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The functions and applications of organoids in rheumatic immune diseases

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ABSTRACT

Rheumatic immune disorders are a group of conditions that affect the immune system, leading to various clinical symptoms. These diseases can cause pain, reduce the quality of life, and increase the risk of death in severe cases. Diagnosis and treatment are very complex due to the different types of disease and individual differences and the unknown pathogenesis of the disease. Further research is necessary to provide new clues for disease treatment. Organoid technology that makes up for the shortcomings of animal model species differences can better simulate disease onset mechanisms than animal models. It can be used as a screening platform for new therapeutic targets, as well as personalized settings based on patient-derived organoids, promising as an effective tool for the study of rheumatic immune diseases. Therefore, the article summarizes studies related to organoids and their application in rheumatic immune diseases. It also provides an outlook on the potential of organoids in this field and discusses the challenges that need to be addressed, putting new ideas for future research on these diseases.

1. Introduction

Rheumatic immune diseases are chronic inflammatory diseases caused by abnormal immune system activation. They affect joints, bones, organs, and tissues, including rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus.[1] These diseases cause pain, disability, and reduced quality of life to patients, and may even increase the risk of death. Over the past few decades, the mortality rate in patients with rheumatic immune diseases has remained high.[2] Long-term inflammation leads to damage to joint structures, and inflammation may affect multiple organs such as the heart, lungs, and kidneys, increasing the risk of serious complications such as cardiovascular disease and renal failure.[3,4] In addition, patients with impaired immune systems or the use of immunosuppressive drugs have an increased risk of infection, which is also one of the important causes of death. In terms of the current research state, rheumatologic diseases are a broad and complex field, with different disease types and individual patient differences making diagnosis and treatment complex. The pathogenesis remains unclear, so more research is needed to provide safe and effective treatments for the clinic.

Organoids are 3D microstructures made from human pluripotent stem cells (hPSC) or adult stem cells (ASC) and used for organ repair or disease modeling. As shown in Fig. 1, ASCs exist as undifferentiated cells in differentiated tissues and organs, with the potential to proliferate and differentiate. The isolated ASCs differentiate into corresponding tissue cells after adding appropriate cytokines to construct an organoid model. PSCs consist of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) that possess excellent abilities to self-renew, differentiate, and proliferate. They produce various disease-related cell types by mimicking the gradual differentiation scheme of organogenesis in the body, commonly used for the construction of various organoids.[5] Therefore, organoids enable functional studies of immune system-related diseases in a microenvironment similar to in vivo, allowing in-depth understanding of immune tissue structure and function.[6] With this technology, researchers can overcome the lack of preclinical models, reconstruct the structure and physiology of human organs in detail, and provide better opportunities for research on human diseases, overcoming ethical issues in the use of stem cells derived from human embryos. Compared with animal models, organoids can be of human origin and can avoid the impact of species differences, making up for the shortcomings of animal models. Compared with organ models directly derived from humans, organoids are easier to obtain and can be individually designed for specific conditions.[7,8] In addition, organoids can mimic the functions of damaged or nonfunctional organs, improving patient quality

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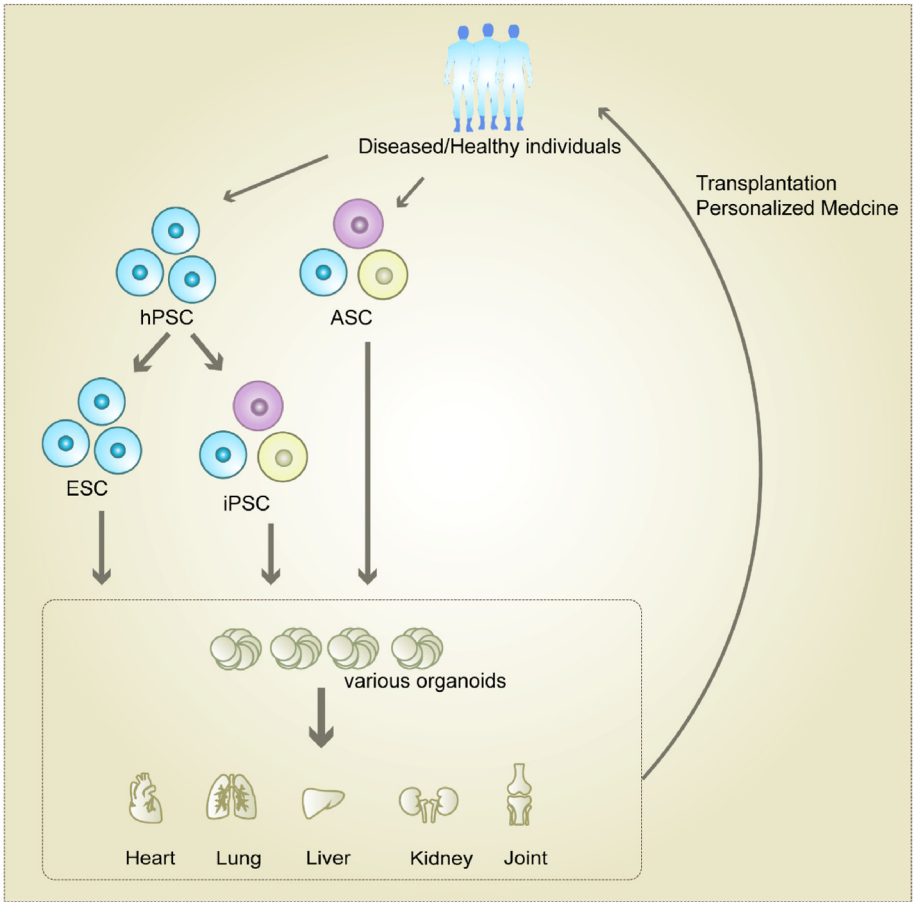

Fig. 1. Organoid differentiation process. hPSC, human pluripotent stem cells; ASC, adult stem cells; ESC, embryonic stem cells; iPSC, induced pluripotent stem cells.

of life and treatment outcomes.

Based on the current difficulties in rheumatic immune disease research and the advantages of organoid technology, organoid technology is applied to the research of various rheumatic immune diseases, such as mechanisms of action, new treatment options, and preclinical research. Therefore, this article summarizes the research on organoids in common organs where rheumatic immune diseases occur, such as the pancreas, kidney, heart, liver, lung, joints and other parts. The article also discusses the challenges of rheumatic immune diseases and proposes ideas for future research.

2. Organoid research related to rheumatic immune diseases

2.1. Pancreatic organoid research

Autoimmune pancreatitis (AIP) is a special type of pancreatic inflammatory disease related to autoimmune factors.[9] It is mostly a focal lesion of the pancreatic head, and can also manifest as acute diffuse pancreatitis. Inflammatory cells can infiltrate and cause fibrosis around the pancreatic ducts. Patients with type 1 AIP have a 7%–40% chance of developing chronic pancreatitis.[10] Long-term chronic pancreatitis can increase the risk of developing pancreatic cancer. This disease is considered one of the risk factors for pancreatic cancer, which is why it is important to monitor and manage chronic pancreatitis carefully.[10,11] Oral prednisone used to stabilize the condition of AIP cannot completely reverse the morphological changes of the pancreas. Therefore, it is particularly important to develop new treatment methods for AIP. The inhibition of protein REST impaired the construction of in vitro organoids featuring pancreatic acinar-ductal metaplasia (ADM), whereas increased REST expression facilitated this process.[12] This indicates that REST can serve as a regulatory factor for pancreatic organoid construction and provide a new way to repair pancreatic tissue damage.

The autoimmune inflammation caused damage to pancreatic β-cells and reduced their number. This led to gradual changes in pancreatic islet structure, which were strongly linked to the development of type 1 diabetes (T1DM).[13] Although inflammation of the pancreas was a key factor in the occurrence and pathogenesis of T1DM, its occurrence had different relationships with the course of the disease. Pancreatic inflammation and β-cell decline might occur years before clinical symptoms of diabetes appeared, leading to poor diagnosis or delayed patient treatment.[14] Therefore, there is a need to explore its mechanism.

Diabetes research often lacks realistic disease models.[15] Organoid technology and its combination with gene editing technology promote studying the development, function, and pathological mechanisms of human islets. Montesano et al. cultured pancreatic islet organoids in vitro using a three-dimensional collagen matrix.[16] Wang et al.[17] found that Procr + islet cells underwent clonal expansion and produced all four types of endocrine islet cells during adult homeostasis. The isolated Procr + cells stably formed islet organoids, and their production was verified to reverse hyperglycemia in a mouse model of streptozotocin-induced T1D. This model investigated the relationship between T1DM and insulitis. Patient-derived beta cells and islet organoids served as information disease models that generalize pathogenesis and phenotypes in specific patients, assessing patient-specific drug responses in screening. The combination of iPSC and CRISPR technologies laid the foundation for autologous transplantation and minimizing immune rejection, suggestive of new treatment strategies.[18] At the same time, the discovery of some biomolecules also promotes the maturation of pancreatic islet organoids and improves their function. Vascular endothelial growth factor A[19] recruited endothelial cells and promoted

vascularization of islet organoids; reduced LIN28B levels[20] and activation of WNT 4[18] promoted organoid maturation; blockade of Fibulin 3, EGFR or CA 19-9[21] prevented excessive activation of EGFR in organoids. These findings promoted the convergence of islet-like organs to natural islets and facilitated modeling studies of insulitis and diabetes mellitus. However, currently established islet organoids are usually not fully mature,[15] and the functionality of islet organoids needs further improvement.

2.2. Kidney organoid research

Glomerulonephritis and systemic lupus erythematosus-related nephropathy have a certain risk of causing renal failure,[22] which requires widespread attention. Renal organoids derived from human pluripotent stem cells have been continuously used for modeling glomerular diseases. They help to explore the mechanisms of progenitor cell damage in glomerular diseases and establish methods for nephrotoxicity screening and drug discovery in vitro systems.

Patient-derived organoids recapitulated the processes involved in glomerular disease abnormalities and provided new insights into disease mechanisms.[23] To bridge the gap of progenitor cells having no proliferative ability, Morizane R et al.[24] induced nephrons in vivo by treating renal progenitor cells with CHIR and FGF9 to form renal vesicles. Taguchi A et al.[25] used a combination of retinoic acid, activin, BMP 4, and moderate concentrations of CHIR to induce posterior neonatal mesoderm, and continued to treat it with FGF 9 and low concentrations of CHIR. They found that renal progenitor cells and tubular epithelium could be generated. Nishinakamura R et al.[26] found that renal tubular epithelial cells in organoids matched the in vivo results of corresponding disease bodies. The ability of renal organoids to model glomerular-related diseases as well as tubular diseases was demonstrated.

Applying nephrotoxic drugs to organoids causes cells to produce the kidney injury molecule KIM1, which can be used as a model to evaluate drug toxicity to the kidneys in real time.[27] Combined with ATP/ADP biosensor, this method was verified.[28] Furthermore, to verify the transport capacity of multidrug resistance MDR1 (also known as P-glycoprotein) and OCT2 in renal organoids, Rizki-Safitri et al.[29] demonstrated that the accumulation of fluorescent substrates in the organoid lumen was regulated by MDR1 and OCT2 Activity-mediated, consistent with a previous study.[30]

However, kidney organoids were unable to construct internal capillary regions and had limited ability to assess their filtration capacity, which can be vascularized via microfluidic devices. For example, Homan KA et al.[31] cultured hPSC-derived organoids on gelatin- and fibrin-coated chips and circulated the perfusion medium. This method effectively increased the richness of the vasculature, and maturation of renal tubular and glomerular cells. At the same time, when culturing kidney organoids, attention must be paid to the culture time. Excessive culture time might lead to loss of vascular gene expression.[32]

2.3. Cardiac organoid research

The immune system can cause damage to the heart, resulting in common rheumatic immune diseases such as rheumatic heart disease and myocarditis. Cardiac organoids can simulate the heart development process, provide an effective pathological model for rheumatoid immune diseases, and provide information on inflammatory responses and potential therapeutic targets.[33]

Developmental injury modeled by localized freezing injury in cardiac organs successfully induced extracellular matrix (ECM) accumulation in endothelial or fibroblast-like cells.[34] And iPSC-derived cardiomyocytes could be used as cell therapy to replenish damaged myocardium.[35] Compared with cardiomyocytes from healthy donors, cardiomyocytes differentiated from iPSCs derived from patients with systemic lupus erythematosus showed high apoptosis, proliferation, and fibrosis rates when exposed to patient serum. Anti-Ro antibodies would aggravate the expression of genes related to metabolism, hypertrophy, and apoptosis, which can be used as a model to study organ damage in SLE.[36] Furthermore, each cardiac organoid had few cells (about 5000) and simple construction process, it had better results than animal models for drug screening.[37] Studies combined hiPSC-derived vasculospheres with direct differentiation of cardiomyocytes to create cardiac organoids that displayed vascularization and chamber-like architecture. It was able to demonstrate the process of cardiac injury and fibrosis in vivo and verified that the drug captopril reduced fibrosis and dysfunction caused by cardiac injury.[38]

In addition, the heart model can also be used to study the impact of other factors on the heart. For example, Lewis-Israeli and others used high concentrations of glucose and insulin to culture cardiac organoids to study the impact of pregestational diabetes on fetal heart development. Due to the diffusion gradient, During the formation of cardiac organoids, cells may differentiate into tissues such as the intestine,[37] pancreas, and liver[39] in cardiac organoids due to gradient diffusion. In this regard, Cakir et al.[40] experimentally found that the transcription factor hETV2 can differentiate hESCs into endothelial cells under three different conditions (including the lack of growth factors for differentiation and maintenance culture), preventing the misdirected differentiation of cells to some extent.

2.4. Liver organoid research

Primary sclerosing cholangitis (PSC) is a liver disease caused by the immune system attacking the bile ducts, which results in inflammation, fibrosis, and narrowing of the bile ducts. This can lead to impaired bile flow, liver damage, and dysfunction. Additionally, patients with PSC are at an increased risk of developing liver cancer, as the inflammatory environment of PSCs, including the cytokine IL-17A, can promote cancer development.[41,42] The cholangioid cells derived from the patient's iPSCs senesced rapidly and had increased secretion of the extracellular matrix molecule fibronectin and the inflammatory cytokine interleukin-6. An in vitro model with disease-specific characteristics can be successfully constructed using the iPSCs derived from patients.[42]

Liver organoids were used to explore the mechanisms and influencing factors of the disease. Reich et al.[43] found that reduced TGR 5 levels caused biliary damage and promoted the progression of PSC mechanisms. Yao[44] et al. further verified this view and affected the transcription of downstream nuclear factor κB by regulating the binding of TGR 5 and Pellino 3, thus affecting the inflammatory phenotype of cholangiocytes. Patient-derived colon epithelial organoids showed downregulation of LGR5 after stimulation with interferon γ, and higher expression of OLFM4 after stimulation with interleukin IL-22. The expression of IL-22 receptor IL22RA1 was also induced by IFNγ, indicating a complex interplay between cytokines. The effect might increase the possibility of canceration in PSC-related colitis.[45] Organoid transplantation was discovered to provide healthy cells needed to repair damaged epithelium and rescue damage. For example, Sampaziotis[46] found that the expression of core single-tube markers in transplanted cells was similar to the expression level of mouse natural cholangiocytes, without expression of other hepatic lineage markers. This finding informs the principle that cholangiocyte-like organs can be used to repair human epithelial cells. In addition, Namoto et al. jointly reported a selective LATS kinase inhibitor that stimulates YAP signaling and accelerates liver regeneration after hepatectomy in mice, promoting research on the regenerative potential of liver organoids.[47]

Liver organoids can be used for drug screening and model validation. Li et al.[48] proposed a new method for identifying genotoxic impurities by exposing complex samples to an in vitro nucleoside incubation model and then mapping complete DNA adducts to infer the structure of potentially genotoxic impurities. Subsequently, genotoxicity was confirmed in humans using 3D bioprinted human liver organoids.

2.5. Lung organoid research

Rheumatic immune diseases can affect the lungs, leading to interstitial pneumonia. Hermansky-Pudlak syndrome (HPS) is a recessive autosomal disease that affects platelet function and causes eye and skin albinism. Among the 11 causative genes of the disease, mutations in HPS1, AP3B1, and HPS4 can lead to fatal HPS-associated interstitial pneumonia (HPSIP),[49] for which the only treatment is lung transplantation. Idiopathic pulmonary fibrosis (IPF), the most lethal interstitial lung disease (ILD), is characterized by fibrotic occlusion of alveoli and interruption of gas exchange, leading to respiratory failure. The treatment for this disease is also lung transplantation.[50] hPSC-derived 3D lung organoids simulate models of HPSIP and IPF to facilitate the identification of disease mechanisms and therapeutic targets. proteomic analysis of AOS from IPSC-derived alveolar type 2 cells and primary lung fibroblasts found mitochondrial dysfunction in HPS1 patient-specific alveolar epithelial cells.[51] Fibrotic features appeared only after deletion of HPS 1, HPS 2, or HPS 4. They were not detectable in organoids depleted of HPS 8, indicating the genetic phenotype associated with the clinical incidence of HPSIP.[52] Deletion of IL-11 prevented fibrosis in HPS 4-deficient organoids, suggesting it as a therapeutic target for fibrosis.[52] However, hPSC-derived 3D lung organoids also have limitations. Since this is an in vitro organoid model at the fetal development stage, it may not fully exhibit all the characteristics of IPF and HPS.

2.6. Joint organoid research

Rheumatoid arthritis (RA) is a prevalent autoimmune disease that affects the immune system. It is associated with long-term inflammation, which causes invasive synovitis and damages the articular cartilage and bone tissues.[53] Fibroblast-like synovial cells (FLS) are continuously produced in the joints, which release enzymes that break down the extracellular matrix and facilitate cell movement to maintain the stability of the joint's internal environment. The pro-inflammatory cytokines, IL-1 and TNF-α, trigger the expression of other cytokines and degradative enzymes in fibroblast-like synoviocytes.[54] This leads to the destruction of articular cartilage. The pathogenesis of RA (rheumatoid arthritis) has not yet been fully understood. Therefore, continuous experiments and research are still required to further explore the mechanism and treatment methods. However, replicating the complex microenvironment of joint tissue in vitro to simulate joint pathology and RA pathogenesis is challenging.

In 2006, Hans et al.[55] constructed RA-FLS-derived synovial organoids that formed a lining layer in vitro by inducing cadherin-11. Later, Lin et al.[56] constructed synovial organoids to evaluate the potential clinical application value of sesamol in the treatment of rheumatoid arthritis. In 2020, Rothbauer et al.[57] developed a chip-based synovium 3D model containing an embedded optical sensor array, and cultured patient-derived synovium-like organoids on a single-chip platform. The inflammation-induced structural changes at the 3D tissue level were observed immediately after two days of incubation. In 2021, FSL was embedded with a 3D synovial microglobule model in Matrigel, which spontaneously formed a synovial structure. It successfully constructed a scaffold-free triculture drug screening model consisting of FLS (SW982 cell line), lipopolysaccharide-activated macrophages, and primary isolated knee chondrocytes.[58]

In addition to these models of great research significance, there have been some discoveries about co-culture technology. Kim et al.[59] conducted a study where they combined chondrocytes with synoviocytes, resulting in an increase in the expression of chondrogenic markers in the micro mass. These markers include type II collagen, proteoglycans, and Sox9 transcription factors, leading to a reduction in IL-6 and IL-8 levels in the co-culture model. Osteochondrocyte progenitor cell microspheres were stimulated with TGFβ for 3 weeks and then mixed with osteogenic medium. This method produced hybrid microspheres with bone and cartilage structures in the periphery, recruited hematopoietic stem cells, and reconstructed hematopoiesis.[60]

Extracellular vesicles that were derived from induced pluripotent stem cells (iPSCs) have been found to reduce the expression of matrix-degrading enzymes, such as matrix metalloproteinases and IL-6. They have also been found to lessen chondrocyte death, which is mediated by IL-1β, and regulate macrophage polarization. As a result, they are able to rescue damaged chondrocytes in the inflammatory microenvironment.[61] iPSCs express pluripotency markers like OCT4, SOX2, and NANOG. Once they differentiate into mesenchymal cells (MSCs), they express typical MSC markers, such as CD29, CD44, CD90, CD105, and HLA-ABC. This makes them a potential source of chondrocytes.[62] Compared with exosomes secreted by synovial MSCs, exosomes secreted by iPSC-derived MSCs stimulated chondrocyte migration and proliferation with better therapeutic effect.[63]

In 2014, Willard et al.[64] successfully constructed a mouse iPSC-derived cartilage model, used IL-1α to simulate the inflammatory environment, and screened the therapeutic drugs. In addition to some common effects, only the NF-κB inhibitor SC-514 effectively reduced IL-1α-induced cartilage loss, suggesting the usability of the iPSC-constructed model for clinical drug screening.

Research on cartilage organoids continues to increase, studying stable models and new possible mechanisms for treatment. During the process of chondrogenesis, TRPV4 was both a marker of chondrogenesis and a regulator of chondrogenesis. The expression of Trpv4 in iPSCs increased significantly, along with the chondrogenic gene markers Sox9, Acan, and Col2a1.[65] iPSCs were placed in a nutrient medium containing BMP2, TGF-β1, and GDF5, which was beneficial to the purification of chondrocytes and the formation of scaffold-free hyaline cartilage.[66,67] Studies showed[68] that if the FN1 gene was mutated, the combination of fibronectin and type II collagen would be reduced, which would lead to a weakened chondrogenesis ability and affect the construction of cartilage organoids. Differentiation of iPSC-derived cartilage organs after transplantation led expression of PRG4, which was essential for joint lubrication.[69] ST2825 (a highly specific inhibitor of MyD88 dimerization) reduced fibroblast proliferation by arresting cells in the G0/G1 phase of the cell cycle. At the same time, it downregulated genes encoding mediators of pain, inflammation, and joint catabolism.[70]

An increasing number of drug-related studies are promoting the development of cartilage organoids. A novel RGD–SF–DNA hydrogel microsphere was reported to induce chondrogenesis through the integrin-mediated adhesion pathway and glycosaminoglycan biosynthesis.[71] The combination of Oroxylin A and amorphous calcium carbonate ACC synergistically inhibits osteoclast formation and activity, demonstrating therapeutic promise for cartilage involvement in rheumatic immune diseases.[72]

The above findings can provide a theoretical basis and reference for the construction of cartilage organoids and the treatment of bone involvement in rheumatic immune diseases.

2.7. Skin organoid research

There are several immune diseases related to rheumatism, such as systemic lupus erythematosus, systemic sclerosis, and psoriatic arthritis, which can cause skin lesions resulting in symptoms like rashes and erythema. Skin organoid technology is useful for creating in vitro skin models and studying the mechanisms behind skin homeostasis and skin repair at a molecular level. Studies showed[73] that genome editing of mutations on iPSCs through the CRISPR/Cas9 system, edited the mutated OASL 202Q into wild-type 202R or edited the wild-type OASL 202R into mutated 202Q. This led to reduced or enhanced type 1 IFN secretion by dendritic cells, facilitating the study of the pathogenesis of SLE. Psoriatic skin studies found[74] that Glut1 deficiency impaired keratinocyte proliferation and migration. Glut1 inhibition reduced the expression of pathology-related genes in human psoriatic skin organs.

Skin organoids are used to investigate new targets and methods for the treatment of rheumatic immune diseases. Induced pluripotent cells

from patients' fibroblasts differentiated into hematopoietic and mesenchymal cells in vitro, without transgenes. Expression of collagen, integrin α, and β in systemic sclerosis fibroblasts Up-regulated, while collagen and integrin β levels were normal in fibroblasts differentiated from iPSCs derived from the patients.[75] This demonstrates that it is possible to distinguish between normal tissue cells and integrated iPSC cells, which can be used as a potential source for stem cell therapy in clinical settings. iPSC-derived skin organoids can also be used to create disease models, which can help to confirm the efficacy of drugs. For example, it has been discovered that estrogen receptor modulator drugs can treat fibrosis in systemic sclerosis disease models.[76]

2.8. Vascular organoid research

Vascular disease is the basis and common ground of rheumatic immune diseases and cardiovascular diseases. Especially, systemic lupus erythematosus and systemic sclerosis are rheumatic immune diseases that can easily cause vascular dysfunction. Systemic inflammation can lead to dyslipidemia and dysfunction. These lesions promote the proatherosclerotic environment and increase the risk of cardiovascular disease, and vasculopathy is the key cause of cardiovascular disease.[77,78] Therefore, preventing vasculopathy in patients with rheumatoid arthritis is crucial for preventing disease progression and cardiovascular disease. The establishment of vascular organoids and vascularization of multiple organoids will enable more in-depth studies of vascular inflammation in rheumatic diseases.

Organoids such as kidneys, pancreas, heart, and lungs are affected by vascularization. Dense blood vessels increase the complexity of their models and help them simulate the functions of the corresponding organs. Regarding the maturation and vascularization of renal organoids, studies found[31] that under static conditions, the area of blood vessels generated by renal organoids in a high-concentration FSS environment was five times greater than that generated by lower concentrations of FSS. It showed that FSS is a key factor in promoting the vascularization of renal organoids in vitro. On the contrary, culturing organoids under dynamic conditions in vitro has been found to support the maturation of tubular epithelial cells in renal organoids. To achieve the optimal effect, it is important to consider the aforementioned factors comprehensively. In the study of cardiac organoids,[79] hPSC-derived cardiomyocytes, ventricular cardiac fibroblasts, and human umbilical vein endothelial cells were cultured at the cell ratio of the naturally developing heart (5:4:1) while maintaining appropriate biological and physical parameters. They formed a microvascular network that exhibited contractile and electrophysiological properties when mature. However, two strategies are usually used for the vascularization of most organoids. One is to co-culture hPSC-derived target cells with endothelial vascular cells and MSC to promote the formation of tissue and microtubule networks in vitro;[80] the other is to transplant organoids into the animal body to truly simulate the in vivo microenvironment to promote the differentiation and maturation of organoids.[81,82]

The microphysiological environment in which vascular organoids form is used for pharmacological studies, including drug distribution and toxicity modeling. Many studies share a common goal of conducting pharmacological research and standardizing model vascular organoids.[48] Microfluidic models were used to assess the ability of MSCs to induce vasculogenesis and vasculogenesis, potentially predicting their ability to contribute to wound healing and revascularization in vivo.[83] Models that collectively include lymphatic vessels, vasculature, and adipocytes can be used to examine the subcutaneous bioavailability of drugs.[84] In addition, multi-organ combined models can be used to simulate vascular transport between organs.[85] These models are used in pharmacokinetic and pharmacodynamic studies to facilitate the identification of biomarkers similar to cardiotoxicity.

3. Limitations and challenges

At present, there are still few specific examples of organoids being applied to rheumatic and immunological disease research, and the application of organoids has certain limitations.

3.1. Immaturity

Although organoids can replicate the basic structure and function of some organs, they often cannot fully mimic the complexity of an entire organ, which is similar to the development of fetal organs in the first or second trimester of pregnancy and cannot fully simulate the human microenvironment. For example, the levels of solute carriers OAT and OCT and related pathways in adult kidneys are significantly different from those in renal organoids.[86] Organoids cultured in vitro are prone to lack of vascularization. These vascular networks are abundant in native organs but are sparse in vitro derived products, such as kidneys, livers, and hearts.[15,27,87] Without blood vessels, organoids are limited in size and function because oxygen and nutrients cannot be efficiently transported inside the organoids, and metabolic waste cannot be removed, preventing them from achieving similar size and functional complexity to actual organs.

3.2. Batch-to-batch reproducibility and standardization

Differentiation of organoids is subject to batch-to-batch variability, which reduces reproducibility. For example, residual undifferentiated cells in culture, clonal differences in pluripotent stem cells, and variability in experimental reagents will all affect organoid differentiation results.[39,88] Depending on the cells' ability to self-organize, different batches of organoids can vary significantly in size, shape, and function. Therefore, developing uniform preparation and evaluation standards is a major challenge for applications such as drug testing and disease modeling.

3.3. Security issues

Although the use of patient-derived iPSC-derived organoids can avoid the problem of species translation in the clinic, there is the possibility of tumorigenesis. The probability of tumor formation from iPSC derivatives is affected by many factors, such as the tissue source of iPSCs, the assessment of iPSC residuals after construction,[84] and their use for reprogramming, differentiation, or transplantation methods.[85] At the same time, it is important to ensure the safety of culture components and prevent contamination of the cell microenvironment.

3.4. Cost issues

The culture conditions of organoids are complex. Maintaining the growth and function of organoids requires precise control of the environment, including specific growth factors, oxygen and nutrient balance. The high experimental cost may limit its popularity in scientific research and clinical applications. For example, the use of organoids for predictive evaluation of compounds in clinical and pharmacological laboratory environments is high cost and low throughput. In response to this situation, Shrestha et al. developed a microarray 3D bioprinting method based on droplet-printing technology, which reduces costs and increases evaluation throughput to a certain extent.[89] This is conducive to promoting the research progress of organoids.

4. Conclusion

Various types of organoids provide opportunities to study the

development mechanisms and potential treatments of different types of human diseases. Their application in rheumatic immune diseases helps to establish safe and effective in vitro models and gain an in-depth understanding of immune tissue structures. This method promotes the excavation of pathogenesis, the construction of in vivo drug response models, and the discovery of clinically feasible biomarkers.[90] Cultivate organoids from individuals for specific patient groups, establish specialized disease models based on patient characteristics, and develop a set of individual-specific precision medicine treatments, which will promote the research and treatment of a variety of rheumatic and immunological diseases. Despite the current limitations and challenges in the construction of organoids as well as in their clinical application, organoid technology is constantly improving and the challenges are being accomplished. For example, microfluidic device perfusion was performed by vascularization of organoids, and contamination due to uncertainty in culture composition was addressed by considering the culture of clinical-grade collagen.[91]

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CRediT authorship contribution statement

Huaijuan Huang: Writing – original draft, Conceptualization. **Aimin Yan:** Writing – original draft, Conceptualization. **Hesong Wang:** Supervision. **Heng Xu:** Methodology. **Ruhang Li:** Methodology. **Kai Yuan:** Writing – review & editing. **Guangrui Huang:** Writing – review & editing.

Declaration of competing interest

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

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