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REVIEW ARTICLE

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The application of organoid models in research into metabolic diseases

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Abstract

Metabolic diseases have become a major threat to human health worldwide as a result of changing lifestyles. The exploration of the underlying molecular mechanisms of metabolic diseases and the development of improved therapeutic methods have been hindered by the lack of appropriate human experimental models. Organoids are three-dimensional in vitro models of self-renewing cells that spontaneously self-organize into structures similar to the corresponding in vivo tissues, recapitulating the original tissue function. Off-body organoid technology has been successfully applied to disease modelling, developmental biology, regenerative medicine, and tumour precision medicine. This new generation of biological models has received widespread attention. This article focuses on the construction process and research progress with regard to organoids related to metabolic diseases in recent years, and looks forward to their prospective applications.

KEYWORDS
metabolic diseases, organoid, stem cells

1 | INTRODUCTIONIN

Metabolic diseases are caused by abnormal metabolic function in the human body that seriously endangers human health. Marked by obesity, blood lipid disorders and abnormal glucose metabolism, the underlying cause of metabolic disease may be congenital inherited enzyme deficiencies or acquired metabolic organ failure.[1] Since the end of the twentieth century, morbidity associated with metabolic disease has been on the rise, which has made it one of the most prominent world public health issues.[2] Achieving early diagnosis and clinical treatment by conducting in-depth research on biochemical metabolism in vivo, as well as understanding the aetiology and pathogenesis of metabolic disease, are therefore of great importance.

Nowadays, research into metabolic disease mainly focuses on constructing animal models and two-dimensional (2D) cell-line models. The relative lack of appropriate models that mimic the human body has restricted current research, both in terms of understanding pathogenic mechanisms and developing rational treatments. Cell models are simple, inexpensive, and most used. However, it is impossible to mimic the complex three-dimensional (3D) environment in vitro because cultured cells are likely to lose their heterogeneity and characteristics in vitro.[3] In addition, the growth patterns of monolayers lack cell–cell and cell–cell matrix interactions and communication, which are critical for maintaining in situ phenotype and biological function.[4]

Various animal models have been successfully applied in the biomedical field, with the aim of improving the understanding of cell signalling, finding potential drug targets, and elucidating the basic

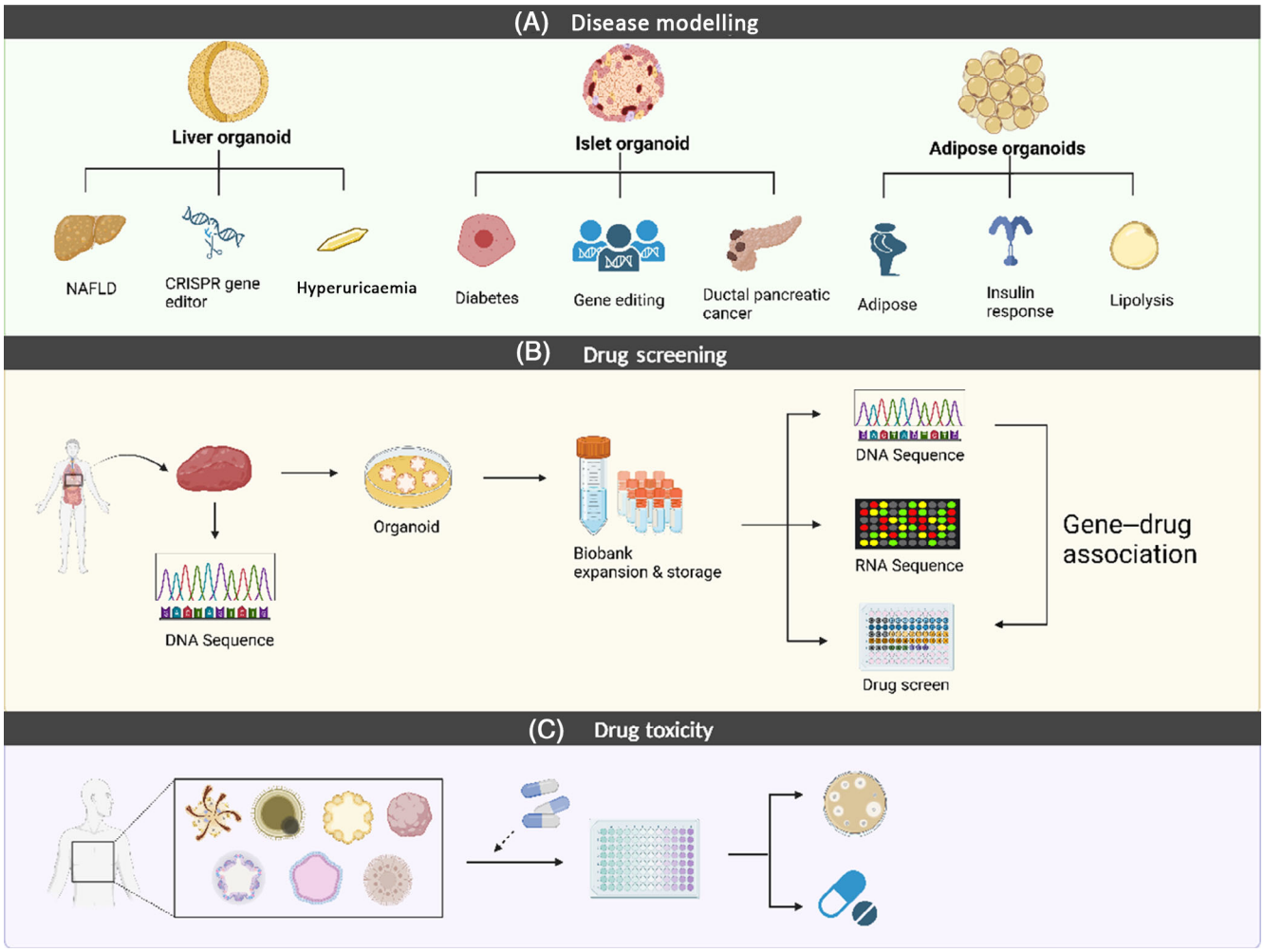

FIGURE 1 General models and applications of organoids in metabolic diseases. (A) Liver organoids are suitable for a variety of metabolic disease construction models, such as nonalcoholic fatty liver disease (NAFLD) and hyperuricaemia, and can be used in conjunction with CRISPR technology to establish new screening platforms. Islet organoids are indicated for diabetes and pancreatic ductal cancer, among others. Adipose organoids provide a powerful platform for studying obesity, insulin resistance, and lipolysis. (B) Patient tissue-derived organoids show great potential for drug prediction. (C) Organoids can be used to predict drug toxicity.

pathological mechanism of diseases. Species differences with regard to genetic characteristics and phenotype hinder the application of animal models. For example, animals that are used in preclinical trials tend to be small, fast in development, short in lifespan, and are also quite different from the human body in terms of energy expenditure, drug absorption and metabolism.[5]

The establishment of new models that can reproduce the pathophysiological characteristics of the human body without recruiting human participants is therefore a path-breaking strategy to investigate the pathogenesis of metabolic diseases and to screen related drug targets. In Figure 1, we summarize the most common metabolic disease-related organoid models, and cite the basic application of metabolic organoids. Generally, liver organoids, islet organoids and adipose organoids are the most used and mature models in research. Drug screening and drug toxicity are the most common application in clinical research.

Since the formation of human stem cell technology, an important research method has been the simulation of human development and disease, with its own multi-directional differentiation potential and strong regenerative ability. Along with an understanding of the biological function of the extracellular matrix (ECM), which has led to advances in 3D cell culture technology, stem cell-derived organ-like miniature-structured organoids, built in a self-organizing manner, have emerged.[6] Compared with traditional cell culture or animal models, organoids can better simulate the living environment of cells in vivo and signal communication between multiple cells. They can also maintain genetic stability during the expansion process, making the model closer to real physiological patterns in the human body. Moreover, organoids reduce the possible impact of physiological and genomic differences between species on assessment of clinical outcomes.

This article reviews the progress made in research into metabolic diseases using stem cell-driven human organ models. The advantages

and limitations of current metabolic organoids in translational applications are discussed, as well as new clues as to a more in-depth understanding of the pathogenic mechanism of metabolic diseases.

2 | ORIGIN AND DEVELOPMENT OF ORGANOIDS

Mammalian cells can self-replicate and even specialize into their own tissues under specific circumstances. Bissel et al. demonstrated that interaction with the ECM improves liver function and regulates the proliferation and differentiation of epithelial cells in rats.[7] In the 1960s, developmental biologists used the term 'organoid' to describe organogenesis through cell dissociation and reaggregation experiments.[8] Previous studies used the 3D self-organizing ability of primary tissues until 2009, when Hans Clevers et al. obtained microstructural complexes resembling crypt villi using a ternary matrix gel containing Noggin, epidermal growth factor, and R-spondin as an ECM. This three-dimensional structural complexe resembles the small intestinal villi and successfully constructed an intestinal organoid system based on adult embryonic stem cells (ESCs) derived from endogenous mouse intestine cultured in vitro by ECM, leading to the improvement of systems in the field of organoids, which is an engineering and conceptual breakthrough.[9] They then further investigated this system by embedding a single leucine-rich repeat-containing G-protein-coupled receptor 5^+ ($Lgr5^+$) intestinal stem cell into ECM and showed that these cells were also able to form tissues similar to those specified above, while maintaining $Lgr5^+$ features. It is evident that the organoids created in this system are capable of mimicking the function and structure of human intestinal tissues while maintaining the characteristics of stem cells.

Based on literature combining common physiological and histological perspectives, Lancaster et al. proposed a more explicit definition of organoid,[10] specifying that successful organoids should meet three basic criteria[1]: cells isolated from the organoid can potentially differentiate into organ-specific cell types[2]; cells in the organoid have a cytological spatial structure similar to that of the original organ; and[3] the organoids possess the specific function of the original organ they mimic. Based on these criteria, researchers are constantly improving and optimizing culture systems for organoids, which has greatly improved the success rate of organoid cultures. Since then, organoids have sprung up in the epithelia of other systems,[11] and organoid technology was selected by Nature Methods as the 'Life Science Technology of the Year' in 2017, one of the most influential technologies in scientific studies of recent years, for its enormous promise in life science research.

3 | ORGANOIDS IN METABOLIC DISEASES

3.1 | Diabetes

Diabetes is a metabolic disorder characterized by chronic hyperglycaemia, resulting from various factors such as autoimmunity, infection, stress and obesity. Presently, approximately 537 million individuals globally are affected by diabetes, and an estimated 6.7 million deaths worldwide in 2021 can be attributed to complications caused by this condition. The International Diabetes Federation projects that the number of individuals with diabetes will rise to 783 million by 2045, making it a global public health concern.[12–14] As β-cell deficiency and inadequate insulin secretion are the ultimate outcomes of all diabetes progression, the development of replaceable functional β cells from renewable resources is anticipated to be a novel therapeutic approach to this disease. In recent years, researchers have conducted extensive investigations into an in vitro expansion-induced generation of islet β cells. It has been discovered that ESCs and induced pluripotent stem cells (iPSCs) may alleviate the bottleneck of islet transplantation donor shortages and immunological rejection. As a result, they have the potential to become a new source of islet β-cell regeneration due to their potential for proliferation and differentiation into insulin-secreting cells or islet organoids in vitro.

Montesano et al. first reported in 1983 that they had isolated rat pancreatic endocrine cells capable of reassembling into islet-like organs in vitro. With matrix gel enhancing their expression, their discovery led to the initiation of fundamental research into islet organoids.[15] The cellular composition and endocrine function of the pancreatic islets are preserved by this cellular dispersion and repopulation to form 'pseudoislets'. After in vivo transplantation, glucose-stimulated insulin secretion remains. Numerous researchers have reported advances in genetics, genomics, islet cell transplantation, and the study of intra-islet signalling and islet cell interactions in pseudoislets, which have been used as a disease research model.[16] In 2006, D'Amour et al. successfully established an in vitro induction protocol for ESCs to form islet endocrine cells, with insulin expression levels of the derived cells approaching those of mature islets.[17] However, the cells obtained through this method lacked the production of key β-cell transcription factor and secreted low levels of both C-peptide and insulin in response to high glucose stimulation, which is a hallmark function of true β cells, indicating the apparent immaturity of these cells. In 2014, Pagliuca et al.[18] and Rezania et al.[19] independently described a protocol for the in vitro differentiation of human pluripotent stem cells (hPSCs) by pancreatic progenitor cells as a means of generating functional β cells. Pagliuca et al. improved the in vitro induction of pancreatic progenitor cells for the generation of mature endocrine cells by testing the incorporation of up to 150 combinations of more than 70 compounds in order to generate high levels of NKX6.1+/PDX1+ co-expressing pancreatic progenitor cell clusters; this protocol produced approximately 33% stem cell derived β (sc-β) cells that resembled primary β cells at ultrastructural, molecular and functional levels. Functionally, these cells repeatedly increase intracellular Ca^{2+} levels and secrete insulin during successive glucose changes in vitro, and are able to respond to multiple rounds of glucose stimulation, much like natural islet cells. However, unfortunately, few other non-β endocrine cells were detected compared to human islets. In the same year, Rezania et al. optimized an induced differentiation protocol to efficiently obtain NKX6.1+/INSULIN+ cells from human ESCs (hESCs). To prevent premature differentiation of precursor cells to

generate polyhormonal cells, a gas–liquid biphasic culture environment was used to generate Stage 7 (S7) cells with similar expression profiles to those of mature β cells (INS, MAFA, NKX6.1). Transplantation into streptozotocin (STZ)-induced acute islet β-cell deficiency diabetes model mice reversed diabetes within 40 days. However, S7 cells have a slower and weaker response to glucose compared to primary β cells, and are not yet able to fully mimic islet cell function.

In contrast to mature β cells that cannot produce insulin secretion in suspension culture or in gas–liquid interface culture, islet organoids can be generated by transforming the two-dimensional pattern of PSCs into three-dimensional structures. This process re-establishes communication between β cells and non-β cells, allowing hPSCs to differentiate into endocrine cell clusters that release insulin in response to glucose stimulation and promote islet functional maturation. Consequently, researchers have commenced exploring 3D organoid culture models in order to restore cells to their natural in vivo state.[20] In 2013, Grapin-Botton's team successfully constructed the first in vitro islet organoid using both mouse and human embryonic islet cells which continued to expand for up to 2 weeks.[21,22] The cultured islet organoids exhibited a physiological structure akin to the human pancreas in vivo, encompassing a surrounding acinar vesicle with endocrine cells and ducts growing centrally.

It was shown that cell aggregation culture enhances the differentiation of hPSCs into physiological functional pancreatic lineages.[23] Thus, one approach to producing islet organoids is to first generate endocrine cells through a 2D culture platform, then aggregate the cells into clusters and extend the culture time to increase differentiation efficiency through a multistep differentiation protocol[24] to allow islet cell maturation.

Hence, a viable approach for islet organoid generation involves initially producing endocrine cells through a 2D culture system, then aggregating these cells into clusters, and extending the culture duration to facilitate islet cell maturation. In 2015, Shim et al. applied a step-wise differentiation protocol to generate hormone-expressing pancreatic endocrine cells.[25] Employing a four-step protocol that utilizes growth factors and small molecule inhibitors, the researchers induced hESCs to generate 3D islet cell clusters that are responsive to glucose stimulation, secreting insulin, and capable of being implanted into STZ-induced diabetic mice for effective blood glucose regulation. Concurrently, the authors unveiled an unprecedented finding that dual inhibition of proteoglycan sulfation and Notch signalling pathways considerably enhanced neurogenin 3 (Ngn3) and neural differentiation factor 1 expression levels, thereby promoting pancreatic progenitor cell differentiation and maturation. Kim et al.[26] first reported the pioneering generation of functional islet organoids derived from hPSCs in 2016 (Figure 2). They induced hPSCs to differentiate into endocrine cells expressing pancreatic endocrine hormones. Subsequently, the isolated endocrine cells autonomously aggregated into endocrine cell clusters analogous in size to pancreatic islets. Following the transplantation of endocrine cell clusters into a diabetic mouse model, the blood glucose levels of these mice returned to normal within 3 days. Mice that underwent transplantation

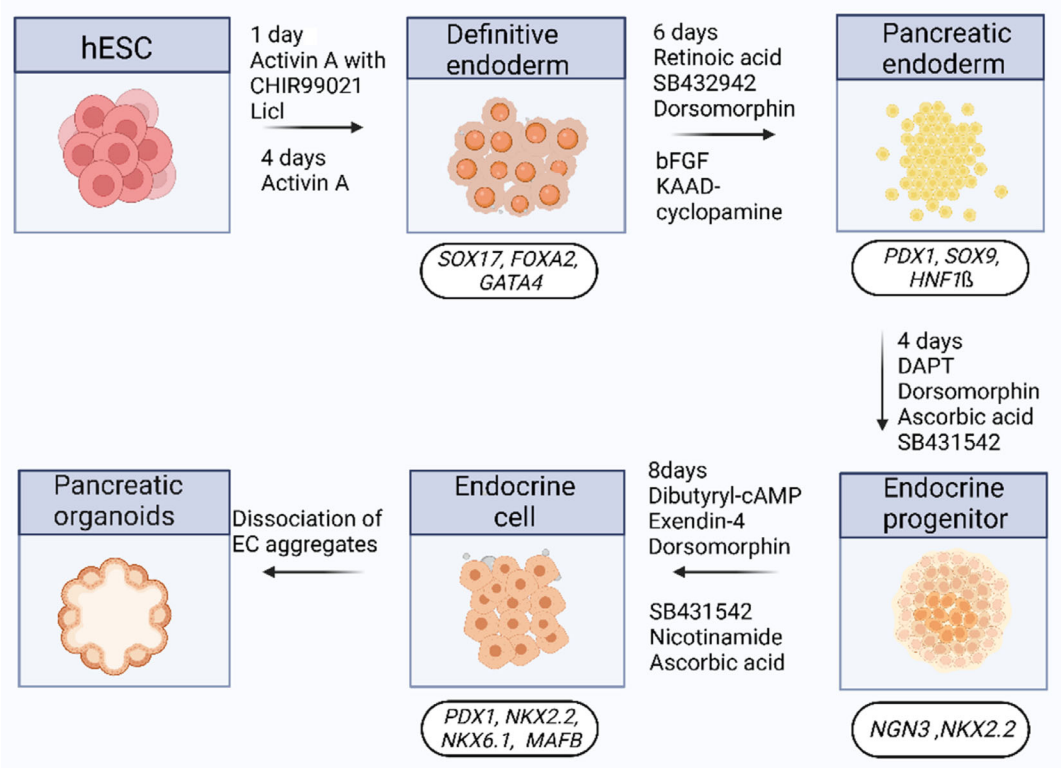

FIGURE 2 A stepwise differentiation scheme from human embryonic stem cells to pancreatic endocrine cells.[26] EC, endocrine cell; hESC, human embryonic stem cells.

survived beyond 40 days, whereas the control mice succumbed to hyperglycaemia within 3 days after insulin treatment cessation. In addition, endocrine cell clusters in this research were found to express elevated levels of mature islet cell markers, including β-cell-related marker genes (*PDX1*, *MafA* and *NKX6.1*), increased expression of glucose sensor genes (*GCK* and *SLC2A1*). They also rapidly released insulin upon high glucose stimulation in vitro, successfully replicating islet organ function. In 2019, Nair et al.[27] concluded that cell aggregation promotes metabolic maturation of β-cell mitochondria. Compared to pre-aggregation, cells in islet-like organs significantly upregulated the expression of genes associated with oxidative phosphorylation, protein secretion and TCA cycling, and upregulated the expression of genes associated with β-cell maturation, *NEUROD1*, *PAX6*, *MAFB* and *SLC30A8*, and showed dynamic insulin secretion similar to that of human islets in response to glucose stimulation. Mechanistically, β-like cell clusters induce metabolic maturation by driving mitochondrial oxidative respiration, which is central to the stimulus-secretion coupling of mature β cells. In a study by Millman et al., the use of type 1 diabetes-derived human induced pluripotent stem cells (hiPSCs) produced C-peptide+/NKX6.1+ cells, insulin-secreting capacity, and responsiveness to glucose stimuli that did not differ from healthy human-derived hiPSCs.[28]

To improve the controllability of the culture environment, in addition to the improvement of the differentiation culture protocol, the optimization of the culture substrate and the application of biomaterials combined with embryonic stem cell-induced differentiation of islet organoids showed a more mature performance in terms of function. It has been shown[29] that the current cause of sc-β-cell immaturity is mainly premature expression of NGN3 triggering premature induction of the precursor developmental process. To inhibit the premature expression of NGN3 and its downstream molecules NEUROD1 and NKX2.2, Rezania et al. found a significant increase in the number of insulin+/NKX6.1+ and KCl-responsive cell populations after the addition of vitamin C during induced differentiation of pancreatic endoderm.[19] Jin et al.[30] found that CD133+ Sox9+ pancreatic progenitor cells were able to expand to form clones in stromal gels but had difficulty in giving rise to mature endocrine cells, and also that the organoids were more inclined to differentiate towards endocrine cells after transfer to hydrogels containing laminin. Bi et al.[31] found that stimulation of hPSCs using tissue-specific decellularized rat pancreatic ECM (dpECM) increased the expression of key transcription factors (PDX-1, NKX6.1, UCN3 and ARX) in islet organoids. These islet organoids secrete insulin and glucagon in response to glucose levels, similarly to human islets. The iPSC-derived islet organoids displayed multiple types of endocrine cells in proportions similar to those of adult islets, including four major islet cell types, α, β, δ and PP cells, which have unique interactions. Candiello et al.[32] used amikacin and polyethylene glycol diglycidyl ether to construct a novel 'Amikagel' 3D scaffolding structure, which induced pancreatic progenitor cells to polymerize with endothelial cells to further differentiate into functional islet organoids with a vascular network, which exhibited high insulin and C-peptide expression as well as glucose-responsive insulin secretion at maturity. Additionally, Amikagel-generated 3D cell aggregates exhibited increased co-expression of NKX6.1/PDX1, and islet organoids cultured in microporous scaffolds displayed superior control over organ volume and cell–cell interactions. This technique has been shown to be more efficient than utilizing conventional matrixgel structures, offering a novel strategy for constructing organoid models with consistent performance and higher reliability in vitro.

Type 1 diabetes mellitus is characterized by absolute insulin deficiency due to damage to pancreatic β cells, and people with type 1 diabetes need to rely exclusively on exogenous insulin therapy. Human stem-cell-derived pancreatic islet-like organoids have the potential to replace pancreatic islet function in patients with type 1 diabetes mellitus. Currently human stem-cell-derived pancreatic islet organoids still produce off-target cell types and cells that are not present in real human islets with the presence of hormones other than C-peptide and glucagon.[27] To improve the differentiation protocol, it has been found that the timing and type of WNT signalling during differentiation can largely influence the size and maturation of SCislets.[33] In addition, in allogeneic islet transplantation, graft-induced immune rejection and the recipient's autoimmune attack may affect the survival of the transplanted cells, and microencapsulation prevents immune rejection through a physical barrier to enhance the survival of the graft. Currently, companies such as ViaCyte and Semma/Vertex have started clinical trials to promote the clinical application of SC- islet organoids. Modulation of the expression of immune recognition-related molecules in pancreatic islet organoids by gene editing techniques also offers the possibility to circumvent immune rejection. The immune checkpoint programmed death ligand-1 (PD-L1) binds to PD-1 on cytotoxic T cells, thereby inducing their programmed death and negatively regulating immune attack. Yoshihara et al.[34] used lentivirus overexpressing PD-L1 to infect human islet-like organs constructed from human umbilical vein endothelial cells, adipose-derived ctem cells, and iPSCs, and transplanted them into immunocompetent diabetic mice, the PD-L1-overexpressing islet organoids were able to normalize the blood glucose of the mice for more than 50 days, while the blood glucose of control mice began to rise 10 days after transplantation. Now, with the development of single-cell sequencing technology, a better understanding of the differentiation pathway of SC islets in vitro has opened up new ideas for researchers to grasp and regulate SC islets.[35]

3.2 | Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) represents the most prevalent chronic liver disease globally, and is characterized by widespread hepatocellular steatosis. As hepatocellular steatosis advances, it may ultimately progress to nonalcoholic steatohepatitis (NASH), cirrhosis and even hepatocellular carcinoma. Thus, NAFLD potentially causes significant liver damage and poses a serious risk to human health.[36] In recent years, China has experienced a rising prevalence of NAFLD, reaching approximately 25% within the general population. In decades to come, NAFLD may emerge as a leading cause of end-stage liver

disease.[37] Despite these alarming developments, there are currently no efficacious pharmacological interventions to combat NAFLD. Consequently, there is an exigent need to develop efficient and precise in vitro human cell systems to elucidate disease pathogenesis and drug response. Emerging organoid culture technologies present newfound potential for such research.

The use of liver organoids as a tool to simulate chronic liver diseases such as NAFLD and liver fibrosis is a promising area that has not yet been effectively explored. In recent years, cell-based bioculture systems have become increasingly sophisticated, but still cannot fully simulate the complex multicellular environment of NAFLD. Previous techniques for co-culture with PSCs have been performed by parallel differentiation, where different hepatocytes are isolated and cultured, but this technique only mimics inflammation and fibrosis, therefore, its application in NAFLD is somewhat limited.

The liver is a heterogeneous organ composed of multiple cell types. The development of various liver diseases is caused by the interaction between genes from different cells and the environment, for example, malfunctioning of the interaction between different type of cells within the liver and between cells and the environment. Therefore, the development of multicellular types of liver organoids has great potential for liver disease that exhibits phenomena such as altered tissue structure and dysfunctional intercellular communication, such as NAFLD.[38] Kupffer cells are macrophages inherent in the liver, accounting for 80% to 90% of macrophages in vivo,[39] and their activation not only releases a variety of proinflammatory cytokines and chemokines, but also regulates the phenotype and function of the surrounding cells, and in particular, the interactions between Kupffer cells and other cells can affect the disease process. The transition of the hepatic stellate cell phenotype from a resting to an activated proliferative phenotype is central to the formation and progression of liver fibrosis. The interaction between Kupffer cells and hepatic stellate cells, two important non-stromal cells in the liver, may play an important role in the initiation and persistence of inflammation and fibrosis in chronic liver injury.[40]

In search of an efficient and accurate in vitro model of NAFLD, Ouchi et al. used iPSCs to derive human liver organoid models composed of liver epithelial cells and non-parenchymal cells(stellate cells, bile duct stem cells and Kupffer cells) that recapitulate focal features of hepatic steatosis and fibrosis.[41] Exposure of human liver organoid models to oleic acid (OA) increased lipid accumulation, with the degree of accumulation positively correlating with OA concentration, and NAFLD characteristics such as inflammatory response and fibrosis were observed. After 5 days of incubation under OA conditions, hepatocytes showed typical balloon-like changes, accompanied by elevated expression of type III procollagen aminoterminogenic peptide, interleukin-8, α-smooth muscle actin and deposition of collagen, while treatment with farnesyl X agonist FGF19 for 2 days significantly improved these conditions. It has been found that hepatobiliary organs with organized and functional bile ducts are more sensitive to free fatty acids (FFAs). FFA-induced organ-like gene expression profiles are similar to those of the liver in NAFLD patients and can trigger attenuation of the biliary network and ductal response[42] (Figure 3). Wu et al.[43] added 25% mTeSR to the liver organoid differentiation medium to mildly inhibit the early differentiation of the liver, and at the same time turned on biliary regulation by activating the transforming growth factor-β and NOTCH2 signalling pathway, which led to the co-differentiation of hepatocytes and cholangiocytes, and successfully cultured mature liver and biliary organoids with certain functional attributes. Hepatocytes in the organoids were able to accumulate lipids and glycogen, secrete albumin and urea, and have drug-metabolizing ability as determined by CYP3A4 activity and inductivity. The biliary system was also found to have rhodamine and bile acid storage ability, and higher gamma glutamyltransferase activity was detected in the organoids. Guan et al.[44] developed a culture system that can induce differentiation of iPSCs into liver organoids according to changes in the order of growth factor addition, and bile acids were detected abundantly in the supernatant of the organoids, suggesting that the liver organoids produced by this system are functional and capable of synthesizing bile acids in vitro. The liver organoid is expected to be a testing ground for drug testing, helping to identify new drug targets and providing a new platform for finding potential treatments for liver disease. Given the multiple cell types involved in the development of NAFLD, the ideal liver organoids used to study this disease should be based on a multispectral model.

In addition to the metabolic environment, individuals have different susceptibilities to the development of steatosis due to genetic factors.[45] Genome-wide association studies have identified several polymorphisms associated with the development of NAFLD. PNPLA3 I148M is currently recognized as a risk variant locus for NAFLD.[46] Accordingly, 3D spheroids composed of hepatic stellate cells (LX-2) and a hepatocellular carcinoma cell line (HepG2), both homozygous for the PNPLA3 I148M variant, were constructed.[47] Incubation of FFAs (500 μmol/L, palmitic acid: OA, 1:2) for 48 h in the abovementioned 3D spheroids showed an accumulation of lipids, increased levels of type I collagen and increased expression of α-smooth muscle actin. Cultures were subsequently incubated with liraglutide or elafibranor for 48 h. Both drugs were found to rescue this phenotype, suggesting that these drugs may be potential treatments for NAFLD. The same approach was used to explore the correlation between NAFLD and transmembrane 6 superfamily member 2 (TM6SF2) Glu167Lys.[48] Without overlaying environmental factors, a large number of organic analogues with different genetic compositions is an ideal way to assess the direct impact of NAFLD to NASH progression in vitro by genetic variation. The latest study by Hans Clevers et al. established liver organoid models of NAFLD induced by three different factors associated with NAFLD (FFAs, PNPLA3 I148M genetic variant and familial hypo/non-β lipoproteinemia *APOB* and *MTTP* monogenic lipid disorders) for drug screening.[49] Compounds that effectively alleviate steatosis were identified in these models. The study also produced several interesting findings. PNPLA3 I148M reduces the efficacy of drugs to resolve steatosis. Analysis of the entire transcriptome based on pharmacological treatment revealed that inhibition of de novo lipogenesis is a common mechanism for effective reduction of steatosis compounds. The researchers then established a CRISPR-based FatTracer screening platform using APOB and MTTP mutant-

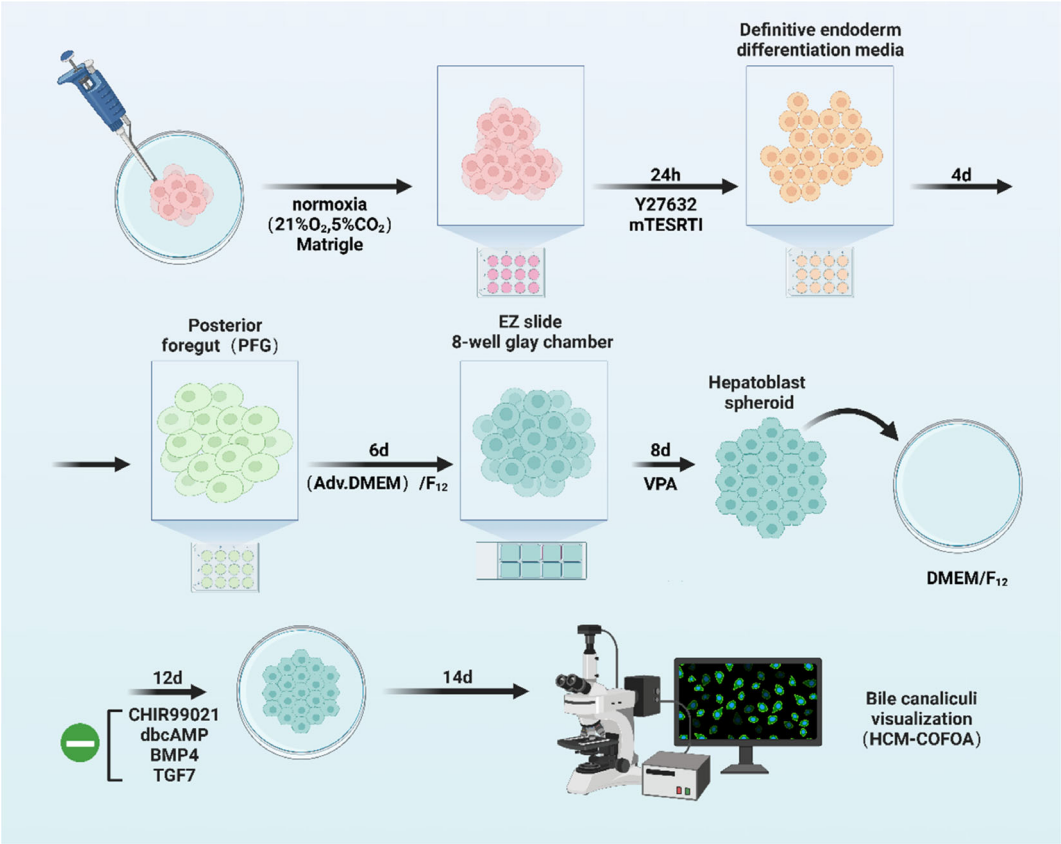

FIGURE 3 Step-wise generation of hepatic organoids (summarized by Nair et al.[27]).

like organoids targeting 35 regulatory factors/targets closely associated with NAFLD development. They identified that fatty acid desaturase 2 (FADS2) is a determining factor in the process of hepatic steatosis. These organoid models are useful for studying the aetiology of steatosis and drug targets, and are another major discovery in NAFLD organoid models.

Nevertheless, the lack of functional vasculature in current organoid cultures makes it a challenge to obtain more accurate results. Without blood circulation, nutrients and oxygen cannot be delivered comprehensively, and metabolic waste continues to increase, causing extensive cell death and premature differentiation at late stages of culture. Moreover, the absence of relevant developmental signals leads to small organoid morphology, immaturity and core necrosis, seriously impeding the research and application of organoids.[50] Tsang et al. used hiPSCs-derived hepatocytes, endothelial cells, hepatic stellate cells, and mesenchymal stem cells to cultivate constructed vascularized liver organoids that mimicked the metabolic effects and spatial structure of hepatocytes. Consistent with the results described above, these organoids also undergo inflammation, steatosis and fibrosis after FFA treatment, realistically reflecting the pathological characteristic changes that occur in NAFLD.[51] However, whether hepatocyte organoids derived from iPSCs can reflect the progression from NAFLD to NASH, and from NASH to cirrhosis and hepatocarcinoma, needs further investigation.

In conclusion, the emergence of liver organoids provides an excellent opportunity to study NAFLD in humans and to test the efficacy and toxicity of drugs. The way forward is to develop organoid models that more closely reflect the histology and pathophysiology of NAFLD. Such a model is promising for the study of molecular mechanisms and processes of NAFLD in a systematic and in-depth manner, and to provide new therapeutic targets for NAFLD.

3.3 | Obesity

Obesity is a major public health problem. According to the latest statistics, the number of overweight and obese people continues to grow worldwide, affecting more than 30% of the world's population.[52] It has been reported that obesity is an important risk factor for chronic and metabolic diseases, and is significantly associated with an increased risk of developing a variety of tumours.[53] Obesity reflects an increase in adipose tissue with collagen deposition and fibrosis within the fat depots, leading to imbalanced secretion of cytokines, FFAs and leptin at the level of individual adipocytes, and ultimately adipose tissue dysfunction.[54,55] Notably, the 3T3-L1 cell line has the ability to differentiate fibroblasts into adipocytes and has a standardized and well-established culture protocol, making it one of the most commonly used cell lines today.[56] However, mature adipocytes are

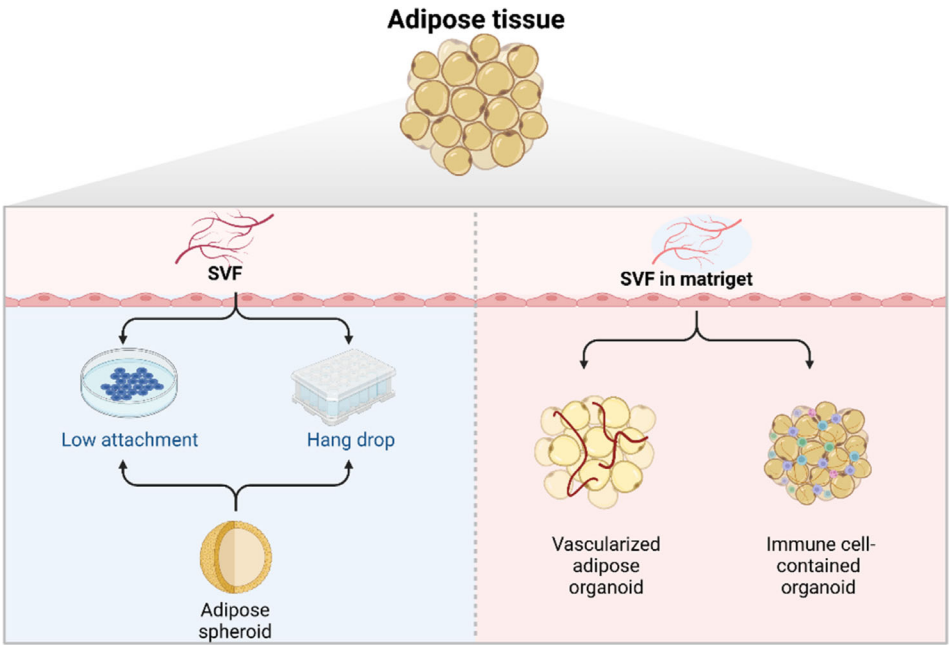

FIGURE 4 Developed into vascularized fat organs or fat organs containing immune cells under different culture conditions. SVF, stromal vascular fraction.

rich in lipids and are fragile and prone to floating and dedifferentiation when cultured in vitro. It is also difficult for adipocytes to reach a fully differentiated state in a 2D mode culture, especially to form large single-chambered lipid droplets in white adipocytes. Using rodent models to study adipose tissue is also a common method, but compared to that of humans, mouse brown adipose tissue differs in origin and heterogeneity.[57] Therefore, it is necessary to establish mature in vitro adipocyte or organ systems to simulate the complex relationship between different cell types and to explore the role of pathological adiposity in obesity-related metabolic diseases. An in-depth understanding of the pathogenic mechanisms brought by such research has important basic and clinical value for treating obesity-related metabolic disorders.

Recent studies have aimed to obtain mature adipocytes capable of producing and storing lipid droplets as well as having the ability to secrete various cytokines such as leptin through co-culture techniques of silk scaffolds and liquefied adipose tissue.[58] Klingelhutz et al. disengaged scaffolds to generate 3D adipose suspension droplet spheroids from human or mouse adipose precursor cells maintained in long-term culture in low-attachment dishes.[59] High-throughput screening to identify regulators of adipogenesis was successfully performed by secreting proinflammatory adipokines more readily in response to culture or screening for toxin-related stress. Alessandra et al. also used adipose precursor cells to construct 3D spheroids in brown adipose tissue that maintained the expression of their cellular markers better than 2D cultures obtained from the same tissue.[60] Muller et al. further updated the cultivation system of adipose tissue organoids in order to give adipocytes a more natural environment by creating a 3D system composed of adipocytes and endothelial cells using stromal vasculature from human subcutaneous white adipose tissue.[61] After induced differentiation, the resulting intra-tissue adipocytes exhibited dense and abundant vasculature and unicellular lipid droplets. After transplantation into immune-deficient mice, integrated chimeric vasculature was formed between the organoid endothelium and recipient circulatory system, which could be maintained in vivo for a long time (Figure 4). Recently, Robledo et al.[62] generated complex adipose-like organs from mouse interscapular adipose tissue stromal vascular fraction, capable of sustaining a prolonged adipose differentiation programme in vitro and secreting leptin and undergoing lipolytic processes in response to insulin and β-adrenergic agonist signalling. Unfortunately, no hPSCs have yet been used to simulate models of diseases related to fat metabolism, and there is a need to develop functional human adipose organoids. Development of these organoids enables the construction of adipose pools from different patients, and facilitates investigation regarding the mechanisms of disease occurrence, treatment options and prognosis. Organoids of adipose tissue orginating from a real patient cohort lays the foundation for individualized medicine.

3.4 | Hyperuricaemia

Hyperuricaemia (HUA) is a metabolic disease involving impaired purine metabolism. Elevated blood uric acid (UA) levels are the main clinical manifestation. HUA causes saturation of low water-soluble urate which precipitates as monosodium urate crystals. These crystals often accumulate in the synovial fluid and cause gout.[63] In addition, HUA was significantly associated with the development and severity of metabolic syndrome, and meta-analysis showed that the risk of metabolic syndrome was higher with higher serum UA levels, regardless of the characteristics of the study, and that there was a linear relationship with its content effect.[64] Epidemiological surveys show that HUA is a metabolic disorder prevalent worldwide in recent years, and it has been widely examined alongside hyperglycaemia,

hyperlipidaemia and hypertension.[65] Lowering serum UA levels is an important initiative to prevent and treat HUA, but the pathogenesis is not yet clear. The first-line UA-lowering and anti-inflammatory drugs are likely to produce various adverse effects such as exfoliative dermatitis, urinary tract stones, bone marrow suppression and hepatorenal toxicity. Limitations and delays in the development of new drugs further exacerbates such problems.[66,67] Therefore, the establishment of more mature in vitro models is important to accelerate new drug development as well as further explore the pathogenesis of HUA and its complications.

Currently, rodents are still commonly used for the construction of HUA animal models due to various advantages such as their rapid reproduction, low cost, and sensitivity to drugs. However, rodents possess an enzyme uricase which metabolizes UA directly into allantoin for excretion,[68] whereas the gene for uricase was inactivated during evolution and UA became the final metabolite of purine substances in humans. Therefore, commonly used experimental animals generally do not develop HUA, which is a major difficulty in the preparation of experimental animal models for this condition. Some studies have established HUA cell models using HK-2 and human-derived LO2 cell lines, which have significant efficiency advantages in large-scale screening of compounds from food that reduce UA levels.[69] However, the traditional 2D culture model cannot simulate the complex mechanisms of purine metabolism in real human physiology. In addition, HK2 cells are derived from the kidney, while human liver is the main organ responsible for purine anabolism. To arrive closer to a model that mimics human UA production and purine metabolism, Ho et al. established a 3D organ culture system that simulates HUA in vitro by culturing human-derived liver organoids. Liver organoids are generated from hepatocytes that are able to retain key morphological features, purine metabolic functions and intact gene expression capabilities. To a certain extent, they are also able to reproduce the functional and morphological characteristics of human hepatocyte tissue and maintain good stability.[70] The organoid can differentiate into a population of hepatocytes highly expressing maturation markers, including hepatocyte nuclear factor (HNF4α), epithelial calmodulin, and albumin. Of interest is that organoids can produce large amounts of UA following xanthine induction. Furthermore, the team also validated the model's potential for preclinical application by testing allopurinol and geraniol, a bioactive substance with anti-UA effects. Hyperuricaemia bioactive compounds can be subjected to high-throughput screening using this novel organoid model.

4 | CONCLUSION

The organoid system has shown unique advantages and potential in the study of metabolic diseases due to its foresight, precision and efficiency. The glandular organoid system has a broader application in the field of regenerative medicine due to its secretory and metabolic functions. However, current organoid technology still faces many challenges, and translational metabolism studies are largely limited by the lack of reliable in vitro models that accurately reflect human physiological characteristics. Due to the small size of the organoid and the lack of components such as blood vessels, a nervous system, and an immune system similar to those of human organs, it is difficult to include all types of cells that constitute the simulated organ and their complete structural features. Therefore the complex physiological regulatory mechanisms inside the body and organs cannot be fully reproduced for the time being. In response to the shortcomings of organoids, some studies have proposed the idea of cross-fertilizing them with multidisciplinary aspects such as organ-on-a-chip technology in the discipline of bioengineering.[71] Organ-on-a-chip is a kind of bionic system that can simulate the main functions of human organs by using microfabrication technology to fabricate bionic systems on microfluidic chips, aiming to mimic the functional units of human organs in vitro.[72] The combination of organoid and organ-on-a-chip technologies and integrating multifunctional analysis methods, such as biosensing and big data analytics, can help to dynamically monitor the culture environment at multiple scales and also to realize complex biological processes and organ functions, which can provide powerful technical support and guarantee for the development of tissue and organ repair, disease treatment and regenerative medicine.

Nevertheless, the strong development trend of organoid technology has made it one of the research hotspots in the biomedical field. With the improvement of organoid technology and the construction of different types of organoids, it provides a good platform to study the pathogenic mechanisms and potential therapeutic strategies of metabolic diseases. The booming development of organoid technology has brought basic research and translational medicine into a new era. It also provides a handy tool for personalized diagnosis and treatment in precision medicine.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article.

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