Living biobank: Standardization of organoid construction and challenges

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Abstract

In multiple areas such as science, technology, and economic activities, it is necessary to unify the management of repetitive tasks or concepts by standardization to obtain the best order and high efficiency. Organoids, as living tissue models, have rapidly developed in the past decade. Organoids can be used repetitively for *in vitro* culture, cryopreservation, and recovery for further utilization. Because organoids can recapitulate the parental tissues' morphological phenotypes, cell functions, biological behaviors, and genomic profiles, they are known as renewable "living biobanks". Organoids cover two mainstream fields: Adult stem cell-derived organoids (also known as patient-derived organoids) and induced pluripotent stem cell-derived and/or embryonic stem cell-derived organoids. Given the increasing importance of organoids in the development of new drugs, standardized operation, and management in all steps of organoid construction is an important guarantee to ensure the high quality of products. In this review, we systematically introduce the standardization of organoid construction operation procedures, the standardization of laboratory construction, and available standardization documents related to organoid culture that have been published so far. We also proposed the challenges and prospects in this field.

Keywords: Living biobank; Organoid; Standardization; Guidelines; Laboratory construction

Introduction

Standardization refers to the orderly process applied to activities in various fields such as science, technology, economy, and management. To obtain the best order within a certain range, the unified standards for repetitive processes or concepts are produced by authorized associations so as to obtain the best order and high efficiency. The main characteristics of standardization include purpose, repeatability, unity, coordination, and advancement. The purpose describes the goal of achieving the best economic and social benefits through standardization. Repeatability means that standardization is based on repetitive processes or production. Unity refers to the requirement that the essence of the standard is agreed upon by all. Coordination means that the standard needs to harmonize the knowledge of multiple disciplines and fields. Advancement means that the standards should be continuously modified to fully reflect the current achievements of science and technology.

Standardization of the biomedicinal field mainly involves bioinformatics and biomanufacturing. Bioinformatics standardization stems from the rapid development of life science, which has produced a massive amount of biomedical data. These data can be integrated and shared through standardized processes to facilitate insights into disease mechanisms and life sciences. The standardization of biomanufacturing refers to the use of bioengineering technology to produce chemicals, pharmaceuticals, food, and other products. This ensures controllability and stability of the production process through standardized technology to improve production efficiency and quality. Therefore, standardization is necessary to promote the development of life sciences, improve product quality, and promote the integration and sharing of data.

The international standardization system is formulated for the construction and implementation of relatively uniform standards in technology, quality, environment, and safety across different countries, aiming to facilitate international trade and economic exchanges. The international standardization system has become an important basis for

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international cooperation between enterprises and governments. The International Organization for Standardization (ISO) is headquartered in Geneva, Switzerland, and is responsible for organizing and formulating the standard system of various industries, including quality management systems, environmental management systems, food safety systems, and so on. The products from different countries compete fairly on a global scale following the international standards set by ISO, which not only helps to improve product quality but also helps to protect consumers' rights. More importantly, it can eliminate the technical barriers between industries and simplify trade transaction procedures worldwide. For example, the first international standard in the field of biobank was the ISO 20387, General Requirements for Biobanking, which was published by ISO in 2018. The ISO 20387 defines the content of biobank capacity building as well as general requirements such as sustainable operation and quality control.[1] Looking at the development history of the standardization system, it is not difficult to find that serious implementation of standards can bring obvious benefits, and the lack of unified standards is not conducive to the development of enterprises.

Usefulness of Organoids

The biopharmaceutical industry is one of the more rapidly developing fields. The growth of high-grade biomedicine, especially the diagnosis and treatment of cancers or research on disease mechanisms, places an increasing demand on reliable experimental models.[2,3] Conventionally used cell lines have experienced selection challenges under the pressure of genomic variation in long-term culture *in vitro*, resulting in the difficulties of accurately reflecting the biological behavior of the original cancer tissues and the response to drug treatment. Therefore, drug screening results on cancer cell lines are effective *in vitro*, but sometimes fail in subsequent clinical trials.[4] The animal experiment is another commonly used model in cancer research, including the immunodeficient mouse model and patient-derived xenograft (PDX) model.[5–9] Although tumor tissues transplanted into immunodeficient mice reflect the genotype and phenotype of tumors to a certain extent, and play a role in pathogenesis, novel molecular target finding, or drug sensitivity evaluation preclinically, the PDX model lacks an intact immune system. In addition, the long preparation period and high cost of PDX models also limit their widespread use.

Since 2009, the organoid model has been useful in three-dimensional (3D) cell culture *in vitro*, cryopreservation, and recovery for repetitive utilization.[5] Organoid models well retain the morphological phenotypes, cell functions, biological behaviors, and genomic profiles of the parental tissues, which makes them favorable to many biomedical researchers.[10–12] As a kind of renewable biological sample *ex vivo*, organoids are known as "living biobanks".[13] At the end of 2022, the US Food and Drug Administration (FDA) approved legislation that stated that organoids or organoid chips could be used as alternatives to animal experiments in preclinical drug research, demonstrating the increasing importance of organoids in drug development.[14] With the devolopment of organoids in biomedical research, clinical translation, and the biopharmaceutical industry, technical standardization and implementation issues emerged within this field. The standardization of organoid construction plays an important role in promoting the development and application of organoid technology.

Organoids cover two mainstream fields: adult stem cell (ASC)-derived organoids (also known as patient-derived organoids [PDOs]) and induced pluripotent stem cell-derived (iPSC) and/or embryonic stem cell-derived (ESC) organoids. The first provides a valuable tool for enhancing personalized medicine, while iPSC-derived organoids are generated from reprogrammed iPSCs, which can differentiate into various cell types representing different organ systems.[15–17] The ESC-derived organoids are established using human ESCs and can differentiate into organ-like structures from all three germ layers, faithfully mirroring early embryonic development toward fetal stages.[18,19] This article focuses on the standardization issues of organoids constructed from ASC.

Standardization of Organoid Construction

The standardization of organoid construction refers to the uniform operation and management of all aspects of organoid development, such as tissue collection, 3D cell culture, organoid passage, organoid cryopreservation, organoid recovery, disposal of waste organoids, profiling of organoid molecular characteristics, and organoid quality control, to ensure the quality and stability of the organoids products.[20–23] The standardization of organoid construction is conducive to the industrial development of organoid technology.

Standardized operating procedures should be followed at each step of organoid preparation. In addition, the construction of organoids involved in the collection and preservation of human biological samples, requires the approval of the ethics committee of the sample provider and the informed consent of the sample donor. The informed consent includes, but is not limited to, the preservation of samples, the conduct of scientific research, drug susceptibility testing, and the potential translational application of the research results. The key steps [Figure 1] of organoid construction have been described in detail in other articles,[24] and are not repeated here.

Tissue collection and pretreatment

All matters involving the collection and preservation of human biospecimen in the construction of organoids should comply with the "*Ethical requirement of human biobanking*" (No. GB/T 38736-2020) in China. The collection and processing of human tissue samples should be carried out by trained physicians, and the tissue collection should be as fresh as possible. The surgically resected specimen should be harvested within 30 minutes (min). The collected tissue should not be less than 5 mm × 5 mm in size and be immediately put into a tissue preservation solution of advanced Dulbecco's

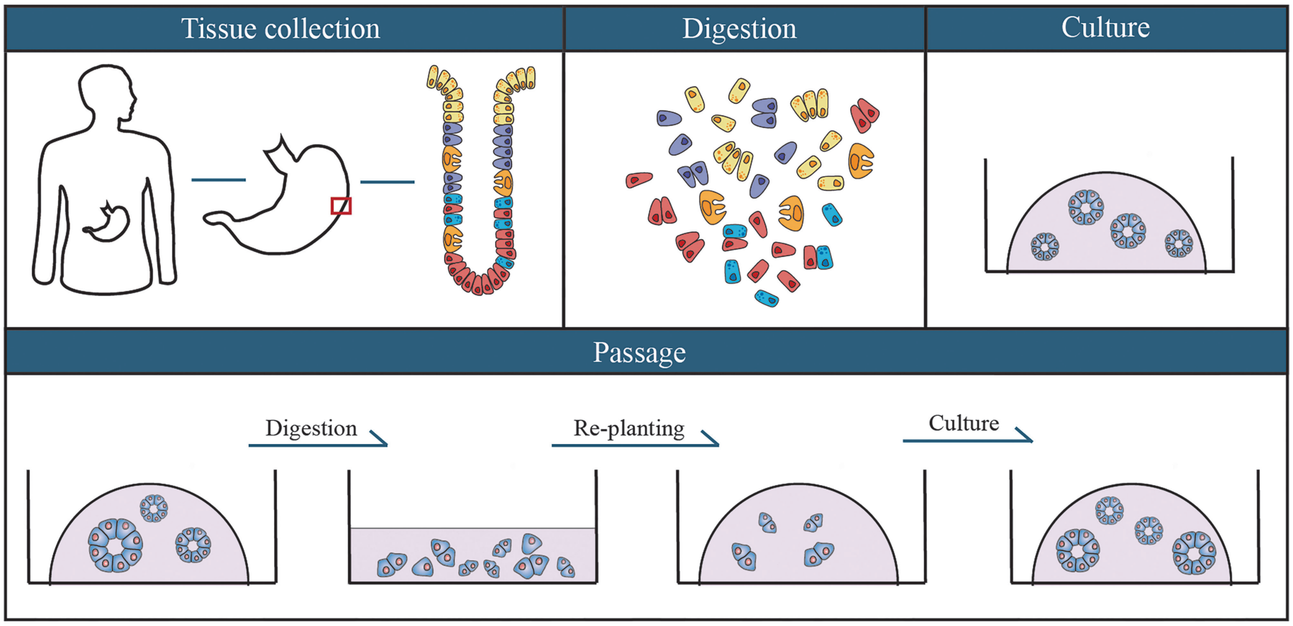

Figure 1: The key steps of organoid construction include tissue collection, tissue digestion, 3D organoid culture, and organoid passaging. The passaging procedure includes organoid digestion, re-planting, and 3D organoid culture. 3D: Three-dimensional.

Modified Eagle Medium (DMEM)/F12 containing 1% penicillin/streptomycin and 100 μg/mL primocin. If the fresh tissue cannot be cultured on the same day, the tissue should be stored in the tissue preservation solution with 10 μmol/L Y27632 overnight. In principle, the organoid culture should be processed within 24 h to maintain cell vitality.[25] The pretreatment of the tissue is a key step for successful construction of organoids. Thorough cleaning of the tissues is a core factor during the pretreatment step, which should include two washing steps. The first step is preliminary cleaning, in which the tissue is washed in no less than 5 mL of PBS washing buffer containing 1% penicillin/streptomycin and 100 μg/mL primocin by shaking on an oscillator every 5–10 min four times. After cutting the tissues into 0.5 mm × 0.5 mm in a 1.5 mL eppendorf (EP) tube with ophthalmic scissors, the tissue should be thoroughly washed in no less than 5 mL PBS washing buffer by shaking on an oscillator every 5–10 min. The solution is to be then centrifuged at 1500 r/min for 5 min. The turbid solution should be discarded, and then the phosphate buffered saline (PBS) washing buffer should be added to continue washing for a total of three times.

Organoid culture

The digestive enzymes and digestion time should be tailored to the tissue type. For example, for gastrointestinal tissues, we use 1 mL digestion solution containing type IV collagenase (1.5 mg/mL) and hyaluronidase (20 μg/mL) to the tissue precipitate obtained from the aforementioned pretreatment in 37°C washing bath and shake it on the oscillator every 15–30 min for 15–30 s until the tissue is digested into a viscous shape. The digestion time depends on tissue types. According to our experience, the digestion time is about 1 h for gastrointestinal tissues, esophageal tissues, and lung tissues, but 30 min for liver tissues and 15 min for kidney tissues. The digested solution is transferred to a 15 mL centrifuge tube and filtered with a 70–100 μm filter to remove undigested residues. The cell suspension is transferred to a 15 mL centrifuge tube for centrifugation (1500 r/min for 5 min) to obtain the cell pellet. Advanced DMEM/F12 medium is added and mixed with matrigel at a 1:1 ratio. The mixed cell solution is seeded into a 24-well plate and incubated at 37°C for 20–30 min. A 500 μL complete medium is added for cell culture. Generally, the complete medium is changed every 3–5 days. The organoid passaging could be carried out after 2 weeks of culture.

Organoid passage

After 2 weeks of cell culture, organoids are digested by a recombinant animal origin-free trypsin (TrypLE™ ExpressEnzyme) into single cell suspension. The cells are resuspended in advanced DMEM/F12 medium mixed with Matrigel at a 1:1 ratio and seeded into a 24-well plate. Generally, the passaging ratio can be 1:2 or 1:4. Organoids of less than 10 generations are considered early passages. Organoids between 10 and 20 generations are called intermediate passages, while organoids over 20 generations are called later passages.[26]

Organoid cryopreservation

To establish a living biobank *in vitro*, about two-thirds of cell sediment from the cellular passaging procedure is cryopreserved. The cell pellet is added to an organoid CryoStor® CS10 cryopreservation solution and placed into programmed cryopreservation boxes, which are placed in a deep freezer at –80°C overnight. Then, the cryopreservation tube is transferred into a liquid nitrogen tank the next day for long-term storage.

Organoid recovery

The basic principle of organoid recovery is instant melting. The cryopreservation tube should be immediately placed into a 37°C water bath with shaking from time to time to melt it as quickly as possible. The cells are aspirated into a 15 mL centrifuge tube with 10 times the volume of advanced DMEM/F12 culture medium for centrifuging (1500 r/min for 5 min). Then, the supernatant is removed and the precipitate is retained. The cells are resuspended in advanced DMEM/F12 medium mixed with Matrigel at a 1:1 ratio and seeded into a 24-well plate. The 24-well plates were incubated in an incubator at 37°C for 30 min, and 500 μL complete medium was added for cell culture. The detailed methods are described previously.[24,26]

Disposal of discarded organoids

It is difficult to avoid contamination during organoid culture. In general, if the matrigel or culture medium becomes cloudy within the first 3 days, it may indicate fungal and/or bacterial contamination and should be discarded in time.[27] In addition, mycoplasma can show clumpy or small globular growth, which is easily confused with organoids. However, the human epithelial organoids reveal tissue structure, not mycoplasma, under the microscope. If mycoplasma contamination is suspected, a mycoplasma removal agent could be added to rescue the cell culture. If the mycoplasma contamination could not be removed, it should be discarded.[28] The contaminated organoids should be discarded at the designated site according to the biomedical waste disposal regulations.

Molecular Characteristics and Quality Control of Organoids

Organoids have become a star of modern biomedical research. The greatest advantage is that they can retain the genetic characteristics and biological functions of their parental tissues.[29,30] However, there is still a lack of systematic follow-up studies on the effects of long-term passaging, cryopreservation, recovery, and re-culture of organoids on molecular characteristics. Yan *et al*[31] analyzed and compared the difference in mutation spectrum between early and late (cultured over 6 months) passages of colorectal cancer organoids with their primary tissues, and found that the drift rate of genetic mutations in short-term passage organoids was less than 20%, while the drift rate of genetic mutation in long-term passage organoids could be up to about 50%. Our team examined the differences in genetic mutations between gastric cancer organoids and their primary tissues and found that the sharing rate of genetic mutations between early passage organoids (<10 passages) and their primary tissues could exceed 90%. However, the sharing rate of genetic mutations between the mid-passage organoids (between 10 and 20 passages) and the primary tissues was about 50–60%.[26] Several genomics methods could be used for molecular profiling, such as whole genome sequencing (WGS), whole exome sequencing (WES), RNA sequencing (RNA-seq), and single-cell RNA sequencing (scRNA-seq). In addition, karyotype analysis and short tandem repeat (STR) detection could be used for the identification of organoid lines.[26,32]

There are several methods for evaluating organoid quality. Morphologically, under 20× magnification, about 60 organoids per field could be observed on the 10th culture day. The average diameter of organoids is 160–200 μm. When organoids grow too large (i.e., more than 1000 μm in diameter), they need to be passaged as soon as possible to avoid necrosis due to lack of nutrients. Currently, different laboratories use different methods to assess cell viability.[33,34] The author's laboratory performed hematoxylin and eosin (HE) staining, immunohistochemical (IHC) staining, trypan blue staining, senescence-associated β-galactosidase staining (SA-β-GAL), Giemsa staining, and artificial intelligence (AI)-assisted evaluation.[24,32] Since adenosine triphosphate (ATP) is an indicator of living cells' metabolism, detection of ATP content by chemiluminescence method is also used to evaluate organoid vitality[35,36] [Figure 2].

Laboratory Standardized Construction

Constructing high-quality organoids depends on a well-established laboratory. In China, the requirements for laboratory establishment are quite different according to experimental purposes. The construction of cell culture rooms is typically divided into three tiers [Figure 3].

Cell culture room for basic research

The laboratory construction should follow the standard of "*Code for the design of clean room*" (No. GB50073-2013). The environmental clean grade of the laboratory is based on the "*Design standards for clean workshops in the pharmaceutical industry*" (No. GB50457-2019), which at least meets the requirements of grade D + A. That means it is grade D in the clean zone and grade A in the clean bench. Only the necessary incubators and shelves are placed in the laboratory. The separated material entrances shall be provided to ensure the shortest path for material delivery. The materials should be cleaned before entering the clean zone. If the space permits, a separate sample entrance path should be set up. At the same time, there should be special arrangements for sample exporting or medical waste exporting. In principle, the researchers enter the laboratory through three-grade buffer zones, including the shoe exchange room, the coat exchange room, and the air showering room. For qualified laboratories, a separate exit route should be prepared to avoid cross-contamination.

The clean grade of the cell culture room for basic research is as follows: the number of dust particle (≥0.5 μm) at static condition is no more than 3,520,000/m^3. The number of dust particle (≥5.0 μm) at static condition is no more than 29,000/m^3. The number of dust particle at dynamic conditions is not specified. The standard for planktonic bacteria is less than 200 colony forming units (CFU)/m^3. The standard of sediment bacteria (Φ90 mm) is less than 100 CFU/4h. The air temperature is set at 18–26°C with relative humidity of 45–60%. The ventilation rate of the cell culture room is set to ≥15/h. All experimental procedures need to be carried out on a clean bench. The staff should turn on the air purification system

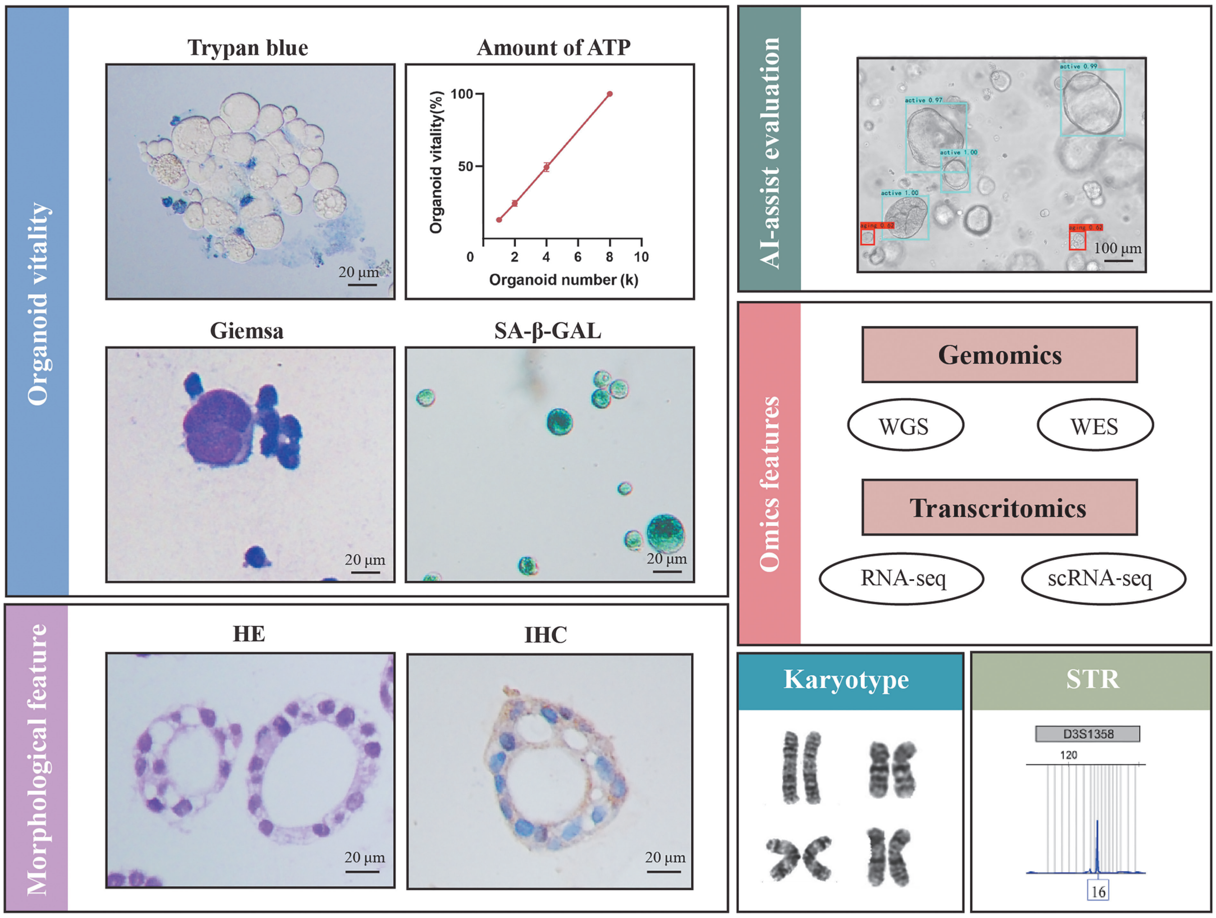

Figure 2: Several evaluation methods are used for organoid evaluation, such as organoid vitality evaluation, morphological evaluation, AI-assisted evaluation, genomic features evaluation, karyotype analysis, and STR identification. Organoid samples used here are from gastric cancer. Organoid vitality evaluation (top left) covers trypan blue staining, ATP detection, Giemsa staining, and SA-β-GAL staining. The morphological evaluation (low left) includes HE staining and IHC staining. AI-assisted evaluation (top right) can produce organoid numbers, organoid diameters, and the ratio of active organoids. The karyotype analysis can evaluate chromosome numbers. The STR identification is used for evaluating the uniqueness of organoid lines. AI: Artificial intelligence; ATP: Adenosine triphosphate; HE: Hematoxylin and eosin; IHC: Immunohistochemical; RNA-seq: RNA sequencing; SA-β-GAL: Senescence-associated β-galactosidase staining; scRNA-seq: Single-cell RNA sequencing; STR: Short tandem repeat; RNAseq: RNA sequencing; WES: Whole exome sequencing; WGS: Whole genome sequencing.

at least 1 h before they enter the cell culture room and turn on the ultraviolet (UV) lamp.

Cell culture room for drug development

This type of cell culture room is designed for new drug screening, drug sensitivity testing, drug safety evaluation, and preclinical research. The clean grade refers to the "*Design standards for clean workshops in the pharmaceutical industry*" (No. GB50457-2019), which at least meets the requirements of grade C + A. That means it is grade C in the clean zone and grade A in the clean bench. The standard of grade C in a clean zone refers to: At static conditions, the number of dust particle (≥0.5 μm) is no more than 352,000/m^3. The number of dust particle (≥5.0 μm) at static condition is no more than 2900/m^3. At dynamic conditions, the number of suspended particles (≥0.5 μm) does not exceed 3,520,000/m^3 and the number of suspended particles (≥5.0 μm) does not exceed 29,000/m^3. The standard of planktonic bacteria is less than 100 CFU/m^3. The standard of sediment bacteria (Φ90 mm) is less than 50 CFU/4h. The air temperature should be 20–24°C with relative humidity of 45–60%. The room ventilation frequency is ≥25 times/h.

It should be noted that the stand for the cell culture room for drug development is significantly higher than that of basic research. For example, all experimental operation is performed on biosafety cabinets with a grade A cleaning grade. The staff should turn on the air purification system at least 1 h before entering the cell culture room and turn on the UV light. The researchers enter the laboratory through three-grade buffer zones, including the shoe exchange room, the coat exchange room, and the air showering room. Meanwhile, a separate exiting route should be prepared to avoid cross-contamination.

Cell culture room for production of biopharmaceuticals at a therapeutic level

The clean grade refers to the "*Design standards for clean workshops in the pharmaceutical industry*" (No. GB50457-2019), which at least meets the requirements of grade B + A. That means it is grade B in the clean zone and grade A in the clean bench. Grade B refers to: At static conditions, the number of dust particle (≥0.5 μm) is no more than 3520/m^3. The number of dust particle (≥5.0 μm) at static conditions is no more than 29/m^3. At dynamic conditions, the number of suspended particles

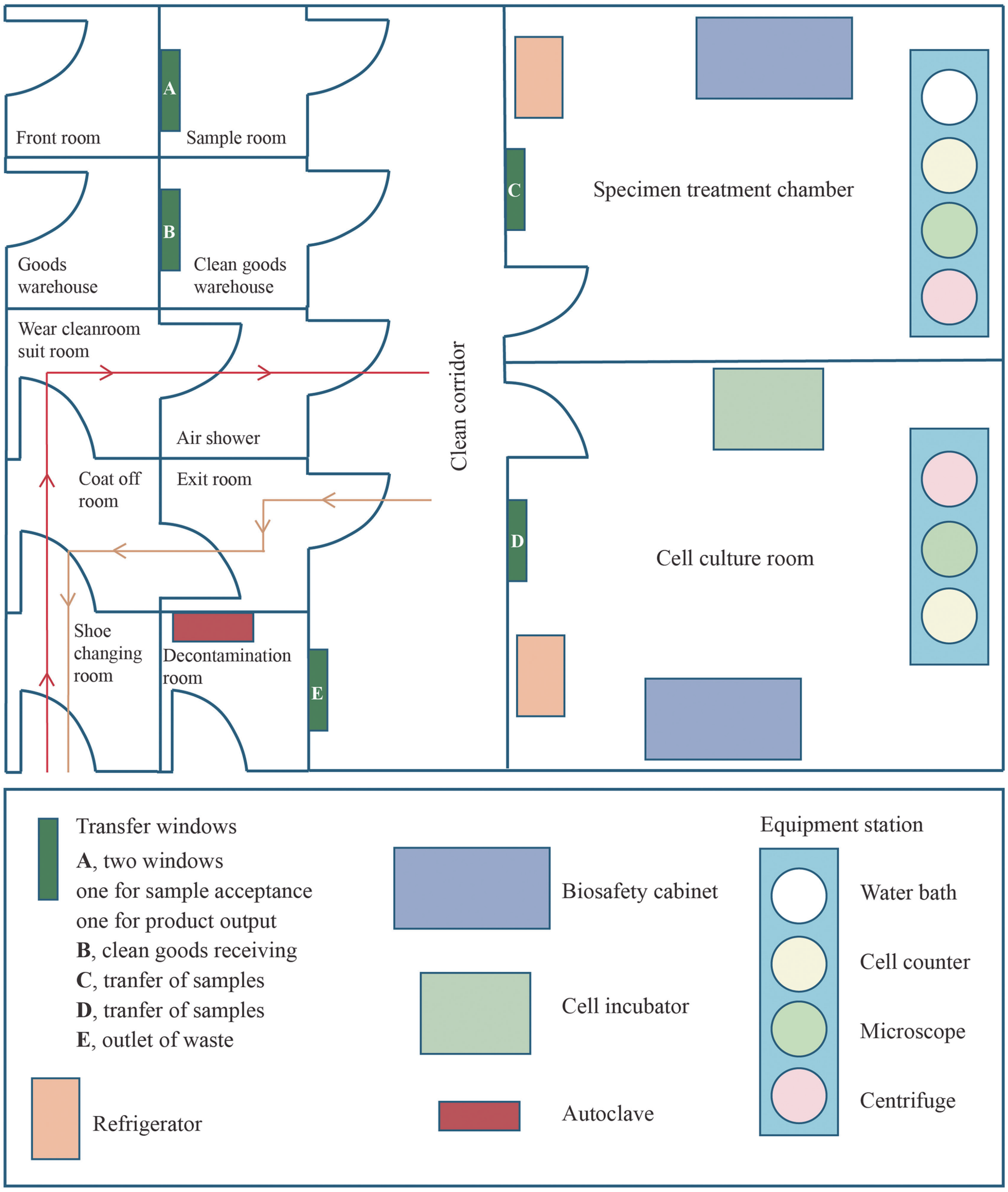

Figure 3: Top: The layout of the standardized cell culture room. The red arrows indicate the entering path, including the shoe changing room, coat off room, wear cleanroom, and air shower room. The orange arrows indicate the exiting path, including the exit room, coat off room, and shoe changing room. Others include the front room, goods warehouse, sample room, clean goods warehouse, clean corridor, specimen treatment room, and cell culture room. Down: The captions describe the functions of different zones and devices, including transfer windows, refrigerator, biosafety cabinet, cell incubator, autoclave, and equipment station (wash bath, cell counter, microscope, and centrifuge).

(≥0.5 μm) does not exceed 352,000/m^3 and the number of suspended particles (≥5.0 μm) does not exceed 2900/m^3. The standard of planktonic bacteria is less than 10 CFU/m^3. The standard of the sediment bacteria (Φ90 mm) is less than 5 CFU/4h. The air temperature should be 20°C–24°C with relative humidity of 45–60%. The experimental procedures should be carried out in biosafety cabinets of grade A for cleaning grade. The staff should turn on the air purification system at least 1 h before entering the cell culture room and turn on the UV light. The researchers enter the laboratory through three-grade buffer zones, including shoe exchange room, the coat exchange room, and the air showering room. Meanwhile, a separate exit route should be prepared to avoid cross-contamination.

The cell culture room is the key space for the successful construction of organoids. Therefore, it should be regularly sterilized, including weekly sterilization and sterilization before experiment (using 0.1% benzalkonium bromide, 2% cresol solution, 5–20 times diluted iodopor solution, 1:50 diluted sodium hypochlorite disinfectant, and 75% ethanol) to wipe the operating bench and possible contaminated spaces. The specific disinfection method is to wipe the entire inner and top surfaces of the clean bench or biosafety cabinet with a disinfection solution. The floor, transfer window, and door handle of the room should also be wiped. The principle of cleaning is followed from the inside to outside, from the high cleaning zone to the low cleaning zone until exit the room. After sterilization, turn on the air filter and UV lamp for 1 h to kill the remaining microorganisms. In addition, at the end of each experimental operation, it is necessary to use the above-mentioned disinfectant to wipe the bench surface, remove indoor moisture, and sterilize with a UV lamp for 30 min.

To facilitate the understanding of cleaning grades and the relationship between different cleaning systems, we list the main parameters in Table 1.

Available Standardization Documents for Organoids

In addition to ISO, standardization-related organizations have been established in different countries. In China, the standardization system covers five levels: National standards, field standards, administrative district standards, academic community standards, and enterprise standards. The national standards are technical requirements that are unified throughout the country and are formulated and issued by the State Council of the People's Republic of China. The field standards are unified technical requirements in a specific field and are approved by the competent ministry, commission, or bureau. The administrative district standards are technical requirements that are unified in an administrative district and are formulated and issued by the administrative department of provinces, autonomous regions, or municipalities. The academic community standards are formulated and issued by an academic community. The enterprise standards are technical requirements that are unified within the enterprise and are formulated and implemented by the enterprise itself.

In recent years, Chinese academic organizations have placed great importance on the development of standards for organoid construction. In 2022, an academic community standard on the *Guideline of construction and preservation of organoids of gastrointestinal epithelial tissues* (No. T/CMBA 017-2022) was released by the China Medicinal Biotech Association (CMBA),[24,37] which provided a guiding document for the standardized construction of gastrointestinal epithelial and tumor organoids. In September 2022, *Technical specification for the culture of patient-derived lung cancer organoids* (No. T/SHSYCXH 12-2022) was released by the Shanghai Genetics Society. In December 2022, *Technical specification for the construction of brain tumor organoid models* (No. T/SHDSGY 167-2023) was released by the Shanghai Urban Industry Association.

In 2023, an academic community standard on *Guideline for preparation, cryopreservation, recovery and identification of organoids of human normal breast and breast cancer tissue* (No. T/CMBA 020-2023) was issued by CMBA.[38] In 2023, *Standard: human intestinal organoids* (No. T/CSCB 0005-2022) and *Standard: human intestinal cancer organoids* (No. T/CSCB 0006-2022) were released by the Chinese Society for Cell Biology (CSCB).[39,40] Meanwhile, *Guidelines for the establishment, quality control and storage of human hepatic progenitor cell derived organoids* (No. T/CRHA 017-2023), *Guidelines for the establishment, quality control and storage of human hepatobiliary tumor organoids* (No. T/CRHA 018-2023), and *Guidelines for the establishment, quality control and storage of biliary epithelial tissue organoids* (No. T/CRHA 019-2023) were issued by the Chinese Research Hospital Association (CRHA). In March 2024, *Culture and identification of patient-derived organoids for intrahepatic cholangiocarcinoma* (No. T/QMHIPA 001-2024) was issued by the Qingdao Medical and Health Industry Promotion Association. In May 2024, an *Evaluation of drug-induced hepatotoxicity based on human liver organoids* (No. T/CRHA 052-2024) was issued by the CRHA. In June 2024, the *Group standard for the construction, quality control, and preservation of human gastric cancer organoids* (No. T/1984CACA 1-2024) and the *Establishment and quality control system for organoids derived from human sarcomas* (No. T/1984CACA2-2024) were issued by the Chinese Anti-Cancer Association (CACA).[41]

In the field of organoid construction, beyond ASC-derived organoids, iPSCs are also used for organoid construction, especially when fresh tissues are difficult to collect.[42–48] Although the construction of organoids from iPSCs is still in the exploratory stage, several related standards have been published recently.[49–52] These standards provided an important reference for the organoid community on the construction and application of organoids from iPSCs.

In addition, guidelines and expert consensus are widely used as guiding principles and recommendations for research and biomedical practice. These are usually

Table 1: The maximum number of suspended particles of cleaning grades for cell culture room.

Cleaning grades	New grades (pc/m^3)				Traditional grades (pc/ft^3)
	Static condition		Dynamic condition		
	Dust particle $\geq$0.5 μm	Dust particle $\geq$5.0 μm	Dust particle $\geq$0.5 μm	Dust particle $\geq$5.0 μm	
A	3520 (ISO 5)	20	3520 (ISO 5)	20	100
B	3520 (ISO 5)	29	352,000 (ISO 7)	2900	100
C	352,000 (ISO 7)	2900	3,520,000 (ISO 8)	29,000	10,000
D	3,520,000 (ISO 8)	29,000	Not specified	Not specified	100,000

ISO: International Organization for Standardization.

formulated by authorized organizations, such as the Ministry of Health and the Chinese Medical Association. Guidelines or expert consensus emphasize the role of expert experiences. For example, the *Expert consensus of clinical application about tumor precision therapy guided by organoid-based drug sensitivity testing* was published in *Chin J Oncol Prevent Treat* in 2022.[53] In the same year, the *Chinese expert consensus on quality control standards for tumor organoids diagnosis and treatment platform* was published in *China Oncol*.[54] The guidelines and expert consensus also play a promoting role in drug sensitivity testing based on organoid platforms [Figure 4].

Opportunities and Challenges in Standardization Process of Organoids

At present, the research and development of organoids is still in the early stage. The standards are currently lacking in many fields. Multiple organoid-related standards have been issued by the academic community and have not yet been updated to the national level. Meanwhile, there is also a lack of international standards for organoids. Therefore, the standardization process of organoids presents both opportunities and challenges. For example, epithelial tissue-derived organoids are relatively homogeneous in cellular composition and lack tumor microenvironment (TME, such as fibroblasts, vascular endothelial cells, immune cells, and pathogenic microorganisms). However, the cells of TME play an important role in the carcinogenesis and progression of tumors, and even in regulating drug resistance.[55] The interaction between immune cells and tumor organoid cells, as well as drug screening for tumor immunotherapy, relies heavily on the establishment of co-culture systems involving tumor organoids and immune cells. Our group used the co-culture system of gastric cancer organoids and lymphocytes to screen and verify that dexamethasone of low-dose mediates the down-regulation of both programmed cell death ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase 1 (IDO1) targets through the GR/STAT3 pathway, inhibiting the immune evasion of cancer cells.[56] Moreover, by means of a co-culture system of PDOs and macrophages, we found that the resistant mechanism of diffuse-type gastric cancer to aurora kinases inhibitors (AURKi) is involved in the cell senescence induced by up-regulation of MCP-1/CCL2, which further induces the M2 polarization of macrophages of TME and reduce the killing effect on cancers.[32]

It is well known that the carcinogenesis of certain malignancies is closely associated with infection of pathogenic microorganisms. *Helicobacter pylori* (*Hp*) plays an important role in gastric carcinogenesis.[57] Some researchers conducted the interaction study between *Hp* and gastric organoids and found that the expression of PD-L1 on gastric organoids significantly increased 48 h after *Hp* infection. Increased expression of PD-L1 is a self-defense mechanism against *Hp* infection, which is mediated by the Sonic Hedgehog signaling pathway.[58] Similarly, to elucidate the molecular events driving hepatocellular transformation and carcinogenesis mediated by the hepatitis B virus (HBV), liver organoids interacting with recombinant viruses or sera from patients infected with HBV can serve as experimental tools. This kind of model can also be used for anti-HBV drug screening.[59] The above-mentioned co-culture models using organoids and microenvironmental components are all in the exploratory stage. In the future, standardization and clinical application of co-culture systems for exploring organoids with microenvironmental components hold significant potential for growth.

The road to standardization in organoid construction still faces some challenges. In ASC-derived organoids, it is difficult to maintain homogeneity of organoids because the organoids constructed from patient-derived tissues contain multiple types of cells.[60] Therefore, the homogeneity of organoid number or growth speed varies depending on primary tissues. Based on our experience, experimental procedures significantly impact the success rate of organoid construction. Since we have strictly implemented the academic community standard on the *Guideline of construction and preservation of organoids of gastrointestinal epithelial tissues* (No. T/CMBA 017-2022), the success rate for gastrointestinal tumor organoid construction has increased from 50% to 90% or more. Standardization improves the stability and consistency of organoids. AI-assisted organoid evaluation also confirmed that organoid number and diameter at specified passaging date maintain high consistency.[61]

Besides the ASC-derived organoids, iPSC/ESC-derived organoids also face standardization issues. It involves multiple steps of stem cell culture, evaluation of cell vitality, cell purity, genetic stability, differentiation capability, organoid identification, and etc.[49] This section is not covered in this article. Nevertheless, iPSC/ESC-derived organoids are very useful in elucidating the pathogenic mechanisms of rare genetic monogenic diseases and in drug target screening.[62,63]

In summary, there are still numerous challenges in the standardization of technical operation of organoid construction. The cost of producing organoids is still high. It is necessary to strengthen the research and development of organoids technology to improve the quality and stability of organoids and promote wide application and industrialization. In the future, standardized organoid technologies combined with AI can promote automatic 3D culture and quality evaluation. Meanwhile, organoid technologies combined with clinical big data or genomic data may realize better prediction of drug sensitivity and clinical outcomes. Recently, ISO/TC 276 committee has initiated the draft of organoid-related standards through international cooperation. Our team, as a group member, engaged in the draft work and the first round of seeking feedback. With the collective efforts from different countries, organoid-related standards will undoubtedly be successively established, thereby driving the sound and sustainable development of this field.

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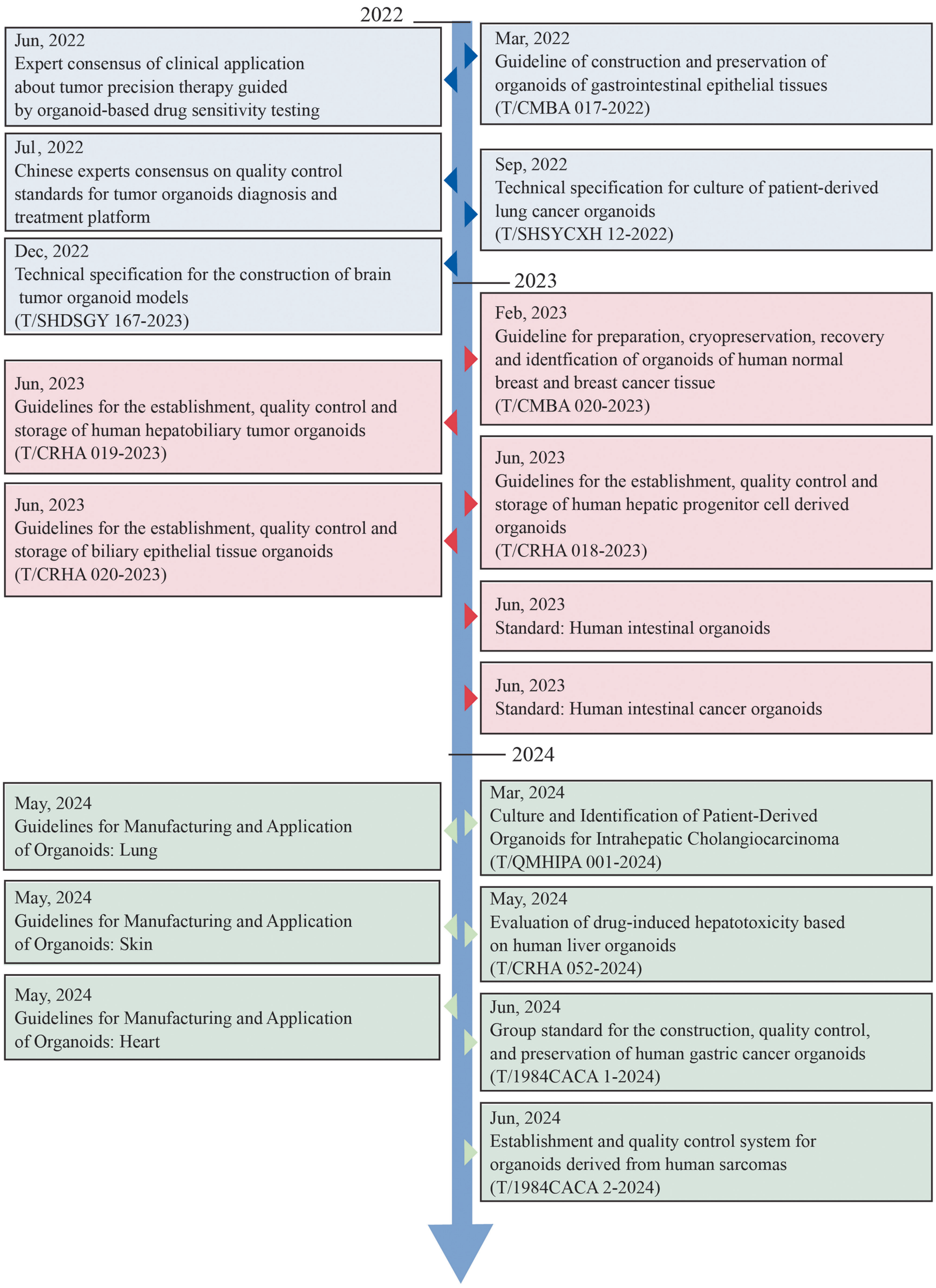

Figure 4: The timelines of organoid-related standards or guidelines. The publication time, title, and ID number of the standard are listed following the title name. CACA: Chinese Anti-Cancer Association; CMBA: China Medicinal Biotech Association; CRHA: Chinese Research Hospital Association; QMHIPA: Qingdao Medical and Health Industry Promotion Association.

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Conflict of interests

None.

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