradient, periodic stress and the spatial distribution of a variety of cell types in vitro, and simulate the microenvironmental characteristics of specific organs. By combining virtual organs with organ chips, on the one hand, we can first explore the impact of different chip structure design and operating parameters (such as channel geometry, flow rate, oxygen tension, etc.) on organ growth and drug exposure in the model, and screen out schemes that are more likely to be stable and close to the state of the body. On the other hand, the continuous imaging and multimodal reading of the chip experiment can be fed back to the model in real time, which is used to update the parameter estimates of the process of substance transport, cell behavior and tissue remodeling.

In terms of scale and automation, virtual organs are expected to become the core decision-making module of intelligent laboratories. By integrating the organ-like chip platform with high-connotation imaging, automatic liquid processing and robot system, a large number of condition combinations can be tested in parallel in a single experiment, and the virtual organ-like model can carry out online learning and active experimental design based on real-time or batch return data, and automatically recommend the most informative in the next round of experiments. Drug combination, dose range or microenvironmental conditions. This high-throughpult platform of virtual-entity collaboration can significantly improve the efficiency of parameter space exploration. It is especially suitable for tasks such as complex combined drug use, dose sequencing optimization and toxicity threshold definition, and is highly compatible with the new methods advocated by regulatory authorities at the technical level.

5.4. Ethical, regulatory and translational considerations

Virtual organs have circumvented the relevant controversies that physical organs may cause to a certain extent, but it does not mean that ethical issues can be ignored. First of all, the construction and application of virtual organs are highly dependent on patients’ multi-group data and clinical information. How to achieve strict privacy protection and data desensitization in the process of model development and sharing is the prerequisite for its entry into clinical scenarios. Secondly, the training data itself often has biases in population composition, medical resources, testing methods, etc. If it is not identified and corrected, the model output may show systematic deviations among different groups of people, thus aggravating health inequality. Transparent disclosure of model training data sources, performance evaluation results and their applicable boundaries is particularly important to avoid over-reliance on technical black boxes in clinical decision-making [145].

At the regulatory level, once virtual organs are used for efficacy prediction, toxicity assessment or clinical decision-making support, they are no longer just scientific research tools, but fall into the regulatory framework of medical AI and medical device software (SaMD). The EU AI Act classifies most medical-related AI systems in high-risk categories, requiring risk management covering the entire life cycle, well-representative training data, complete technical documents and logging, traceability, and necessary transparency and manual supervision. If virtual organs are used to guide treatment choices or safety judgments, in principle, they should be designed in compliance with reference to this level. In the clinical decision support software guidelines and AI/ML SaMD action plan, the U.S. FDA emphasizes regulatory ideas centered on the overall product life cycle (TPLC), including pre-agreed algorithm change plans, continuous performance monitoring after listing, and clear indications and limitations. Define clearly. At the same time, the FDA Modernization Act 2.0 and the subsequent roadmap for reducing

animal experiments integrate advanced in vitro models, organ chips and mechanism-driven or AI-driven computational models into the non-clinical evidence system, and participate in the safety and effectiveness of drugs for virtual organs. Sexual evaluation provides space, but it also means that its development process must meet the engineering standards that can be reviewed and quality controlled.

Corresponding to the requirements of these regulations, the virtual organ-like platform needs to be equipped with a complete set of operable engineering practices at the technical and governance levels. On the one hand, strict version management and audit trajectory are required: every update of model structure, parameters or training data should have a unique version number and detailed records, so that any prediction used for clinical or regulatory declaration can be traced back to specific models and data snapshots afterwards. On the other hand, a standardized bias test process should be established to evaluate the performance of the model in different subgroups (such as gender, age, ethnic group, complication lineage, medical center), and set early warning thresholds that trigger retraining, limit the scope of application or go offline. For high-risk applications (such as individualized drug delivery and toxicity threshold definition), it is also necessary to meet the minimum degree of interpretability requirements, such as providing feature importance analysis, counter-fact examples or simplified proxy models to help clinicians understand which data patterns mainly drive model prediction. Overall, these practices are consistent with the current consensus on trusted medical AI and good machine learning practices (GMLP), but in the virtual organ-like situation, the mapping relationship between virtual experimental settings and physical organ experiments must also be additionally tracked.

At the level of data management, the requirements of multimodal data ecology on which virtual organs rely on FAIR principles (discoverable, accessible, interoperable, reusable) are also different from traditional single-group learning or imaging databases. VO data usually contain both single-cell and spatial comics, high-connotation imaging, three-dimensional mechanics or transmission field simulation results, as well as clinical outcomes and intervention information that change over time. If only the original matrix and image files are uploaded, it is difficult to support real reuse and reproduction. A more reasonable approach is to build a unified identification system at the data level, bind cells, organs, patients and virtual experimental scenes to the same ID space, and use standard ontology to semantically label cell types, anatomical sites, stimulation conditions and reading indicators; at the same time, for the virtual experiment itself (Such as drug delivery scheme, boundary conditions, finite element grid, proxy model rules) Provide machine-readable metadata and version information, so that others can not only reproduce the experimental data, but also play or modify the corresponding simulation experiments locally. In this way, the FAIR principle extends from data sets to the overall constraints on data-model-virtual experimental conditions, laying the foundation for the construction of a shareable and verifiable virtual organ-like ecology.

Economy and fairness are issues that cannot be ignored in the process of promoting virtual organs. The training of high-quality models depends on large-scale sequencing, imaging and computing resources. If they are only concentrated in a few high-resource centers, it may create a digital divide in technology and service access. Therefore, it is necessary to explore the construction of open data sets and open source models, so that researchers in different regions can retrain and calibrate on the basis of local small samples. At the same time, through a variety of business models such as public-private cooperation and pay-as-you-go, part of the proceeds will be used to feed back data contributors and vulnerable groups to avoid virtual organ technology. Art has become a factor that further exacerbates health inequality [146,147].

In the clinical transformation path, the growth of virtual organs from experimental research tools to regulatory and recognized medical-assisted decision-making systems also requires the long-term cooperation of interdisciplinary teams, including clinicians, computational scientists, bioinformatics, ethicists and regulatory experts to participate in model design, evaluation and updating. Only by forming a synergy in terms of reliable technology, clear supervision and social trust, can virtual organs play a sustainable and controllable role in real-world clinical practice.

5.5. Promote the development of organoids

Solid organs reconstruct tissue-specific structures and functions outside the body through 3D self-organization or assembly process, which has been widely used in the intestines, liver, nervous system and other fields. In recent years, bone organs have gradually emerged as a new model for simulating bone tissue development, remodeling and disease. They can be used to study a series of disease processes such as osteoporosis, osteoarthritis, bone marrow disease and tumor bone metastasis, and have shown application potential in drug screening and regenerative medicine. However, compared with soft tissue organs, the construction of bone organs faces more challenges, such as complex cell composition (osteoblasts, osteoclasts, bone marrow matrix, blood vessels and immune cells, etc.), significant mechanical load dependence, and the long-term culture process required for mineralized matrix formation [148–150].

Virtual organs provide an important reference for the development of bone organs. The existing bone remodeling model has been able to predict bone volume changes and fracture healing process under the conditions of given strain distribution, signal molecular concentration and drug action. New bone organ research further attempts to integrate cell-matriate interaction, angiogenesis and local immune response into the three-dimensional in vitro system. The virtual bone organs built on this basis can uniformly represent the biological process of bone tissue with material and mechanical factors, so that researchers can predict the impact of different stent materials, hole structures, stiffness gradients or mechanical stimulation schemes on the formation and maturity of bone organs in the computer, so as to screen out the design stage. Reasonable combination of conditions.

In terms of experimental design and optimization, virtual bone organs can significantly reduce exploration experiments. Bone organs often rely on a variety of materials (such as ceramics, porous metals, polymers or hydrogels) to provide mechanical support and cell adhesion sites. Different pore sizes, connectivity and surface topology will significantly affect the distribution and function of bone cells and vascular endothelial cells. Virtual models based on finite element analysis and cytodynamics can predict the stress, strain and trophic diffusion field inside organs under given load and culture conditions, and thus infer potential bone formation hotspots and bone absorption regions [151].

Researchers can first screen out a number of material combinations and mechanical stimulation parameters in the virtual space, and then carry out key verification on the solid bone organ platform, so as to reduce costs and shorten the cycle under the premise of ensuring scientific rigor.

In terms of disease modeling and drug evaluation, virtual bone organs help to link the photypic reading of bone organs with the course information at the patient's level. Bone organs can reproduce characteristics such as bone loss, trabecular structural disorder or abnormal mineralization in a specific disease state, which is suitable for evaluating anti-osteosorbent drugs, promoting bone drugs and combined treatment strategies. Further combined with imaging (such as μCT) and clinical follow-up data, virtual bone organs can simulate the long-term evolution of bone microstructure under different drug delivery schemes at the individualized level, providing auxiliary information for assessing fracture risk and implant bone integration. Patient samples are used to build individualized bone organs. Organ experiments provide calibration data for virtual models, and virtual models undertake the tasks of large-scale program screening and medium- and long-term result prediction [152].

6. Future perspectives and challenges

6.1. Technical challenges

A central technical challenge for virtual organoids is the completeness, quality and accessibility of the data on which they are built. High-fidelity VOs require large, diverse and well-annotated datasets that capture dynamic cell and tissue states across multiple modalities, including genomes, epigenomes, transcriptomes, proteomes, metabolomes, spatial imaging, clinical information and environmental factors [153]. In practice, data are strongly imbalanced: common cancers such as lung and breast cancer [154] are represented by large cohorts, whereas rare diseases such as high-grade glioma have only small and heterogeneous datasets. Differences in assay platforms and sample handling further limit generalizability. To address these gaps, more comprehensive observational technologies and standardized acquisition pipelines are needed, together with multi-centre collaborations that pool clinical trial and real-world data. Generative models can help augment rare subgroups and fuse heterogeneous sources, but their outputs must be carefully validated.

A second challenge lies in computational efficiency and model scalability. Virtual organoids must simulate the dynamics of many interacting virtual cells while integrating high-resolution single-cell and spatial measurements. As multi-modal and longitudinal data accumulate, naive scaling of current models leads to prohibitively high training and inference costs, which are difficult to sustain in routine research and especially in clinical environments with limited hardware. Future work needs to explore more efficient architectures and numerical schemes that maintain predictive accuracy while reducing complexity, for example through principled dimension reduction and model reduction. High-performance computing and cloud infrastructures will remain important, and in the longer term, emerging hardware such as quantum processors and neuromorphic chips may offer new options for multi-physics and multi-scale simulation at organ or whole-body levels.

A third technical issue concerns model interpretability and trustworthy AI. Many VO implementations rely on deep neural networks that behave as black boxes, making it hard to trace how specific inputs-such as mutations, microenvironmental factors or drug regimens-lead to particular predictions [155]. This lack of transparency can undermine clinical trust and hinder biological insight. Developing explainable AI approaches and causal inference frameworks tailored to VOs will be essential. For example, attribution methods, mechanistically constrained architectures and counterfactual simulations can help reveal which features drive predicted responses. Visual analytics tools that display feature contributions, uncertainty and decision pathways in a user-friendly way will also be important to support communication between data scientists, biologists and clinicians.

6.2. Ethical and societal impacts

Because virtual organoids are often linked to patient-level information, including genomic profiles, medical images and electronic health records, they raise significant questions about privacy protection and data security. VO development must comply with legal and ethical requirements for informed consent, data minimization and secure storage. Technical strategies such as federated learning and differential privacy can help by allowing models to be trained across institutions without sharing raw data, while still capturing population-level patterns. These approaches should be accompanied by clear data-use agreements, consent management interfaces and audit trails that protect patient rights and make data flows transparent.

Algorithmic fairness is another key concern [156]. If training data under-represent certain demographic or clinical groups, VO-based predictions may be systematically less accurate or less safe for those populations, reinforcing existing inequities in care. Ensuring diversity in the underlying datasets and explicitly monitoring subgroup performance are therefore essential. Bias detection and fairness evaluation should be built into the VO development pipeline from the outset, and mitigation strategies—such as reweighting, resampling or group-specific calibration—should be applied where needed. More broadly, the social acceptability of VO technologies will depend on transparent communication about their capabilities and limitations, and on realistic framing of their role as decision-support tools rather than autonomous decision makers.

Regulation and public engagement will play a decisive role in how VOs are deployed. Clinicians, researchers, regulators and patient groups will need to develop a shared understanding of what constitutes a “safe and effective” VO in different contexts, such as preclinical drug testing versus bedside decision support. Educational efforts aimed at both professionals and the wider public can help manage expectations and foster informed debate about benefits and risks. In parallel, regulatory bodies will need to update existing frameworks for medical devices and AI tools to cover VO-specific issues such as continuous model updating, data provenance and responsibility sharing when model outputs influence care.

6.3. Standardization and collaboration

The development and deployment of virtual organoids are not limited by algorithms alone, but also by the lack of common platforms, tools and standards. At present, many studies focus on optimizing models for a single dataset or data type, and there are relatively few efforts to create interoperable infrastructures that connect datasets, models and analysis workflows across groups and borders. Building open platforms for data and model sharing will be crucial. Such platforms could host curated multi-modal VO training datasets, standardized benchmarks, and reference implementations of key models, lowering the barrier to entry and enabling reproducible comparison of methods.

Cross-disciplinary alliances are equally important. Effective VO research requires input from organoid biologists, clinicians, computer scientists, engineers, ethicists and legal scholars. Formal consortia and networks that bring these communities together can accelerate progress, for example by defining common use cases, harmonizing protocols and coordinating multi-centre validation studies. Public–private partnerships involving academia, hospitals, industry and non-profit organizations can support the development of robust, clinically relevant VOs while addressing issues of cost, access and sustainability. At the international level, coordination with regulatory agencies and standards bodies will be needed to define how VOs are certified, validated and monitored in practice. This includes setting minimum requirements for data quality, model documentation, performance reporting and post-deployment surveillance. Common guidelines for VO use in drug development, precision oncology and public health could help align expectations across jurisdictions and simplify the pathway from research prototypes to widely adopted tools.

6.4. Future directions

Looking ahead, several directions appear particularly promising for virtual organoids. One is the interconnection of VOs representing different organs and systems, moving towards virtual humans that capture cross-organ interactions, systemic treatment effects and off-target toxicities. Such multi-organ frameworks could support the design of combination therapies and help anticipate adverse events that are not apparent in isolated organoid or organ-on-chip models.

Another direction is the integration of VOs with emerging technologies [157]. Advances in quantum computing, synthetic biology, nano-robotics and advanced sensing may enable more detailed and more efficient modelling of complex, multi-physics phenomena such as electromechanical coupling, immune–tumour interactions and drug distribution in heterogeneous tissues. Combining generative AI with active

learning can help expand training data in low-resource settings and guide experimental design by identifying the most informative organoid or VO experiments to perform next.

Finally, future VO systems are likely to move from pure prediction towards intervention. In a closed-loop setting, VOs could be updated in near real time with new measurements and used to recommend adjustments to drug dose, treatment schedule or culture conditions, which are then applied and fed back into the model. Such "mixed reality" workflows, in which virtual and physical organoids co-evolve, could support personalized dosing, nutritional interventions and long-term health management. Realizing these scenarios will require not only technical advances, but also robust ethical and regulatory frameworks and sustained international collaboration. If these conditions are met, virtual organoids have the potential to become a central tool in future drug development, disease research and precision medicine.

7. Conclusion

VOs mark a decisive transition in organoid research from physical constructs to digital, intelligence-enabled systems. By integrating multi-omics, imaging, and clinical data with machine learning, generative AI, and mechanistic models, VOs show substantial promise for drug discovery, disease modeling, and precision medicine. Fulfilling this promise will require advances in data quality, model transparency, computational efficiency, and ethical governance, alongside cross-disciplinary collaboration and internationally harmonized standards, to convert the potential of this emerging field into tangible scientific and clinical gains.

CRediT authorship contribution statement

Long Bai: Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Jiacan Su:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Ethics approval and consent to participate

Confirm that no ethical issues are involved.

Declaration of competing interest

Long Bai is an early career editorial board member for Bioactive Materials and was not involved in the editorial review or the decision to publish this article.All authors declare that there are no competing interests. All authors declare no conflict of interest.

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