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Perspective

hiPSC-Driven Organoid Construction and Application Prospects

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Abstract: Induced pluripotent stem cell (iPSC)-derived organoid platforms can simulate various target tissues and hold broad application prospects in personalized medicine, disease modeling, drug screening, organ transplantation, and understanding organ development mechanisms. Currently, the development of human iPSC (hiPSC) organoids is gradually shifting towards Matrigel-free and scaffold-free systems, promoting precise control over the composition and structure of these systems and establishing induction protocols for specialized organoids. Researchers are also exploring the construction of multifunctional systems with complex structures and material exchange channels through vascularization, segmented induction, and assembly technologies, though further breakthroughs are needed. In the future, hiPSC organoids are expected to advance towards personalized precision treatment, high-throughput module detection systems, multi-organ integration, and automation. Additionally, when combined with large artificial intelligence models, there is potential to establish hiPSC data and medical platforms, providing support for drug development and clinical decision-making. Moreover, the development of medical AI is anticipated to foster collaboration rather than competition, promoting coordinated growth in the field. For hiPSC-derived platforms, it is crucial to further enhance the ethical review framework to balance radical scientific exploration with conservative public attitudes. Researchers must also optimize or develop new induction protocols to reduce genomic instability and tumorigenic risks, while avoiding the emergence of non-target cells and insufficient functional maturity.

Keywords: hiPSC; organoid; multi-organ integration; vascularization technology; AI; personalized medicine

1. Introduction

Induced pluripotent stem cells (iPSCs) are a type of cell obtained by reversing somatic cells through genetic reprogramming technology, capable of differentiating into almost all types of cells in the body. Organoids are millimetre-scale tissue models formed by the self-organization of stem cells under specific culture conditions, possessing structures and partial functions similar to those of organs, enabling researchers to study organ development, disease mechanisms, and therapeutic responses in a controlled environment. Utilizing human iPSCs (hiPSCs) for organoid construction presents several significant advantages over traditional embryonic stem cells (ESCs). One of the primary benefits is the avoidance of ethical concerns associated with the use of embryos.

Furthermore, hiPSCs can be derived from easily accessible adult tissues, enhancing their availability for research and clinical use. Another critical advantage of hiPSCs is their potential to minimize immune rejection issues. Since hiPSCs can be generated from a patient's own cells, the resulting organoids are more likely to be immunologically compatible with the patient, paving the way for personalized disease modelling and targeted treatment strategies. This individualized approach not only improves the efficacy of therapies but also reduces the risk of adverse reactions, ultimately advancing the field of regenerative medicine and personalized healthcare [1].

Except for the central nervous system (CNS), most human organs consist of parenchyma, extracellular matrix (ECM), capillaries, and peripheral nerves. In certain simpler organs, such as cartilage, the cornea, and the epidermis, the parenchyma and matrix are closely integrated. The parenchyma is a specialized structure composed of functional cells organized to perform the physiological functions of an organ. The ECM provides essential structural support for the parenchyma, facilitating signal transmission between cells and participating in processes such as cell growth, migration, and differentiation. Capillaries, formed by a single layer of endothelial cells, create thin walls that facilitate the exchange of gases, nutrients, and waste products. At the capillary ends, endothelial cells are loosely arranged to form pores, which play a critical role in blood storage and immune cell function. These capillaries are prevalent in organs such as the spleen, liver, and brain. Additionally, the axons of neurons are interwoven, protected by Schwann cells and satellite cells, which together form bundles that constitute the peripheral nerves. Consequently, achieving the unique spatial organization and arrangement of diverse cell types within an organ, along with the construction and maintenance of the matrix, cannot be accomplished solely through the use of multipotent or unipotent stem cells. This complexity underscores the necessity of employing hiPSCs and multi-step differentiation protocols. And to achieve a complex material exchange architecture, researchers need to further focus on vascularization and assembly.

Traditional two-dimensional (2D) cultures fall short in replicating the complex three-dimensional (3D) structures of tissues found in the body. The growth patterns of cells on a flat surface differ significantly from their native environments, often leading to behavioral distortions, such as altered gene expression and abnormal functions [2]. Additionally, these 2D cultures lack the regulatory interactions between the ECM and cells. In contrast, organoids provide a more accurate simulation of real organs' 3D structure and organizational hierarchy. They can replicate the body's microenvironment by incorporating various cell types and ECM components, and they can exhibit some organ-specific functions, such as secretion and metabolism, through functional cell partitioning. Primary tissues also come with challenges, including difficulties in obtaining samples, limited proliferation capacity, and high cell heterogeneity. Traditional Organ-on-a-Chip (OOC) systems, which are essentially multi-channel 3D microfluidic cell culture platforms, typically focus on a single cell type and function, making it challenging to strike a balance between simplicity and physiological complexity. Combining organoid technology with OOC systems holds great promise for advancing multi-cascade organ platforms. As micro-physiological systems, organoids serve as a robust platform for simulating the characteristics of human organs and tissues in an in vitro setting, closely resembling the physiological complexity of the human body more accurately than animal models [3]. Additionally, the enhanced reproducibility and consistency of organoids facilitate more reliable data analysis and the evaluation of results in high-throughput screening, thereby advancing research and therapeutic applications.

The development of iPSC-derived organoids has been driven by key technological breakthroughs across multiple fields, enhancing their complexity, functionality, and applications in disease modeling, drug screening, and regenerative medicine [4]. For example, single-cell sequencing can track the origins and differentiation pathways of different cell types in organoids, analyze the composition and state of cells, and reveal key regulatory mechanisms in development and disease processes [5]; bioprinting technology can also precisely control the spatial distribution of cells and biomaterials, improve the structural complexity and functionality of organoids, and support the cultivation of larger and more complex organoids through internal scaffold structure optimization [6]; microfluidic systems can provide continuous nutrient supply and improve waste discharge efficiency, and also simulate mechanical forces in the body to affect cell development behavior; gene editing technology can not only improve the efficiency and safety of reprogramming, but also enhance the specific functions of organoid cells, such as the corresponding anti-hypoxia ability, which is of great significance for increasing the size of organoids; machine learning algorithms can help analyze a large amount of experimental data, assist in optimizing experimental conditions, and even predict results. This article investigated the technical methodologies, application potential, and challenges of using hiPSCs for organoid preparation. It also highlight the prospects of hiPSC-derived organoids in fundamental research and clinical translation. The relevant explanation and summary was summarized in Figure 1 [7].

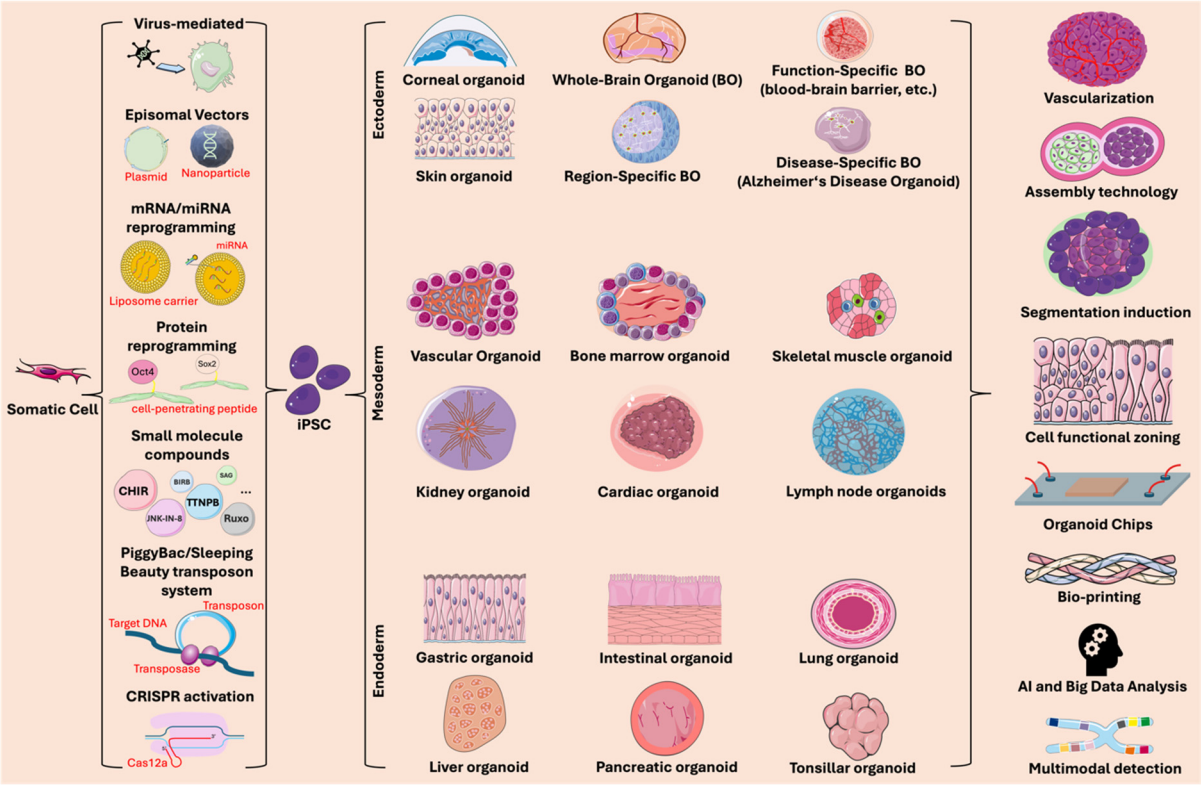

Figure 1. The induction methods, classifications and development of hiPSC-derived organoids. Currently, the predominant method for generating iPSCs remains the use of viral vectors. This approach typically involves the introduction of key transcription factors, such as Oct4, Sox2, Klf4, and c-Myc, into cells via RNA viruses (Sendai virus), retroviral or lentiviral systems. Non-viral vector methods have also been developed, including plasmid transfection, transposon systems, and mRNA/miRNA transfection, which offer alternative pathways for reprogramming. Additionally, CRISPR technology has emerged as a powerful tool to enhance both the efficiency and safety of the reprogramming process. However, there is a notable lack of evidence or supporting reports to support the safety and efficiency of protein transduction methods for generating iPSCs. Meanwhile, the use of small molecule compounds remains largely auxiliary, primarily employed in conjunction with other reprogramming methods to boost efficiency and potentially reduce the number of required transcription factors. But the prospect of developing induction systems that rely solely on small molecules is indeed very appealing for the future.

2. Materials in hiPSC-Driven Organoid Construction and Development Trend: Matrigel-Free and Scaffold-Free System

Matrigel is a matrix extract derived from mouse tumors, rich in various ECM components, including collagen, laminin, fibronectin, and glycoproteins [8]. It also contains essential growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor (TGF), which facilitate the attachment of hiPSCs and help maintain their undifferentiated state [9]. Matrigel is a temperature-sensitive gel that remains liquid at 4 °C and transitions to a gel-like consistency at 37 °C, allowing for straightforward organoid preparation by simply heating the droplets to 37 °C and incubating for 10 to 15 min (Matrigel dome). In recent years, research on the preparation of hiPSC-derived organoids using Matrigel has expanded to include various tissues such as the heart [10,11], liver [12], intestine [13,14], mammary [15], blood vessels [16], and spinal cord [17]. However, these systems often lack the specific mechanical properties and inducible capacities necessary to optimize functionality, leading researchers to supplement them with additional proteins or compounds. Moreover, the incorporation of animal-derived components in clinical applications raises significant ethical and safety concerns, necessitating the development of xeno-free alternatives [8].

Researchers are actively exploring alternatives to Matrigel for constructing organoid architectures. They are utilizing mechanical devices [18,19] to promote hiPSC self-assembly and experimenting with other materials such as GelMA [20], alginate [21], collagen [22,23], gum Arabica [24], agarose [25], polyethylene glycol (PEG) [26], polycaprolactone-bisurea (PCL-BU) [27], and synthetic self-assembling peptides [28]. Additionally, they are leveraging microwell geometries—manipulating curvature and resolution—to facilitate the formation of organoids from hiPSCs [29]. The development of precisely engineered, xeno-free microenvironments and controllable

engineered ECM (EECM) using artificial or natural materials is crucial for advancing personalized organoids. For instance, the elastic modulus of neural tissue ranges from approximately 0.1 kPa to 10 kPa, significantly exceeding that of Matrigel, which is only 3–20 Pa [30]. The mechanical properties of the microenvironment influence receptor perception, such as integrins, and appropriate stiffness is essential for supporting the growth, differentiation, and function of nerve cells [31]. Suitable EECM can enhance neurogenesis and glial maturation over extended periods, with reports indicating durations of nearly seven months [32]. When hiPSCs are encapsulated in Matrigel, the organoids tend to aggregate due to shear stress during long-term culture, leading to a gradual loss of structural integrity and functional variation. Furthermore, certain proteins abundant in Matrigel are not essential for the maturation of spinal cord organoids and may even prompt cells to express non-specific markers, such as forebrain and midbrain markers like FOXA2, FOXG1, and OTX2, rather than maintaining spinal cord-specific markers like homeobox genes and choline acetyltransferase (ChAT)-related genes [21]. This may occur because laminin can indirectly influence hiPSC differentiation through the PI3K/AKT pathway, while type IV collagen, nestin, and heparan sulfate proteoglycans regulate FOXA2, FOXG1, and OTX2 expression via Wnt and BMP pathways. Additionally, growth factors such as TGF, EGF, and FGF may activate related markers through the MAPK/ERK pathway. Consequently, EECM offers several advantages over Matrigel, including lower cost, standardized production suitable for high-throughput screening, controllable and transparent ingredient profiles devoid of animal-derived components, and adjustable mechanical properties with potential for further modification and optimization. Therefore, exploring EECM can focus on utilizing polymer materials in conjunction with recombinant proteins or functional peptides. This exploration should also emphasize the development of scaffolds with complex spatial structures and dynamic response capabilities that can self-regulate their mechanical properties or chemical signal release.

Different EECM combinations can effectively enhance the differentiation and functionalization of hiPSCs. For instance, Zeleniak et al. encapsulated hiPSCs in sodium alginate microspheres with a diameter of approximately 1000 μm [33]. Within these microspheres, the cells developed into hiPSC aggregates measuring around 50 μm. Subsequently, these aggregates were induced to differentiate into thymic epithelial progenitor cells. These differentiated progenitor cells were then injected into a decellularized mouse thymus scaffold, along with $CD34^+$ hematopoietic stem cells, to generate thymic organoids. This innovative organoid model successfully simulates the crosstalk between epithelial cells and T cell progenitors, providing a supportive environment for the development of early T cells within the thymic microenvironment in vitro. This construction strategy can also be applied to pancreatic islet organoids [34]. However, the goal of establishing a fully functional and self-tolerant adaptive immune system remains a significant challenge. Currently, the in vitro products are primarily thymic epithelial progenitor cells. To obtain functional thymic epithelial cells, these organoids must be transplanted into the body and cultivated for several months [35]. At the same time, for organoid systems, the addition of EECM should be purposeful and strategically designed to enhance the function and performance of the product, rather than being added arbitrarily. Lee et al. found that when using hiPSCs to generate lymphatic endothelial cells for wound repair, the use of the OP9 feeder layer system had advantages in differentiation efficiency and cell survival rate over the gelatin system containing the same lymphatic growth factor and epidermal growth factor [36].

The design with higher bionic requirements is the scaffold-free system. Here, scaffold-free means that the final product does not contain artificially prepared EECM, not that EECM is not used in the entire preparation process. Relying on hiPSCs self-assembly, the complex spatial structure of organoids is difficult to accurately control, and without the assistance of material mechanical properties or other dynamic response regulation, it is difficult to achieve physiological or pathological processes such as vascularization [37], immune microenvironment simulation [38] and mechanical stress response [39] by relying on cell-cell self-regulation or chemical regulation of the culture environment. Therefore, this application direction aims to utilize EECM to provide biophysical and functional support during the early stages of hiPSC organoid formation. Subsequently, the scaffold can be gradually degraded to promote hiPSC self-assembly or facilitate the construction of their own secreted ECM, simulating the in vivo environment. This approach helps to avoid biocompatibility and degradation issues associated with materials in the human body, significantly enhancing the potential for clinical translation.

It is important to note that when constructing an organoid capable of building its own ECM environment, using collagen, hyaluronic acid, fibrin, or decellularized ECM as preliminary scaffold components may not be appropriate. These materials are intrinsic to the cellular ECM environment, and the enzymes required to dissociate them could potentially damage the target organoid. Therefore, alternative materials such as sodium alginate (which can be degraded through metal complexation), thermosensitive hydrogels (including poloxamers [40,41], PNIPAM [42], block copolymers [43,44], and blends of chitosan and sodium glycerophosphate [45]), and photosensitive polymer hydrogels should be considered for scaffold construction. The schematic diagram was shown in Figure 2i.

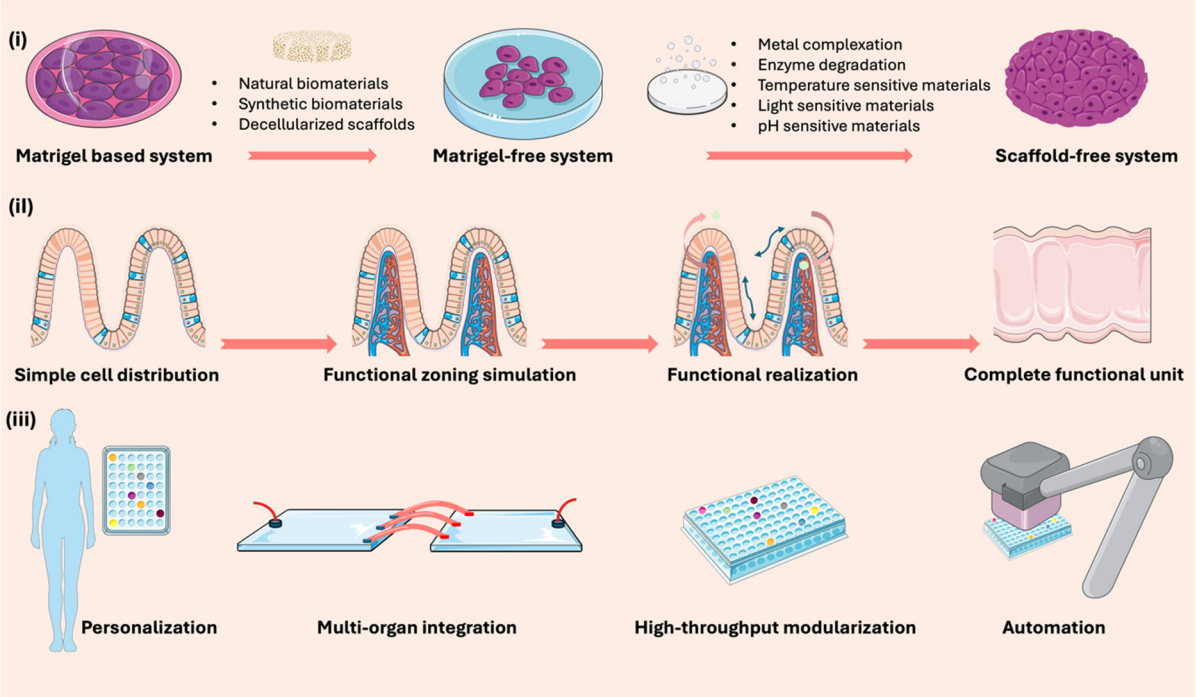

Figure 2. Schematic diagram of material development, structural development and technical direction of hiPSCs-derived organoids. (**i**) The development trend of material systems; (**ii**) The idea of constructing organoids, starting with the imitation of simple cell distribution, followed by inducing vascularization and segmented induction of cells with different functions, followed by the ability to reproduce some functions of the target organ in vitro or in vivo. The ultimate goal is to construct a complete functional unit with a complex structure and the synergy of multiple cell types; (**iii**) Technological development trends of hiPSC-derived organoids.

3. Innovations in the Construction of hiPSCs-Derived Organoid Structures: Vasculature and Cell Functional Zoning

hiPSCs can be utilized to generate more complex organoid systems derived from multiple germ layers through a multi-step differentiation process. This simulation of organ development provides a valuable platform for studying developmental biology, with the ultimate aim of creating organoids suitable for clinical transplantation to address organ shortages. Tissue interactions—such as those between blood vessels and epithelial tissues, and among functional cells in different compartments—are crucial for organ formation [46]. However, current organoids often resemble stem cell spheres and lack intrinsic functional vascular or compartmental structures. The widely used hiPSC-derived organ buds typically require transplantation into animal models to achieve vascularization and functional maturation, which necessitates stimulation by host hemodynamic [47,48]. Currently, directly regulating the zoning of stem cell clusters to induce regenerative functions is not feasible. Instead, a more common approach involves sub-packaging and integration: hiPSCs are separately induced to form their own cell clusters, which are then integrated to construct complex structure organoids. This method facilitates the assembly of diverse cell types and enhances the functional capabilities of the resulting organoids [49].

Recent breakthroughs have demonstrated the successful generation of bile ducts within liver organoids using hiPSC-derived progenitors [23]. In this method, collagen is combined with hiPSC-derived liver progenitors to create precursors with tubular structures, which are then coated with liver organoid sheets composed of hiPSC-derived liver endoderm cells, mesenchymal cells, and endothelial cells. After three weeks, liver progenitor cells differentiate into bile duct cells, acquiring essential epithelial characteristics, such as cell-cell connections, microvilli on the apical membrane, and secretory functions. The integrated organoid also exhibits the capability to alleviate symptoms associated with cholestatic injury, highlighting its potential for therapeutic applications. Some researchers also induced hiPSC-derived hepatoblasts to differentiate into hepatocytes and bile duct epithelial cells, and then coated them with mouse embryonic fibroblasts to promote the maturation of bile duct epithelial cells, constructing a structure in which hepatocytes are covered by bile ducts, and achieving the flow of bile from hepatocytes through the bile canaliculi into the bile ducts [50]. Future research can concentrate on integrating Notch signaling regulation [51] with system construction methods, alongside the stimulation of fibrosis-related cytokines [52] and inflammatory factors [53].

Brain organoids are increasingly used to simulate brain development and diseases, necessitating the incorporation of functional vascular structures [54]. Current in vitro bio-engineering methods include: co-culturing (brain organoids are co-cultured with vascular or mesodermal spheroids to induce vascular invasion) [55]; endothelial cell mixing (blood vessel regeneration is promoted by mixing hiPSCs with endothelial cells) [56]; vascular endothelial growth factor (VEGF) stimulation (adding VEGF can induce stem cells in embryoid bodies to differentiate into endothelial cells and enhance vascularization [57], and microfluidic technology can also be employed to regulate VEGF levels [58]); ETS variant 2 (ETV2) overexpression (inducing the overexpression of ETV2 in stem cells can further promote vascularization) [54]. ETV2 is an essential transcription factor that plays a pivotal role in endothelial cell development and angiogenesis. It regulates the expression of genes critical for blood vessel formation. VEGF, a key signaling protein, drives angiogenesis by enhancing endothelial cell proliferation, migration, and the formation of new capillaries. Microfluidics technology enables precise control over the physical microenvironment of cultured cells at the microscale, replicating dynamic physiological conditions. Current technologies have successfully reproduced critical processes such as neural tube angiogenesis, the formation of neurovascular unit-like structures, and the establishment of early barriers in vitro [59]. The current bottleneck in the development of brain organoids is that blood vessels invading from the outside are unable to form a neural vascular plexus within the core cell group. Additionally, blood vessels that are induced and differentiated internally often lack functionality. The construction of the blood-brain barrier (BBB) also necessitates the stimulation of physiological flow, and the deficiency in effective vascularization has further complicated advancements in BBB-related research [60]. Therefore, it remains essential to explore whether short-term perfusion can effectively induce the necessary vascular structures and functionality within brain organoids.

The vascularization scheme for pancreatic islet organoids can draw inspiration from the design by Quintard et al. [61]. In this approach, a mixture of human umbilical vein endothelial cells (HUVEC) and fibroblasts is placed into a fibrin hydrogel, which is then injected into a serpentine geometry chip. The chip features a recessed trap designed to capture pancreatic islet organoids, effectively confining the organoids that are pushed by the gel to a specific position. After allowing the system to gel, an I-shaped structure is formed, with the organoid positioned in the central pipeline and both ends containing the HUVEC-fibroblast mixture. This design enhances endothelial network formation under dynamic perfusion, promoting the differentiation of the cells into vascular-like structures, thereby improving the integration and functionality of the pancreatic islet organoids.

The kidney is indeed a highly vascularized organ, and when hiPSC-derived kidney organoids were first reported in 2015, the primary focus was on achieving morphological maturation and extensive vascularization [62]. Currently, the differentiation of nephrons and collecting ducts has been successfully accomplished, but the goal remains to create an organotypic kidney structure that includes nephrons, collecting ducts, ureters, ECM, and vascular flow within a single organoid [63,64]. Recent research efforts have concentrated on enhancing vascularization and structural maturation through the use of induction factors [65], EECM scaffolds [66], and microfluidic technologies [67]. Existing platforms have been effective in simulating early-onset diseases that affect glomeruli and renal tubules [68]. A significant area of discussion in the field is the integration of induction schemes for renal progenitor cells and branched ureteric buds (precursors of the collecting ducts). Researchers aim to simulate the developmental stages of human kidneys to achieve complex structures with mature functional partitions in organoids. This challenge underscores the reasons why kidney organoids have developed more slowly compared to other organoid systems. Kidney regeneration necessitates the induction of over 20 different cell types, each playing a vital role in regulating pH, metabolites, and the water-electrolyte balance, as well as participating in filtration, absorption, and secretion processes during excretion [62]. The functionality of these cells is highly dependent on their precise arrangement and interactions within complex three-dimensional structures. In contrast, while the liver and brain also consist of diverse cell types, their functions often rely more on the collective behaviour of predominant cell types—such as hepatocytes in the liver and neurons in the brain—rather than on strict structural arrangements. This difference in functional dependence has driven the next wave of innovation in organoid research: achieving complex functional partitioning among multiple cell types.

For such a system, the diversity of cell types differentiated from hiPSCs is ensured, integrated with an in vitro perfusion system, functional biomaterials, and 3D printing. Subsequently, growth factors, cytokines, or mechanical stimulation are introduced to achieve a biophysical simulation of the microenvironment [69]. Related attempts can begin with simpler tissue types, such as cartilage structures that lack blood vessels and nerves, or corneas without vascularization. Recent reports highlight several advancements, including the creation of repair materials with a compressive modulus similar to that of natural cartilage by combining hiPSC-derived cartilage with 3D printing [70]; the preparation of cartilage by using GelMA loaded with hiPSC-derived chondrocytes and coupled with microenvironment regulation [71]; and the use of thermosensitive hydrogels combined with hiPSC-derived human corneal endothelial cells to participate in corneal repair [72]. The current challenges that need to

be addressed include the potential production of non-target cell types, such as osteoblasts or adipocytes, during the differentiation of hiPSCs into chondrocytes. Additionally, there are issues related to the integration of the construct with the surrounding host tissues. ECM components secreted by the cells, such as type II collagen and proteoglycans, may not be as abundant as those produced by natural chondrocytes. Furthermore, the long-term efficacy of the repair remains unclear. The hiPSC-derived cornea also faces significant tissue integration challenges [73]. The induced differentiated cells struggle to connect tightly and secrete properly, which hinders the orderly arrangement of collagen fibres. This deficiency greatly compromises the barrier function and transparency of the organoids. Currently, there are no reports on the effective construction of the epithelium, stroma, and endothelium. Additionally, the innervation of the cornea is crucial for the functionality of corneal organoids, yet current technologies have not achieved this essential physiological input [74]. Future research may focus on the integration of neural, immune, and various cell types, as well as exploring the use of patients' own hiPSCs to treat conditions like limbal stem cell deficiency (LSCD) [75].

For bone marrow, which exhibits higher tissue complexity, Klein's team employed a segmented induction method to differentiate hiPSCs into hematopoietic cells, mesenchymal cells, and endothelial cells [76]. The resulting bone marrow organoids comprised 39.3% hematopoietic cells, 41.3% mesenchymal cells, 6.0% endothelial cells, 1.42% hematopoietic stem/progenitor cells, and 0.96% mesenchymal stem/progenitor cells. These organoids displayed vascular, hematopoietic, and mesenchymal structures akin to those found in natural bone marrow. Notably, $CD45^+$ cells were embedded within a network of $CD31^+$ vascular structures and $CD271^+$ stromal cells, reflecting the complex architecture of native bone marrow. The development of hiPSC-derived organoids for the digestive system has successfully resulted in the differentiation into specialized cell compartments. hiPSC-derived organoid development in the digestive system has successfully achieved the differentiation of specialized cell compartments. For instance, hiPSCs were differentiated into foregut and hindgut spheroids, which were then chimerized to simulate the continuous patterning and dynamic morphogenesis of liver, bile duct, and pancreas structures. These constructs evolved into distinct multi-organ primordia [77]. Additionally, hiPSCs were differentiated into mid/hindgut spheroids, which were subsequently combined through low-speed centrifugation to create an hiPSC-derived human vagus nerve system, effectively integrating the enteric nervous system [78]. Breakthroughs in recent years have been the generation of organ-specific mesenchyme [79] and stem cell integration [80–82]. It is a pity that current research on intestinal organoids often emphasizes physiological and pathological aspects—such as nerves, microorganisms, pathogen-host interactions, immune regulation, and inflammation—while neglecting the crucial mechanisms of intestinal development [83] and in vitro reproduction of intestinal epithelial cell functions. Exploring the mechanisms of hiPSC differentiation into different intestinal epithelial cell types (such as absorptive cells, goblet cells, and Paneth cells), identifying the signaling pathways and cell fate determination encountered during development, and studying the regeneration mechanisms and potential targets of intestinal epithelium after injury are, instead, the direct and effective methods for clinical translation.

There are several types of region-specific brain organoids. Cortical organoids achieve a preliminary simulation of cortical stratification, including the distribution of deep and superficial neurons [84]. Midbrain organoids, rich in dopaminergic neurons [85], are powerful tools for modelling late-stage Parkinson's disease [86,87] and exploring treatments for sporadic progressive supranuclear palsy [88,89]. Hippocampal organoids, divided into cornu ammonis (CA) and dentate gyrus (DG) types [90], are effective in investigating Alzheimer's disease and schizophrenia [91]. Thalamic organoids serve as important models for understanding nuclei-specific development [92,93]. Cerebellar organoids serve as models for studying electro-physiological properties and neural network activity, enhancing our understanding of the human body's role in motor coordination, learning, and memory [94]. Research on hypothalamic organoids focuses on the regulation of the endocrine system, human metabolism, appetite, and energy balance, shedding light on the mechanisms underlying these processes and their connections to metabolic diseases like obesity and diabetes [95]. Additionally, some studies explore behavioural regulation, emotions, and physiological responses [96]. Striatal organoids from the basal ganglia are important tools for investigating mental illnesses, including addiction, autism, obsessive-compulsive disorder, Tourette syndrome, and Huntington's disease [90].

Regarding the establishment of functional zoning, the current challenge lies in addressing the problem of multiple specific neuron types [97]. Taking the hypothalamus as an example, the internal neuron types include those that promote appetite, suppress appetite, regulate energy balance, respond to stress, control biological rhythms, and support reproduction. It is crucial to develop differentiation protocols for neurons with distinct functions [98]. Once differentiation is achieved, rational assembly of these neurons is necessary, which requires a comprehensive understanding of the shaping and developmental time windows of neural networks at various stages of brain development, including both environmental and endogenous factors. Ultimately, this brings us back to a

fundamental point similar to that in intestinal organoids: the driving force behind organoid research is the understanding of organ development, rather than the function of scientific research products.

Therefore, at present, organoids lag behind in terms of cell functional division and cannot compare with animal models. While organoids have made significant strides in mimicking the structural and cellular complexity of human tissues, they still face limitations in achieving the precise spatial organization, functional maturation, and intercellular interactions observed in vivo. However, with the advancement of assembly technology, bioprinting technology, and gene editing technology, researchers will have a deeper understanding of the overall biological responses and systemic interactions, and the technological singularity will also come. The schematic diagram was shown in Figure 2ii.

4. hiPSC-Driven Organoid Platforms: Personalization, High-Throughput Modularization, Multi-Organ Integration and Automation

Drugs used in clinical practice are widely adaptable, often with relatively few side effects and good accessibility. However, this adaptability can lead to unpredictable treatment outcomes and time-consuming drug testing due to individual differences among patients. The development of 3D organoids based on specific diseases holds great potential for drug screening, evaluating drug toxicity, and even integrating existing drugs to enhance efficiency. This approach is a key driving force behind "off-the-shelf" manufacturing strategies [38]. Takeuchi et al. used patient pancreatic ductal adenocarcinoma (PDAC) to integrate hiPSC-derived mesenchymal and vascular endothelial cells to create fused pancreatic cancer organoids (FPCO) in an air-liquid interface to study the recurrence treatment of PDAC [99]; Wang et al. prepared hiPSC-derived CAR T cells with a traditional T cell phenotype ($CD8\alpha\beta^{+}$) through an organoid platform, which had stronger cytotoxic killing and Th1 cytokine secretion activity than the innate-like phenotype hiPSC-CAR T cells ($CD8\alpha\alpha^{+}$) generated by the traditional extrathymic culture system [38]; the organoid platform can also be used to verify the treatment of tumor immune escape based on CAR-NK cells differentiated from hiPSC [100]; the researchers also tried to use collagen-Matrigel to coat hiPSC-derived bone marrow organoids to simulate TGFβ-induced bone marrow fibrosis in leukaemia and screen for suitable inhibitor drugs such as SB431542 (TGFβ superfamily type I activin receptor inhibitor) and JQ1 (BET bromodomain inhibitor) [101]. There are also cases of exploring personalized viral infections based on organoids, providing general guidance for drug testing and transplantation therapies [102–104]. In particular, after the outbreak of SARS-CoV-2, there have been cases of exploring viral mutations and host responses based on hiPSC-derived organoids (heart, alveoli, cortex, intestine, etc.) [105–110].

To achieve clinically relevant drug discovery and in vitro models of human diseases, two key requirements must be met: high-throughput module detection and the simulation of the actual physiological environment as closely as possible. These requirements correspond to two technical approaches: organoid chips and multi-type organoid integration. Organoid chips require an initial design of the geometry, channel layout, and chamber structure of the microfluidic chip based on the simulated object. Subsequent innovations focus on creating physiological structures rather than merely arranging geometric space channels. This includes microfabrication technology that simulates physiological submicron structures, which encompass the regulatory relationships between cells, and between cells and the ECM. Additionally, chemical modifications of the inner surface can simulate tissue interfaces through protein or cell membrane structures. There is also a need for sensor detection of multiple parameters or detailed indicators, along with simulations of fluid dynamics environments, such as blood, lymph, bile flow, breathing, intestinal peristalsis, and joint movement. Applications can also be expanded from drug metabolism to the effects of environmental toxins, health products or cosmetics on the human body, thereby expanding commercial influence. In terms of materials, we can also try to go deeper into silicon-based materials, using lithography techniques such as electron beam, nanoimprinting, femtosecond, etching and other micro-processing techniques combined with nanoparticles to simulate real physiological conditions.

Organoid chips can also be integrated into multi-organ systems to simulate the interactions and system-level physiological functions of multiple organs on a single platform, communicate with each other through a perfusion system, and simulate blood flow and material exchange [111]. For macroscopic assembly and integration, the interface can be considered as a simulation of a multi-organ cascade. For example, the gas-liquid interface and tissue-liquid interface in the culture system, or the use of centrifugation and other methods to assemble different organoids to construct a tissue-tissue interface, and induce vascularization to achieve close connection and functional integration between different tissues. The brain-gut axis model can be built by combining nerves and intestines, and the in vivo metabolism of drugs can be simulated through the liver-gut-kidney cascade. There are also similar immune tissue-tumor interfaces and lung-heart interfaces to explore the systemic pathological effects of viral infections. These can be combined arbitrarily to adapt to different functional evaluations.

In recent years, there have been notable advancements in multi-organ combinations and coupling. For example, Koenig et al. successfully utilized closed microfluidic circuits to connect hiPSC-derived models of the BBB, cerebral cortex, and liver [112]. In this model, the BBB was constructed from differentiated brain microvascular endothelial cells, astrocytes, and human brain pericytes. The liver spheroids consist of a mixture of hepatocyte-like cells (80%), endothelial cells (15%), and mesenchymal stromal cells (5%), all differentiated from hiPSCs and incubated for 72 h using 3D rotation. This setup allows for the biological signal exchange between each organoid through the culture medium's supernatant, facilitated by the microfluidic system. Interestingly, the model demonstrated that atenolol and propranolol exhibited permeability characteristics similar to those observed in vivo within the BBB. However, a notable limitation of this model is the absence of kidney organoids. Without kidney integration, it becomes challenging to achieve reasonable regulation of drug concentration, particularly regarding the rapid metabolism and excretion of drugs. Novak et al. successfully maintained the viability and organ-specific functions of eight vascularized dual-channel organ chips, which included models for the intestine, liver, kidney, heart, lung, skin, BBB, and brain [113]. By employing an automated design, they were able to simulate the dynamic physiological characteristics of these organs, such as drug transport and metabolism across the endothelial-tissue interface, and subsequent in vitro-in vivo conversion.

The above technologies can also be combined with automated systems to efficiently perform cell culture, culture medium replacement, real-time monitoring, and parameter measurement, thereby reducing operating costs and avoiding batch differences caused by manual operations while enhancing repeatability [114]. Additionally, automation allows for the expansion of experimental scale, ensuring that products meet regulatory standards through consistent record-keeping and tracking [111]. The schematic diagram was shown in Figure 2iii. In addition, with the rapid development of artificial intelligence, the organoids has also had a new driving force, namely intelligent medical care.

5. Reshaping hiPSCs Development under the Wave of AI

The construction of complex functional organoids necessitates the parallel differentiation of hiPSCs into multiple specific cell types. This process can be enhanced by integrating spatial transcriptomics, classical transcriptomics, epigenomic, and genomic data with artificial intelligence (AI) models to identify key factors influencing organoid development. Current methodologies enable high spatial resolution analysis of gene expression mechanisms, including techniques such as CuRVE [115], DBiT-seq, Spatial-ATAC-seq, Spatial-CUT&Tag, Spatial-CITE-seq, Spatial ATAC–RNA-seq, and Spatial CUT&Tag–RNA-seq [116]. However, the existing bioinformatics tools for multi-omics analysis lack an open-source AI platform for joint analysis, which hampers researchers' ability to gain deeper insights and efficiently develop complex biological systems. Moreover, the technological breakthroughs of AI platforms are only reflected in financial or basic medical research, and the profit returns in this area are enough to make up for all costs. How much value AI models can play in clinical practice is not a concern for the stakeholders. Thus, AI models remain predominantly restricted to the domain of scientific research, lacking effective integration into clinical application scenarios. This disconnect also further raises key questions about the actual value of AI models for clinical practice. Relevant discussions at the 2025 Paris AI Summit also focused on competition and investment. Perhaps in the future we need to think more about cooperation, security, and promoting the upgrading and development of the pharmaceutical industry.

Drug screening models based on organoid development should be integrated with AI to create personalized organoids for testing various drugs and simulating biological responses across different disease states in the early stages of research. This approach can help establish a robust database of high-quality drug candidates for training AI models. In later stages, AI can predict drug effects and cellular responses based on a patient's genomic and clinical data, effectively analyzing and interpreting these datasets to uncover potential therapeutic modalities. While hiPSC-driven organoids pave the way for personalized precision medicine, it is essential to recognize that personalization does not eliminate the need for standardized protocols. Without universal programming operations, controlling operational costs becomes challenging. Here, AI can assist in defining the modularity of personalized treatment processes. The modular process can commence with a patient's organoid chip, which, due to its miniaturized design, facilitates high-throughput experiments and allows for the simultaneous processing of multiple samples. This improves experimental efficiency and reduces costs. Data generated from the organoid chip (also known as OOC) can then be utilized to tailor drug candidates or assess the patient's physio-pathological state using AI models trained on diverse datasets. Furthermore, subsequent treatment records and real-time feedback from patients can be integrated back into the AI model, enhancing its adaptability. This feedback loop can also monitor side effects and adverse drug reactions, enabling timely adjustments to research directions or treatment plans. This iterative process not only improves patient outcomes but also optimizes the overall personalized service

pipeline. In 2025, a major breakthrough in the relevant industry will be the AI co-scientist released by Google based on Gemini 2.0. And it's possible that drug screening prediction software for organoids, akin to AlphaFold, could emerge soon.

Currently, generative AI (e.g., ChatGPT, DeepSeek) is capable of assisting researchers in designing reprogramming protocols, cell differentiation strategies, and hierarchical organoid structures. For beginners, generative AI serves as a powerful tool to enhance productivity by offering foundational knowledge, learning resources, and actionable experimental plans. It can also interpret complex experimental data, such as single-cell sequencing results, and guide subsequent analysis. Additionally, generative AI aids in diagnosing and troubleshooting experimental issues, while suggesting potential research directions or innovative topics based on field trends and user interests. As model architectures are optimized and inference efficiency improves, the complexity of instructions needed to obtain desired answers will correspondingly decrease. AI transcends its role as a mere back-end tool for aggregating experimental data and automating data analysis [117]. By screening large datasets, AI models excel at capturing multidimensional interactions among variables such as gene expression, signaling molecules, biochemical reactions, and physical microenvironments, enabling the identification of correlations between variables and performance outcomes. For instance, AlphaFold demonstrates remarkable precision in predicting the three-dimensional structures of proteins. By identifying potential regulatory targets for differentiation, AlphaFold can provide a foundation for designing small-molecule drugs or growth factors tailored for organoids. This capability aids researchers in understanding key signaling pathways and protein interactions, ultimately optimizing organoid differentiation protocols. AI-based single-cell data analysis can accurately identify cell types, reconstruct developmental trajectories, and predict functions. Analysis plans can also be customized through generative AI without the need for cumbersome programming languages.

Therefore, in the wake of AI advancements, hiPSC-driven organoid development urgently requires the construction of comprehensive knowledge graphs, updated genealogical organoid drug datasets, and a robust feedback data-sharing platform. At the same time, organoid chips can play a crucial role in multimodal data integration, assisting AI in performing multidimensional analyses and updating information effectively. Moreover, the personalized treatment of organoids should embrace modularity, facilitating the operational processes of precision medicine based on patient datasets. This approach aims to reduce practical costs and ensure that the benefits of advanced therapies are accessible to the broader public. We also hope that the development of medical AI platforms will move more towards co-creation and cooperation rather than zero-sum competition. The relevant AI workflow diagram is shown in Figure 3.

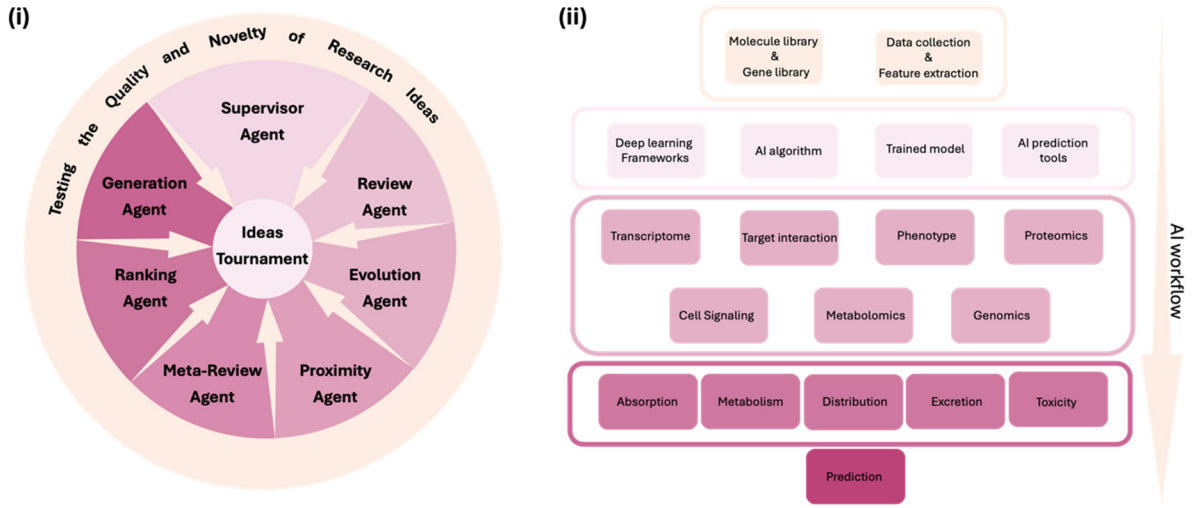

Figure 3. AI workflow diagram. (**i**) Overview of Google's AI co-scientist system; (**ii**) Schematic diagram of AI medical workflow for drug screening or small molecule induction prediction of hiPSCs-derived organoids.

6. Difficulties Faced during Promotion

The public's general fear of biotechnology, including gene editing and synthetic biology, significantly influences perceptions of hiPSC-driven organoids. Many individuals worry that these technologies could lead to uncontrollable outcomes or ethical dilemmas, such as unintended genomic alterations, cloning, or even the creation of biological weapons [118]. These concerns encompass both the inherent risks associated with the technology itself and the derivative risks stemming from its uncontrolled application [119]. Additionally, there is a belief that

the advancement of organoid technology may exacerbate social inequality, allowing only a privileged few to access cutting-edge medical innovations, which could widen existing health disparities. Compounding this issue, regulatory frameworks have not kept pace with technological developments. In China, for instance, relevant regulations are gradually improving and strengthening, as evidenced by the "Ethical Guidelines for Human Genome Editing Research" issued by the Ministry of Science and Technology in 2024. The United States relies on existing food and drug laws for oversight, while the European Union employs a stricter GMO legal framework but suffers from a lack of coordination, leading to potential regulatory arbitrage.

The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) consider stem cell-related therapies as cell and gene therapies (CGT) and advanced therapeutic medicinal products (ATMPs), while China's National Health Commission (NHC) or the National Medical Products Administration (NMPA) consider them as medical technologies. In many cases, they are not regulated as biological drugs [120,121]. The increase in cases and investments in stem cell therapies does not necessarily indicate regulatory enthusiasm; rather, it may reflect a relaxation or lack of regulatory oversight. Many products exploit stem cell therapy as a marketing tactic, circumventing FDA, EMA, or NMPA supervision under the pretext of medical practice, often involving practitioners who have not undergone rigorous training. This situation poses potential risks to users [121]. Currently, the FDA, EMA, and NMPA stress the importance of ethical review, patient informed consent, and testing safety, which is why no hiPSC therapies have been authorized [122]. The scientific community remains concerned about issues such as cell genetic stability, tumorigenicity, and immune rejection, indicating that iPSCs have not yet gained sufficient trust among scientists. In the realm of chimeric research, major scientific powers have banned certain high-risk studies, including the mixing of human and animal genes, to prevent potential misuse of synthetic biology. Similar to nuclear technology, the aim is to guide research in a direction that aligns with the future welfare of humanity. While regulators may not predict the emergence of new technologies, they can play a critical role in managing and mitigating their risks.

This public distrust towards hiPSCs can be viewed as a form of neo-Luddism, reflecting concerns and criticisms about the impact of technology. However, it tends to represent a cautious stance towards specific technologies rather than a wholesale rejection of all technological advancements. Addressing these concerns through improved public education and communication is essential for alleviating distrust and fostering the healthy development of science and technology. In the future, the construction of the regulatory system should begin from the perspective of external oversight. A multi-tiered ethical review system should be developed based on different review subjects to effectively avoid conflicts of interest, identify stakeholders, and facilitate conflict resolution. The future ethical review framework should emphasize the alignment of rights and responsibilities, establish the obligation of the ethics committee to conduct diligent reviews, and use negligence as the standard for determining accountability.

Currently, hiPSCs obtained through existing technologies have several limitations. Low reprogramming efficiency can lead to high costs, while suboptimal differentiation efficiency and stability may result in outcomes that do not fully replicate the maturity of in vivo cells. Additionally, concerns about genomic instability and the potential for tumorigenicity persist. The composition and proportion of cell types in organoids derived from hiPSCs often differ from those found in real organs, which can impact their physiological characteristics and responses. Furthermore, issues related to standardization and variability between batches complicate their application. Over time, hiPSC organoids may age and lose functionality during long-term culture, which affects their suitability for extended research. Addressing these challenges is crucial for advancing the use of hiPSCs and their derived organoids in biomedical applications.

The primary methods for inducing hiPSC reprogramming involve several approaches to introduce reprogramming factors into cells. These methods include using viral vectors, liposomes, and plasmids to deliver DNA; activating endogenous reprogramming factors or inserting exogenous genes via CRISPR/Cas9 technology; transfecting cells with synthetic mRNA or proteins; and employing direct chemical reprogramming using small molecule compounds as assist in reprogram efficiency. Future developments in this field should focus on balancing efficiency and universality while avoiding genome integration, ultimately promoting clinical applications and industrial advancement. This requires optimizing exogenous factor-free reprogramming technologies, such as enhancing the efficiency of Cas12 and Cas13 in activating endogenous reprogramming factors while minimizing off-target effects. Additionally, improving the reprogramming efficiency of combinations of small molecule compounds is cost-effective and scalable. So developing efficient nano-delivery systems and optimizing the processes for proteins or mRNA will also be important. Finally, integrating multiple reprogramming technologies could standardize organoid production processes, paving the way for more consistent and effective applications in research and therapy.

When constructing organoids with complex structures, researchers usually use partition preparation and then combine them by physical collage or wrapping. This technique allows for precise control over the cell types and structures within each segment. However, it can lead to unnatural interfaces, which may negatively impact cell interactions. Co-culturing different cell types within a single system can better simulate the initial stages of development, yet it poses challenges in accurately controlling the proportions and spatial distributions of each cell type. This imbalance can lead to issues such as over-proliferation of certain cell types, ultimately affecting the organoid's functionality. A promising trend in organoid development combines genetic engineering and bioprinting techniques. By manipulating stem cell development through gene silencing or activation, and by printing various cell types and biomaterials layer by layer, researchers can create highly customized organoid models. Despite these advancements, current organoid hierarchies and structures often fail to accurately replicate original human organs or tissues, particularly sub-millimeter structures.

Long-term in vitro cultivation of organoids presents additional challenges. Key issues include the lack of fluid material exchange systems—such as blood vessels—necessary for nutrient delivery and waste removal. Furthermore, maintaining a balance between cell proliferation and apoptosis in the deeper layers of organoids is complex. Current strategies to address these challenges include the use of porous hydrogel systems, which enhance nutrient and oxygen permeability and facilitate waste elimination, and dynamic culture conditions that mimic blood flow by continuously perfusing culture medium into the organoids. However, these interim solutions can limit the complexity and functionality of the organoids. For the in vitro construction of organoids, cells only develop into precursor cells. Subsequent functional differentiation often necessitates in vivo conditions, particularly for complex systems such as pancreatic islets and the thymus. Therefore, there is an urgent need for further discussion and development of in vitro protocols for functional cell differentiation to replicate the essential biological processes better.

7. Conclusions

The organoid platform derived from hiPSCs encompasses a wide range of target tissues. Unlike ESCs, hiPSCs do not face ethical pressures since they are induced from somatic cells of patients, and they exhibit greater multidirectional differentiation potential compared to multipotent and unipotent stem cells. The development of hiPSC-derived organoids is increasingly shifting toward Matrigel-free and scaffold-free systems, advocating for the creation of specific induction and maintenance systems tailored to different tissues to minimize the interference of unknown substances in the microenvironment. To construct organoids with complex material exchange systems, either vascularization techniques are used, or different types of cells are induced in segments, or multiple different types of organoids are integrated through assembly technology. Currently, vascularization technology has limitations, such as insufficient vascular invasion to reach the internal functional areas or the presence of non-functionalized blood vessels, primarily sinusoids. Additionally, segmented induction for different functional subtypes of the same cell type has yet to be realized, and the development of new assembly technologies is crucial. The future of hiPSC-derived organoids is poised for personalization, high-throughput modularization, multi-organ integration, and automation, driven by AI. There is also hope for more collaborative efforts on medical AI platforms, fostering cooperation over competition. For hiPSC-derived platforms, the ethical review framework needs refinement to balance scientific advancement and public concerns. Researchers must also enhance or develop new induction methods to mitigate genomic instability and tumorigenic risks while improving differentiation efficiency, quality, and cost control.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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