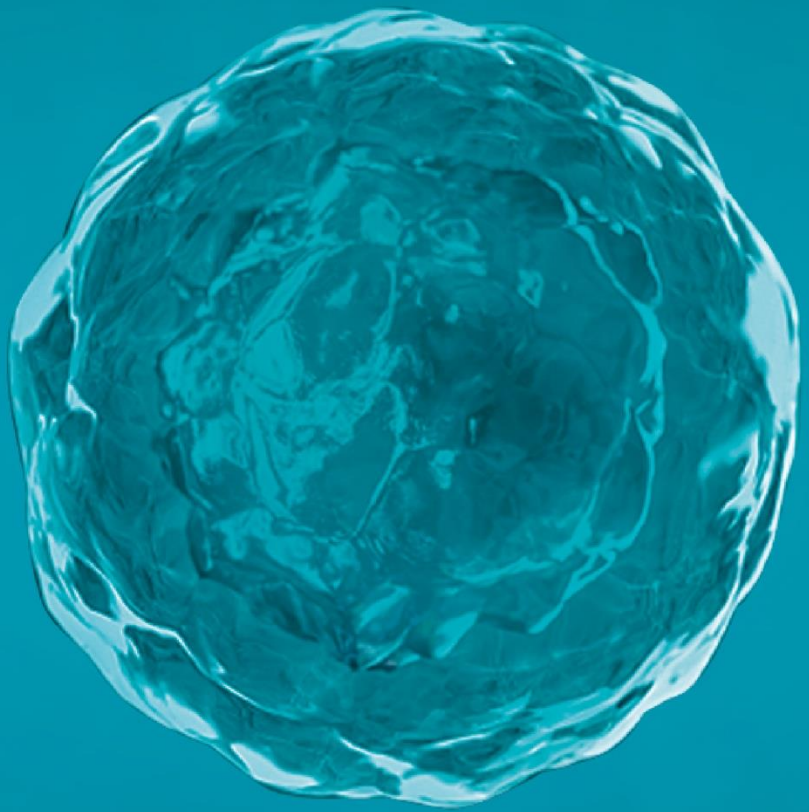
ISOR International Society of Organoid Research

White Paper

The Next Frontier: Exploring Organoids and Organ-on-a-Chip



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1 Terminology

1.1 ASC

Adult stem cells (ASC) are multipotent stem cells present in various tissues. These cells possess the remarkable capacity for self-renewal and can differentiate into diverse mature cells with specific phenotypes and functions. Through these capabilities, ASCs contribute to the maintenance of overall somatic functions and actively participate in physiological cell renewal as well as the repair of tissue damage.

1.2 CIVM

Complex *In vitro* Model (CIVM) refers to an experimental construct comprising multiple cell types and/or tissues designed to replicate physiological or pathological processes within the human body or other organisms in an *in vitro* environment.

1.3 CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a repetitive genomic sequence found in prokaryotic organisms, enabling them to defend against foreign pathogens. This remarkable feature has been extensively harnessed in gene-editing technology.

1.4 EGF

Epidermal Growth Factor (EGF), a vital growth factor naturally secreted by cells, exhibits potent physiological activity.

1.5 FDA

United States Food and Drug Administration (FDA).

1.6 GWAS

Genome-Wide Association Study (GWAS) is a comprehensive examination of genetic variations (polymorphisms/markers) across the entire genome in multiple individuals. This involves subjecting genotypes to statistical analysis at the population level, correlating them with observable traits or phenotypes. The aim is to identify genetic variations (markers) most likely to influence the trait, determined by statistical significance (p-value), and to identify genes associated with the trait variant.

1.7 HTS

High Throughput Screening (HTS) technology involves experimental methods at both the molecular and cellular levels. It utilizes microplates as carriers, automated systems for test implementation, sensitive and rapid detection instruments for collecting experimental data, and computer analysis for processing the data. This approach allows testing of large numbers of samples in a single experiment, generating a comprehensive database to support the technical system. In essence, HTS enables the extraction of a substantial amount of information from a single experiment and the identification of valuable insights.

1.8 KRAS

The *KRAS* gene, a proto-oncogene, is commonly found to be mutated in various human cancers.

1.9 MPS

Microphysiological Systems (MPS) represents a bioengineering technology designed to replicate the structure and function of human organs through *in vitro* models. Integrating diverse biocellular technologies such as cell culture, differentiation, organoids, microfluidics, and more, MPS can emulate different physiological environments within the human body. This innovative approach offers a novel research model for both basic research and drug development.

1.10 NCATS

National Center for Advancing Translational Sciences (NCATS), USA.

1.11 NIH

National Institutes of Health (NIH), USA.

1.12 PDMS

Polydimethylsiloxane (PDMS) is a soft, transparent elastomeric polymer widely utilized in small-scale biological applications. Readily accessible from commercial sources, PDMS is cost-effective, optically transparent, highly elastic, breathable, and biocompatible for prolonged use in cell culture devices. However, drawbacks include susceptibility to small molecule uptake, autofluorescence, and the potential for causing microchannel swelling.

1.13 PDO

Patient-Derived Organoids (PDOs), cultivated from surgical or biopsy samples, are cultured to generate organoids with specific structures and functions in a corresponding culture medium,

replicating the histopathological characteristics of the patients. Offering high clinical relevance, a short experiment duration, and the capacity for high-throughput drug screening, PDOs serve as an ideal model for drug development and drug sensitivity testing.

1.14 PDX

The Patient-Derived Tumor Xenograft (PDX) model involves transplanting tumor tissues or progenitor cells from patients into immunodeficient mice to create a transplanted tumor model. The PDX model preserves the key characteristics of the original cancer at histopathological, molecular, and genetic levels, demonstrating predictive clinical efficacy.

1.15 PSC

Pluripotent stem cells (PSCs) possess a remarkable capacity for self-renewal through division and differentiation into three primary germ cell layers of an early embryo. As a result, they can give rise to all cell types present in an adult body, excluding extra-embryonic tissues such as the placenta. Examples of pluripotent stem cells include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

1.16 SFEBq

Serum-free floating culture of Embryoid Body-like aggregates with quick reaggregation (SFEBq). To obtain rapid aggregating blastoid-like structures, pluripotent stem cells are initially dissociated into homogeneous single-cell suspensions to mitigate any endogenous induction signals. Subsequently, these cells are promptly aggregated for cultivation in a serum-free and growth factor-free medium.

1.17 Microfluidics

Microfluidics is a technology that enables precise control and manipulation of microscale fluids, particularly in sub-micron scale. It is also referred to as Lab-on-a-Chip or microfluidic chip technology.

2 Overviews of Organoids and Organ-on-a-Chip Technology

2.1 Significance

The conventional drug development process usually entails a series of sequential assessments, starting with *in vitro* cell culture experiments, progressing to *in vivo* animal models, and culminating in clinical trial validation. This traditional methodology is both time-consuming and costly. It often requires more than a decade and may surpass a billion dollars for the development of an innovative drug.

Cell culture experiments deviate from authentic tissue structures and physiological conditions. whereas animal models, aside from ethical concerns, often inaccurately predicts human responses, with an error rate exceeding 90%. Drug candidates identified through cell culture and animal models frequently encounter high failure rates in clinical trials, resulting in significant waste of time and financial resources. These limitations have subsquently contributed to escalating costs in pharmaceutical research, particularly in drug development. Consequently, addressing the urgent need to enhance the preclinical assessment of drug efficacy, to identify more clinically relevant drug candidates, to improve the success rate of drug development, and to reduce the costs associated with late-stage drug development has emerged as a critical imperative for the pharmaceutical industry.

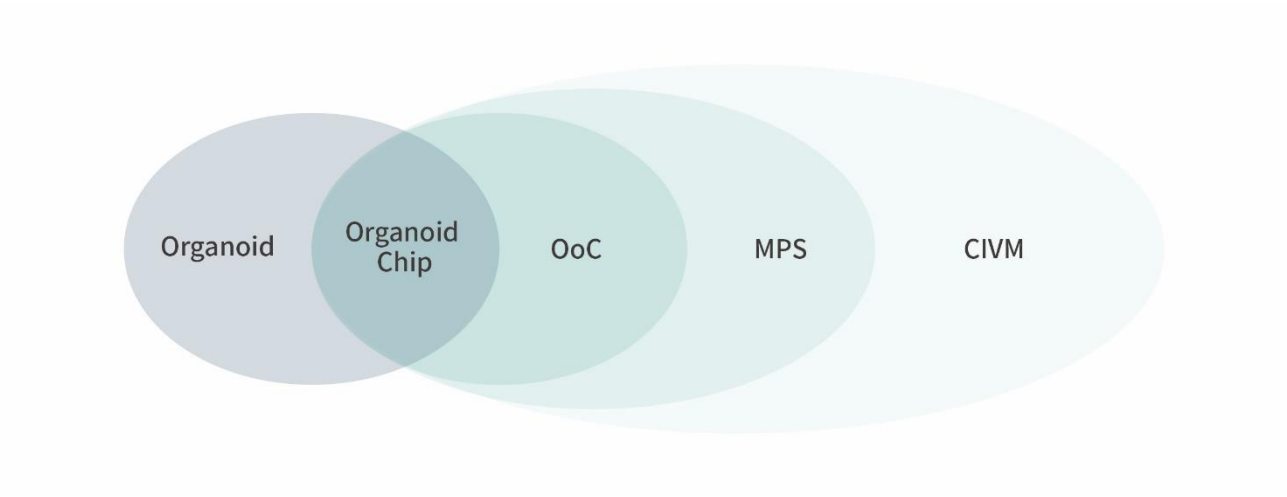
Organoid and Organ-on-a-Chip (OoC) technology proficiently overcome the constraints of conventional pharmaceutical research methods by simulating human tissue and organ structures. This innovative technology has significantly impacted various medical domains, encompassing disease modeling, drug development, oncology research, regenerative and precision medicine. Notably, in 2013, the journal *Science* recognized organoids as one of the top ten technological breakthroughs of the year. Furthermore, in early 2018, the journal *Nature Methods* named it the best method of the year for 2017.

Over the past decade, regulatory authorities worldwide have championed the advancement of organoid technologies. Notably, in the United States, key agencies such as the National Institutes of Health (NIH), the Food and Drug Administration (FDA), and the National Center for Advancing Translational Disease Science (NCATS) have provided substantial support. In China, backing has come from entities including the Ministry of Education, the Ministry of Science and

Technology, the Drug Administration, the Chinese Anti-Cancer Association, and the Chinese Society of Genetics. Furthermore, the European Union's Seventh Framework Program has contributed to this global effort. Additionally, the European Union's policy prohibiting the use of animals in cosmetic testing has indirectly fostered the development of organoid technology.

In a significant stride, in April 2017, the FDA entered into an agreement with Emulate Inc. to assess the efficacy of OoC technology. Subsequently, in August 2022, the FDA granted approval for clinical trials of a new drug (NCT04658472) solely based on preclinical data derived from OoC studies. Notably, this marks the world's first clinical approval of a drug using organoid data. According to the NIH, there are currently over 100 clinical trials related to organoids and OoC, and the China Clinical Trial Center has documented nearly 50 related trials, primarily focusing on targeted cancer therapy and precision medicine. The outcomes of these clinical trials hold promise for benefiting a larger patient population in the future. In summary, organoid and OoC technologies have already exerted a profound impact on the evolution of the pharmaceutical field and clinical treatment management.

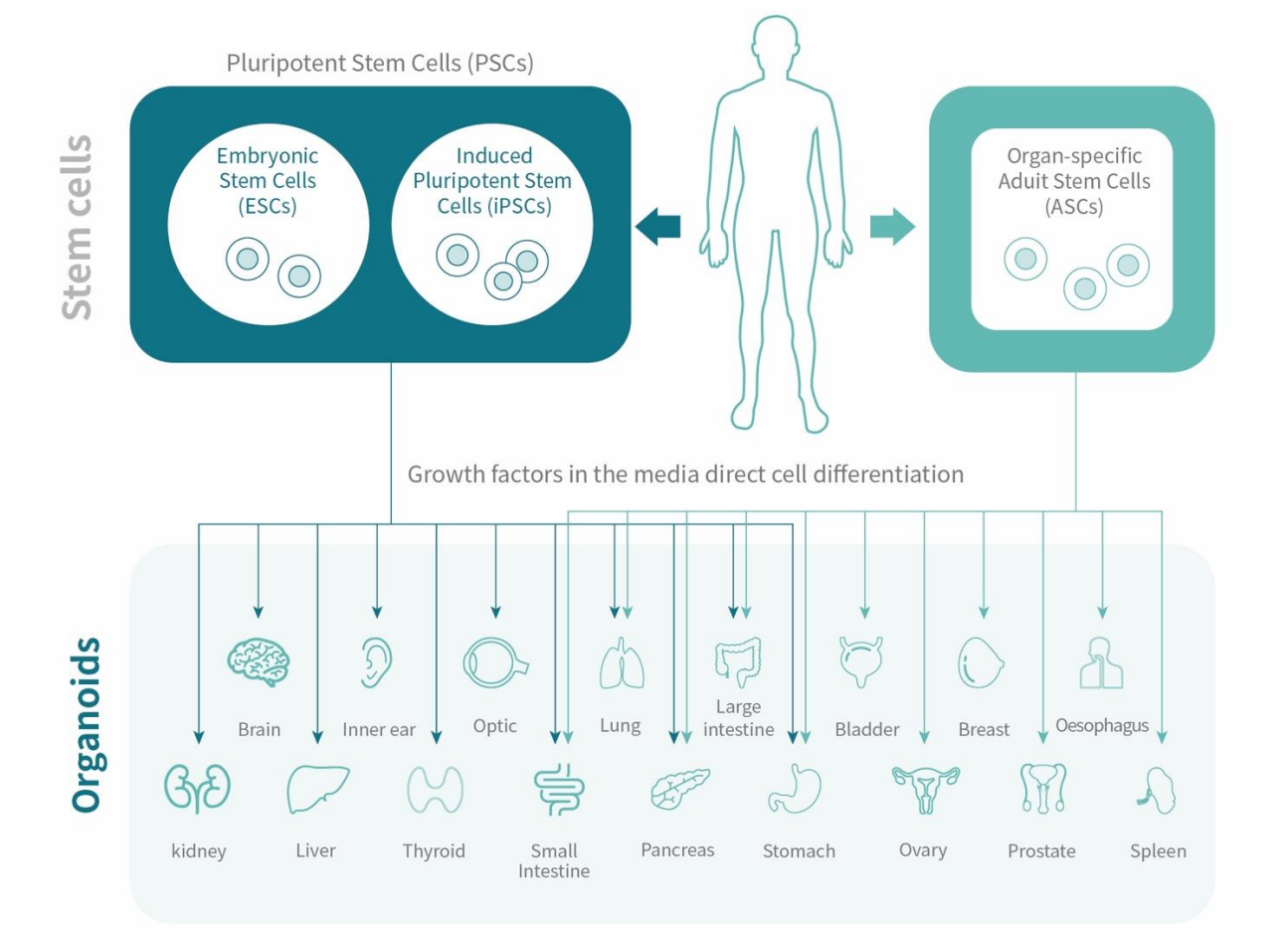
2.2 Definition



Before delving into the specific definitions of organoid and organ-on-a-chip, it is crucial to clarify the foundational concepts associated with these fields. Both organoid and organ-on-a-chip technologies share the common objective of simulating human body tissues and organ structures, creating some overlap in their conceptual frameworks. However, in terms of emphasis, organoids lean more towards biological principles, aligning closely with concepts in the field of biology. On the other hand, organ-on-a-chip places a stronger focus on engineering, constructing micro-physiological systems, and developing intricate *in-vitro* models.

2.2.1 Definition of Organoid

An organoid refers to a tissue analog characterized by a complex spatial structure, created through *in vitro* three-dimensional (3D) culture utilizing adult stem cells (ASC) or pluripotent stem cells (PSC). Organoids have the capacity to manifest interactions among cells, as well as interactions with the surrounding matrix, demonstrating distinct spatial location patterns of the original tissue.



Organoids typically need to fulfill certain criteria: (1) they should consist of more than one cell type identical to the source organ, (2) they must exhibit functional characteristic of the source organ, and (3) the organization of cells should resemble the histological characteristics of the source organ.

In 2014, a *Science* article defined an organoid as a collection of organ-specific cell types that originate from stem cells or organ progenitors and self-organize in an *in vivo*-like manner through cell sorting and spatially restricted lineage orientation.

2.2.2 Definition of Organ-on-a-Chip

Organ-on-a-chip (OoC) refers to a multi-channel 3D microfluidic culture device, initially developed using photolithographic etching of microchips. Its purpose is to emulate the activity, mechanics, and physiological responses of an organ or system at the nanometer to micrometer scale. The primary goal is to synthesize the smallest functional units, reproducing tissue and organ-level functions with precision.

2.3 Difference between Organoid and Organ-on-a-Chip

Organoids result from the self-organization of stem cells (or specific types of adult cells) under specific culture conditions, guided by their inherent developmental biology. These 3D structures can, to some extent, replicate the microstructure and function of authentic organs. The formation of organoids relies not only on the pluripotency of stem cells but also on the precise regulation of extracellular matrix, growth factors, and other signaling molecules.

On the other hand, organ chips are typically miniaturized devices created through microfluidics, designed to replicate functions of human organs by finely controlling fluid dynamics and the cellular growth environment. Cells on organ chips can be derived from various sources, including stem cells, adult cells, cells from specific organs, or organoids. These chips can imitate not only the function of a single organ but also the interactions between different organs.

At its core, organoid technology leans towards the biological concept, prioritizing attention to intricate details such as cell composition, proteins, genes, signaling pathways, and functions. Conversely, the OoC aligns more with engineering principles, focusing on the construction of microchips and microfluidic systems to replicate the precision and spatial control found in real tissues or organs. This technology is particularly well-suited for studying mechanisms related to nutrition, drug delivery, pathogen pathogenesis, and other aspects.

Over the past five years, the distinctions between these two technological concepts have become increasingly blurred. A *Science* article published in June 2019 (DOI: 10.1126/science.aaw7894) even introduced the notion of organoid microarrays. This term broadly encompasses microprocessed cell culture devices engineered to replicate the functional units of human organs *in vitro.*

2.4 Development Process

2.4.1 The History of Organoid Development

The evolution of organoid technology extends over more than a century. Initial experiments with *in vitro* organoid regeneration date back to Wilson's attempts in 1907. However, it wasn't until 1975 that the field truly took root, with Howard Green *et al*.'s groundbreaking work. They discovered that co-cultures of primary human keratinocytes and 3T3 fibroblasts could give rise to stratified squamous epithelial colonies resembling the human epidermis. Subsequently, researchers delved deeper into the exploration and development of organoid technology. In 1987, researchers initiated experiments involving the 3D culture of primary cells on EHS tumor recombinant basement membranes. By the turn of the 21st century, significant breakthroughs had been achieved in this realm of research. In 2008, Sasai *et al*. successfully generated cerebral cortex tissue from embryonic stem cells through 3D aggregation cultures. Additionally, in 2009, Hans Clevers *et al*. demonstrated that adult intestinal stem cells expressing a single leucine-rich repeat sequence of Lgr5 could form 3D intestinal-like organoids within matrix gels. Since then, the term organoid was widely accepted.

Between 2011 and 2021, the research and application of organoid technology significantly expanded, yielding remarkable outcomes. A diverse array of human organs, including the esophagus, retina, brain, liver, kidney, and pancreas, along with organoids representing various diseases such as prostate, lung, colorectal cancer, pancreatic cancer, glioma, cervical cancer, and nasopharyngeal cancer, have been successfully established.

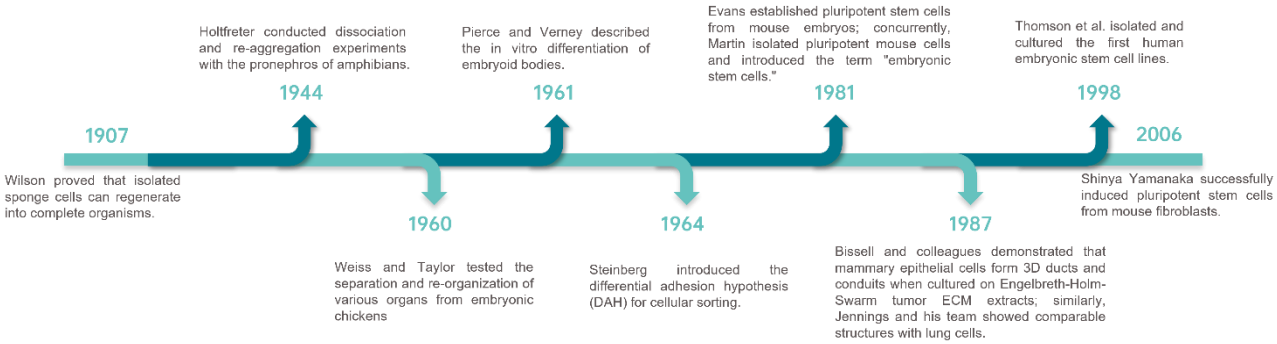
1) Unveiling *In vitro* Regenerative Processes Leading to Induced Pluripotent Stem Cell Technology

1907-2006

- In 1907, Wilson proved that isolated sponge cells can regenerate into complete organisms.
- In 1944, Holtfreter conducted dissociation and re-aggregation experiments with the pronephros of amphibians.
- In 1960, Weiss and Taylor tested the separation and re-organization of various organs from embryonic chickens.
- In 1961, Pierce and Verney described the *in vitro* differentiation of embryoid bodies.
- In 1964, Steinberg introduced the differential adhesion hypothesis (DAH) for cellular sorting.
- In 1981, Evans established pluripotent stem cells from mouse embryos; Concurrently,

Martin isolated pluripotent mouse cells and introduced the term "embryonic stem cells".

- In 1987, Bissell and colleagues demonstrated that mammary epithelial cells form 3D ducts and conduits when cultured on Engelbreth-Holm-Swarm tumor ECM extracts; Similarly, Jennings and his team showed comparable structures with lung cells.
- In 1998, Thomson *et al*. isolated and cultured the first human embryonic stem cell lines.
- In 2006, Shinya Yamanaka successfully induced pluripotent stem cells from mouse fibroblasts.



2) Establishment of Each Organ Organization With the Corresponding Organoid

- In 1907, Wilson first attempted the *in vitro* regeneration of a complete organism.
- In 1975, Howard Green *et al*. discovered that primary human keratinocytes and 3T3 fibroblasts co-culture formed stratified squamous epithelial colonies resembling human epidermis.
- In 1987, attempt of 3-dimensional culture by cultivating primary cells on the reconstituted basement membrane of EHS tumors.
- In 2008, Sasai *et al.* developed cerebral cortex tissue from embryonic stem cells through 3D aggregate culture.
- In 2009, Hans Clevers *et al*. demonstrated that single Lgr5-positive adult intestinal stem cells, characterized by leucine-rich repeat sequences, could form 3D intestinal organoids in a matrix gel.
- In 2011, Esophageal organoids were successfully cultivated.
- In 2012, retinal organoids derived from human pluripotent stem cells were successfully cultivated.
- In 2013, brain, liver, kidney, and pancreatic organoids derived from human pluripotent stem

cells were successfully cultivated.

- In 2014, prostate, lung, colorectal cancer, prostate cancer, salivary gland, and taste bud organoids were successfully cultivated.
- In 2015, breast, fallopian tube, stomach, pancreatic cancer, and rectal organoids were successfully cultivated.
- In 2017, corneal and liver organoids were successfully cultivated.
- In 2018, esophageal cancer organoids were successfully cultivated.
- In 2019, breast cancer organoids were successfully cultivated.
- In 2020, glioblastoma organoids were successfully cultivated.
- In 2021

  Cervical cancer and nasopharyngeal cancer organoids were successfully cultivated.

  The simulation of brain neurons and glial cells, as well as the construction of a bone marrow atlas, was accomplished;

  Photoreactive brain organoids were constructed;

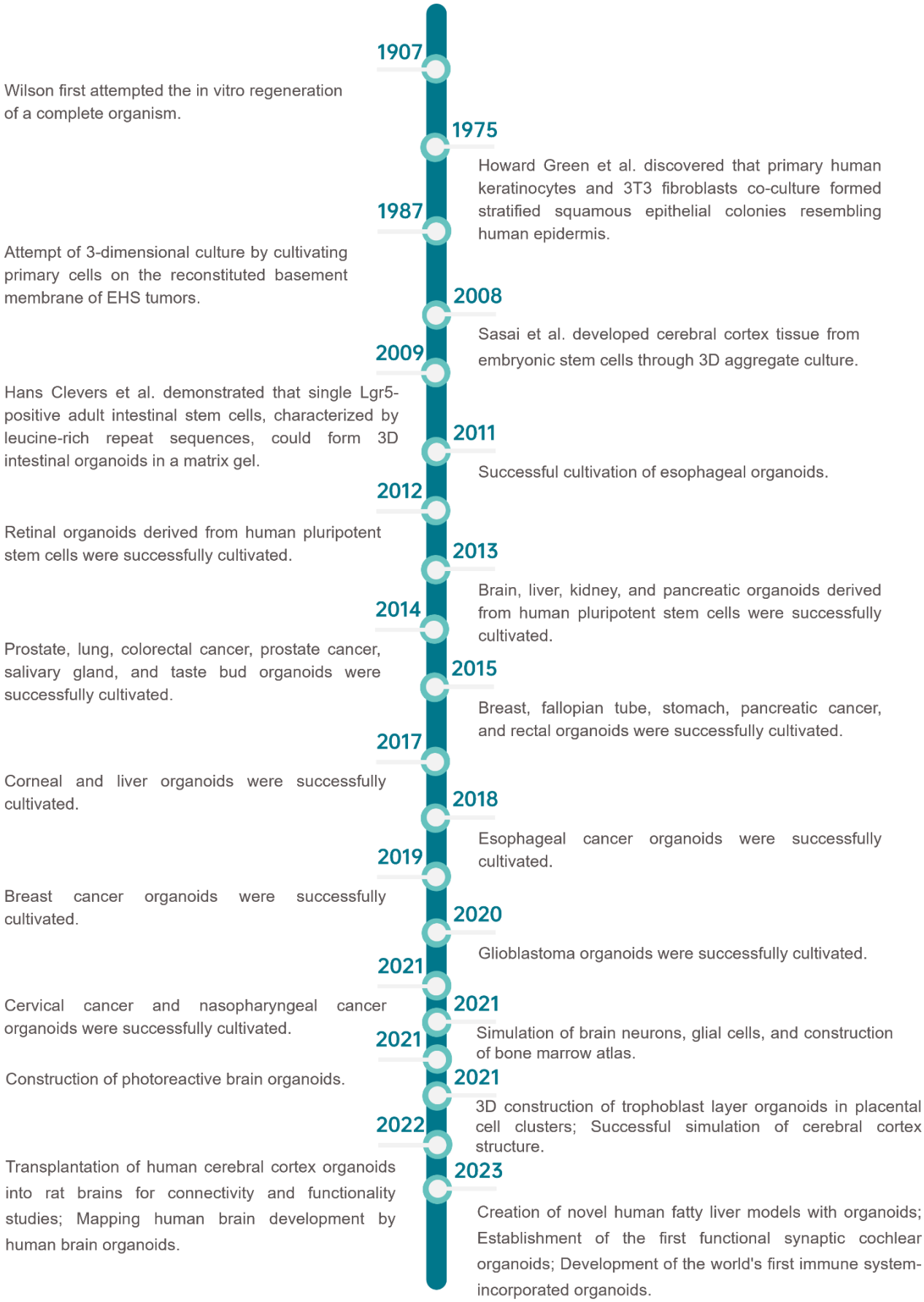
  3D construction of trophoblast layer organoids in placental cell clusters was achieved.

  The successful simulation of the cerebral cortex structure was realized.
- In 2022, human cerebral cortex organoids were transplanted into rat brains for studies on connectivity and functionality. Human brain development was mapped using human brain organoids.
- In 2023

  Novel human fatty liver models were created with organoids;

  The first functional synaptic cochlear organoids were established;

  The world's first immune system-incorporated organoids were developed.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Retinal organoids derived from human pluripotent stem cells were successfully cultivated.
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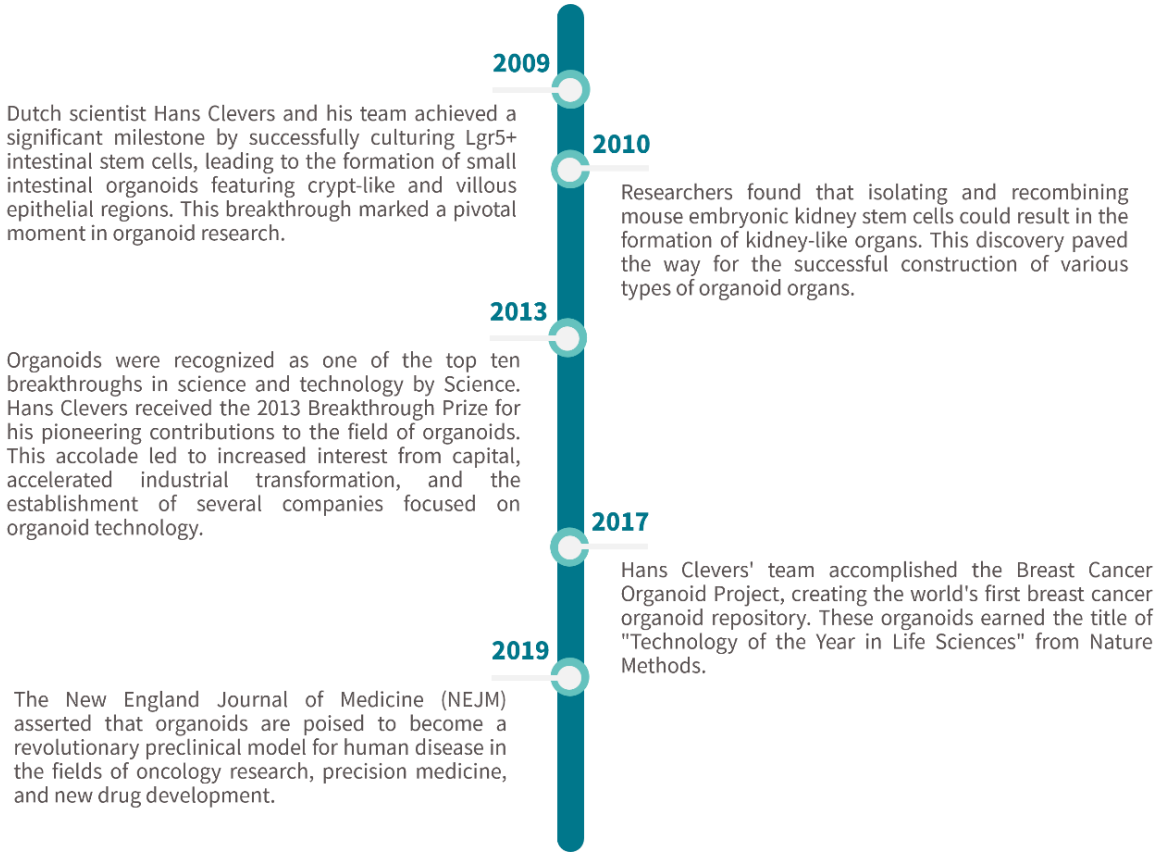
Brain, liver, kidney, and pancreatic organoids derived from human pluripotent stem cells were successfully cultivated.
2014
Prostate, lung, colorectal cancer, prostate cancer, salivary gland, and taste bud organoids were successfully cultivated.
2015
Breast, fallopian tube, stomach, pancreatic cancer, and rectal organoids were successfully cultivated.
2017
Corneal and liver organoids were successfully cultivated.
2018
Esophageal cancer organoids were successfully cultivated.
2019
Breast cancer organoids were successfully cultivated.
2020
Glioblastoma organoids were successfully cultivated.
2021
Cervical cancer and nasopharyngeal cancer organoids were successfully cultivated.
2021
Simulation of brain neurons, glial cells, and construction of bone marrow atlas.
2021
Construction of photoreactive brain organoids.
2021
3D construction of trophoblast layer organoids in placental cell clusters; Successful simulation of cerebral cortex structure.
2022
Transplantation of human cerebral cortex organoids into rat brains for connectivity and functionality studies; Mapping human brain development by human brain organoids.
2023
Creation of novel human fatty liver models with organoids; Establishment of the first functional synaptic cochlear organoids; Development of the world's first immune system-incorporated organoids.

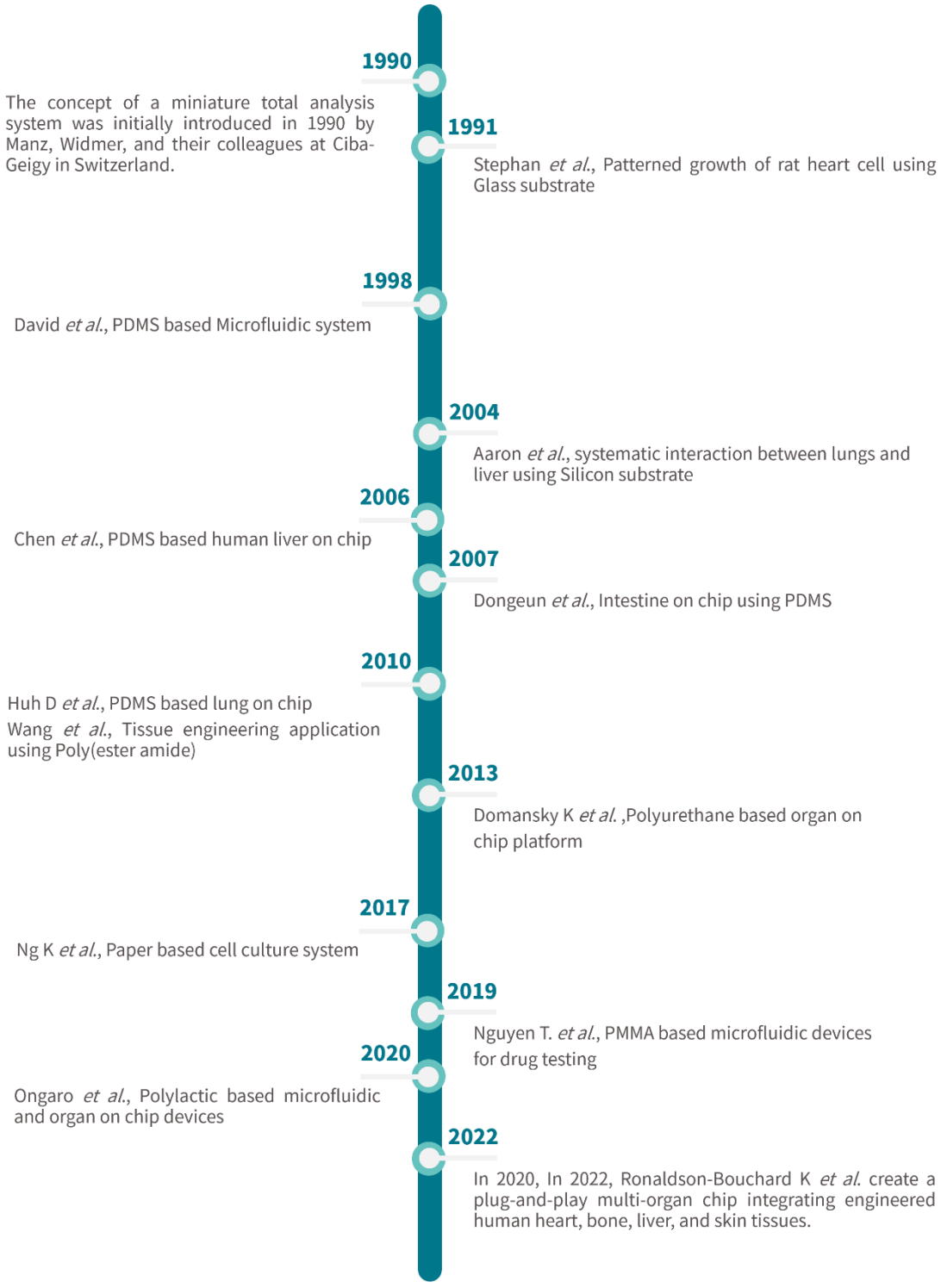
3) Crucial Milestones

- In 2009, Dutch scientist Hans Clevers and his team achieved a significant milestone by successfully culturing Lgr5^{+} intestinal stem cells, leading to the formation of small intestinal organoids featuring crypt-like and villous epithelial regions. This breakthrough marked a pivotal moment in organoid research.
- In 2010, researchers found that isolating and recombining mouse embryonic kidney stem cells could result in the formation of kidney-like organs. This discovery paved the way for the successful construction of various types of organoids representing the organs of origin.
- In 2013, organoids were recognized as one of the top ten breakthroughs in *Science*. Hans Clevers received the 2013 Breakthrough Prize for his pioneering contributions to the field of organoids. This accolade led to increased interest from capital, accelerated industrial transformation, and the establishment of several companies focused on organoid technology.
- In 2017, Hans Clevers' team accomplished the Breast Cancer Organoid Project, creating the world's first breast cancer organoid repository. These organoids earned the title of "Technology of the Year in Life Sciences" from *Nature Methods*.
- In 2019, the *New England Journal of Medicine* (NEJM) asserted that organoids are poised to become a revolutionary preclinical model for human disease in the fields of oncology research, precision medicine, and new drug development.



2.4.2 Development of Organ-on-a-Chip

1) Crucial Milestones



- In 1990, the concept of a miniature total analysis system was initially introduced by Manz, Widmer, and their colleagues at Ciba-Geigy in Switzerland.
- In 1991, Stephan *et al*. employed spherical glass substrates for the *in vitro* cultivation of murine cardiac cells. This pioneering work established the first physiological model that not only achieved patterned cell growth but also provided insights into cardiac conduction block.

- In 1998, David *et al*. pioneered the fabrication of the initial microfluidic system designed for drug screening and clinical diagnostics. This groundbreaking system, constructed using polydimethylsiloxane (PDMS) materials, enables the simultaneous execution of multiple experiments. This approach minimizes variability, effectively emulating real physiological environments under tissue culture conditions. Subsequently, microfluidic systems have been tailored to address practical challenges in human organ-level function. Organ chips, designed to replicate the environments of the liver, lungs, intestines, and kidneys, have since been developed in succession.
- In 2004, Aaron *et al*. employed silicon substrates to facilitate systematic interactions between the lung and liver.
- In 2006, Chen *et al*. engineered a human liver microarray using PDMS.
- In 2007, Huh *et al*. created intestinal microarrays using PDMS.
- In 2010, Huh D *et al*. developed a human lung microchip using PDMS, successfully achieving organ-level functionality. Concurrently, in the same year, Wang *et al*. achieved a milestone in tissue engineering by pioneering the application of polyester-amide in microfluidic scaffold construction.
- In 2013, Domansky K *et al*. established an OoC platform based on polyesteramide.
- In 2017, Ng K *et al*. developed a paper-based cell culture system.
- In 2019, Nguyen *et al*. engineered a microfluidic device for drug testing against lung adenocarcinoma, utilizing polymethylmethacrylate (PMMA) attached to polyethylene terephthalate (PETE) chips.
- In 2020, Ongaro *et al*. developed microfluidic and OoC devices using polylactic acid (PLA) materials.
- In 2022, Ronaldson-Bouchard K *et al*. create a plug-and-play multi-organ chip integrating engineered human heart, bone, liver, and skin tissues.

2) Iteration of Materials

Generation I: Silicon/Glass (1991~)

Initially employed for the laboratory construction of organ models, it was swiftly supplanted due to its inability to simulate elastic tissue, coupled with a complex and costly preparation process.

Generation II: Synthetic Materials (1998~)

Synthetic materials continue to dominate the field of organ chips and are primarily classified into elastomeric and thermoplastic materials.

Elastomeric materials have become prevalent in constructing OoC platforms and flexible

microfluidic devices. They are characterized by ease and cost-effectiveness in fabrication, optical transparency, high elasticity, breathability, and extended biocompatibility in cell culture devices.

Elastomeric Materials：PDMS, SU-8, Polyester, PICO, PLLGA, FEMP, Polyurethanes, POMaC, PPS, PGS.

Thermoplastic materials belong to a category that allows for remolding after deformation, presenting potential applications in microfabrication and microelectromechanical systems (MEMS). These materials are characterized by optical transparency, cost-effectiveness, rigidity, reduced susceptibility to monomer leaching, biocompatibility, and resistance to the uptake of small hydrophobic molecules.

Thermoplastic Materials：PMMA, COC, Polystyrene, Polycarbonate, PPE, PEGDA, Peek, PTFE

Generation III: Natural Materials (2017~)

Natural polymers are well-suited for creating a stable environment conducive to stem cell development, migration, and proliferation. Derived from natural sources, these materials closely mimic the real physiological environment and offer enhanced environmental friendliness.

Common natural polymers include: collagen, gelatin, fiber, hyaluronic acid, chitosan and alginate.

Generation IV: Paper (2017~)

Paper is commonly employed in microarrays featuring non-enclosed channels. Its advantages include the absence of pumps, high surface area ratios, straightforward preparation of multilayer microfluidic devices, and benefits in chromaticity analysis owing to its white background. Nonetheless, the fabric matrix of the channel may lead to sample dilution, and the material exhibits drawbacks such as lower sensitivity and susceptibility to liquid evaporation.

Generation V: Composites (2006~)

Composites amalgamate the benefits of both natural and synthetic biomaterials, making them well-suited for research focused on topics such as enhancing cellular interactions, signaling, and regulating cellular behavior.

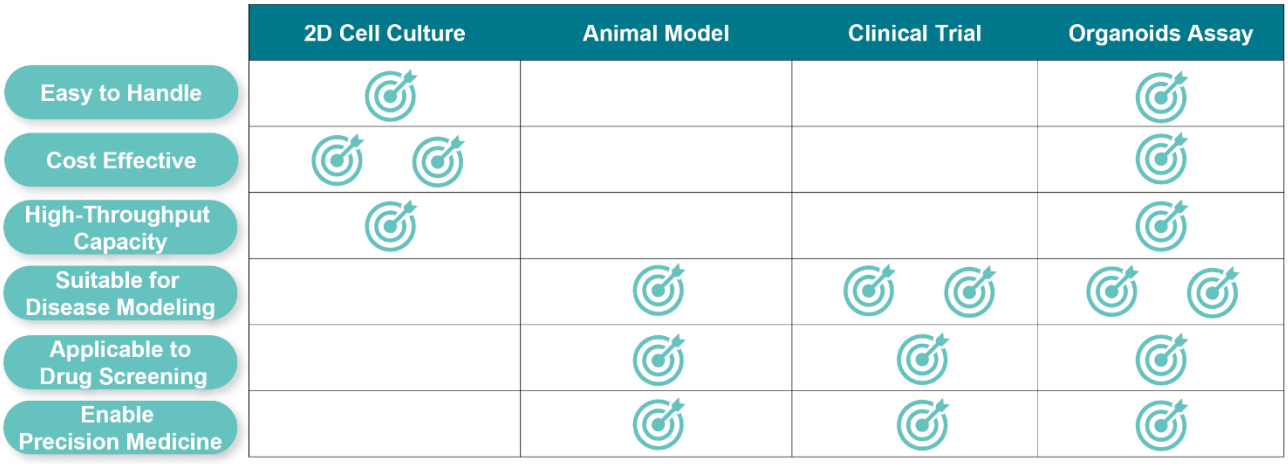
The composites primarily encompass modified ceramic materials, such as polyethylene glycol-

fibrinogen, polylactic acid-chitosan-gelatin, and chitosan-siloxane.

Composite biomaterials offer a clear advantage by combining the optimal properties of both natural and synthetic biomaterials. The evolution of hybrid biomaterials has significantly contributed to the creation of innovative products, delivering the desired performance. The materials' degradation rate produces natural components, fostering enhanced cell affinity. The development of degradable and controllable hybridized polymeric biomaterials holds promising potential for mimicking extracellular protein structures in biomedical applications. Future research directions include functionalization to enhance cellular interactions, signaling, and modulation of cellular behavior. Notably, polyethylene glycol fibrinogen is utilized as a hybrid biomaterial in tissue engineering, along with compositions such as PLA-chitosan-gelatin and chitosan-siloxane.

2.5 Advantages

2.5.1 Key Advantages of Organoids



Conventional drug development and mechanism studies typically rely on either 2D cell culture or animal models, both of which come with inherent limitations. In the case of 2D cell culture models, the realistic simulation of the 3D structure of human tissues and the tissue environment is challenging. On the other hand, animal models, aside from ethical concerns, face challenges in correlation with clinical data due to species differences. Based on pertinent reports, current preclinical *in vitro* experiments often fall short in offering effective guidance for clinical trials. Therefore, organoid technology, which has evolved and matured from induced pluripotent stem cell technology, is poised to offer a novel *in vitro* culture method for preclinical experiments.

Drawing from existing research findings, organoid culture technology demonstrates several advantages over traditional models:

Less Sample Requirement and Shorter Experiment Duration

Organoid culture techniques facilitate the extraction and cultivation of required cells from a relatively small number of samples. Moreover, the growth and maturation cycle is typically short, eliminating the prolonged waiting periods required for development and maturation seen in animal models.

Cost Effective

Organoid models present a considerably lower economic cost compared to animal models in research or drug screening. Animal models demand significant investments of time, money, and resources for maintenance and experimental manipulation, while organoid models can be more easily established and sustained under laboratory conditions.

Exceptional Predictive Accuracy

Organoid culture technology can partially replicate the microenvironment of the human body, encompassing pathological, genetic, and epigenetic features of the patient. As a result, it offers a more realistic representation of individual patients, enabling precision medicine.

High-Throughput

Organoids enable large-scale cultures and parallel experiments, facilitating simultaneous screening and testing of multiple variables or drugs. This high-throughput capability renders organoid modeling highly advantageous, especially in areas such as drug development and toxicity screening.

Significant Clinical Relevance

Organoid models excel at replicating the human biological environment and exhibit a stronger correlation with clinical situations, thus offering superior guidance for drug development. In contrast, the predictive accuracy of animal models may be compromised due to inter-species differences, while 2D cell culture models often lack the necessary biological complexity.

2.5.2 Key Advantages of Organ-on-a Chip

Precision in Simulating Biomechanical and Chemical Gradients

OoC technology enables precise control of cellular and tissue structures, mimicking blood flow, oxygen and nutrient delivery, and cell-to-cell signaling. This capability allows organ chips to faithfully reproduce biomechanical and chemical gradients in the human body, providing highly realistic conditions for disease research and drug testing.

Modeling Interactions in Multi-Organ Systems

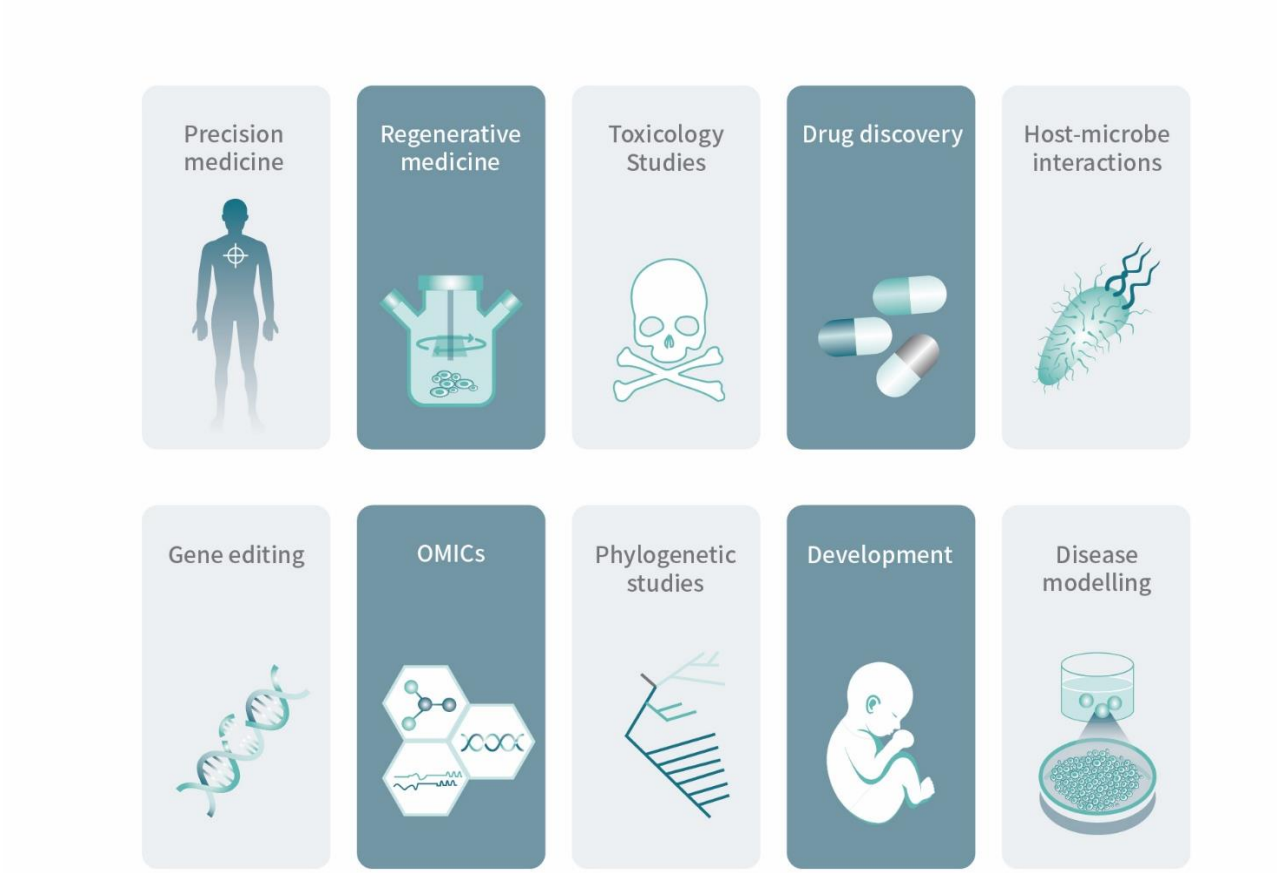
Multi-OoC technology enables the concurrent development of multiple organ models, facilitating the simulation of organ interactions and overall biological responses. This capability is crucial for comprehending complex biological processes and systemic diseases, such as cancer and cardiovascular disease.

Applicability to Rare Diseases and Personalized Medicine Research

Due to the ability of organ chips to use cells from specific patients, they provide a new avenue for rare disease research and open doors for the development of personalized medicine. This technology can help researchers better understand individual responses to drugs, thus providing more customized treatment plans for patients. Based on current research data, the involvement of organoids and OoC in the drug development process typically increases the success rate of clinical trials by 30% and helps save half of the costs[1-4].

3 Applications of Organoids and Organ-on-a-Chip Technology

3.1 Applications of Organoids in Medical Research



3.1.1 Drug Screening

Ideal drug screening platform must satisfy three requirements: good clinical correlation, short experiment duration, and high throughput capacity. In comparison to traditional two-dimensional cell line models and animal models, human-derived organoids have demonstrated distinct advantages in drug screening. They offer high clinical relevance, shortened experimental duration, and enhanced throughput. The clinical relevance of organoid based drug screening has been extensively demonstrated in various studies. For instance, Broutier *et al*. utilized hepatocellular carcinoma organoids to screen 29 anti-tumor compounds, including drugs currently in use or under development in clinical settings. They observed that hepatocellular carcinoma organoids with the *CTNNB1* mutation exhibited resistance to the Porcupine inhibitor LGK974, whereas Wnt pathway-dependent organoids showed sensitivity to

LGK974. Additionally, wild-type *KRAS* organoids exhibited sensitivity to the EGF receptor inhibitor AZD8931, whereas *KRAS* mutant organoids demonstrated resistance to it[5]. These results underscore the clinical relevance and effectiveness of organoid-based drug screening.

Considering the advantages of organoids in drug screening, prominent pharmaceutical companies have actively engaged in collaborations with organoid companies to conduct related research. The pivotal capabilities required for organoid companies to offer drug screening service encompass the ability to establish pan-cancer organoids culture and the consistence of experiment among multiple batches. Achieving these demands rigorous quality control and standardization processes, complemented by a transition towards automation in the instruments employed for both culture and analysis of organoids.

Time	Pharmaceutical Company	Organoid Company	Collaboration
2018	AstraZeneca	Emulate	Integration of organoid chip technology into AstraZeneca's IMED drug safety labs.
2018	Pfizer	Hubrecht Organoid Technology	Joint development of a human intestinal organoid platform for the identification of drug targets and screening in Crohn's disease and ulcerative colitis.
2018	Pfizer	Systeml Biosciences	Development of cerebral organoids.
2021	Sanofi	Hesperos	Preclinical study NCT04658472.
2022	Crown Bioscience	Crown Bioscience	Screening and characterization of cell therapy using the organoid platform.
2022	Bristol Myers Squibb	Prellis	Development of high-affinity human antibodies against human proteins based on human lymph node organoids.
2022	Sanofi	Prellis	Utilization of lymph node organoids to reconstruct immune responses in vitro to aid in the development of antibody therapeutics.

Corporate Collaboration Overview

3.1.2 Disease Modeling

Organoid models, owing to their functional and structural similarity to *in vivo* organs, prove invaluable in replicating diverse disease processes. These encompass a wide range of conditions, including infectious diseases, genetic disorders, rare diseases, and degenerative conditions. For instance, researchers can infect gastric organoids with *Helicobacter pylori* to gain insights into the mechanisms of infection[6]. Organoids, used to represent pathological features, can be generated through gene editing techniques or directly established from patient samples. Notably, researchers have successfully employed the CRISPR/Cas9 knockout system to create pathological models of kidney organoids. Moreover, cortical organoids derived from

human iPSCs have been developed to mimic Miller-Dieker syndrome[7].

Furthermore, in the realm of rare disease research, where mature animal models are often lacking, organoids showcase a distinctive advantage. They enable the construction of corresponding models based on humanized clinical samples within a relatively short timeframe, significantly expediting the development of related drugs. For instance, in collaboration with Hesperos, Sanofi's research involved the generation of organoid chip models that mimicked two rare autoimmune demyelinating diseases. These models were created using neurons differentiated from human iPSCs and human Schwann cells. Presently, there are no animal models that faithfully replicate the symptoms of both diseases, rendering them unsuitable for evaluating potential therapies. To address this, researchers employed an organoid chip model to recreate the autoimmune attack on myelin sheaths, resulting in a reduction in the conduction rate of neuroelectric signals. Notably, the use of TNT-005, an antibody developed by Sanofi targeting the complement system, demonstrated the recovery of nerve function in this model.

This is the first time that the FDA has approved an investigational therapy for entry into clinical trials based solely on preclinical efficacy data obtained in a human organoid chip study, combined with available safety data. This approval was not reliant on efficacy data derived from traditional animal studies. This decision not only underscores the drug developer's confidence in the data produced by organoid studies but also signifies the FDA's acknowledgment of the credibility of organoid research. This breakthrough holds the potential to open new avenues for supporting clinical research for thousands of diseases that lack suitable animal models.

3.1.3 Regenerative Medicine

The primary objective of regenerative medicine is to substitute a functionally or structurally impaired organ with healthy tissue, striving to achieve goals such as eliminating the need for immunosuppression, minimizing complications and toxicity, and preventing tissue rejection. Despite the increasing sophistication of allograft technology, challenges such as a limited number of donors and persistent issues with tissue rejection remain unresolved. The capability of amplifying organoids using patient-identical tissue for autologous transplantation offers a sustainable resource for organ replacement strategies. Autologous organ transplants are less vulnerable to magnetic fields or physical attacks compared to implantable medical devices and carry a lower risk of rejection than allogeneic organ transplants. Moreover, organoids can undergo genetic correction in cases of genetic abnormalities, facilitating the replacement of damaged organs with restored tissue[8].

3.1.4 Precision Medicine

Organoids offer a personalized treatment strategy for individual patient, encompassing *in vitro* drug screening and genotyping. This strategy aims to formulate therapeutic drugs and methods customized to each patient's unique disease profile. When integrated into precision medicine, the drug sensitivity of Patient-Derived Organoids (PDOs) can be assessed by analyzing alterations in cell viability and morphology. This information serves as a valuable resource for guiding the clinical prescription of drugs to patients. Current studies indicate a remarkable level of concordance between the drug sensitivity results from PDOs and those observed in clinical medication.

For instance, the assessment of cystic fibrosis transmembrane conductance modulator function in rectum organoids derived from individuals with cystic fibrosis enables the identification of those who may benefit from cystic fibrosis treatments[9]. In a groundbreaking study published in *Science*, Vlachogiannis G and his team conducted *in vitro* drug sensitivity testing of tumor organoids to guide clinical medication strategies. The study involved a collection of 110 tissues from 71 cases of metastatic gastrointestinal cancers for organoids construction. A comprehensive analysis encompassed a testing of a total of 55 anticancer drugs. The study yielded compelling outcomes, demonstrating the remarkable effectiveness of organoid drug screening with 93% specificity, 100% sensitivity, 88% positive prediction rate, and 100% negative prediction rate[10]. These results underscore an exceptionally high clinical relevance of the approach. Furthermore, PDOs offer a promising avenue for developing novel drugs targeting both passive and acquired tolerance[11]. Notably, PDOs exhibit heightened sensitivity to cytotoxic drugs, enhancing their capacity to predict patients' responses to pharmaceutical interventions with greater precision.

3.2 Organoid-on-a-Chip in Medical Research

3.2.1 Drug Discovery

Organ-on-a-chip technology emerges as an ideal tool for diverse applications such as drug screening, toxicity assessment, drug discovery, pharmacokinetic studies, phenotypic screening, and efficacy assessment. This technology possesses notable attributes, including exceptional reproducibility, high-throughput screening capabilities, seamless integration, and superior adaptability and functionality. For example, a study by Skardal *et al* in 2017 demonstrated the power of organoid chip technology. They assembled organoid chip representing the liver, heart,

and lungs and introduced bleomycin to induce pulmonary toxicity. Remarkably, they observed that bleomycin triggered the release of the inflammatory factor IL-1β from the lungs. Subsequently, this led to morphological damage and cessation of beating in heart-like organs, revealing clinically undetected cardiotoxic adverse effects. The study unveiled the mechanism by which bleomycin-induced inflammation in the lungs affects cardiac function. This research highlights the potential of OoC in modeling complex human organ interactions and drug side effects[12].

3.2.2 Disease Modeling

OoC possess the capacity to replicate diverse disease states, encompassing cancer, cardiovascular disease, and neurological disorders. By constructing microenvironments that closely resemble real organs through the integration of living cells (derived from patients or healthy individuals), these chips enable researchers to observe the progression of disease processes and evaluate the impact of drug treatments *in vitro*. For instance, employing organoid chip to emulate a tumor microenvironment facilitates the study of cancer cell growth, metastasis, and their response to various drug interventions. This not only aids in comprehending the pathogenesis of cancer but also serves as a platform for testing novel anti-cancer drugs, thereby expediting the drug development process[13]. For instance, in cardiovascular disease models, OoC technology can emulate heart tissue function, facilitating the study of heart disease pathogenesis and the impact of drugs on cardiac health. This is crucial for advancing the development of new drugs to address cardiovascular diseases[14]. Furthermore, OoC has proven valuable in simulating neurological diseases such as Alzheimer's and Parkinson's. By replicating brain tissue, researchers gain insights into the development of these diseases and can evaluate potential treatments more effectively[15].

OoC platforms currently employed for disease modeling are presented in the table below:

Organ or system	Functional component	Preclinical application
 HEART	· Contractible 3D conformation · Electrical stimulation · Regulated mitochondrial distribution	· Simulation of Frank-Starling mechanics in cardiomyocytes · Demonstration of auxotonic contractions · Cardiotoxicity tests · Study of physiological phenomena involved in cardiac functioning
 KIDNEY	· Barriers formation · Permeability modulation · Functional assessment under mechanical stress · Separation of tubular flow and interstitial fluids	· Selective filtration in kidney models · Simulation of type II diabetes mellitus nephropathy · Evaluation of drug-induced nephrotoxicity · Research on the physiological role of specific proteins · Study of viral infections
 LUNG	· Simulation of the alveolus/capillary interface · Modulation of cell permeability · Presence of intercellular proteins · Simulation of air-water interfaces with or without the presence of airflow	· Breathing simulation using cyclic mechanical stress · Evaluation of resistance mechanisms in lung cancer · Lung proteins study · Drug Metabolism · Systemic toxicity studies
 GUT	· Differentiation of the intestinal epithelium · Reproduction of peristalsis · Incorporation of extracellular proteins · Reproduction of intracellular and paracellular transport	· Microbiota interactions studies · Pathogenic bacteria models · Study of viral infections · Simulation of barrier function loss · Drug absorption and metabolism evaluation
 LIVER	· 3D cultures with specific liver substructures · Kupffer cell incorporation · Secretion and production of high levels of urea and albumin · Enzymatic activity · Production of bile and formation of gallbladder	· Drug metabolism studies · Hepatotoxic effect of drugs and toxicity effects on other organs (including the influence of liver metabolites) · Glycogenesis studies (and inhibition by drugs) · Fatty liver drug development
 BRAIN/CENTRAL NERVOUS SYSTEM	· Blood-brain barrier (BBB) formation · Formation of tubular vessels · Brain folding · Replication of the neurovascular unit	· Study of neurodegenerative diseases · Neural inflammatory disease · Determination of permeability of drugs and nanoparticles through BBB
 PLACENTA	· Formation of the placental barrier · Permeability modulation for high molecular weight proteins · Division of fetal and maternal chambers with fluid flow · Transfer of nutrients and glucose to fetal compartments · Evaluation of placental responses	· Simulation of drug transport across the placenta using · Study of the influence of drugs in the prenatal period
 ADIPOSE TISSUE	· Creation of adipose spheres that simulate adipose tissue in vivo · Vascular-adipose tissue interface · Fatty acid absorption	· Glucose uptake studies · Obesity models · Development of cell retention methods
 RETINA	· Retinal pigment formation · Development of the epithelium-choroid structure · Presence of structural proteins	· Pathologies models · Reproduction of imaging processes
 MUSCLE	· Contractile units through chemical or electrical stimuli	· Evaluation of the effect and toxicity of drugs · Study of degenerative diseases and muscle physiology

3.2.3 Toxicological Evaluation

The utilization of OoC technology in the realm of toxicological assessment represents a significant research avenue, offering a novel approach to safety risk assessment for chemical substances, including chemicals, drugs, pesticides, food additives, and cosmetics. By mimicking the microenvironment of human organs, this technology can provide a more precise representation of the human body's response to hazardous elements, thereby enhancing the scientific and economic efficiency of toxicity testing[16]. OoC exhibit the capability to simulate a diverse array of toxicological test scenarios, including exposure to environmental pollutants, chemicals, nanoparticles, biotoxins, and physical radiation[17]. These chips recreate microenvironments closely resembling real organs by incorporating living cells, allowing researchers to observe authentic reactions of compounds, bacteria, toxins, and more on human organs *in vitro*. For instance, OoC prove invaluable in evaluating drug toxicity. By simulating the metabolic processes of drugs within the human body, researchers can more precisely anticipate side effects and assess drug safety. This holds significant importance in expediting research, development, and market introduction of new drugs, while simultaneously mitigating risks and reducing costs in the drug development process[18].

OoC play a crucial role in evaluating the toxicity of chemicals and environmental pollutants. Through modeling the human body's response to these substances, researchers gain insights into their potential impact on human health. This understanding facilitates the development of robust safety standards and protective measures[19].

3.3 Other Applications

3.3.1 Integration of Organoids and Artificial Intelligence in Drug Discovery and Development

The integration of organoid technology's precise simulation capabilities with the robust data processing capabilities of artificial intelligence enables automated, high-throughput, multimodal data generation and analysis. This synergy produces accurate experimental data that closely mimics human physiological responses, offering an innovative platform for drug discovery and development. Artificial intelligence plays a pivotal role in expediting various facets of drug development by processing and analyzing vast amounts of intricate data derived from organoids. This includes the identification of interactions between drug molecules and specific disease markers, prediction of drug effects and potential side effects, and optimization of drug design[20]. Moreover, this integration facilitates personalized drug development. Through the integration of patient-specific genetic information and organoid data, artificial intelligence contributes to the

development of personalized therapeutic regimens tailored to an individual's unique genetic background[21].

The integration of AI is pivotal across the entire spectrum of organoid research. In the initial phases, AI plays a key role in aiding the establishment of organoid culture, assisting in tasks such as selecting suitable substrate materials and constructing models. In a multifaceted process encompassing intricate data analysis and pattern recognition, AI algorithms swiftly sift through extensive sets of candidate materials to identify the most suitable ones for a specific experimental purpose. Following the generation of experimental data, AI leverages its robust data processing capabilities to analyze complex datasets and extract valuable insights. This information not only enhances comprehension of experimental results but also offers guidance for the ongoing design and optimization of the organoid chip. The synergistic interplay between organoid and AI creates a mutually reinforcing cycle[22]. On one hand, the copious data produced by organoid experiments serves as valuable training material for AI, enhancing its analytical and predictive capabilities. Conversely, the efficient data processing and analysis capabilities of AI contribute to the advancement and application of organoid technology.

3.3.2 Personalized Human Genetics and Pharmacogenomics

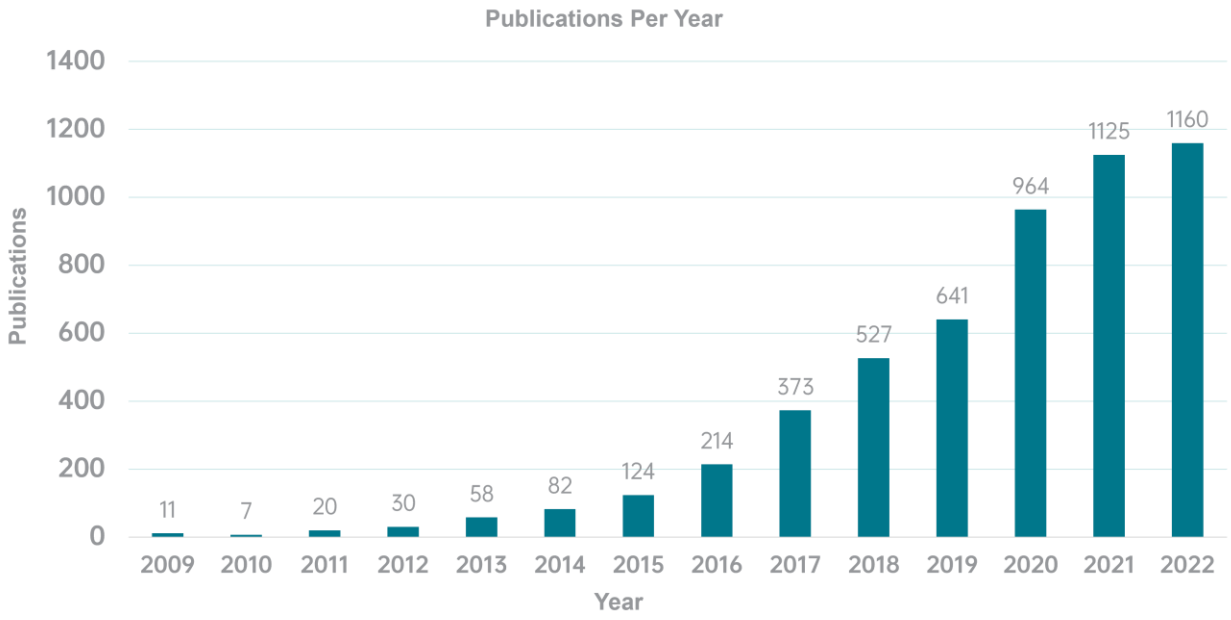
Organoids can be synergistically integrated with bioengineering techniques, such as iPSCs and CRISPR technology. This integration enables the modeling and exploration of intricate genetic diseases, as well as the study of the impact of the microbiome and other environmental factors on the human body[23]. Utilizing iPSCs, scientists can culture stem cells derived from a patient's somatic cells, demonstrating the capability to differentiate into a diverse array of different cells. Enhanced by CRISPR technology, these stem cells undergo precise editing to replicate specific genetic variants, enabling the recreation of models for particular genetic diseases with organoids. Additionally, breakthroughs in Genome-Wide Association Studies (GWAS) contribute vital genetic insights for the implementation of organoid[24]. GWAS form the foundation for personalized drug therapy by analyzing the correlation between hundreds of genetic variants and complex inherited diseases, as well as drug efficacy. This valuable information guides the design of organoid experiments, enhancing their ability to faithfully model the genetic background of specific patients. Furthermore, progress in molecular and nanotechnology has significantly streamlined the evolution organoid platforms[25]. These advancements empower organoids to deliver distinctive and personalized solutions for investigating the impacts of host genetics and environmental factors on organ physiology.

4. Current Status of Organoids and OoC

4.1 Frontier in Organoid and OoC Research

4.1.1 Overview of Organoid Models

From a research perspective, the volume of publications related to organoids and OoC technology in the PubMed database has steadily increased in recent years. It is noteworthy that in terms of the countries and regions contributing to published literature, China has now ascended to the second position after the US, emerging as a significant research force in this field. In terms of developmental stages, organoid technology remains in its early phases, with the primary focus still centered on elevating the level of biomimicry. This entails achieving more precise simulations of the morphology, structure, and physiological functions of organs under both normal and disease states. Building upon this foundation, researchers are exploring the integration of various organoids to establish multi-organoid composite systems[26].



Intestinal organoids were among the best characterized organoids in the field. Following the successful construction of the first small intestine organoid in 2009[27], researchers systematically refined the culture conditions for various intestinal cell types. In 2023, researchers achieved another milestone by creating the first functional immune system within an intestinal organoid, increasing complexity in the structure and functionality of organoids[28].

Brain organoids are currently at the forefront of research interest. Since Austrian scientists achieved a breakthrough in brain organoid construction in 2013[29], researchers have

progressed from culturing of whole-brain organoids to region-specific organoids corresponding to specific brain regions. To date, researchers have successfully developed brain organoids with regional features such as the cerebral cortex and midbrain, significantly enhancing the precision of brain function simulation and drug development[30]. In 2022, American scientists implanted human brain organoids into rat brains, successfully establishing connections with rat neurons and achieving control over rat behavior[31].

Constructing heart organoids presents a formidable challenge due to the extraordinary complexity of the heart as an organ. Nevertheless, researchers have surmounted critical challenges related to the differentiation of cardiac muscle cells, non-cardiac muscle cells, and neurons. In 2021, Austrian scientists achieved a major breakthrough by successfully cultivating the first self-organizing heart organoid using human pluripotent stem cells[32]. This year, a team from the Austrian Academy of Sciences, Institute of Molecular Biotechnology accomplished the creation of the first multi-chambered heart organoid, shedding light on how interacting heart chambers coordinate contractions[33]. This model will greatly aid scientists in studying heart development, advancing drug development, and enhancing toxicology research.

Furthermore, the long-term survival and stable function of organoids *in vitro* hinge on the transport of oxygen, nutrients, and metabolites, primarily relying on the establishment of vascular structures[34]. Currently, researchers have made significant strides in constructing vascular networks within various organoids, including the brain[35], intestines[36], heart[37], kidneys[38], and more. This accomplishment has led to the development of complex functional vascular networks *in vitro*, structures resembling the blood-brain barrier, and vascular networks capable of connecting with the host vascular system. This ability to mimic drug transport and absorption across blood vessels facilitates the evaluation of pharmacokinetics of drug candidate.

Construction of Disease-Specific Organoids

Organoids possess the unique capability to preserve the pathological, genetic, and epigenetic characteristics of samples and allow gene manipulation and editing *in vitro*. Researchers can express disease-specific genes within organoids or directly employ patient-derived samples to construct organoid models tailored to specific diseases. Currently, researchers have achieved success in building disease-specific organoids for conditions such as Alzheimer's disease[39], Parkinson's disease[40], Crohn's disease[41], inflammatory bowel disease[42], among others.

Tumor organoids have garnered widespread attention due to their potential in personalized cancer therapy. In 2014, a research team at Memorial Sloan Kettering Cancer Center in the

United States cultivated the first organoids derived from human prostate tumors[43]. Since then, researchers have successfully developed tumor organoids representing various tissues, including the intestines[44], stomach[45], liver[46], pancreas[47], breast[48], prostate[49], bladder[50], and brain[51]. Moreover, thanks to technological advancements, tumor organoids now could offer comprehensive simulations of tumor characteristics and faithfully replicate the tumor microenvironment, encompassing tumor-associated immune cells and fibroblasts. This is pivotal for gaining insights into tumor initiation, progression, and metastasis.

Construction of Multi-Organoid Composite Systems

The intricate interplay between different organs underscores the limitations of studying individual organs in isolation when striving to comprehend true physiological or pathological conditions. Consequently, researchers have shifted their focus toward constructing organoid systems capable of reflecting the intricate interactions between multiple organs. Research teams have effectively harnessed iPSC (induced pluripotent stem cell) technology to establish co-culture systems featuring organs like the liver, bile ducts, and pancreas. Furthermore, they have created systems that mimic structural connections between the brain and optic cup, as well as embryo models containing both brain and heart components.

Beyond conventional co-culture methods, scientists are actively exploring alternative technological avenues to facilitate communication between organoids. In 2020, a collaborative effort between American and South Korean scientists led to the development of a groundbreaking technology known as "organoid assembloids"[52]. This pioneering approach employs a unique "assembly" technique, enabling the construction of organoid systems with highly intricate spatial structures. Consequently, researchers gain greater precision in simulating the complex regulatory pathways that govern interactions between various systems within the human body. An exemplary application of this technology comes from research conducted at Stanford University in the United States. The research team successfully assembled iPSC-derived organoids representing the cerebral cortex, spinal cord, and skeletal muscle, thereby creating a model of the "cortico-motor pathway"[53].

4.1.2 Current Status of Organoid Culture Methods

The growth of organoids relies heavily on a variety of cytokines and a 3D extracellular matrix (ECM) environment that provides structural support. Cytokines encompass growth-promoting factors, pathway-activating agents for differentiation, inhibitors, and hormones. They also

include cytokines that facilitate cell proliferation and those added to enhance the successful generation of organoids.

In 2009, Sato and colleagues successfully cultivated organoids with crypt and villus-like structures in basic culture media by adding Wnt pathway agonist R-spondin, transforming growth factor-beta inhibitor Noggin, EGF, and more[27]. This breakthrough laid a solid foundation for the development of organoid technology. Building upon this, the same laboratory further improved the formulation by incorporating compounds such as nicotinamide, transforming growth factor-beta receptor inhibitor A-8301, prostaglandin E2, and p38 inhibitor SB202190. These additions enabled the establishment of human colon tumor organoids. Subsequently, various labs developed organoid systems for diverse tissues by modifying the composition of cytokines.

Regarding 3D system construction, early efforts often employed ECM gel as a three-dimensional scaffold. However, the use of ECM gel as a substrate could limit the exchange of gases and metabolites between organoids and their surrounding microenvironment, thereby inhibiting organoid growth and maturation. Consequently, the exploration of alternative scaffold materials has become a hotspot in organoid research.

For example, Robertson and colleagues employed the internal vascular network of the liver as a scaffold, significantly improving nutrient transport within organoids and enhancing their *in vitro* survival rate. These groundbreaking scaffold innovations have greatly propelled the continuous advancement of organoid technology.

To better retain patient heterogeneity and mimic the human tumor microenvironment, a variety of organoid culture systems have been established, ranging from the most basic embedding or droplet methods to more advanced air-liquid interface and co-culture techniques. In recent years, several studies have successfully constructed tumor-immune organoid systems using holistic or reductionist approaches. Neal and colleagues, using a holistic approach, introduced an air-liquid interface organoid model containing distinct epithelial and stromal compartments along with specific tumor-infiltrating lymphocytes. In addition to the base culture medium, they added extra T cell activators such as interleukin-2 to support immune cell growth. Consequently, various types of immune cells, including $CD8^+$ T cells, $CD4^+$ T cells, B cells, natural killer cells, and natural killer T cells, were able to survive in this system for several days. In the reductionist system, organoids derived from patient surgical or biopsy specimens were co-cultured with immune cells from the same patient's peripheral blood, enabling long-term co-culture. As a

result, the diverse range of organoid-based systems developed can cater to various organoid growth and amplification needs, providing a robust research platform for studying the tumor microenvironment and individualized cancer immunotherapy.

4.2 Market Demand

4.2.1 Drug Development: More Effective and Economical Drug Development Models

As an increasing number of new drugs receive regulatory approval, the standards for pharmaceutical research and development are continuously escalating. This trend has led to heightened challenges, greater research and development expenditures, diminished return on investment, and an exacerbated predicament in drug development. Consequently, the pharmaceutical industry is urgently seeking novel methods, paradigms, and tools to enhance the success rate of drug development.

At present, organoids and OoC technologies can be seamlessly integrated with CRISPR/Cas9 gene editing techniques for tailored model construction. They present a cost-effective approach, facilitating real-time precision manipulation, supporting high-throughput screening, enabling the observation of cellular and organ interactions, and demonstrating strong clinical relevance. Additionally, they are capable of replicating tissue vascularization and the immune microenvironment, thereby providing substantial clinical reference value.

Within the pharmaceutical companies, the primary concerns on drug development revolve around cost, the success rate of clinical trials, and project efficiency. Currently, the cost of progressing from the discovery of a drug candidate to clinical trials, excluding compound synthesis and non-primate animal experiments, hovers around 7 million USD. Should primate experiments be included, the costs rise even further. However, when organoids are employed for these purposes, expenses can be reduced to approximately 3.5 million USD. In terms of clinical relevance, organoids simulate patient-specific responses to treatments, complementing traditional 2D cell lines, GEMM, and PDX models[54]. They serve as a bridge between drug discovery and clinical trials and have become invaluable tools in drug development and screening.

Recent research has already demonstrated the precise prediction of drug responses in colorectal cancer (CRC) patients using CRC organoids, serving as preclinical models to validate drug safety and effectiveness[55]. Oral cancer organoids effectively screen drugs for oral cancer

treatment by emulating the structure, function, and microenvironment of oral tissues[56]. The development of *in vitro* models for liver function is crucial for accurately assessing drug toxicity. Complex *in vitro* liver organoids, constructed using HepaRG liver cells, bile duct-like cells, LX-2 cells, and human primary macrophages, closely mimic most liver functions and serve as relevant *in vitro* models for non-alcoholic fatty liver disease (NAFLD) drug metabolism, drug toxicity, and adverse drug events[57].

Moreover, organoids enable high-throughput drug screening, significantly reduce experimental timelines, and greatly enhance drug development efficiency compared to animal experiments. Furthermore, organoids can provide *in vitro* models for rare diseases that lack animal counterparts and offer insights for indication expansion. The growing complexity of drug and treatment strategies is propelling the rapid expansion of the organoid and OoC industry.

4.2.2 Personalized Medicine: Providing Individualized Disease Treatment and Prevention Strategies

Precision medicine has emerged as a focal point in the global healthcare landscape in recent years, particularly in developed countries that allocate substantial funding to advance this field. The utilization of organoids for drug sensitivity testing, especially in the context of tumor patients, offers a powerful means to accurately predict how individual patients will respond to medications. This predictive capability empowers patients to make informed decisions, avoiding drugs with toxic side effects and minimal therapeutic benefits while opting for treatment plans that effectively target their specific diseases. Consequently, this approach reduces the risk of drug resistance, lowers the likelihood of tumor recurrence, and significantly enhances the overall quality of life for patients. Given the substantial and continually growing population of cancer patients, organoids have become increasingly relevant in the realm of drug sensitivity testing.

In a groundbreaking study published in 2018 in the journal *Science*, Vlachogiannis and colleagues compared a library of gastrointestinal tumor organoids with corresponding clinical trials. Their findings unequivocally validated the remarkable predictive capabilities of tumor organoids when it comes to assessing drug efficacy. The results revealed that tumor organoids boasted an impressive performance with 100% sensitivity, 93% specificity, 100% negative predictive value, and 88% positive predictive value[10]. Recent research has further underscored the striking resemblance between drug responses observed in organoid models and clinical outcomes. This underscores the pivotal role played by organoid-based *in vitro* drug sensitivity testing in the arena of precision medicine[58-60].

Country & Region	Period of Time	Amount	Purpose	Scope
European Union	2007-2013	€ 1 billion	Research on Personalized Medicine in FP7	International
	2008-2013	€ 2 billion	First phase of the Innovative Medicines Initiative (IMI)	
	2008	€ 50 billion	Pan-European Biobank and Biomolecular Resources Research Infrastructure (BBMRI)	
	2012	€ 160 million	Advancement of patient cohort stratification techniques, enhancement of omics data statistical methods, validation of biomarkers, etc.	
	2014-2020	€ 80 billion	Personalized Medicine Research	
	2014-2024	€ 3276 billion	Second phase of the Innovative Medicines Initiative (IMI)	
	2016	€ 343 million	Horizon 2020 Personalized Medicine funding projects	
	2017	€ 315 million	Horizon 2020 Personalized Medicine funding projects	
America	2007	$ 277 million	Funding Personalized Medicine Projects	National
	2008	$ 352 million	Funding Personalized Medicine Projects	
	2008	$ 330 million	Financing research on the cost, effectiveness, and quality of healthcare, fostering the integration and innovation in personalized medication across various fields, and investing in medical information technology	
	2009	$ 1.1 million	The Million Veteran Program	
	2011	$ 7.5 million	Precision Medicine Initiative	
	2016	$ 215 million	Comparison of treatment efficacy for specific diseases and specific patients	
	2016-	$ 1.5 billion	Comparison of treatment efficacy for specific diseases and specific patients	
United Kingdom	2006	£ 61 million	UK Biobank Project	National
	2007	£ 17 million	Biomarker Research Program	
	2011	£ 130 million	Support for the development and commercialization of stratified healthcare	
		£ 60 million	Support for enterprise-led stratified medicine projects	
		£ 200 million	Stratified Medicine Innovation Platform	
		£ 60 million	Funding for non-cancer stratified medicine application projects	
	2011-2015	£ 50 million	Facilitating the connection between cancer drug discovery and early development	
		£ 300 million	Supporting industry collaboration with UK higher education and research institutions	
	2013	£ 100 million	Whole-genome sequencing of 100,000 cancer and rare disease patients	
	2014	£ 300 million	100,000 Genomes Project	
	2015	$ 375 million	100,000 Genomes Project	
	2015-2019	£ 50 million	Stratified Medicine Incubator	
	Each Year	£ 500 million	Infrastructure development at the UK Health Research Institutes; supporting experimental drug development and clinical trials within the UK NHS	
Germany	2013-2016	€ 360 million	Personalized Medicine Action Plan	National
France	2016-2025	€ 670 million	Genomic Medicine Program 2025	National
Sweden	2017-2020	CHF 80 million	Personalized Medicine Action Network Plan	National

Funding Status for Personalized Medicine and Related Fields (By Country/Region)

4.3 Policy and Regulatory Environment

4.3.1 Supportive Policies and Key Events

United States

- In 2011, the U.S. government initiated the Human-on-Chip program, led by NIH, FDA, and DARPA, to advance the development of safety assessments before conducting human trials for drugs.
- In 2012, NIH, FDA, and DARPA jointly launched the "Organs-on-chips" project with a total investment exceeding $2 billion.
- In 2017, CASIS, in collaboration with NCATS and NIH, established multiple funds to support experiments on human organ chip technology at national laboratories. In the same year, Emulate announced a "Collaborative Research and Development Agreement" with the FDA to evaluate and identify the potential use of Emulate's organ-on-chip technology as a platform for toxicology testing.
- In 2018, the FDA accepted some experimental data based on liver chips as part of pediatric drug development proposals and encouraged drug development organizations to submit supplementary data.
- In 2019, the EPA announced its goal to significantly reduce mammalian research by 2035 and proposed alternative methods such as "computational modeling" and "organ-on-chip" technologies.
- In 2021, organoids were encouraged as models for pediatric cancer. The FDA's Oncology Center of Excellence encouraged the use of patient-derived xenograft models and organoid models in the research and drug development for most pediatric cancers, where research models were lacking.
- In 2022, the FDA approved the world's first drug for clinical trials based entirely on "organ-on-chip" research. The approval of Sutimlimab, which obtained preclinical data, signifies a breakthrough for organoid technology in clinical research for thousands of diseases without animal models. Additionally, the U.S. Senate passed the FDA Modernization Act 2.0, aiming to eliminate federal requirements for animal testing in the development of new drugs and generics, with the goal of significantly reducing the use of animal experiments in the coming years.

The year 2022 marked a milestone for organoids and OoC technologies. The FDA's approval of a drug for clinical trials based entirely on preclinical data from OoC experiments symbolized the acceptance and recognition of this technology by authoritative regulatory agencies. In the same year, both the U.S. House of Representatives and the Senate passed the FDA Modernization Act 2.0, with the aim of eliminating mandatory federal requirements for animal testing in the development of new drugs and generics. This initiative is expected to significantly reduce the use of animal experiments in the coming years, further expanding the application and potential of organoid technology.

European Union

- In 2010, the Berlin Institute of Technology in Germany received support from the GoBio fund for research in related fields.
- The European Union's Seventh Framework Program, with a total budget of €50.521 billion, included the "Human-on-Chip" project.
- Initiatives such as the EU-Tox Risk project, starting in 2016, also include support for organ-on-chip technology. In the same year, organoid chip technology was listed as one of the "Top Ten Emerging Technologies" by the World Economic Forum in Davos.
- On July 25, 2023, the European Union outlined legislation and policy frameworks related to animal experimentation, aiming to gradually phase out animal testing and improve animal welfare. This includes preserving and strengthening the ban on animal testing for cosmetics. This ban is expected to greatly promote the application of organoids and organ-on-chip technology in preclinical trials

Asia and Other Regions (Using China as an Example)

- In 2013, the Chinese Ministry of Science and Technology initiated the "Key Technologies for New Drug Research and Development Based on Microfluidic Chips" as part of a major research project.
- The establishment of the "Organ-on-Chip" Higher Education Discipline Innovation Introduction Plan (known as the "111 Plan") by joint efforts from Southeast University, Harvard University, Cambridge University, and others.

- On January 28, 2021, the Chinese Ministry of Science and Technology issued a notice entitled "Solicitation of Opinions on the Guidelines for the 2021 Project Proposal of 6 Key Special Projects under the '14th Five-Year Plan,'" which listed "Malignant Tumor Disease Models Based on Organoids" as one of the first key tasks under the '14th Five-Year Plan' national key research and development program.
- On November 30, 2021, the Center for Drug Evaluation of the China National Medical Products Administration (NMPA) issued "Technical Guidance Principles (Trial) for Non-Clinical Research and Evaluation of Gene Therapy Products" and "Technical Guidance Principles (Trial) for Non-Clinical Research of Gene-Modified Cell Therapy Products." For the first time, these guidelines included organoids as part of the guidance for gene therapy and gene-modified cell therapy products.
- In February 2022, the Chinese Ministry of Science and Technology released a draft of the "2022 Guidelines for the Key Special Project on Stem Cell Research and Organ Repair," emphasizing the significant value of organoid disease models in cancer research.
- In June 2022, the Chinese Anti-Cancer Association's Multidisciplinary Diagnosis and Treatment Committee and Endocrinology Committee, in collaboration with the Guangdong Organoid Engineering Technology Research Center and the Guangdong Precision Medicine Application Society's Organoid and Organ-on-Chip Division, jointly published the "Expert Consensus on Clinical Applications of Tumor Organoid Drug Sensitivity Testing on the Tumor Diagnosis and Treatment Platform (2022 Version)."
- In September 2022, under the guidance of the Chinese Stem Cell Standards Committee and Academician Ye-Guang Chen, dozens of Chinese institutions jointly formulated the first group standards for intestinal organoids: "Group Standards for Human Intestinal Cancer Organoids" and "Group Standards for Human Intestinal Organoids."
- On September 20, 2022, the Shanghai Genetics Society and the Shanghai Metrology and Testing Society jointly released the "Technical Specifications for the Cultivation of Human Lung Cancer Organoids."
- In 2022, a national draft standard titled "General Technical Requirements for Skin Chips" was initiated, led by Professor Zhongze Gu from Southeast University.
- In February 2023, the China Association for Medical and Biotechnology released the "Operation Guidelines for the Preparation, Cryopreservation, Revival, and Identification of

Human Normal Mammary Gland and Breast Cancer Organoids."

4.3.2 Milestone Cases

Milestone Cases in Europe and America

- The First Study solely Based on OoC Data to Submit and Obtain Investigational New Drug (IND) Approval

In 2022, the FDA approved the world's first new drug (NCT04658472) to enter clinical trials solely based on preclinical data obtained from "organ-on-a-chip" research. This experiment was conducted in collaboration between Sanofi and the organ-on-a-chip company Hesperos, aimed at treating two rare autoimmune demyelinating neurological diseases, namely Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) and Multifocal Motor Neuropathy (MMN). It became the first study to submit and obtain IND approval based on OoC data.

- Organoids Guide Precision Treatment of Rare Diseases

Cystic Fibrosis (CF) was one of the earliest diseases to be studied using organoids and also one of the first to incorporate organoids into clinical research. The Dekkers team in the Netherlands collaborated with the American company Vertex to use intestinal epithelial organoid technology to study cystic fibrosis. They conducted drug sensitivity tests *in vitro* using organoid models to predict the efficacy of drugs, becoming the first case where organoid technology was used as a companion diagnostic for efficacy prediction.

Milestone Cases in Asia (Taking China as an Example)

- Cardiac Organoid Chip Data support Cardiovascular Drug Approval for Clinical Trials

At the end of May 2023, Hengrui Pharmaceuticals's drug HRS-1893 was approved to conduct clinical trials. HRS-1893 is a drug that inhibits excessive myocardial contraction through a special mechanism, primarily intended for the treatment of hypertrophic cardiomyopathy and heart failure caused by myocardial hypertrophy. During its development, a subsidiary of Hengrui Pharmaceuticals used cardiac organoid chip technology from Avatarget for *in vitro* screening, simulating the microenvironment of the human heart and providing more accurate data support for drug development.

- Organoid chip data help Bispecific-Antibody Immunotherapy get IND clearance

In July 2023, the website of the Center for Drug Evaluation (CDE) of the National Medical Products Administration showed that Qilu Pharmaceutical's class I new bi-specific antibody, QLF3108, received IND clearance for clinical trial. This was the first bi-specific antibody to enter clinical trial using organoid chip data. The indication for QLF3108 is advanced solid tumors. During its development, Daxiang Biotech used the IBAC O2 chip to construct a tumor organoid immune co-culture model, providing key data support for the development of QLF3108. This model more accurately simulates the tumor microenvironment within the human body, offering a more effective method for drug development and evaluation.

- China's First Cell Therapy Drug Aided by Organoid Chips Data Enters Clinical Trials

In June 2023, Imunopharm Technology developed a new generation anti-cancer drug, IM83 Chimeric Antigen Receptor T-cell Injection (IM83 CAR-T Cell Injection), which received the clinical trial approval notice from the National Medical Products Administration for the treatment of advanced liver cancer. Notably, its strategic partner, DaXiang Biotech, provided Imunopharm Technology with an evaluation service for CAR-T drug efficacy based on the tumor chip model. This service efficiently and accurately screened potential CAR-T drug candidates, with the relevant data incorporated into the IND (Investigational New Drug) submission package. It is worth mentioning that IM83 CAR-T Cell Injection has become the first cell gene therapy (CGT) drug in China to utilize organoid chip data and receive IND approval.

4.3.3 Ethics and Compliance

Ethics

Ethical Considerations for Alternatives to Animal Testing: Organoid and OoC technologies are considered potential alternatives to animal testing. The development and application of these technologies reduce dependence on animal experiments, addressing some ethical concerns. However, the effectiveness and reliability of these technologies still need further validation to ensure they can truly serve as alternatives to animal experiments.

Use of Human Materials: Organoid technologies typically involve the use of human cells and

tissues, raising ethical questions about their sourcing, informed consent, and privacy. Ensuring that the procurement of human materials meets ethical standards and patient consent is crucial. Risk of Technology Misuse: With technological advancements, there is a risk of misuse, such as conducting human enhancement or non-medical research without appropriate oversight.

Compliance

Regarding compliance, currently, the United States and other Western countries primarily rely on standardized systems established by companies like HUB for conducting targeted scientific research. The global organoid industry is developing rapidly, but many countries need to enhance their capabilities in terms of organoid culture quality control and sample compliance. Work on addressing compliance and ethical issues related to organoid sample sourcing is still in progress, and some models in organoid libraries have not yet reached applicable standards, lacking relevant quality control criteria. In terms of compliance, the ultimate goal is to achieve downstream compliance for organoids, including GMP-grade reagents, ethical compliance for human-derived organoids, commercial compliance for organoid libraries, and the integration of new medical technologies into clinical practice. Human genetic property of organoids is of particular concern. As derivatives of primary cells or tissues, it remains unclear whether organoids qualify as human genetic resource materials, and specific management regulations are gradually being improved.

4.4 Capital Markets

4.4.1 Financing Status of European and American Organoid Companies(Partial)

It has been nearly a decade since the establishment of the first organoid or OoC companies in the US or Europe. As a result, there is a noticeable trend of maturation in both their business operations and financing status. Many companies have successfully progressed to series A funding rounds or beyond and have established stable collaborations with pharmaceutical companies. Additionally, there is a growing preference for companies that have integrated automation and machine learning, utilizing AI-assisted techniques to streamline high-throughput sample handling and data processing.

Financing Status of European and American Organoid Companies(Partial)

Company	Country	Year Founded	Latest Funding Date	Latest Funding Amount	Latest Deal Type	Latest Investors	Total Funds Raised
Cellesce	United Kingdom	2013	June, 2019	-	Seed	Tangram Partners Limited	--
Cortical Labs	Australia	2019	April, 2023	Nearly $10 million	Seed	Horizons Ventures, Life Extension Ventures, In-Q-Tel, Radar Ventures, Blackbird Ventures	--
HeartBeat.bio AG	Austria	2021	November, 2023	€ 4.5 million	Pre-A	i&i Biotech Fund, Tensor Ventures	€ 6 million
Herophilus	America	2017	January, 2021	$ 1.6 million	Seed	KF Ventures, Kinled Holding, Luxor Capital Group & 515 Ventures	--
JangoBio	America	2015	January, 2018	$ 112, 500	Seed	--	Nearly $ 2.51 million
NextVivo	America	2021	December, 2021	$ 7.9 million	Seed	Khosla Ventures, Wilson Sonsini Goodrich & Rosati, Alexandria Venture Investments	--
Parallel Bio	America	2021	December, 2022	$ 4.3 million	Seed	Refactor Capital, Breakout Ventures etc.	--
Prellis Biologics	America	2016	August, 2022	$ 35 million	Series C	Avidity Partners, Celesta, Lucas Venture Group, True Ventures, SOS Ventures, Khosla Ventures	$ 60 million
SEngine Precision Medicine	America	2015	July, 2022	$ 10 million	Series A	Vincere Capital, Alethea Fulcrum Fund, Washington Research Foundation, Bangarang Group	$ 18.1 million
Xilis	America	2019	July, 2022	$ 19 million	Series A+	FPV Ventures, Alix Ventures, Felicis Ventures etc.	Nearly $ 100 million

In addition to financing, major players in the life sciences sector have entered the organoid field through acquisitions or collaborations. Companies like Johnson & Johnson, AstraZeneca, Pfizer, and others have been establishing partnerships with organoid companies since 2015, integrating organoid technologies into their in-house laboratories.

Furthermore, over 20 top pharmaceutical companies, including AbbVie, Merck, Novartis, and others, have jointly established a non-profit organization called the Innovation and Quality

Consortium (IQ Consortium). This consortium is dedicated to promoting the standardized application of organoid technologies to accelerate the drug development process.

In March 2022, Hans Clevers, known as the "international pioneer of organoids," officially joined Roche as head of Research and Early Development Department. He focuses on using organoids to better predict the safety and effectiveness of candidate drugs.

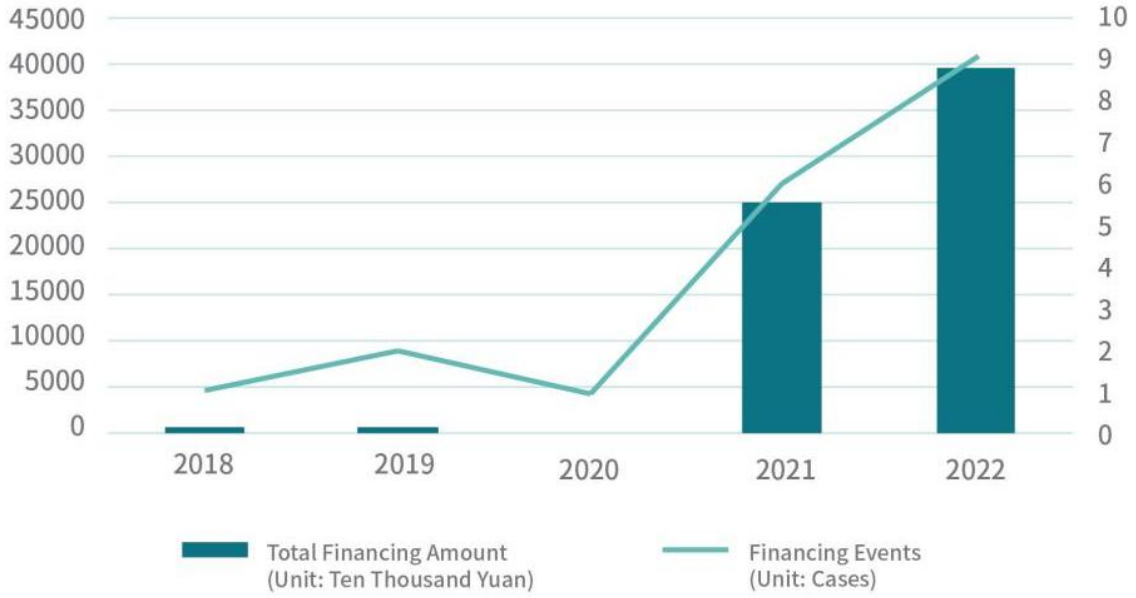
Acquired Organization (Abbreviation)	Acquiring Organization	Acquisition Date	Purpose of Acquisition
Cellesce	Molecular Devices	2022	Development of Automated Organoid Screening Machine
Epistem	Foresight Group	2018	Support for Epistem's independent operation and growth
OcellO B.V.	Crown Bioscience	2021	Expansion of Preclinical In Vitro Drug Development Platform
TARA Biosystems	Valo Health	2022	Construction of Cardiovascular Drug Development Platform
Trevigen	Bio-Techne	2017	Enhancement of Drug Discovery and Toxicology Product Portfolio

Overview of Mergers and Acquisitions in Organoid Companies

4.4.2 Overview of Financing in the Organoid and Organ-on-a-Chip Industry in Asia (Taking China as an Example)

In 2021 and 2022, the organoid and organoid chip industry in Asia witnessed rapid growth in financing trends. Both the number of financing events and the total financing amounts reached new highs. In 2021, there were a total of 6 financing events in the Chinese organoid and organ-on-a-chip industry, with a total financing amount of 250 million RMB (about 35 million dollars). In 2022, there were 9 financing events in the Chinese organoid and organoid chip industry, with a total financing amount of nearly 400 million RMB (about 56 million dollars).

Financing Status of Chinese Organoid Companies (Partial)



Company	Year Founded	Latest Funding Date	Latest Funding Amount	Latest Deal Type
AimingMed	2020	November, 2022	Tens of millions of RMB	Pre-A
Avatarget	2021	July, 2022	Nearly 100 million RMB	Pre-A
Biogenous	2022	January, 2023	Nearly 100 million RMB	Series A
Accurate International	2018	October, 2022	100 million RMB	Pre-B
Daxiang Biotech	2018	November, 2022	Nearly 100 million RMB	Pre-B
D1Med	2019	October, 2021	120 million RMB	Series A
Guidon Pharmaceutics	2017	March, 2023	-	Series A
HeHu Technology	2020	January, 2022	50 million RMB	Angel
HonrayMed	2021	July, 2022	Tens of millions of RMB	Pre-B
Asia Regenerative Medicine	2018	February, 2023	Tens of millions of RMB	Pre A+
Hunter Biotech	2010	August, 2019	-	Angel
Hopstem Biotech	2017	November, 2021	Hundreds of millions of RMB	Series B
kingmed Pharma	2019	November, 2022	-	Series A+
Regenovo	2013	2018	-	Series B
Jingke Biotech	2015	April, 2018	-	Series A
Cancerfree Biotech (Taiwan, China)	2018	April, 2021	$ 428, 600	Pre-Seed
K2 Oncology	2016	August, 2022	Tens of millions of RMB	Series B
Puheng Technology	2021	April, 2022	Nearly 10 million RMB	Angel
Probro	2020	March, 2020	-	-
Puissan Biotech	2019	2020	-	Angel
Cyberiad Life Sciences	2020	2021	Tens of millions of RMB	Angel
TulymBio	2016	October, 2021	Tens of millions of RMB	Series A
OneTar Biomedicine	2020	November, 2021	-	Angel
Signet Therapeutics	2020	August, 2023	-	Pre-A
Xellar Biosystems	2021	August, 2022	Tens of millions of dollars	Angel
ChexMed	2021	January, 2022	-	Angel

Financing Status of Chinese Organoid Companies (Partial)

5. Business Models and Industrial Chains of Organoids and Organ Chips

5.1 Industry Overview

The organoid industry chain is mainly divided into three parts: upstream, midstream, and downstream. Upstream companies focus on providing reagents and materials required for 3D cell culture, such as cell scaffold materials, extracellular matrix, cell growth factors, culture medium, and bioreactors. They contribute to material innovation and iterative upgrades in culture technology. Some of them have expanded from upstream to the midstream in recent years. Companies in the midstream are product- or service-based, offering microphysiological systems, organoid chips, related equipment, or customized services for precision medicine or drug development. The downstream comprises those who use organoid or OoC technology for various applications, including pharmaceutical companies, contract research organizations (CROs), universities, hospitals, and other scientific research institutes engaged in drug research and development.

Organoid companies mainly have four business models: scientific research cooperation, reagent supply, precision medicine, and assistance in drug development. Due to the relative maturation of tumor organoid techniques and easier access to samples, tumor organoid-based drug sensitivity testing for precision medicine is the major type of service for organoid companies. Most companies include this service in their business, which allows them to access clinical samples, opportunities for culture condition iteration, and data accumulation. Those with mature products/services and accumulated data/samples could directly sell products to academic or industrial customers or cooperate with pharmaceutical companies to assist in drug development. Some of them even carry out their own drug discovery based on their know how in relative disease field. Those with expertise in degenerative diseases also employ organoids as a means of regenerative medicine.

In the drug development business, organoid companies are more like preclinical CROs. Mayjor requirement of Pharmaceutical companies are the consistence and heterogeneity of organoids, as well as whether the organoid library is comprehensive and whether needed disease models are available. But in the precision medicine business, customers are more focused on operational details and quality control. Currently, organoid culture for drug screening is fast and has a high success rate. Drug screening can usually be conducted in just one week, and it only takes 2-3 weeks from sample collection to results. In this process, using organoid technology , multiple drugs or different concentrations can also be screened on multiplex plates. At present, the drug screening business of organoids has not yet reached the level of *in vitro* diagnosis (IVD) products, and services can only be provided in the form of laboratory developed test (LDT).

5.2 Panoramic View of Industrial Chain

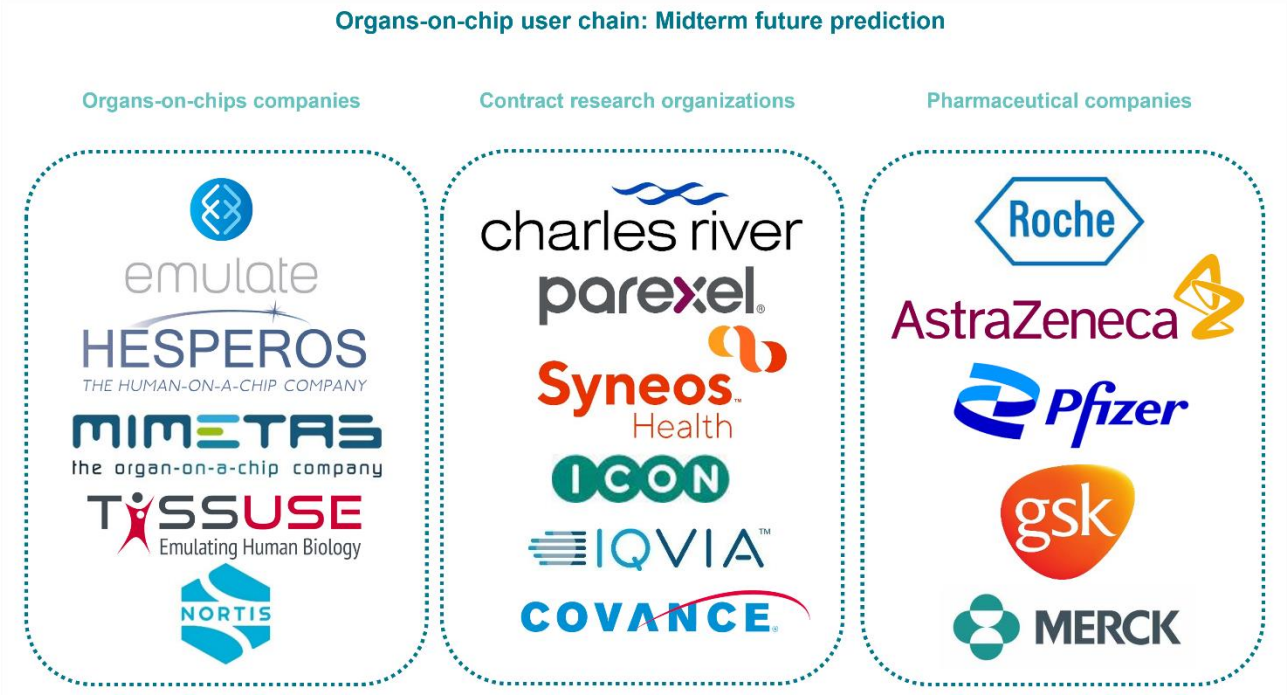
5.2.1 Upstream of the Industrial Chain

Upstream companies in the organoid industry, such as Thermo Fisher, Sigma-Aldrich, and STEMCELL Technologies, mainly focus on providing reagents and experimental materials for 3D cell culture, including providing cell scaffold materials, extracellular matrix, cell growth factors, culture media, and biological reactors *etc*. According to Meticulous Research Analysis, the global cell analysis and testing market value of 3D tissue culture in 2017 was US$818.1 million, and will reach US$1.2426 billion in 2022. Among them, the United States contributes

approximately 34.8% of the major share of the global 3D cell market, ranking the first in the world. China is expected to grow at a compound annual growth rate of 11.8%, becoming the country with the highest annual compound growth rate and possessing market development potential.

5.2.2 Midstream and Downstream of the Industrial Chain

The middle reaches of the industrial chain mainly provide services for the culture of human or animal organoids, the cryopreservation and storage of organoids, and the passage of organoids. The revenue point of mid-stream and downstream companies in the organoid industry mainly lies in providing *in vitro* drug testing solutions and disease models to major new drug testing companies, that is, clinical trial outsourcing services. Several pioneer companies have made collaborations with pharmaceutical companies for drug screening. As an important driver of new drug research and development, the downstream customers of organoid and OoC companies mainly include CROs and pharmaceutical companies.



5.2.3 Overview of Midstream and Downstream Cooperation in Recent Years

Hubrecht Organoid Technology (HUB), founded by the team of Hans Clevers, the originator of organoids, is the world's first organoid R&D center, and HUB has also helped the first batch of organoid companies emerge such as Epistem , Cellesce , Crown Bioscience, and STEMCELL

Technologies through technology licensing. This year, HUB officially transitioned from a foundation to a company.

In addition, technology companies established by university research institutes are also the mainstay of this type of OoC company, such as Emulate, a biotechnology company established in 2013 by the Wyss School of Bioengineering at Harvard University.

Previously, most OoC companies indirectly provided services to pharmaceutical companies by selling products to CROs. After 2015, more and more pharmaceutical companies have entered the market directly through purchasing products, cooperating with licensing, and investing, becoming another force in this field.

- In 2015, J&J purchased thrombosis chips from Emulate to assess the procoagulant properties of marketed drugs or drugs under development .
- In 2016, Merck, Seres Therapeutics and others purchased Emulate's organoid chips for new drug development and testing.
- In 2018, AstraZeneca reached an agreement with Emulate to integrate its organoid chip technology into AstraZeneca's IMED drug safety laboratory; AstraZeneca is also the first big pharma to integrate organoid chip technology into its internal laboratory.
- In May 2018, Pfizer collaborated with HUB to develop a human intestinal organoid platform to study Crohn's disease, ulcerative colitis and other diseases.
- In September 2018, System1 Biosciences, a neurotherapy start-up company, received US$25 million in Series A financing led by Pfizer to develop its own brain organoids to develop new methods of neurotherapy.
- In 2021, Sanofi cooperated with Hesperos to jointly conduct preclinical research for trial NCT04658472.
- In January 2022, Bristol-Myers Squibb entered into a collaboration with Prellis to create high-affinity human antibodies targeting human proteins based on its human lymph node organoid platform.
- In March 2022, Sanofi reached a cooperation with Prellis to use its platform to reconstruct immune responses *in vitro* to provide antibodies with significant genetic diversity.

  In addition, more than 20 top pharmaceutical companies including AbbVie, Merck, and Novartis jointly established the non-profit organization IQ Alliance (Innovation Alliance and Quality Consortium), committed to promoting the standardized application of organoid chips to accelerate the drug development process. A series of industry standards have been published for liver, kidney, lung and other models.

6. Challenges and Future Perspectives

6.1 Challenges

6.1.1 Challenges of Organoids

Despite the revolutionary impact of organoids on the field of medicine, their development still faces numerous challenges, including high costs, complex vascularization, and the simulation of immune environments. In the future, the key trends in organoid development will involve systematization, standardization, and automation of the technique.

Cost Efficiency

Organoids, as a next-generation research model, offer cost advantages compared to animal models like Patient-Derived Xenograft (PDX) models but are still significantly more expensive than traditional cell lines. The main consumables required for culturing organoids, such as matrix gels, culture mediums, Transwell, or ultra-low attachment plates, are costly and can vary between batches, especially matrix gel. Therefore, developing matrix gels with consistent batch-to-batch quality and reducing the cost of core consumables are critical factors for the industry's future development. Organoid chips, involving chip manufacturing and microfluidic systems, present greater challenges in cost reduction.

Vascularization Challenge

Most organoids do not develop a complete vascular structure during formation, leading to inadequate oxygen supply and poor removal of metabolic waste as they increase in size, potentially resulting in tissue necrosis. To address this issue, researchers have started incorporating endothelial cells into the microenvironment of tumor organoids to promote the formation of vascular structures.

Immune Environment Simulation

Simulating the immune environment is another major challenge in organoid research, particularly in replicating the interaction between tumors and immune environments. In recent years, scientists have begun co-culturing tumor organoids with immune cells to more accurately

mimic the tumor microenvironment. For example, by adding activated immune cells to the culture medium, growing tissues alongside immune cells, or incorporating recombinant cytokines in the extracellular matrix (ECM), researchers can effectively reshape the interaction between organoids and immune cells. These research methods hold significant potential for simulating and understanding immune responses in the tumor microenvironment.

Systematization

Systematization is a key direction for the development of organoid research, aiming to construct multiple interconnected organoids to comprehensively assess drug efficacy and potential toxicity. While current organoid models can detect the inhibitory effects of drugs on tumors, they cannot predict potential side effects or safety risks on other organs and tissues. To address this, researchers have started constructing organoid systems that include multiple organs (such as heart, lungs, and liver) integrated into a closed loop. These systems can comprehensively demonstrate the effects and toxicity of drugs on different organs, providing more comprehensive and accurate data support for drug development.

Standardization and Automation

Standardization and automation are crucial factors in advancing organoid research toward industrialization. Currently, each step of the process, from sampling, culturing, and testing to data reporting, relies heavily on manual operations. This reliance on manual processes means that the quality of operations and quality control at each step can impact the final test results. Therefore, achieving full industrialization of organoids requires support from industry policies, along with technical advancements in standardization and automation to enhance operation accuracy and efficiency, reduce errors, and ensure the accuracy and reliability of test results.

6.2 Future Perspectives

6.2.1 Future Outlook for Organoids

The field of organoid technology is poised for significant advancements in the future, particularly in terms of the quantity and diversity of organ tissue models. It is anticipated that more organoid cell atlases and biobanks will be established, enriching the variety of organoids available. As

culturing methods for the complex microenvironments of multiple tissues and organs continue to improve, the simulations of real physiological environments will become more accurate.

Moreover, regulatory frameworks and ethical guidelines established by authoritative organizations are expected to evolve further, fostering the industrialization, commercialization, and automation of high-throughput processes in organoid technology. The integration of organoid technology with existing technologies such as Next-Generation Sequencing (NGS) and immunotherapy will become more seamless, expanding its applications in precision medicine.

Organoids will play an increasingly prominent role in simulating complex organs and inter-organ interactions, exploring pathogenic mechanisms, and providing personalized treatment strategies tailored to various age groups, genders, and racial backgrounds. Ultimately, the aspiration for organoid technology is to achieve the complete *in vitro* cultivation of intricate bodily organs, contributing significantly to advancements in regenerative medicine and organ transplantation.

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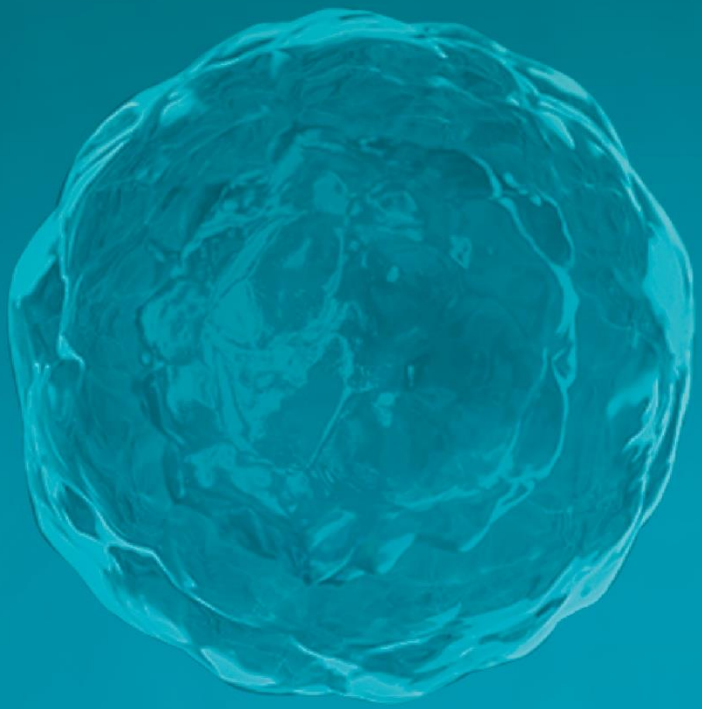

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