Frontier Interdisciplinary Strategy

Research Report on Organoids and Organ Reconstruction

Organoids and Organ Reconstruction Research Team

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Abstract

Organ failure caused by diseases, aging, and injuries poses a major threat to human health. Challenges such as slow or insufficient self-repair, organ shortage for transplantation, and safety issues in xenotransplantation have made organ dysfunction repair a persistent focus and difficulty in the fields of life sciences and health research. Organoids, which can simulate key structures and specific functions of corresponding organs well *in vitro*, hold significant implications for organ development, disease modeling, drug screening, precision medicine, and more. Leveraging organoid technology and interdisciplinary integration at the forefront, the recreation of organ structure and function *in vitro* is crucial for organ reconstruction, transplantation, disease research, and functional restoration.

This project systematically reviews the overall development trends, current status, significance, and value of organoids and organ reconstruction in China. Combining a general overview of the field, the project integrates cutting-edge technologies in organoid research and categorizes developments and strategic values in nine aspects: digestive system, respiratory system, nervous system, endocrine system, urinary and reproductive system, musculoskeletal system, circulatory system, embryoid body models, and tumors. It summarizes the scientific and technical challenges faced by each area and outlines the research foundations and conditions to drive the forward-looking development of advanced technologies. Building upon the development of organoids, the project also assesses interdisciplinary integration in the field of organ reconstruction, including the role of organ chips in promoting the integrity and stable function of reconstructed organs, vascularization of organ tissues for substance exchange and long-term maintenance, 3D bioprinting for cellular diversity and precise structure construction, material science support for biological materials, and the synergies between organoids and organ transplantation for organ repair and regeneration. The project concludes with a summary of these interdisciplinary advantages. In addition to disciplinary development and interdisciplinary assessments, the project provides a

summary of relevant theoretical issues such as ethical status and dignity, privacy protection, and commercialization. Furthermore, the project offers insights and policy suggestions for the development of this field, encompassing capacity building, institutional development, and regulatory construction.

In summary, starting from the development of organoids and incorporating interdisciplinary approaches, the project enhances the maturity, applicability, efficiency, and complexity of organoid models. It aims to propel the field of regeneration from local repair to overall reconstruction, from single-function evolution to achieving multi-functional organs, and from *in vitro* cultivation to the significant breakthrough of *in vivo* transplantation. By summarizing and organizing the interdisciplinary organoid industry, *in vitro* organ reconstruction, and *in vivo* organ regeneration, the project provides research directions and scientific guidance, offering policy suggestions for the development of organoids and organ reconstruction.

Content

Chapter 1: Introduction

The escalating prevalence of degenerative disorders, injuries, and organ failures has gradually emerged as a major threat to human health. The insufficient supply of transplantable donor organs, compounded by safety concerns related to allogeneic transplantation, interspecies organ disparities, and immune rejection issues, has impeded the widespread application of organ transplantation, jeopardizing public well-being and safety. In response to the urgent demand for population health, organ reconstruction has emerged as a pivotal focus within the life sciences and health research. However, the complexity of organ cellular composition and long-term *in vitro* survival challenges render conventional biomedical approaches inadequate for organ reconstruction. Likewise, singular engineering strategies struggle to mimic the intricate physiological structures and functions of organs. Therefore, organ reconstruction aims to integrate multiple modalities such as biomedical and engineering techniques to achieve the reconstitution or reassembly of organs both *in vivo* and *in vitro*, with a primary focus on addressing tissue and organ loss or functional impairments. By partially reconstructing the structure and function of organs *in vitro* and subsequently safely and effectively transplanting them *in vivo*, the goal is to restore the functionality of failing organs and mitigate organ dysfunction arising from damage or aging, thereby sustaining normal life activities.

In recent years, the rapid advancement of organoid research has notably propelled the progress of organ reconstruction. Organoids are multicellular three-dimensional (3D) structures spontaneously self-assembled *in vitro* from various cell types, including stem cells. These constructs encompass diverse cell types specific to the respective organs and simulate key organ structures and specific functions. Organoids hold substantial importance across multiple fields, including organogenesis, disease modeling, drug screening, and precision medicine. Due to their capacity for self-renewal and tissue organization, organoid transplantation offers a promising avenue for restoring and repairing impaired organ structures and functions. Notable milestones have been

achieved, such as the successful integration and repair of injured murine intestinal tissue using human intestinal organoids, substantial restoration of damaged liver in a murine model via transplantation of human fetal liver organoids, improved glycemic control in diabetic mice through the transplantation of pancreatic islet organoids derived from C-peptide receptor-positive cells, and the observation of skin-like structures and hair growth upon transplantation of human skin organoids to murine skin defects. Furthermore, in the realm of human organ transplantation, a landmark publication in 2021 by *Science* reported the transplantation of *in vitro*-cultured biliary organoids into human liver tissue under *ex vivo* conditions, demonstrating the potential of biliary organoids for repair and regeneration of damaged bile ducts. These preliminary cases and foundational researches underscore the unique advantages of organoids in reconstituting organ functionality, showcasing robust and important prospects for application within the field of regenerative medicine.

1.1 Global Trends in Organoids and Organ Reconstruction

In 2009, the research team led by Hans Clevers at the Utrecht Institute in the Netherlands achieved a notable breakthrough by successfully cultivating adult intestinal stem cells into the similar crypts and villi structures of the small intestine *in vitro*, marking the inception of a major developmental direction known as organoid technology. In 2013, researchers from Japan, the United States, and Austria independently managed to construct organoids resembling the liver, kidney, and brain, respectively, further elevating the prominence of this field. As a result of these advancements, the organoid technology was picked as one of the top ten breakthroughs of the year by *Science* and was selected as “Method of the Year 2017” by *Nature Methods*. Subsequently, the organoid technology led to a rapid increase in research achievements within the global organoid field. From 2009 to the present day, over 6,000 papers related to organoids have been published worldwide. In recent years, the organoid field has experienced an explosion of accomplishments.

1.2 Comparison of the Development Status of Organoids and Organ Reconstruction in China and Globally

1.2.1 Publication Output

The initiation of organoid research in China occurred relatively later, resulting in a certain disparity with the international forefront. However, in this field, the pace of development in China has accelerated gradually. Since 2012, a total of 794 papers related to organoids have been published in China, accounting for 3.23% of the global output in this field. This proportion surged to 22.78% in 2022, catapulting China to the second-highest rank in terms of paper quantity globally. Among the top five countries in terms of organoid-related paper output worldwide, China's compound annual growth rate for the past five years (2017-2021) stands at an impressive 55.34%, significantly surpassing the growth rates of the other four countries (United States: 23.64%, Germany: 37.24%, Japan: 20.47%, United Kingdom: 28.07%). The average citation frequency of our country's published papers is 17.16. Among the top ten countries in terms of paper quantity, our country ranks last. This indicates that the influence of our country in the relevant fields still needs improvement.

At the level of research institutions, among the top ten institutions globally in terms of organoid-related paper output, three are from the United States, two from the Netherlands, two from France, and one each from Germany, China, and the United Kingdom. Only the Chinese Academy of Sciences ranks among the top ten institutions from China. This places China in the eighth position globally, with an average citation frequency ranking seventh among the top ten countries.

Numerous research institutions and universities in China have begun to focus on the development of organoids, achieving a series of groundbreaking accomplishments. For instance, our country was a pioneer in identifying stem cell populations within mouse pancreatic islets and constructing mouse pancreatic organoids. Moreover, our country introduced the hiHeps technology for hepatocyte transdifferentiation, utilizing these cells to fabricate liver organoids and simulate the occurrence of liver cancer. Our

country has been at the forefront of integrating cutting-edge organ-on-chip technology with organoid techniques, and successfully constructed various functional organoids, including brain, liver, and pancreatic organoids, on chips. These organoids have been employed in research on tissue and organ development, and disease modeling. Zhejiang University furthered its achievements by constructing bone callus-like organoids that resemble developmental processes. Experimental tests involving bone defects in mice and rabbits confirmed their ability to achieve rapid *in situ* bone regeneration. Fudan University successfully developed the world's first human organoid model of COVID-19 infection. Additionally, organoid models of lungs and colons developed by Shanghai Jiao Tong University have provided valuable resources for screening potential therapeutic drugs for COVID-19. Leveraging a strong foundation in stem cell research, Tsinghua University established an automated organoid platform. Utilizing this platform, they successfully cultivated organoids of normal tissues as well as organoids representing various tumors in cancer patients, including lung, kidney, stomach, and rectal tumors. The Army Medical University has developed a series of physiological culture matrices for vascular-like organs and integrated organ chips, providing a new approach for researching vascular diseases and vascularization of organoids.

1.2.2 Patent Output

Analyzing the patent landscape in the field of organoids, it is evident that since 2009, the United States has filed 776 patents in this field, holding the top global position. In comparison, China has filed a total of 117 patents in this field, ranking fourth globally.

From the perspective of IPC (International Patent Classification) categories, both global and Chinese patents in the field of organoids primarily focus on the cultivation and maintenance of organoids. Patents falling under the IPC category C12N 5/00 (Undifferentiated human, animal, or plant cells, e.g., cell lines; tissues; their cultivation or maintenance; culture media) constitute 56.75% of the global patents and 70.94% of Chinese patents. In addition, a small portion of Chinese patents also involve directions

such as A61L 27/00 (Materials for prostheses or for coating prostheses), C12M 3/00 (Apparatus for enzymology or microbiology), etc. However, there are essentially no patents related to functional organoids and their applications (disease research, drug screening, drug toxicity testing) in China, while internationally, there has been a layout for such directions, aiming to manufacture functional human tissues (such as neural, retinal, pancreatic islets, liver, heart, airway tissues, etc.), and subsequently utilize organoids or their derived cells for tissue repair or conduct drug toxicity/adverse reaction screening, evaluation of new therapeutic methods for diseases, etc.

Among the top ten institutions globally in terms of patent applications in the field of organoids, there are four from the United States, two from Japan, and one each from the Netherlands, Singapore, Germany, and Australia. Notably, no Chinese institution is among the global top ten. From a patent perspective, China's overall research and development level in the field of organoids is at the forefront internationally. However, there exists a significant gap between the technical research and development capabilities of Chinese institutions and the international leading level.

In addition to paper and patent output, the industry related to artificial organs is also in its initial stages of development. The establishment of enterprises in the artificial organ field in our country is concentrated after 2016. These enterprises are mainly distributed in regions such as Beijing, the Yangtze River Delta, and Guangdong. Some of them have technical backgrounds from universities and research institutes, and they promote the development of this field through further collaboration between industry, academia, and research. In terms of research and development models, domestic enterprises in this field primarily focus on the construction of tumor-like organ models and the development of organ-on-chip technology. Furthermore, they provide technical support for biological mechanism research, disease studies, drug efficacy, and toxicity research.

1.3 Domestic Policy Support for Organoids and Organ Reconstruction

During the "13th Five-Year Plan" period, China began to systematically layout the field of organoids. The "13th Five-Year Plan for Biotechnology Innovation" proposed the development of new-generation vascular stents, neural repair conduits, artificial bone tissue repair materials, and other products to promote the application and transformation of tissue engineering products and bio 3D printing products. Additionally, there were explorations into the artificial construction of tissues and organoids such as valves, liver, and kidneys, aimed at fostering the leapfrog development of related industries. Furthermore, the national key research and development programs during the "13th Five-Year Plan" and "14th Five-Year Plan," have planned a series of relevant thematic directions to advance the field. These include "Establishment of *In Vitro* Organoids Based on Stem Cells," "Stem Cell-Based Organ Chips," "Stem Cell and Organoid Models for Diseases and Humanized Animal Models," "High-Throughput Preparation and Application of Stem Cell-Derived Organoids," "Intelligent Multi-Organ Chip Systems Based on Stem Cells," and "Digital Assessment of Functionality of Stem Cell-Derived *In Vitro* Organs and Organoids," among others. In recent years, the National Natural Science Foundation of China (NSFC) has also provided substantial support for the organoid field. Multiple major research programs and guidelines have encouraged the development and utilization of organoid technology, particularly in the context of various mechanistic studies in the field of biomedical research.

In terms of relevant legal and policy aspects, although organoids are not explicitly listed as clinical testing items, from a legal standpoint, organoid experiments fall under the category of "cytological examinations of various specimens extracted from the human body" and may be applied in the "diagnosis or treatment of diseases." Therefore, the operational requirements outlined in the "Notice on Issues Concerning the Management of Clinical Testing Items" released by the former National Health and Family Planning Commission in 2016, as well as the revised "Measures for the

Management of Clinical Laboratories in Medical Institutions" issued by the National Health Commission in 2020, are equally applicable to organoids. Additionally, as cutting-edge clinical-related experimental bioproducts, guidelines such as the "Measures for the Clinical Application of Medical Technology," "Technical Guidelines for Non-Clinical Research and Evaluation of Gene-Modified Cell Therapy Products," "Technical Guidelines for Clinical Trials of Human Stem Cell and Derived Cell Therapy Products," and "Technical Guidelines for Clinical Trials of Immune Cell Therapy Products" have provided guidance for the development and application of organoids. These guidelines include procedures for utilizing organoid models to provide assessments of efficacy and safety. Lastly, the establishment of organoids and organoid libraries also falls within the scope of constructing biological sample libraries for human genetic resources. Thus, the establishment of organoids and organoid libraries must comply with the regulations stipulated in the "Regulations on the Management of Human Genetic Resources" and the "Guidelines for Biological Sample Libraries of China Medical Biotechnology Association (Trial)." Compliance with these regulations ensures the legitimate and standardized operation of organoid resource preservation and organoid library management. The promulgation of these normative documents is beneficial to the healthy development of the organoid field.

1.4 Significance of Development in the Field of Organoids and Organ Reconstruction

1.4.1 Value in Meeting National Strategic Needs

Currently, in addition to challenges posed by industrialization, urbanization, and an aging population, China also faces new challenges in terms of changes in disease patterns, ecological environment, and unhealthy lifestyle, which impact public health. Among these challenges, organ damage and functional failure are crucial threats to public health. When severe organ dysfunction necessitates replacement, the current solutions include organ donation transplantation or xenotransplantation. However, both

approaches encounter issues of immune rejection and insufficient supply. Organoids, derived from the individual donors themselves, offer a potential solution that mitigates these concerns.

Specifically, the development of organoids and organ reconstruction technologies holds several aspects of value. (1) Organoids serve as excellent model systems to study early human organ development, ushering in a new era in developmental biology research. They can mimic and simulate early human organ development events and key features *in vitro*, bypassing ethical constraints associated with human sample collection and deepening the understanding of the mysteries of human life. (2) Organoids can be utilized to construct complex disease models, especially for rare diseases, enabling dynamic simulation of disease progression and prediction of drug treatment responses. This can bring disruptive changes to the paradigm of biomedical research and revolutionize the understanding of disease mechanisms. (3) Functional organoids provide diverse sources of multi-tissue cells for tissue regeneration and organ repair, partially addressing the challenges and demands in organ transplantation. (4) Research on central nervous system organoids contributes to establishing cutting-edge core technologies in brain science, fostering breakthroughs in brain science; their integration with information technology and engineering science can greatly promote the development of emerging industries such as artificial vision and artificial intelligence. (5) Organoids carrying patient genetic information hold vast potential in the fields of biomedical research, personalized precision medicine, and pre-clinical drug testing. Organoid transplantation also brings hope for the treatment of neurodegenerative diseases. (6) The integration of organoid technology and engineering systems will realize the development of biologically inspired technology for precise three-dimensional assembly and structural formation, providing new research methods and platforms for organ reconstruction, regenerative mechanisms, and the study of tumor development and personalized therapy.

1.4.2 Value in Driving National Economic and Social Development

The development of organoids and organ reconstruction not only has a significant impact on life sciences and technology but also yields positive effects on industrial upgrading, transformation, and application. Its value in driving national economic and social development is demonstrated as follows: (1) The establishment of organoid models contributes to resolving issues related to species differences and ethical limitations in life sciences. (2) Utilizing organoids or their derived cells for tissue repair, drug toxicity/adverse reaction screening, assessment of new disease therapies, and effectiveness evaluation fulfills the strategic needs of the nation in fields such as life and health, new drug development, and biotechnology. (3) The application of organoid technology in drug screening and precision medicine drives the development of related industries, diversifying the national economy in the field of life and health. (4) With changing demographic trends and residents' health status in China, the demand for engineered tissue organs is becoming increasingly urgent, and related products have significant market value, economic benefits, and societal demand.

Organoid and organ reconstruction technologies have also propelled relevant industries in China, including organoid production, product application, innovative drug development, and precision medicine. Currently, the establishment of enterprises in the field of organoids in China is concentrated after 2016, and the industry is in its initial stages. These enterprises are mainly located in areas such as Beijing, the Yangtze River Delta, and Guangdong. Some of them have strong technological backgrounds from universities and research institutes, and they are further promoting the development of the field through industry-academia-research collaboration. For instance, the Institute of Medical Devices (Suzhou), jointly established by Suzhou New District Area, Southeast University, and Jiangsu Industrial Technology Research Institute, has achieved breakthroughs in tumor organoids, cardiac organoids, organ-on-chip technology, and upstream materials and equipment automation. It has also incubated the organ-on-chip company AVATARGET. Founders and chief scientists of companies like bioGenous and

D1Med have strong research backgrounds. Guangzhou HUAYI REGENERATION, together with Guangzhou Lab, Guangdong Provincial People's Hospital, and Kingmed, has jointly established an internationally leading organoid big data biological sample library.

Chapter 2 Organoid Research Frontiers

While two-dimensional cell models have been used in the field of biology for decades, they have nonnegligible limitations in simulating the structure, function, and surrounding environmental information of real tissue organs. Organoid technology has emerged as scientists explore three-dimensional cell culture. In the past decade, China has achieved a series of research outcomes in various organoid research fields, with publication proportions exceeding 10% in various areas. This chapter will focus on the cutting-edge exploration of organoid technology related to tissue systems such as the digestive system, respiratory system, endocrine system, as well as the development and applications of embryoid bodies and tumor organoids. Other disease models, such as fibrosis, aging, metabolism, etc., will be further complemented with the development of artificial organ models.

2.1 Digestive System

2.1.1 Introduction to the Field and Its Significant Strategic Value

The digestive tract organs primarily include the esophagus, stomach, intestines, and other accessory digestive glands such as the liver and pancreas. These organs play a pivotal role in nutrient digestion, absorption, and metabolism within the human body. Digestive tract diseases mainly encompass the emergence of tumors, infections, and inflammations, involving interactions among components of the microenvironment, such as stroma, immune cells, pathogens, and epithelial cells. The development and intervention models of digestive tract organoids offer novel avenues for simulating and treating digestive tract diseases, including complex organoid models for simulating the formation of digestive tract tumors, infections, and inflammations. Analyzing the mechanisms of disease formation and developing new therapeutic approaches will become a focal point for future clinical translation applications, facilitating the translation of organoid models into clinical applications.

The liver is responsible for the metabolism of substances and energy within the

body, including the therapeutic processes of most drug metabolism. The liver also serves as a central regulator of physiological homeostasis, with the development and progression of many major chronic diseases directly or indirectly linked to liver function. Furthermore, there are approximately 90 million carriers of hepatitis B virus in our country, with an annual death toll of 400,000 due to viral hepatitis, cirrhosis, and liver cancer. However, our country's research and development of innovative drugs and therapies for liver diseases significantly lags behind developed nations. Liver and liver disease research should become a pivotal focus of support in the fields of biomedical and new drug development in our country. Leveraging liver organoids for liver disease and liver biology research will comprehensively elevate our nation's research capabilities in liver diseases, enhance new drug development, and alleviate the substantial burden of liver diseases on our national economy.

2.1.2 Key Scientific and Technological Challenges

The scientific and technological challenges associated with digestive tract organoids include: (1) The relatively homogenous cell types within digestive tract organoids. How to construct multi-cellular digestive tract organoids containing epithelial, stromal, vascular, immune, and neural cells, as well as interconnected multi-organoid systems. (2) Investigation and application of digestive tract organoids in organ formation, stem cell fate determination, tissue homeostasis maintenance, and regenerative repair mechanisms. (3) Utilization of digestive tract organoids to simulate human infections or inflammatory diseases, and the development of prevention and treatment strategies. (4) Elucidating the mechanisms underlying tumor initiation and progression using digestive tract organoids, deciphering the regulation and mechanisms of different cellular components in tumor formation, and developing corresponding treatment approaches. (5) Establishing drug screening systems based on physiological and pathological digestive tract organoids for drug discovery and efficacy evaluation.

The scientific and technological challenges related to liver organoids include: (1) Constructing complex liver organoid structures and functionalities, encompassing

vascularization, neuralization, and immunization. (2) Developing liver disease models of various types and establishing large-scale liver disease organoid platforms for studying disease mechanisms and treatments. This involves creating organoid models with typical pathological features for conditions such as fatty liver, viral hepatitis, liver fibrosis, cirrhosis, and liver cancer. (3) Establishing standardized and scalable production processes for liver-like organs to meet clinical demands in regenerative therapies, such as acute liver failure, severe hereditary metabolic liver diseases, cirrhosis, etc.

2.1.3 Research Foundations and Conditions

Some universities and research institutes in China possess research teams with solid experience, achieving certain research outcomes in the field of digestive tract organoids. These accomplishments include the construction of digestive tract organoids and tumor organoids, testing of drug sensitivity indicators, and *in vitro* animal transplantation of digestive tract organoids. These cutting-edge studies have laid a solid foundation for subsequent development. The current drawback is that China's research on the role of digestive tract organoids in human development, organ homeostasis maintenance, regenerative repair, inflammatory diseases, tumor formation mechanisms, and drug development is still in its early stages. Furthermore, considering China's high prevalence of digestive system diseases, overall research investment in this field is relatively lacking.

Chinese scientists have made pioneering contributions in these areas, accumulating rich research experience and a solid foundation. For instance, using hepatocyte-like cells derived from pluripotent stem cells, co-cultured with adult mesenchymal stem cells and human umbilical vein endothelial cells, "liver bud" structures can be generated *in vitro*. Additionally, scaffold-containing organ-on-a-chip systems have been employed to facilitate cell self-assembly, 3D bioprinting has been utilized to create complex liver organoids, and the cultivation of liver cells obtained from human fetal liver or primary adult liver cells can acquire organoids. Liver organoids also hold immense potential for

transplantation-based treatment of liver diseases. Overseas researchers have demonstrated that bile duct organoids can integrate into the biliary system, thereby advancing the treatment of related diseases through *ex vivo* whole liver culture systems. In terms of strategies, China has already initiated scientific strategic leading programs in liver research, resulting in the development of a series of new technologies and the cultivation of a cohort of cutting-edge scientific talents.

2.2 Respiratory System

2.2.1 Introduction to the Field and Its Significant Strategic Value

Respiratory system diseases rank as the third leading cause of death globally. These diseases encompass a range of conditions, such as lung cancer, chronic obstructive pulmonary disease, asthma, pulmonary fibrosis, and acute lung injury. Many of these respiratory diseases originate from epithelial cell damage or abnormal proliferation. Evidence suggests that the composition and plasticity of lung epithelial cells differ between humans and mice, resulting in varying responses to injury. Consequently, mouse models cannot fully mimic the occurrence of respiratory diseases in humans. Stem cells play a crucial role in maintaining lung function and repairing damage. In recent years, both domestically and internationally, culture systems for organoids derived from human lung alveoli and airways have been established. Organoid technology has been used to investigate the interaction and fate transition of lung stem cells within the microenvironment, identify novel lung stem cells, and construct multi-cellular organoid models of the lung. The establishment of these systems holds significant reference value for studying the pathogenesis of human respiratory diseases, revealing the function of stem cells in lung injury repair, and consequently developing new therapeutic strategies to promote lung repair. This pursuit carries substantial theoretical innovation and clinical translational potential.

2.2.2 Key Scientific and Technological Challenges

Respiratory organoids face several challenges: (1) Most current respiratory organoids utilize adult lung stem/progenitor cells or directed differentiation of pluripotent stem cells, predominantly composed of epithelial cells. A key scientific question is how to import other cell types and form specific, intricate lung structures. (2) Establishing organoid models for different acute and chronic respiratory diseases to simulate disease progression holds important scientific value for studying lung regeneration and diseases. (3) The core function of the respiratory system is gas exchange. Simulating gas exchange processes, including mechanical force changes, within organoids could provide a platform for studying the effects of mechanical forces on lung development, regeneration, and diseases. (4) Achieving immune barrier function within lung organoids or simulating inflammatory reactions and immune cell infiltration is a core technical challenge in respiratory regeneration and disease researches. (5) Achieving translational applications of lung organoids, such as moving away from or substituting human-derived extracellular matrices with alternative matrices. Addressing these challenges will be instrumental in advancing the field of respiratory organoids, contributing to a better understanding of lung development, regeneration, and diseases, as well as the development of innovative therapeutic strategies.

2.2.3 Research Foundations and Conditions

Multiple research teams in China have conducted in-depth work on various aspects of lung development, injury, regeneration, and infection using lung organoid technology: (1) The team led by Ming Jiang has utilized mouse models and *in vitro* organoid culture systems to discover that signaling pathways regulate the proliferation and differentiation of lung airway stem cells, playing a vital role in airway injury repair. (2) The team led by Nan Tang found that increased mechanical tension in lung alveolar stem cells leads to the formation of progressive pulmonary fibrosis. (3) The team led by Bin Zhou identified multipotent stem cells at the bronchioalveolar duct junction that promote lung

regeneration. (4) The team led by Ying Xi identified a novel type of lung distal airway epithelial stem cells with differentiation potential for both alveoli and bronchi. These cells contribute to abnormal repair of alveolar epithelium during lung injury. (5) The team led by Xinwen Chen utilized lung organoids to discover that remdesivir and neutralizing antibodies effectively inhibit the replication of the novel coronavirus. (6) The team led by Ye-Guang Chen used lung organoids to study the mechanisms of SARS-CoV-2 infection. These studies have leveraged organoid technology to reveal critical mechanisms underlying lung development, homeostasis, injury regeneration, and disease onset. Furthermore, they have provided platforms for drug screening and begun to pave the way for clinical translation of research findings.

2.3 Nervous System

2.3.1 Introduction to the Field and Its Significant Strategic Value

The brain is the most important and complex organ in the human body. However, species differences between model animals and humans, limitations of 2D cell cultures in simulating complex interactions among multiple brain regions, and other factors have constrained the advancement of cutting-edge research in this field. As part of the central nervous system, the retina is a major sensory organ for vision. Brain and retinal organoid technologies have overcome issues related to species differences, individual variability, challenges in obtaining human samples, and limitations of traditional cell cultures. These technologies have been considered significant breakthroughs in the fields of stem cell research and neuroscience over the past decade. Since the establishment of neural organoid cultivation techniques in 2011, organoids have become important platforms for studying critical scientific questions, such as developmental regulation mechanisms of the human brain/retina, neurological or psychiatric disorders, drug discovery for diseases, and human brain evolution. Furthermore, neuro-organoid technology is undergoing rapid development. Transplantation therapy for the brain/retina and chimeric brain technologies have become cutting-edge and highly

sought-after directions on the international stage. These advancements hold significant strategic value in the pursuit of advancing the understanding of the brain, developing new therapeutic approaches, and addressing neurological disorders.

2.3.2 Key Scientific and Technological Challenges

Nervous system-related organoids face critical challenges: (1) Significant differences exist between human brain/retinal organoids and actual human brain/retina, such as fine-tuning cortical structures, precise brain region identity, and vascularization of brain organoids. Addressing these differences and minimizing the gaps between organoids and real organs is crucial. (2) Understanding the developmental patterns of organoids after transplantation and the regulatory mechanisms underlying their integration with native tissues, including the establishment of complex functional neural networks post-transplantation. (3) Decoding and interaction of neural network information in organoids play a guiding role in understanding human central nervous system development, function, and repair. (4) Cultivating human brain organoids is a delicate process. Stable construction of homogeneous neural organoids is advantageous for subsequent applications. (5) Post-transplantation development of neural organoids still exhibits randomness. Developing a mature and reliable transplantation technology system to enhance post-transplantation survival rates, safety, and functional maturity is essential. Addressing these challenges will be pivotal in advancing neural organoid technology, enabling a better understanding of brain development, function, and repair, and potentially leading to breakthroughs in neurological disorder treatment and neurological research.

2.3.3 Research Foundations and Conditions

In addressing the core scientific and technological challenges within the aforementioned field, including neural organoid modeling, *in vivo* transplantation, neural network information decoding, and interaction, relevant research teams in China have established a solid research foundation and have achieved internationally

competitive and even leading results. Techniques for *in vitro* vascularization of brain organoids have been developed, and various brain region-specific organoids have been established for simulating interactions between brain regions. *In vivo* transplantation techniques for brain organoids have been established, and it has been discovered that human brain organoids can establish neural projections in the mouse brain to improve fear memory. Brain organoid transplantation has promoted research and development in major neurological disorders, such as Parkinson's syndrome and stroke. In the realm of neural network information decoding, various flexible electrode and brain-machine interaction technologies have been developed. Techniques have been developed to enrich rod or cone cells in organoids, which have been applied in studying the pathogenesis of uncommon ophthalmic diseases. Pioneering techniques involving photoreceptor-glial cell co-transplantation have been introduced and applied in clinical research for treating various ophthalmic diseases such as retinitis pigmentosa and macular degeneration.

Currently, domestic researches on neural organoids has received supports from key projects such as the Ministry of Science and Technology and fundings from the National Natural Science Foundation. The Brain Initiative, which is being launched, will significantly promote researches on neural organoids. Some institutions have started to establish platforms for stem cell or brain organoid-related study. However, platform construction and talent cultivation are still in the initial stages and have not yet developed into a systematic and large-scale endeavor.

2.4 Endocrine System

2.4.1 Introduction to the Field and Its Significant Strategic Value

In 2021, the number of diabetes patients in China is approximately 140 million cases, and nearly 200 million individuals exhibit pre-diabetic conditions, such as impaired glucose tolerance and impaired fasting glucose. However, current pharmaceutical treatments are unable to fully restore autonomous blood glucose

regulation, necessitating long-term and continuous administration or injection of drugs to maintain blood glucose levels. Clinical investigations have revealed that islet and pancreatic transplantation can effectively supplement the quantity of β cells in critically ill patients, aiding in the maintenance of their blood glucose homeostasis. Yet, due to the limited source of donated islets, the key challenge in diabetes transplantation therapy lies in obtaining a substantial supply of insulin-secreting cells *in vitro*.

The development of pancreatic organoid cultivation technology provides new possibilities for diabetes treatment and potential cost savings in diabetes-related medical expenses. Researchers have established an *in vitro* cultivation system for pancreatic organoids with insulin-secreting capability and morphological similarity to pancreatic islets. Pancreatic organoids can serve as a cellular source for diabetes transplantation therapy and provide an *in vitro* model for exploring pancreatic development, the pathogenesis of diabetes, and treatment strategies. Currently, pancreatic organoids have successfully restored blood glucose levels in diabetic mice and non-human primates. However, large-scale clinical experiments in diabetic patients have not been conducted yet, and the full potential of pancreatic organoids in clinical transplantation awaits further demonstration.

2.4.2 Key Scientific and Technological Challenges

To achieve effective treatment of diabetes using pancreatic islet organoids, several challenges persist: (1) Pancreatic islet organoids induced *in vitro* exhibit significant heterogeneity in the secretion of products. (2) Insulin secretion capacity of *in vitro*-cultured pancreatic islet organoids falls short of that of fresh pancreatic islets. The maturity level of pancreatic islet organoids poses a limitation on their ability to regulate blood glucose post-transplantation. (3) Pancreatic islet organoids derived from pluripotent stem cells contain heterogeneous subpopulations, including pancreatic progenitor cells and endocrine progenitor cells, posing safety concerns post-transplantation. (4) Certain pancreatic islet organoids exhibit inadequate expression of genes associated with mature β cells, resulting in a lack of responsiveness to glucose

stimulation. (5) Pancreatic islet organoids require an extended maturation period post-transplantation to become fully functional. Prolonged oxygen and nutrient deprivation post-transplantation lead to a high proportion of cell loss.

2.4.3 Research Foundations and Conditions

Currently, the generation of β cells from other cell types is a focal and challenging aspect of this field. In this regard, China is positioned at the forefront and leading edge of research. In the path towards differentiating pluripotent stem cells into islet-like β cells, the team led by Hongkui Deng has identified factors such as Activin A and retinoic acid that promote the differentiation of murine pluripotent stem cells into pancreatic lineages. This team has further optimized the preparation of three-dimensional structured, functionally mature pancreatic islet organoids and, for the first time, validated their effectiveness and safety in treating diabetes in non-human primate models. In the pathway of generating pancreatic islet organoids from adult stem cells, the team led by Yi Zeng first identified Procr-marked adult stem/progenitor cells in mouse islets. These cells are capable of differentiating into all types of endocrine cells *in vivo*. Simultaneously, Procr stem cells can be co-cultured with vascular endothelial cells *in vitro* to form pancreatic islet organoids that encompass the entire endocrine cell lineage of the pancreas. Among these, islet-like β cells respond to glucose stimulation, secrete insulin, and are capable of restoring blood glucose levels in diabetic mice post-transplantation. These results have provided important theoretical innovations for islet regeneration, opening up new horizons for obtaining a substantial supply of human pancreatic β cells for diabetes cell therapy in the future.

2.5 Urinary and Reproductive Systems

2.5.1 Introduction to the Field and Its Significant Strategic Value

Prostate cancer, the most common urinary system cancer globally, continues to experience rising incidence and mortality rates, and it drops to approximately 30% in

the late-stage tumor phase. Approximately 80% of new urinary system cancer cases in China occur in individuals aged 60 and above. Given China's status as one of the countries with the fastest aging population growth, prostate cancer will become a nonnegligible medical burden. Currently, systemic therapy remains the primary treatment approach for advanced prostate cancer. However, tumor heterogeneity and interactions with the tumor microenvironment lead to drug resistance, resulting in the transformation of the cancer into an incurable state. In recent years, breakthrough prostate organoid technology has been successfully applied in the research field, including the construction of prostate cancer models used for drug sensitivity testing and other studies. This has propelled breakthroughs in understanding the mechanisms of prostate cancer occurrence and development, as well as in drug efficacy identification for prostate cancer. Therefore, the construction of highly faithful advanced-stage prostate tumor organoid models, and using them as a basis for drug evaluation and screening, holds significant clinical translational value. At the same time, kidney organoids have unique advantages in drug metabolism and toxicity testing, and their development is expected to make important contributions to the field of life and health.

In recent years, artificial abortions have been on the rise in China, reaching approximately 9.5 million cases annually. Abortion can result in damage to the endometrium, causing epithelial cell loss, tissue fibrosis, uterine cavity occlusion, and adhesions. These complications constitute the main reasons for female uterine infertility. However, current treatment methods have limited effectiveness, with a postoperative pregnancy rate of only 22.5%-33.3% for procedures such as hysteroscopic adhesion separation. Additionally, due to ethical concerns, inaccessibility to the implantation process *in vivo*, and a lack of suitable research models, the molecular mechanisms underlying embryo implantation in the endometrium remain incompletely understood. Currently, endometrial organoids have been applied in disease modeling, simulating the effects of aging on the endometrium during embryo implantation, and modeling *in vivo* repair of uterine cavity adhesions. These organoid studies are beneficial for uncovering the mechanisms of the occurrence and development of endometrial-related diseases,

developing new drug development targets, studying interactions at the maternal-fetal interface, and promoting research on the repair of endometrial injuries. This research can provide new insights and approaches for understanding the molecular mechanisms during the occurrence and development of diseases and embryo implantation processes, as well as for developing new treatments for Asherman's syndrome.

2.5.2 Key Scientific and Technological Challenges

Prostate organoids face important challenges: (1) The efficiency of establishing patient-derived prostate tumor organoids is notably low. Current culture conditions do not efficiently establish organoid models containing the "correct" tumor microenvironment components that stably maintain tumor tissue characteristics. (2) Moreover, obtaining representative, highly invasive prostate tumor samples is exceedingly challenging. (3) Epithelial-stromal interactions play a crucial role in prostate development, carcinogenesis, and drug response regulation. Systematically exploring suitable stromal components for optimizing prostate organoid culture is of paramount importance. (4) Prostate organoid models remain *in vitro* culture systems, unable to simulate physiological processes such as angiogenesis, tumor cell metastasis, and tumor dormancy that occur *in vivo*. (5) Inherent competition between epithelial and tumor cells in tumors leads to the loss of tumor cells during passaging, which is a crucial technical bottleneck for establishing human prostate tumor organoids.

The field of endometrial organoids also faces significant challenges: (1) To enhance the fidelity of the models, it is necessary to introduce stromal cells, vascular endothelial cells, immune cells, and other components to better mimic the physiological environment *in vitro*. (2) Mass-scale, standardized production of endometrial organoids is not yet achievable. Combining 3D bioprinting technology with organoid system could generate more realistic 3D structures. Integration with microfluidic chip technology could better simulate physiological changes *in vitro*. (3) Current organoid construction relies on matrix gels as scaffold materials. The development of animal-free, well-defined, batch-stable, and safe scaffold materials for culturing endometrial

organoids is essential to advance their clinical translation.

2.5.3 Research Foundations and Conditions

Since the initial publication of the three major prostate organoid techniques, breakthrough progress in prostate organoid technology-related research has yet to be achieved. China still holds certain advantages in the field of prostate organoid research. China possesses abundant clinical resources. This positions Chinese researchers to rapidly collect rare subtypes of prostate tumor samples. However, researchers in China also face other challenges, including the lack of a rapid autopsy program for systematic collection of metastatic tumor samples from terminal cancer patients, which hampers research efforts. Collecting metastatic specimens from end-stage prostate cancer patients is challenging. Therefore, to leverage prostate organoid technology and advance prostate scientific understanding and disease treatment, it is imperative for the government to provide fundings and coordinate efforts to address these limiting factors.

Since the successful establishment of human endometrial epithelial organoids in 2017, research on endometrial organoids remains no significant breakthroughs achieved yet. In China, several research groups have established endometrial cancer organoid culture systems. These efforts contribute to explore the mechanisms of endometrial cancer and drug treatment for the related disease. Teams from the Department of Obstetrics and Gynecology at Zhejiang University School of Medicine have applied endometrial epithelial organoids in repairing rat and mouse models of intrauterine adhesions. The University of Hong Kong has reported the use of endometrial epithelial organoids to simulate hormone fluctuations during the menstrual cycle and early pregnancy. Collaborative teams from the First Affiliated Hospital and Fourth Affiliated Hospital of Zhejiang University School of Medicine have developed a system for programmatically inducing pluripotent stem cells to become cells resembling the Müllerian duct. These cells can be used to construct endometrial organoids containing both epithelial and stromal cell components through 3D culture and hormone stimulation. As organoid research progresses, the combination of endometrial organoids

with other technologies is necessary to deepen our understanding of the molecular and cellular mechanisms underlying endometrial development, embryo implantation, and disease initiation and progression.

2.6 Motor System

2.6.1 Introduction to the Field and Its Significant Strategic Value

The musculoskeletal system is the largest organ system in the human body. Injuries, disorders, and weaknesses of the musculoskeletal system are the primary causes of disability, such as meniscus injuries, joint damage, muscle and tendon injuries. Bone defects are among the most common injuries, and bone grafting surgery ranks second in frequency globally, after blood transfusion. However, these methods have limitations, such as secondary damage from removing implanted metals, volume restrictions for autograft bone donors, potential complications, disease transmission risks, and difficulties in integrating allografts with host tissues.

The establishment of musculoskeletal organoids can obviously accelerate drug screening, efficacy assessment, safety evaluation, and reduce costs for musculoskeletal diseases. Moreover, musculoskeletal organoids produced *in vitro* can be directly transplanted into the body to support the functional recovery of affected organs. Bone tissue engineering provides various functional scaffold materials and seed cells, accelerating bone regeneration and addressing clinical challenges in repairing extensive bone defects. Bone organoids hold immense potential for applications in skeletal development, drug screening, mechanism studies, tissue regeneration, and open new avenues for understanding bone formation mechanisms, diagnosis, and disease prevention.

2.6.2 Key Scientific and Technological Challenges

Several challenges exist in the construction of musculoskeletal organoids *in vitro*: (1) Reproducing the molecular expression profile and complete functionality of mature

tissue organs is difficult, resulting in limited maturity of musculoskeletal organoids, and real-time monitoring of mechanical function is challenging. (2) The obtained organoids of the musculoskeletal system are far from the scale and fine structure of natural musculoskeletal tissues. The formation and maintenance of large-scale organized tissues pose significant challenges. Currently, it is also challenging to achieve a high level of matching with the body's natural state in this regard, and the functionality of constructed organoids remains relatively limited. (3) Variability in morphology and function between batches of organoids is common. (4) Challenges like immune rejection after transplantation, *in vivo* survival, integration of the organoids with the host, and other compatibility issues need further clarification. (5) Due to the inherent differences in hardness and organization within the musculoskeletal system, it remains challenging to fully replicate the natural variations and diversity in the softness, hardness, and tissue organization of musculoskeletal organs *in vitro*. (6) Musculoskeletal organoids intended for transplantation need to exhibit good compatibility with the body's vascular and neural networks. (7) The cellular composition of musculoskeletal organoids is currently relatively limited, primarily consisting of bone and muscle cells. One of the challenges in musculoskeletal organ research lies in how to mimic the fine assembly of multiple cell types found in the body.

2.6.3 Research Foundations and Conditions

The field of *in vitro* construction of musculoskeletal organoids is still in its early stages. Current construction methods mainly involve self-assembly and material-based ordered stacking. Self-assembly relies on stem cells, introducing key biochemical signals based on developmental cues to induce lineage-specific developmental processes and guide organoid assembly. While self-assembled organoids contain a diverse range of cell types, they struggle to mimic the morphology and mechanically ordered properties. The material-based ordered stacking method provides a fundamental structural unit and anchoring points for mechanical stimuli in the construction of organoids by introducing special materials. Therefore, it exhibits better morphological

and mechanical characteristics. To achieve the construction of biomimetic structures, biocompatible materials are widely used to support the assembly of the musculoskeletal system organoids. Mechanical stimuli also further promote the maturation of organoids. Currently, three-dimensional musculoskeletal organoids, such as human muscles, have been established, forming neuro-muscular junctions and simulating diseases. However, this method still requires improvement in the interaction between materials and cells to better simulate the *in vivo* environment. In addition, technologies such as nanofibers, biochips, and microfluidic chips also make significant contributions to the complexity of musculoskeletal organoids. Furthermore, the large-scale preparation of musculoskeletal organoids is still in its early stages. Effectively increasing the efficiency of organoid formation, reducing heterogeneity, and combining the development of new materials and high-throughput equipment are essential steps to achieve the high-throughput preparation of musculoskeletal organoids.

Chinese scientists have achieved many important results in the field of bone tissue engineering. For instance, the team led by Ping Hu has established a system for long-term *in vitro* expansion of functional muscle stem cells. The muscle stem cells that have been long-term expanded *in vitro* can maintain their full stemness. *In vivo*, they efficiently repair muscle damage and correctly home to their designated locations. These human muscle stem cells have been used to construct muscle organoids, resulting in functional neuromuscular junctions capable of autonomous contractions. Xiaohui Zou's team has successfully isolated mouse myogenic stem cells and induced them to differentiate into mouse muscle organoids with multiple cell types that can contract. Bone organ research, as an emerging field, is still in its early stages domestically. However, there is a large pool of talents in this research direction, and significant progress is expected. In the field of muscle and tendon organoids, there is relatively less investment and fewer researchers in China. Existing research teams are either keeping pace with or leading the international advanced level and are actively cultivating talent for further advancements.

2.7 Circulatory System

2.7.1 Introduction to the Field and Its Significant Strategic Value

Cardiovascular diseases are the leading cause of death among various fatal illnesses, representing a significant global public health issue. Myocardial infarction results in the loss of myocardial cells and the formation of fibrotic tissue, subsequently leading to left ventricular remodeling and heart failure. In adults, the regenerative capacity of myocardial cells is weak, and promoting myocardial cell regeneration in the damaged heart is a forefront and critical scientific and technological challenge in the treatment of heart diseases. Over the past three decades, research with the goal of cardiac regeneration has been attempted in various directions, including the search for stem cells capable of differentiating into myocardial cells, the induction of myocardial cells from human pluripotent stem cells, transplantation of cardiac organoids, induction of non-cardiac cells to differentiate into myocardial cells, and the stimulation of myocardial cell proliferation and regeneration. While these research directions face scientific and technical bottlenecks, they hold promising prospects for addressing the challenge of in situ cardiac regeneration, providing a foundation for the treatment of myocardial infarction, and holding significant importance for socioeconomic development and public health.

2.7.2 Key Scientific and Technological Challenges

This field still faces several challenges: (1) The identification and marker molecules of cardiac stem cells are not yet clear, and the existence of cardiac stem cells in the adult heart is still to be confirmed. (2) While there is a theoretical basis for inducing cardiomyocyte differentiation/proliferation to promote mammalian cardiac regeneration and repair, the mechanisms underlying dedifferentiation and redifferentiation are not well understood. (3) Using induced cardiomyocytes derived from embryonic stem cells or iPSCs for myocardial infarction treatment has shown promise. However, issues related to the immaturity and incomplete integration of the

generated cardiomyocytes, leading to arrhythmias, have not been fully resolved. (4) The strategy of converting fibroblasts into cardiomyocytes has achieved some success in expanding cardiomyocytes *in vitro* and promoting *in vivo* cardiac repair. However, this approach faces challenges of low efficiency and suitability for clinical use. (5) Clinical trials involving xenotransplantation, such as pig heart transplantation, have shown some progress. However, challenges related to immune rejection, organ maintenance, and stability in xenotransplantation still need to be addressed. (6) The development of cardiac organoids in China is still in its early stages, and there is a lack of mature construction systems.

2.7.3 Research Foundations and Conditions

In the field of cardiac regeneration, Chinese scientists have made significant contributions to the study of adult cardiac stem cells. The research teams of Bin Zhou discovered that adult mice do not possess cardiac stem cells. Meanwhile, the team led by Zhongzhou Yang identified p53-positive cardiac stem cells in neonatal mouse hearts. Teams led by researchers like Yihan Chen, Jingwei Xiong, Bin Zhou, and Chunyu Zeng have found that genes or chemical small molecule combinations can regulate cardiac cell proliferation and regeneration. In the directed differentiation of pluripotent stem cells into cardiomyocytes and cardiac organoids, researchers such as Sheng Ding and Huangtian Yang have utilized iPSCs to generate cardiomyocytes for myocardial infarction treatment. Additionally, teams led by Sheng Ding and Xin Xie reprogram fibroblasts into cardiomyocytes, achieving *in vitro* expansion and *in vivo* repair functions. While foreign researchers have already established human and mouse cardiac organoids, China started relatively late in the field of cardiac organoids and is currently in a catching-up stage. In summary, Chinese scientists have achieved important achievements in cardiac regeneration, with most directions still in the process of catching up, and a few directions leading internationally. China’s actual research funding in cardiac regeneration is relatively limited. However, China’s talent reserves in the field of cardiac regeneration are entering a positive cycle. It is expected that within

5-10 years, China will enter the forefront of international research in cardiac regeneration.

2.8 Embryoid Models

2.8.1 Introduction to the Field and Its Significant Strategic Value

Due to the precious and limited resources of human embryos and the inaccessibility of embryos during the implantation period, the developmental events during the period of embryo implantation largely remain unknown. The developmental abnormalities during the period from embryo implantation to formation of the primitive gut are a major cause of early pregnancy miscarriages. Therefore, comprehending the critical events of embryo implantation and elucidating the developmental movements of the primitive streak will help unveil the secrets of early embryonic development and advance the field of regenerative medicine.

Human embryoid models have significantly advanced developmental biology and clinical medicine. Embryoid models established based on stem cells provide an alternative to embryos and can be used to understand the mechanisms of early pregnancy failure and developmental defects. Additionally, the application of embryoid models in drug screening enables the identification of molecular components that may cause embryonic malformations and death. This provides crucial models for drug screening and safety testing, ultimately improving assisted reproductive technologies to reduce miscarriages and birth defects.

2.8.2 Key Scientific and Technological Challenges

Currently established embryoid models still face limitations: (1) The efficiency of obtaining human embryoids through various methods remains quite low. (2) The proportion of cells in embryoids is imbalanced, deviating from the cell lineage proportions compared to normal embryos. (3) Induction efficiency varies due to batch differences, cell line variations, initial conditions, and induction methods. (4) The

induction and differentiation process is lengthy and asynchronous, leading to heterogeneity and poor uniformity of outcomes. (5) Some blastocyst cell states do not match those of human embryos, and undefined cellular subgroups are present. (6) The developmental efficiency of *in vitro*-established embryoids following simulated *in vivo* implantation is low. (7) Culturing the trophoblasts poses a significant challenge, and the assembly of multicellular structures based on the trophoblast layer is also an urgent issue that needs to be addressed.

2.8.3 Research Foundations and Conditions

In the past few years, the establishment of human blastocyst *in vitro* culture methods and the flourishing development of single-cell technologies have opened up new possibilities for investigating early embryo development. These possibilities include *in vitro* blastocyst culture systems, construction of embryoid or blastocyst-like models, human blastocyst *in vitro* studies, construction of human blastocyst-like structures from eight-cell-like cells, and establishment of intermediate cell populations to create human blastocyst models. While various international efforts have led to the *in vitro* construction of blastocyst-like structures and the utilization of embryoid models derived from human pluripotent stem cells to simulate various aspects of real blastocyst development and implantation, China is still in the early stages of research in this field. Relevant technologies and conditions are still being explored. Subsequent efforts should be intensified to drive the development of embryoid models further.

2.9 Tumor-Related Field

2.9.1 Introduction to the Field and Its Significant Strategic Value

The increasing burden of cancer in our country has become a serious threat to the health and lives of our people. The high plasticity and personalized nature of tumors are key factors contributing to the difficulty in cancer diagnosis and treatment. Precise targeted therapies and personalized treatments for cancer patients have become a global

medical research hotspot. The scarcity of cancer *in vitro* models that can faithfully mimic the *in vivo* characteristics of cancer has become a bottleneck in the research of personalized and targeted therapies for cancer patients.

Two-dimensional patient-derived tumor cell lines (PDC), patient-derived xenograft mouse models (PDX), and patient-derived tumor organoid models (PDO) have all played important roles in the fundamental research of tumors and the development of anticancer drugs. However, PDC lacks the *in vivo* characteristics of tumor cells, PDX has a low success rate, long cultivation period, high cost, and is not suitable for late-stage cancer patients who are not suitable for surgical treatment. Therefore, PDC and PDX cannot serve as the foundation for future personalized and targeted therapies for cancer. PDO can effectively simulate the tumor cell microenvironment in the body. It holds a unique advantage in constructing physiologically functional *in vitro* research models. It is foreseeable that in the next decade, PDO will replace PDC and PDX to become the preferred model system for drug development stages such as anti-tumor drug screening, preclinical trial validation, and drug toxicity monitoring.

2.9.2 Key Scientific and Technological Challenges

The field of tumor organoids still faces several challenges: (1) There is a need for further research in optimizing the cultivation systems of tumor organoids, particularly for malignancies that are prevalent in the Chinese population, such as those affecting the urinary, digestive, and respiratory systems. (2) There is currently a lack of large-scale tumor model libraries, which limits both fundamental tumor research and clinical treatment applications. (3) Tumors exhibit high heterogeneity, and tumors originating from different tissues display unique clinical characteristics, treatment responses, and prognostic outcomes. Overcoming these challenges is essential to enhance treatment efficacy and prognosis for cancer patients. (4) Variations in pathogenic factors and driver gene mutations exist across different ethnic groups. Exploring the mechanisms underlying these differences is crucial to deepen our understanding of tumors and their development. (5) The construction of tumor organoid models from various tissues faces

challenges such as low efficiency, poor stability, and high costs. (6) The maintenance of characteristics and the issue of tumor cell purity in the *in vitro* culture of tumor organoids.

2.9.3 Research Foundations and Conditions

Currently, China has established a relatively good foundation in the field of tumor organoid research. China pioneered the construction of pancreatic cancer organoid models, elucidating the mechanisms governing the fate determination of prostate cancer cell lineages. The use of prostate cancer and lung tissue organoids successfully predicted the ineffectiveness of androgen deprivation drugs in treating lung infections caused by the novel coronavirus. Tumor organoid models were also employed to predict the sensitivity of liver cancer to treatment and the chemotherapy sensitivity of colorectal cancer. However, overall, China’s foundation in tumor organoid research is relatively weak. Despite a high volume of published papers, there is still a lack of groundbreaking research.

Currently, there is limited investment in tumor organoid research in China. The initiation of special projects by the country focusing on organoids and diseases such as cancer is expected to play a crucial role in advancing organoid research. China's advantages in conducting tumor organoid research include a large population and a high prevalence of cancer patients, creating an urgent need for both basic and translational research in tumor organoids. However, there are disadvantages, such as the presence of numerous institutions conducting tumor organoid research without effective coordination and integration, along with inadequate policies and regulations in this field.

Chapter 3: Cutting-Edge Cross-Disciplinary Assessment of Organ Reconstruction

In the wake of rapid advancements in the field of life sciences, traditional two-dimensional cellular and animal models have fallen far short of meeting the urgent demands of modern biomedical research and novel drug development. Organoids represent a forefront direction in contemporary life sciences research. They can faithfully recapitulate key structural and functional attributes of source tissues or organs, offering a novel investigative tool in the realm of life sciences with important applications in developmental biology, disease research, and drug discovery. Simultaneously, the progression of *in vitro* organoids has opened avenues for the reconstruction of organ structures and functions, enabling possibilities for organ transplantation, repair of damaged organs, and localized organ replacements.

While existing organoid cultivation systems have seen substantial development, they still face numerous challenges in terms of homogeneity of the culture matrix, controllability of the tissue microenvironment, intricate interplay between diverse cell types, sustainability of vascularized nutritional supply, maturation of organoids, and high-throughput analysis. Moreover, organoids still require optimization in aspects such as size constraints, diverse cellular composition, refined three-dimensional architecture, functional integrity, exchange of substances with the external environment, long-term stability maintenance and flow shear stress. Resolving these critical issues demands not only the inherent advantages of organoids themselves but also the integration of cutting-edge interdisciplines, filling the gaps in organ reconstruction. Among these, interdisciplinary fields such as organ-on-a-chip technology, organoid vascularization, tissue materials and 3D printing, and organ transplantation hold the potential to break barriers within the forefront of organoid research. Simultaneously, they have the prospect of advancing the *in vitro* reconstruction of organs reliant on organoids, offering technological support for subsequent organ function restoration and organ transplantation.

3.1 Organ Chips Drive the Integrity and Stable Functionality of Reconstructed Organs

3.1.1 Introduction to Significant Strategic Value

Organ chips, in conjunction with organoid technology, offer a promising solution to address the limitations of organoid development, such as small size, difficulty in long-term maintenance, and limited cell diversity. And research in this realm has been steadily increasing. Organ chips provide new strategies and technologies for achieving organoid reconstruction and functional reconstitution. They enable the incorporation of various cellular spatial arrangements, dynamic 3D cultivation, biochemical gradient cues, and mechanical stimuli to recreate the tissue microenvironment. By combining principles from the field of tissue materials, organ size and structure requirements can be met, and organ cell composition can be reconstructed through diverse cell seeding techniques. Furthermore, the integration of multiple types of organ chips can simulate the coordinated functions of different organs, such as the brain-gut axis. Hence, the integration of organ chips with organoids holds the potential to enhance the fidelity, precision, controllability, and functionality of *in vitro* organ reconstruction.

3.1.2 Key Scientific and Technological Challenges

There remain key scientific and technological challenges: (1) Further refinement is required in chip-related technologies and material science to meet the specific demands of biology. (2) While organ chips can expand the size of organoids, there still exists a disparity between the dimensions achieved and the actual size of organs. (3) Despite assisting in replicating unique structures and cellular distributions of organs, organ chips still fall short of capturing the intricate architecture and cellular composition of real organs. (4) Achieving partial organ reconstruction, functional performance, and long-term stability through organ chips requires further development. (5) The design of multi-organ interactions on chips involves considerations of organ-specific maintenance, degree of functional implementation, cross-interference of negative factors, and stability.

(6) Implementing real-time monitoring and intelligent information integration based on the biological functions of organ chips *in vitro* presents pivotal challenges.

3.1.3 Research Foundations and Conditions

The organ chip technology has undergone more than two decades of development and has currently entered the clinical trial phase. In this field, China has built a substantial research foundation, ranking among the international leaders. However, there is still some distance to cover to reach the forefront of the global organ chip industry, especially in terms of clinical research and translation. Institutions such as the Dalian Institute of Chemical Physics of the Chinese Academy of Sciences have established various organ chip systems by integrating organ chip and organ-like tissue technologies. For instance, they have employed microchip-constrained structures to guide the in-situ controllable formation, growth, and development of brain-like organs. They pioneered the development of multi-organ chips with interactive characteristics of stem cell-derived pancreatic islets and liver-like organs, applied for diabetes modeling and drug assessment. Innovative technologies and application systems for high-throughput manipulation of organ-like tissues based on droplet microfluidic systems have also been established. Several works on multi-organ chips have been cited and reviewed in specialized journals like Science, contributing early data accumulation for organ reconstruction using organ chips.

In terms of project funding support, in 2017, the Chinese Academy of Sciences took the lead in incorporating the “Construction of Functional Organ Chips” into the strategic pilot A category of the “Organ Reconstruction and Manufacturing” program. This move facilitated the accumulation of relevant talents, platforms, and technologies. The Ministry of Science and Technology also invested in and strategically planned for the direction of organ-like tissues and organ chips in the National Key Research and Development Programs of 2021 and 2022. However, there is a need to further enhance the output of original research findings and develop research institutions and talent teams. All of these aspects require comprehensive national planning and policy

guidance to promote rapid development in this field.

3.2 Organ Tissue Vascularization Drives Organoid Material Exchange and Long-Term In Vitro Maintenance

3.2.1 Introduction to Significant Strategic Value

As a type of miniaturized organ cultivated *in vitro*, another crucial direction for the future application of organoids is their development into small-scale organs or as substitutes for organ transplantation, offering boundless possibilities for disease treatment. To achieve this, vascularization of organoids is imperative. Firstly, the growth of organoid size is constrained by oxygen deficiency and the accumulation of metabolic waste, leading to tissue necrosis. Secondly, rapid anastomosis with the host's blood vessels is essential during organoid transplantation, and only organoids with relatively mature vascular networks can accomplish this. Thirdly, the establishment of vascularization is essential to facilitate material exchange, functional expression, and long-term stability of organoids or organs, laying the foundation for organ reconstruction.

Currently, the cultured organoids are far from simulating actual tissue organs, and there is still a necessary gap in achieving functional blood vessels comparable to those of fully formed organs. Thus, vascularization has become a bottleneck technology constraining the development of organoid techniques and organ reconstruction. In current organoid cultivation, the majority lack vascularization, and even experimental vascularized organoids face challenges like immature vascular networks and the absence of larger vessel structures. Vascularization of organoids contributes to bringing their morphology and functional characteristics closer to those of real organs, addressing issues related to constrained organoid growth, and enhancing post-transplant organ survival. Therefore, the vascularization of organoids is advantageous for breaking through technical barriers in organoid research and driving *in vitro* organ reconstruction, holding important value for their future clinical applications.

3.2.2 Key Scientific and Technological Challenges

The vascularization of engineered organs and organ-like tissues is a critical challenge faced by tissue engineering and organ reconstruction. Key scientific and technical challenges involved include: (1) Structural Complexity: The vascular network comprises multi-level branching structures, with differences in cellular and extracellular matrix composition and structure among vessels of different calibers. Currently, the lumens formed in the vascularization of organ-like tissues are not true blood vessels; they are prone to collapse and lack the viscoelasticity characteristic of natural vessels. (2) Functional Complexity: Blood vessels perform functions such as nutrient exchange and physiological information transmission. Current efforts in vascularizing organ-like tissues have not yet achieved effective perfusion and neural connections. (3) How to utilize directed differentiation systems to generate organ-like structures containing blood vessels, or to form a vascular network through 3D printing, organ chips, or microfluidic technologies. (4) After implanting vascular cells, how to construct a vascular network structure that synchronizes with the growth process of organ-like tissues. (5) Whether engineered blood vessels constructed *in vitro* can maintain stable functionality in the long term and whether they can interact effectively with other functional tissue cells.

3.2.3 Research Foundations and Conditions

Internationally, significant progress has been made in the vascularization of organoids. In China, several patent applications are currently in progress, encompassing the vascularization of mouse pancreas and adipose tissue organoids, as well as biologically active materials related to vascularized organoids. However, no relevant articles have been published yet. The team from the Army Medical University has pioneered the development of physiological matrix materials for cultivating vascular organoids, along with matching organ chips. They have successfully used these vascular organoids for repairing heart and brain infarctions, achieving promising results. Overall, China is in the early stages of engineering blood vessels for *in vitro* tissue organs,

leaving considerable room for future development.

In terms of talent cultivation, due to the interdisciplinary nature of the field, there is an ample supply of researchers from various disciplines who can engage in research in this area, adequately meeting the needs of the field's development. Nonetheless, there is a lack of overall investment and limited participation of personnel. Moving forward, facilitating effective collaboration between these two groups could potentially drive advancements in the direction of vascularizing organoids in China.

3.3 Biological 3D Printing Facilitates Diversification of Organ Reconstruction Tissue Cells and Precise Construction of Fine Structures

3.3.1 Introduction to Significant Strategic Value

Biological three-dimensional (3D) printing involves the fabrication of personalized *in vitro* 3D biological structures and functional models by employing "3D printing" techniques to assemble biological units (cells/proteins/DNA, etc.) and biological materials based on biomimetic morphology, organismal functionality, and cell-specific microenvironments. This technology finds applications in fields, such as regenerative medicine, organ reconstruction, cancer research, new drug development, and advanced medical devices. For instance, 3D-printed organ-like structures can offer the pharmaceutical industry high-throughput, standardized, clinically relevant tools for drug screening. The gradual achievement of complex *in vitro* organ structures and functional reconstruction through organ 3D printing and organ reconstruction holds the promise of individualized, minimally invasive, in-situ regenerative repair. Biological 3D printing is an emerging interdisciplinary field that has emerged at the intersection of engineering, materials science, information technology, and life sciences. It has the potential to lay the scientific, technological, and industrial foundations for the development of the 21st-century biotechnology industry, serving national strategic requirements and the well-being of the economy and society.

Specifically concerning organoids and organ reconstruction, the integration of 3D printing technology with organoids can enhance the uniformity and stability of organoids themselves, refine their fine structures, and enhance their functions. Moreover, the synergy between the two can advance the development of precision medicine for organoids, *in vitro* diagnostic reagents, drug development models, and inter-organ interactions. Presently, the development of bioprinting techniques and equipment capable of precise three-dimensional cellular assembly and structural formation within the cellular space, along with the establishment of advanced three-dimensional biological models mimicking the multi-level release of biofactors in the microenvironment of human tissues, collectively contribute significantly to *in vitro* organ reconstruction.

3.3.2 Key Scientific and Technological Challenges

Biological 3D printing technology provides the basis for breaking through *in vitro* limitations and achieving high-quality biomimicry. Presently, the field faces several significant challenges: (1) Human organs possess intricate heterogenous components, multiscale structures, and functional microenvironments. Some aspects of biomimetic microenvironment materials composition, spatial distribution, and material interactions remain unclear, impacting the progress of the field. (2) Printing multi-level gradient distribution vascular structures presents an important challenge. (3) The biomechanical signals of 3D-printed organoids arise from complex coupled factors, including bioinks, cellular microenvironments, and cultivation conditions. Coordinating the understanding of biomechanical regulation with biochemical regulation for *in vitro* modeling poses another crucial challenge for organ reconstruction. (4) Developing component-controllable, batch-stable engineered humanized biomaterials to enhance the innovation and applicability of bioinks. (5) Balancing the dual advantages of macroscopic spatial-temporal manipulation and microscopic cell self-assembly capabilities in 3D printing. (6) Optimizing stable biofunctional printing processes to drive innovation in organoid 3D printing equipment and facilitate the clinical translation

of bio-printed products. (7) Assessing whether the combination of 3D printing with cutting-edge technologies such as biomaterials and organoids meets the stability maintenance and functional expression requirements of various cell types in an *in vitro* setting.

3.3.3 Research Foundations and Conditions

China's application-based research in the field of 3D bioprinting is at the forefront internationally. China has established specialized international journals in this field, such as Biofabrication and Bio-Design and Manufacturing. China has made groundbreaking achievements in the construction of tumor models using bioprinting. For instance, the Tsinghua University team has developed an integrated 3D printing system tailored for tumor tissues, enabling the construction of human-derived tumor organoids, all of which have been effectively applied for predicting tumor treatment efficacy. The Zhejiang University team has developed various cell manipulation methods based on principles like droplet-based and extrusion-based techniques, achieving stable cultivation of cell lines and organoids *in vitro*, and applying these techniques to study tumor vascularization and drug effects. The Fudan University team has established human-derived tumor organoids for screening various anti-tumor drugs and radiation doses, creating profiles of chemotherapy and radiation sensitivity.

In recent years, departments such as the National Natural Science Foundation of China, the Chinese Academy of Engineering, the Chinese Academy of Sciences, and the Ministry of Science and Technology have all included 3D bioprinting in relevant strategic research reports and planning outlines. At the same time, China has the necessary research conditions in this field, including experimental platform equipment, a high-level research talent pool, and industrial transformation enterprises. Chinese scientists are expected to achieve breakthrough research results in the future, rapidly realizing the industrial application of the aforementioned technologies. This progress aims to keep pace with and lead internationally in the field of 3D bioprinting.

3.4 Meeting the Material Needs for Organ Reconstruction Tissues with Biomaterials

3.4.1 Introduction to Significant Strategic Value

In the mid to late 20th century, the field of biomaterials began to gradually develop. Biomaterials serve as a crossroads between biology and other disciplines and play a crucial role in organ reconstruction, disease treatment, and clinical applications, with their market size increasing year by year. The materials mentioned earlier in the context of organ chips and bioinks in 3D printing all fall under the category of biomaterials. Biomaterials influence the applicability of chips, the success rate of 3D printing, and bring breakthroughs to the specific requirements of organ reconstruction, such as the construction of microvessels and tissue basement membranes. In addition to its integration with other disciplines, biomaterials themselves have extensive applications in both research and clinical settings. Examples include biodegradable sutures, drug-delivering microcapsules, vascular or cardiac stents, biomimetic materials for bone regeneration, nanobiomaterials, and more. Therefore, the development of biomaterials holds significant importance for clinical applications and disease treatment.

3.4.2 Key Scientific and Technological Challenges

While biomaterials have made key progress in their own application fields, challenges remain in the area of organoids or organ reconstruction: (1) Ensuring effective integration of biomaterials with cells or organoids, without adversely affecting their growth or functionality. (2) Meeting the size and fine structural requirements of *ex vivo* reconstructed organs. Current 3D printing capabilities often have limitations in size and precision. (3) Developing biomaterials that can fully replicate the functionality of biomimetic blood vessels or microvasculature. (4) Addressing stability issues of biomaterials under *in vitro* conditions, such as degradation or structural deformities. (5) Incorporating additional functionalities into biomaterials, such as controlled release of growth factors, *in vivo* transplantability, extensibility, biosensing, and bio-intelligent

control.

3.4.3 Research Foundations and Conditions

The enormous demand for biomaterials in China has propelled the rapid development of related industries domestically. During the “Twelfth Five-Year Plan” and “Thirteenth Five-Year Plan” periods, China identified biomaterials as an industry to vigorously develop. Based on this, China has conducted extensive research in various directions of biomaterials and has made significant progress, including membrane biomaterials, nano biomaterials, composite scaffold materials, and more. Corresponding research techniques and methods have also been developed to continuously drive innovation in the field of biomaterials science. In addition to a well-established technological industry, China also demonstrates strong international competitiveness in cutting-edge technologies. For instance, Chinese scientists pioneered the concept of “tissue-inductive biomaterials,” allowing the design of artificial stem cell microenvironments to induce tissue regeneration and repair. The Institute of Biophysics of the Chinese Academy of Sciences successfully bio-synthesized a novel nano tumor diagnostic reagent, enhancing the efficiency of tumor detection. The National Center for Nanoscience and Technology is dedicated to the design and self-assembly of functional nano materials guided by biological molecules, receiving widespread international attention in the research of nano drugs for tumors.

Original research in biomaterials requires a solid theoretical foundation, high-throughput screening and evaluation methods, rational optimization strategies, and rigorous quality control. This poses a challenge for researchers and industry professionals in China. Additionally, in terms of principles and original technologies, the integration of biomaterials with organ-like structures still lags behind the international frontier. These new demands and challenges not only drive the development of the biomaterials industry itself but also intersect with disciplines such as organ-like structures, providing a new breakthrough for *in vitro* organ reconstruction.

3.5 Organoids and Organ Transplantation Drive Organ Repair and Regeneration

3.5.1 Introduction to Significant Strategic Value

Organ dysfunction poses a major threat to human life. Organ transplantation stands as an effective solution to address organ dysfunction. China ranks second globally in terms of both organ donation and transplantation numbers. However, the most substantial challenge in organ transplantation remains the shortage of donors.

In situations where there is a shortage of adult organs, organ repair and functional restoration can be partially achieved through the combination of newly developed organoids and organ transplantation techniques. Organoids can be expanded using genetically matched tissue, enabling autologous transplantation and providing a regenerative resource for organ replacement strategies. This approach also holds potential to circumvent complications associated with current transplantation methods, such as immune rejection, severe infections, and malignant tumors. Various types of organoids have been reported for transplantation purposes, albeit mainly limited to small animals, such as mice and rats. These organoids can grow and maintain limited survival within the host's body for a certain period. Further refinement of organoid technology to enhance the structure and functionality of corresponding organs, and even achieve *in vitro* organ reconstruction, holds substantial promise for organ functional repair and replacement. Therefore, the development of organoids, organ reconstruction, and even organ transplantation techniques carries vital significance in repairing organ function, replacing failing organs, and potentially saving lives.

3.5.2 Key Scientific and Technological Challenges

The application of organoids in the field of transplantation is still in the initial stage, with key gaps to be bridged before clinical implementation. Numerous difficulties and challenges persist in this endeavor: (1) Ethical concerns associated with traditional transplantation techniques also apply to clinical applications of organoid technology,

potentially leading to emotional, personality, and behavioral changes in recipients, thereby raising ethical dilemmas related to identity. (2) The prediction of outcomes for organoid transplantation remains challenging, making it difficult to extrapolate from animal models to clinical effectiveness, leaving a lack of preliminary clinical data. (3) From a technical safety perspective, organoid or *in vitro* reconstructed organ transplantation involves multiple invasive procedures or surgeries, for which evidence predicting human risks and benefits is lacking. (4) Transplantation-related rejection reactions and adjunctive medication treatments also lack relevant experience. (5) Uncontrolled development of stem cells presents considerable risks. (6) Different types of transplantation involve distinct technical methods and evaluation criteria, yet cross-comparisons are presently lacking.

3.5.3 Research Foundations and Conditions

Current organoid-related organ transplantation technologies mainly focus on animal models. China has accumulated some experience in liver organoid transplantation and retinal organoid transplantation, yet achieving clinical safety remains a pivotal distance away. Innovations in organ preservation techniques promote the exploration of organoid technology in human organ transplantation. For instance, normothermic perfusion preservation technology has been clinically implemented, maintaining their physiological functions *ex vivo* over an extended period. Leveraging liver normothermic perfusion preservation systems, researchers have successfully utilized biliary organoids to repair human livers discarded due to biliary injuries, achieving biliary regeneration and improvement in bile properties. This study validated for the first time that lab-cultured organoids can be transplanted onto human organs and exert their functions, opening up new avenues for human organoid transplantation and laying a foundation for clinical applications.

In 2022, the National Medical Products Administration Verification Center officially released the “Guidelines for Quality Management of Cell Therapy Products (Trial).” Organoid technology falls within the ambit of relatively complex cell therapy

techniques, making it eligible for standardized research and translational approval in accordance with the aforementioned guidelines. Furthermore, the evaluation of organ function prior to various organoid transplants is intricate and necessitates tailored assessments based on organ type, including evaluations of functional stability and the standardization certification of *in vitro* cultivation conditions. These measures ensure the stability, safety, and efficacy of organoid transplants. In summary, the *in vivo* transplantation and clinical application of organoids are still in the early stages, requiring long-term tracking of effectiveness and safety to promote the healthy development of related fields.

3.6 From Organoids to Organ Reconstruction: An Overview

3.6.1 Research Foundations

Compared to cells and tissues, organs exhibit significant differences in three-dimensional structure and function. Organs are three-dimensional systems composed of various tissue cells, with their structures satisfying the requirements for growth space maintenance, nutrient supply, material exchange, and functional expression. Functionally, organs differentiate specialized cells based on their unique structures, and these cells interact to maintain the normal functions of the organ. In summary, the reconstruction of *in vitro* organs requires addressing both structural and functional aspects.

Traditional two-dimensional cell culture models are insufficient in meeting the requirements for both structure and function. While animal organs fulfill these criteria, overcoming immune rejection remains a challenge. Given the current limitations, the development of organoids brings new hope to organ reconstruction. First, in terms of structure, *in vitro* cultured organoids possess a certain level of three-dimensional architecture, partially mimicking the spatial structure of organs. For example, *in vitro* cultured kidney organoids can induce differentiation to form mature collecting ducts, and *in vitro* cultured heart organoids exhibit preliminary structures such as ventricles

while exhibiting self-beating. These technological breakthroughs in organoids indicate progress towards achieving the three-dimensional structures of corresponding organs. The functionality of organs relies on cells that perform specific functions. Organoids, while self-renewing, can differentiate into mature cells, partially exhibiting the representative functions of organs. Liver organoids, for instance, possess drug metabolism functions, and intestinal organoids can absorb nutrients in the gut lumen. The expression of these functions demonstrates the representative organ functionality of organoids, representing a qualitative leap in functional expression compared to traditional two-dimensional models.

3.6.2 Challenges Ahead

While the development of organoids is rapidly advancing towards organ reconstruction, there are still several challenges to overcome. In terms of three-dimensional structure: (1) Organoids are significantly smaller in overall size compared to real organs, limiting their capacity for *in vitro* organ reconstruction, typically measured in micrometers or millimeters. (2) Organoids currently lack the ability to replicate the intricate structures found in organs. For example, kidney organoids can differentiate into collecting ducts but lack structures like glomeruli or renal tubules. (3) Organoids lack vasculature, directly affecting their ability to maintain structure, provide nutrients, and facilitate substance exchange. (4) Organoids consist of a limited variety of cell types, leading to structural deficiencies; for instance, intestinal organoids lack the muscular layer found in the outer walls of the intestines. (5) Organoids often lack the specialized structures required for full organ functionality, with most current organoids being spherical or cyst-like rather than closely resembling the complex structures of real organs, such as the luminal structure of the intestines or the alveoli structure of the lungs.

In terms of functional expression: (1) The absence of various cell types in organoids, such as immune cells, muscle cells, and nerve cells, limits their ability to fully express certain functions. For example, intestinal organoids are limited in their

ability to perform functions like gut immunity, peristalsis, and food perception. (2) Organoids often exhibit limited and simplified functions, making it challenging to replicate progressive functional expression. While heart organoids can generate contractions, they may lack the ability to effectively pump fluids. (3) Coordinating the functional interplay between organoids and other tissue systems remains difficult. (4) The stability of functional expression in organoids also presents a significant challenge, as ensuring sustained and stable functionality is essential. (5) Cell therapy and functional repair related to organoids are still in the early stages, and there is a need for strengthened research to develop more stable and realistic application models.

3.6.3 Proposed Solutions

Addressing the challenges highlighted above requires a multidisciplinary approach and a systems-level strategy. In terms of structure: (1) Integrating materials science and 3D printing technology with organoids can lead to improvements in organoid size, such as constructing artificial organ frameworks and combining them with *in vitro* organoid cell seeding techniques to reshape organ structures. (2) The combination of tissue engineering, 3D printing technology, and cell biology can drive the creation of finely detailed and functionally representative organoid structures. (3) Chip technology, coupled with omics approaches, can be used to construct artificial blood vessels to address the issue of vascularization in organoids, supporting the development of neuralization and immunization in organ-like structures. (4) Utilizing genetic engineering to construct gene-edited organoids, aiding in the research of specific disease targets and the diagnosis and treatment of genetic diseases. (5) Considering the construction of organoids from various cellular and tissue sources, such as organoids derived from tumor ascites, and organoids constructed from nodular tissues.

For the multifaceted expression of organoid functions and their interplay, interdisciplinary collaboration holds great potential for breakthroughs: (1) Co-culturing various cell types and organoids can explore the amalgamation of multiple functions. For instance, co-culturing immune cells or mesenchymal cells with organoids can

enhance immunological functions or enable self-regulating signal responses, even in the construction of immune organoids. (2) Combining different types of organoids on chip platforms can facilitate interactive functional cooperation. For instance, co-culturing liver and intestinal organoids could simulate the liver-gut axis. (3) The integration of materials science, 3D printing, and tissue engineering with organoid technology can amplify functional expression based on unique structural foundations. (4) Chip technology combined with organoid cultivation can improve the sustained and stable expression of organoid functions. (5) Strengthening research and application in interdisciplinary areas such as organoid detection methods, high-throughput applications, and the integration of artificial intelligence.

Chapter 4 Ethical Considerations in Organoids and Organ Reconstruction

As discussed in previous chapters, the development of organoid technology has ushered in a new era of developmental biology research, providing excellent model systems for studying organ development and avoiding ethical limitations associated with human sample collection. However, the journey from organoids to organ reconstruction, especially human-derived organoids, and eventually to organ transplantation, still presents significant ethical challenges.

4.1 Moral Status and Dignity

In the realm of embryo research, the internationally recognized "14-day rule" stipulates that human embryos cultured *in vitro* should not be allowed to develop beyond 14 days. The main rationale behind this rule is that embryos undergo neural differentiation after 14 days, indicating the potential for pain perception. Continuing experimentation beyond this point would be deemed ethically unacceptable. While human brain organoids do not possess the complexity of a fully developed human brain's neural network, they exhibit certain neural structures that suggest the potential for perception and consciousness. With advancing technology, the goal of organ reconstruction-oriented organoid technology could potentially lead to the creation of more complex brain organoids with sophisticated neural networks and perceptual capabilities, which might raise ethical questions regarding their moral status. On the other hand, brain organoids, whether current versions or future reconstructions, may not fully represent individual entities like embryos, leading to debates about their moral status. Additionally, as technologies like 3D-printed organs and multifaceted organoid systems on microfluidic chips progress, they will increasingly approximate real individual structures, raising their own set of ethical controversies.

In the research of organoids and organ reconstruction, researchers cultivate human-derived organoids and transplant them into animal hosts to form chimeric

animals. These chimeric animals can serve as vehicles for studying the physiological functions of human-derived organoids. The chimeric animals could potentially be utilized as carriers for human-derived organ reconstruction, with mature human-derived organs transplanted back into the human body. The growth of human-derived tissue or the transplantation of reconstructed organs from animal hosts to humans could potentially challenge human dignity. A more nuanced perspective on dignity in light of scientific developments could aid in the progression of organoid research. The ultimate goal of organoids is organ reconstruction and transplantation, which will inevitably challenge traditional ethical norms concerning human dignity. Therefore, it is important to approach ethical considerations prudently, setting forth rational guidelines that respect ethical principles while avoiding undue restrictions on the advancement of scientific technology.

4.2 Informed Consent and Privacy Protection

A significant portion of organoid samples in repositories is sourced from donated tissues or organs. Therefore, ensuring informed consent from donors is paramount. Donors should fully understand the nature of organoids, be informed that their donated materials will be used for organoid cultivation, and be provided with specifics about the experimental types and potential applications. Importantly, as the applications of organoids are rapidly evolving, with potential new experimental types and fields emerging, informed consent documents should be updated and re-signed with donors accordingly. Additionally, in cases of deceased donors during tissue or organ donation, their next of kin should possess the right to informed consent. However, legal frameworks in this context may be insufficient and should be approached on a case-by-case basis when necessary.

In human-derived organoids, particularly those derived from individuals with diseases, sequencing analysis is often required to determine genetic mutations and related information. This raises privacy concerns not only for donors but also for their

relevant family members. The leakage of such sensitive information could jeopardize individual, familial, and societal interests. While anonymous donation can provide a level of privacy protection, the individual information of donors, such as age, gender, and disease status, remains important for scientific research. Anonymous donation may potentially limit the advancement of organoid research. To safeguard donor privacy and promote scientific research simultaneously, it is essential to ensure informed consent from donors and establish standardized databases for organizing and categorizing all organoid-related information, with dedicated personnel responsible for maintaining and preventing information leaks.

4.3 Commercialization Issues

The unique value of organoid research has led to its transition toward commercialization. Increasingly, businesses and institutions worldwide are focusing on the commercial aspects of the field. The commercialization of organoids will inevitably be linked to economic interests. If organ reconstruction becomes a reality in the future, the question of whether reconstructed organs can be treated as commodities raises important considerations and concerns. Commercialized organoids or reconstructed organs will naturally raise ethical, moral, and privacy issues as mentioned earlier. Furthermore, once the commercialization of organoids or reconstructed organs becomes a reality, aspects such as benefit distribution and risk assessment must be addressed reasonably. Therefore, prior to full-fledged commercialization, relevant legal frameworks should be established to ensure the legitimate and ethical commercialization of organoids, preventing any misconduct or illegal activities.

4.4 Ethical Governance Recommendations

As previously discussed, China has already embarked on a systematic layout of the organoid field during the "13th Five-Year Plan" period and has introduced a series of policies to encourage and support organoid research. However, while conducting

ambitious research in this field, it is important to address the ethical issues related to organoids and organ reconstruction, promptly establish relevant regulations, and ensure the smooth progress of research while safeguarding the fundamental interests of the people.

Firstly, establish an ethical governance framework for organoids and organ reconstruction. This framework can draw inspiration from the guidelines published by the International Society for Stem Cell Research regarding stem cell research. Simultaneously, take into account the specific characteristics of organoids and organ reconstruction and engage a variety of perspectives to formulate a comprehensive ethical governance framework. Secondly, enhance public awareness. Through means such as popular science lectures and online media, disseminate knowledge about organoids and organ reconstruction to the public, thereby strengthening their understanding of ethical principles. Thirdly, proactively develop relevant laws and regulations and institutional policies. Anticipate the achievement of organ reconstruction and establish preemptive policies to safeguard privacy and effectively mitigate ethical risks. Fourthly, promote benefit sharing. Organoid and organ reconstruction research involves donors, researchers, and participants. Sharing experimental results and related benefits with all stakeholders helps protect the rights of informed consent and avoid crossing ethical moral boundaries.

Chapter 5: Development Strategies and Policy Recommendations in Relevant Fields in Our Country

The "Establishment of Malignant Tumor Disease Models Based on Organoid Systems" is listed as one of the initial key tasks of the 14th Five-Year National Key Research and Development Program. The focal point of this initiative lies in the establishment of organoid systems as disease models. Organoid systems can simulate the three-dimensional structure and functionality of real organs in the body. They offer distinct advantages in elucidating the development, homeostasis, and pathogenic mechanisms of diseases. They provide novel research methodologies and therapeutic approaches for precision medicine and hold promising applications. However, in the course of developing organoid systems, further optimization is required in areas such as capacity-building of relevant expertise, establishment of regulatory frameworks, and improvement of legislation. These steps are necessary to drive the advancement of innovative organoid technologies, prevent potential bottlenecks, facilitate the transition of technologies to industries, accelerate the development of industrial chains, address international competition, and ultimately enhance the capacity of organoid systems to better meet the healthcare needs of the population. This will lay a solid foundation for achieving the goals outlined in the "Healthy China 2030" strategic plan.

5.1 Capacity Building

In the forefront and interdisciplinary fields of organoid systems, relevant technological barriers directly impede the development of these systems. Enhancing the scientific research and clinical application capabilities of organoid systems will directly drive the advancement of this field. Recommendations in this regard include: (1) Enhancing the construction of complex organoid systems with multiple cellular lineages, encouraging interdisciplinary fusion, and establishing mature functional organoids. (2) Focusing on the construction of highly simulated complex organoid bodies with tissue structure and functionality, such as the incorporation of vascular systems and immune

microenvironments. (3) Improving interdisciplinary construction, establishing relevant discipline systems at an early stage, and promoting the application of neural organoid technologies, such as advancing the translational applications of organoids in disease research, new drug development, and transplantation therapy. (4) Encouraging the enhancement of preclinical and clinical experiments in cell therapy, utilizing mature organoid systems for transplantation to repair related organs in the body, and further optimizing relevant protocols. (5) Promoting the resolution of existing technical challenges in organoid systems, supporting original work, and fully exploring their research potential. This includes advancing technologies such as vascularization and neuralization of human neural organoids, multifunctional differentiation and assembly, *in vivo* transplantation, and chimeric construction. (6) Focusing on the development characteristics of complex organoid-related technological fields, increasing technological support for stem cell technology, organ regeneration, organ-on-a-chip systems, and 3D printing technology. Additionally, increasing funding support for related basic research and the translation of regenerative medicine, establishing special funds, and promoting the development of talent cultivation systems and research platforms in this field. (7) Establishing phased and achievable goals, implementing step-by-step plans, and adopting a progressively advancing approach to drive the development of organoids.

5.2 Team Building

As organoid technology advances rapidly, team building in various aspects such as tissue sampling, organoid system establishment, organoid cultivation, organoid repository establishment, application, and process management and supervision is equally crucial, directly impacting the healthy and rapid development of the field. Recommendations in this regard include: (1) Suggesting the formation of a well-trained team of junior scientists and clinical physicians to ensure the high-quality collection of specimens, consistent execution of organoid and animal work. (2) For special samples,

including tumor specimens or rare cases, recommend assembling a team of clinically trained physicians and researchers with relevant backgrounds to collaborate closely, ensuring the optimal utilization of clinical samples. (3) Screening and identifying various experimental conditions that promote organoid growth, ultimately establishing standardized and reproducible experimental procedures. (4) Building innovative teams and nurturing talents and reserves in various sub-directions of organoid or organ regeneration and repair, based on key development projects and funding initiatives from the Ministry of Science and Technology, the National Natural Science Foundation, domestic advantage teams, and platforms, contributing to the improvement of talent pool and development of talent tiers. (5) Strengthening the development of a multidisciplinary talent pool and relevant platforms for organoid research. Given the interdisciplinary nature of organoid research and the existing technological barriers, suggest policy-guided initiatives to enhance the cultivation of multidisciplinary talent. (6) Enhancing talent reserves by suggesting the establishment of organoid-focused disciplines at primary and secondary levels. (7) Strengthening the construction of ethics and legal regulation teams for organoid production and application processes, ensuring the safe and regulated use of organoids.

5.3 Platform Construction

With the development and accumulation of organoid technology, organoids are bound to become crucial strategic resources in the life sciences. Therefore, establishing relevant platforms for resource integration, rational planning, and comprehensive management becomes especially important. Recommendations in this regard include: (1) Integrating clinical resources and collaborating across multiple centers to establish standardized, systematic, and comprehensive organoid biobanks, facilitating resource sharing and the creation of clinical sample organoid repositories. (2) Establishing biological resource repositories related to organoid platforms, including information databases, corresponding blood sample repositories, pathology repositories, and more.

(3) Improving the construction of organoid platforms, including the development of standardized facilities, equipment, and personnel qualification training. (4) Establishing comprehensive regulations and rules for organoid platform construction, including usage standards, requirements for intake and output, confidentiality protocols, and safety regulations. (5) Given that organoid platforms are national strategic resources, direct government leadership, standardized management, and joint construction and application promotion involving hospitals, research institutions, universities, and enterprises are necessary.

5.4 Institutional Development

As the field of organoid systems advances, corresponding institutional development is essential. Recommendations in this regard include: (1) Developing quality control standards for applications to promote organoid research in regeneration, disease modeling, and drug development. (2) Establishing relevant regulations to standardize the research and application scenarios of organoid systems. (3) Applying equivalent risk management principles to regulate autologous organoid transplantation technologies similarly to autologous cell tissue engineering and autologous organ transplantation technologies, promoting the accumulation of clinical transplantation experience and research development. (4) Encouraging clinical experiments with seed cells and resulting organoids with lower immunogenicity to explore the patterns of allogeneic transplantation. (5) Actively promoting organoid research through innovative technology pilots, increasing the adoption of companion diagnostics and facilitating the clinical translation of organoids. (6) Regulatory agencies responsible for food and drug supervision should develop policies to support organoid research and issue guiding principles for encouraging innovative application models. (7) Overcoming challenges in *in vitro* organoid reconstruction and functional remodeling requires top-level planning. Suggest establishing national scientific and technological projects to provide increased support for research in this direction, encouraging interdisciplinary collaboration in

biology, engineering, materials science, and more, to facilitate major achievements. (8) Establishing dedicated projects and funds specifically for organoids to strengthen investment and research in this field, facilitating breakthrough advancements.

5.5 Legal and Regulatory Development

In parallel with the refinement of relevant institutional frameworks for organoid system development, it is crucial to establish comprehensive legal and regulatory frameworks to safeguard the interests of all parties involved. Recommendations include: (1) Utilizing the three guidance principles related to gene therapy and cell therapy issued by the National Medical Products Administration's Center for Drug Evaluation, which for the first time include organoids in the verification guidelines for gene therapy and genetically modified cell therapy products. Efforts should be made to ensure the effective implementation of relevant legal and regulatory documents. (2) Gradually initiating a rapid autopsy program for terminally ill patients to provide sustained clinical specimen resources for future scientific and clinical research. (3) Advancing the integration of medical research and public awareness regarding the significance of metastatic tumor specimen donation for research. Considering the implementation of a nationwide tissue and organ donation program, suggesting the establishment of well-coordinated organ collection efforts, standardized operating guidelines, and corresponding regulations for regulation. (4) Due to the complex culture conditions and numerous influencing factors, evaluating research results involving organoids introduces uncertainties. Recommend the development of standardized ethical and policy regulations for organoids, forming a rational resource sharing mechanism and strengthening regulatory standards for clinical organoid research. (5) Developing comprehensive laws and regulations pertaining to the protection and application of human genetic resources, standardizing ethical regulations in the field of regenerative medicine.

5.6 Other Aspects

The development of organoid and organ reconstruction fields also requires support from other aspects, including: (1) For cutting-edge areas such as stem cell technology and organ regeneration, establish more open ethical and management approaches, encouraging bold exploration and exploratory applications of new technologies and methods in regeneration and clinical treatment of major diseases. (2) Enhance supporting facilities for organoid-related research and industries, and increase industry support. Plan ahead, improve various aspects of the industrial chain, break down industry barriers, and promote the value transformation of neural organoids from scientific research to practical applications. (3) Recommend increasing financial support for field projects, establishing special funding, and implementing a system for project application and review, providing timely funding for excellent projects. (4) Establish a streamlined approval process for clinical trials of relevant projects. For research results with potential clinical applications, establish a fast-track approval process for clinical trials to facilitate the translation and application of outcomes. (5) Strengthen the promotion and popularization of basic knowledge about organoids, organ reconstruction, and related fields, enhancing public awareness and understanding of new technologies and applications.