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Advances in Research and Applications of Cardiac Organoids

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Abstract: Cardiovascular disease (CVD) prevalence and mortality have been in an increasing stage globally, which is one of the main causes of death and poses a serious threat to human health. The research related to cardiovascular diseases is mainly based on traditional two-dimensional (2D) cell models and animal models, but due to some limitations, they cannot be fully applied to the research of cardiovascular disease pathogenesis and treatment methods. Organoid technology is a kind of three-dimensional (3D) biological tissue engineering constructed *in vitro*, which can highly simulate the cellular structure and physiological function of organs *in vivo*, providing a new direction and idea for the research of cardiovascular diseases. Therefore, cardiac organoid, as an effective complement to traditional cardiovascular disease models, has great potential in the study of disease mechanisms, drug experimentation, regenerative medicine and many other fields. This paper summarizes the construction methods of cardiac organoids and their applications in cardiovascular diseases, and also presents its development prospects, challenges and directions to be improved.

1. Introduction

Cardiovascular disease (CVD) is the number one killer of human health and one of the main causes of death and disability worldwide [1,2]. In China, the number of people suffering from CVDs has reached 330 million, and deaths caused by CVDs account for more than 40% of the deaths of residents, ranking first among all kinds of diseases [3]. According to the World Health Organization, 230 million people will die of CVDs by 2030.

Due to the variety of CVD symptoms and the complexity of the correlations between the influencing factors, the pathogenic mechanisms of CVDs remain a mystery, and prevention, monitoring, diagnosis, and treatment methods also need to be improved. In today's disease research and drug development, the analysis of disease models is an indispensable part of the process, therefore, the construction of appropriate CVD models is essential to advance the study of CVD mechanisms and therapies. 2D cellular models and animal models have long been used in human disease research. But 2D cellular models lack the natural physiological microenvironment and the interactions between cultured cells, thus failing to reflect the complexity of tissues *in vivo*; and due to the inherent species differences, animal models are harder to be used to study some

developmental and disease mechanisms, in addition, genomic and epigenomic specificity can lead to clinical trial failures [4,5]. Therefore, it is urgent to break through the traditional research mindset and technological limitations to build more bionic research models. In this context, 3D organ culture technology (i.e., organoid) was born and has been widely used in biomedical research. Since the development of the first organoid technology, organoids have made great progress in the field of biology. Human cardiac organoids (hCOs) have become an important bridge between traditional 2D culture and animal/human models. Cardiac organoids are a class of miniature 3D *in vitro* culture models with heart-like structural and functional properties, which mimic the structure and function of the heart to a certain extent, providing a promising research platform for cardiac development and regeneration research, disease modeling, precision medicine research, and also attracting extensive attention in the field of drug research.

This paper describes the construction methods and applications of cardiac organoid and related disease models and introduces their roles and development potentials in disease research.

2. Concept of Organoids

Organoids are commonly defined as 3D tissues generated from stem, progenitor or differentiated cells that self-organize through cell sorting and spatially restricted lineage differentiation [6,7], and are capable of highly mimicking the genetic profile, organization and function of the corresponding organ *in vivo* [8]. Simply put, an organoid is an *in vitro*-constituted micro-organ with self-renewal and self-organization capabilities, which has similar spatial organization as a real organ and is capable of performing original organ functions. Compared with traditional 2D culture models, organoids represent an innovative technology that can summarize the physiological processes of the whole organism, reflecting cell-cell and cell-extracellular matrix interactions, reflecting the function of the organ and its relationship with the surrounding environment, and maintaining individual heterogeneity while preserving genome stability [9]; compared with animal models, they can more accurately simulate the development of the human body and the disease progression, and can realize real-time imaging and dynamic observation, which is also more in line with ethical requirements. Therefore, organoids have a wide range of application prospects in organ development, precision medicine, regenerative medicine, drug screening, gene editing, disease modeling and other fields. So far, 3D organoid culture technology has successfully cultured a large number of organoids with some key physiological structures and functions, such as kidney, liver, lung, intestine, brain, prostate, pancreas , retina and so on [10].

3. Construction of Cardiac Organoids

Cardiac is an organ with an unusually complex structure and a difficult problem in the field of organoids. Researchers have now overcome key problems in the construction of cardiac organoids, such as the differentiation of cardiomyocytes, non-cardiomyocytes and neurons, the formation and annulation of cardiac tubes, and the formation of ventricular chambers. Based on this, in 2021, Austrian scientists used human pluripotent stem cells (hPSCs) to successfully cultivate the first *in vitro* self-organizing cardiac organoid, which is capable of spontaneously forming cavities and beating autonomously, and can also autonomously mobilize cardiac fibroblasts to repair injuries [11], realizing a breakthrough in cardiac organoids.

In the early exploration and development of cardiac organoids, mainly simulating the early differentiation process of human embryo, human or mouse embryonic stem cells (ESCs), human induced pluripotent stem cells (hiPSCs) and so on are often used to construct cardiac organoids by suspension culture into embryoid bodies (EBs), and adopting the strategy of differentiation of EBs. Chen et al. developed a hiPSCs aggregation suspension culture system, which can produce

cardiomyocytes with more than 90% purity by using the addition of small molecules to modulate the WNT signaling pathway and suspending the cultured cells to direct the differentiation of hiPSCs to cardiomyocytes [12]. These *in vitro* production methods of cardiomyocytes have laid the foundation for the study of cardiac organoids. The emergence of hiPSCs has greatly facilitated the study of cardiac organoids.

In addition, it has been found that human engineered heart tissue (hEHT) can further improve the cardiac organoid by restoring its chamber structure, electrical conduction properties, and pumping function as much as possible. Tissue engineering-based hEHT usually relies on an artificial "skeleton" constructed from hydrogel, decellularized mice or human cardiac extracellular matrix [13]. Depending on the experimental purpose, various types of hEHT have been constructed, such as strips, loops, patches, chips, chambers, and so on [14].

In recent years, with further research and exploration of cardiac organoids and the boom in 3D printing technology, 3D-printed artificial hearts have also emerged. Kupfer et al. published research in 2020 that utilized 3D printing technology to construct a perfusable cardiac chamber simulation based on the results of magnetic resonance scans of the human heart, which obtained the specific chamber structure of the heart while ensuring that the large blood vessels were printing allows for continuous, unidirectional flow, making blood supply possible [15]. In addition, ZHANG et al. 3D printed endothelial cells along with microfiber hydrogel as a bio-ink, and then implanted cardiomyocytes into a 3D endothelial scaffold, producing a myocardial organoid capable of synchronized contraction [16].

4. Applications of Cardiac Organoids

4.1. Applications in Disease Models

4.1.1. Myocardial Infarction Model

Myocardial infarction (MI) is the apoptosis or necrosis of cardiomyocytes due to persistent ischemia and hypoxia caused by blockage of coronary arteries for various reasons [17]. Organoid models of MI are the first established cardiac organoid disease models. Richards et al. used different concentrations of Norepinephrine to stimulate cardiac organoids to mimic the structure of the infarcted heart. Analysis of the transcriptome of this cardiac organoid of myocardial infarction revealed a high degree of similarity to the genetic changes in human MI and animal models of acute MI [18].

4.1.2. Cardiac Arrhythmia Model

Cardiac arrhythmia, as one of the typical CVDs in clinical practice, are conditions in which excitation of the sinoatrial node is abnormal or arises outside the sinoatrial node, resulting in slow, blocked, or abnormal conduction of excitation through abnormal channels. Previous studies have demonstrated that hCOs are capable of generating spontaneous and evoked action potentials and have faster conduction velocities compared with 2D cell models [19], suggesting that hCOs are more suitable for cardiac arrhythmia research. Shinnawi et al. found that hCOs can be used to observe more complex electrophysiological phenomena (e.g., conduction and refractoriness) in cardiac arrhythmia syndromes such as short QT interval syndrome (SQTS) and are more sensitive to refractory cardiac arrhythmia. The team also utilized hCOs to evaluate the efficacy of antiarrhythmic drugs [20], suggesting that hCOs have potential for use in drug evaluation.

4.1.3. Heart Failure Model

Heart failure is a common CVD with a high mortality rate, and is the end stage of various CVDs [21]. In recent years, due to the increase in cardiovascular risk factors in the aging population, the prevalence of heart failure has been increasing year by year. In in vitro models, 2D monolayer cell cultures have been studied for 50 years and have produced many results for heart failure; however, 3D organoid cultures better mimic the physiology and pathology of organ tissues and provide a suitable environment for cell-cell interactions compared to traditional 2D culture models. Tiburcy's team successfully constructed cardiac organoids with more mature cytoarchitecture and function by inducing PSCs in embryos under serum-free conditions and deriving a large number of human cardiomyocytes and found that overstimulation of Catecholamines triggers contractile dysfunction, myocardial hypertrophy, cardiac myocyte death, desensitization of adrenergic signaling, and release of biomarkers of heart failure, which are typical manifestations of heart failure. This study lays a theoretical foundation for the use of hCOs in heart failure disease models and the evaluation of anti-heart failure drugs, which may help to develop new therapeutic strategies for heart failure [22].

4.1.4. Congenital Heart Disease Model

Congenital Heart Disease (CHD) due to abnormal heart development is the most common human birth defect [23], but its pathogenesis is still unclear [24]. Although transgenic mouse models are used in usual studies, there is still a great need for human-related disease models for research and translational applications. Drakhlis et al. constructed self-organizing cardiac organoids similar to early embryonic hearts, used NK2 homeobox 5 (Nkx2.5) knockout hESCs-derived cardiac organoids to study the genetic defects and found that the Nkx2.5 deletion displayed a phenotype of cardiac malformations previously observed in transgenic mice [25].

4.2. Applications in Drug Discovery

Using animal models for new drug research often requires a 3~6 month dosing cycle, making it difficult to conduct large-scale experiments, and disadvantages such as racial differences and high costs can also hinder the process of new drug development [26]; while cells cultured under 2D conditions can undergo significant changes in their transcriptome and proteome, which may produce experimental results that do not represent the *in vivo* cellular physiology and affect the complete expression of the cellular function [27], so the choice of which research mode to use has also become a bottleneck in new drug development and evaluation, and the emergence of organoids has undoubtedly provided a new idea for standardized drug screening and safety evaluation.

4.2.1. Drug Screening

Organoid technology has demonstrated its unique advantages in high-throughput drug screening and individualized therapy, and previous studies have found that intestinal organoids can be used for high-throughput drug screening [28]. As for drug screening using cardiac organoids models, Milis et al. established a high-throughput bioengineered cardiac organoids platform, which contains both mature and arrested cardiomyocytes with biological properties similar to those of natural heart tissue. The platform was used to functionally screen 105 proliferative potential small molecules, and found two proliferative small molecules that had no significant effect on cardiac function [29].

4.2.2. Drug Safety Evaluation

Drug safety evaluation is an important reference for drugs entering the clinical stage [30].

Cardiac is one of the important target organs for toxicity of exogenous chemicals, how to evaluate drug cardiac safety and reduce the risk of cardiotoxicity in a more detailed and comprehensive way before new drug development and clinic is now a problem that must be faced and paid attention to. Sallam et al. utilized hiPSCs-derived cardiac organoids to study the adverse remodeling effects of immunosuppressant Tacrolimus and Sirolimus after cardiac transplantation, and the results found that Tacrolimus may be more detrimental to cardiac remodeling compared to Sirolimus [31]. Takeda et al. used 3D artificial heart tissues for *in vitro* testing of drug cardiotoxicity and found that Adriamycin inhibited cell viability and had a more sensitive toxicity response [32].

4.3. Applications in Regenerative Medicine

The main goal of regenerative medicine is to replace a functionally or structurally impaired organ with healthy tissue in vitro, to promote regeneration of the organism, and thus to help the organism recover or establish normal functioning [33]. However, the shortage of organs and immune rejection of tissues have always been the thorny problems faced by global organ transplantation medicine, which seriously constrains the development of regenerative medicine research, and the current research has found that organoids are capable of homogeneous tissue amplification and thus can be used for autologous transplants, which provides a good platform for solving this problem.

Gao et al. found that implanting human myocardial patches (made from hiPSC-induced cardiomyocytes) into pigs with myocardial infarction resulted in a reduction in the infarcted area of the heart, an increase in cardiomyocyte survival, and a significant improvement in cardiac function in pigs [34]. Furthermore, Varzideh et al. developed cardiac organoids that can beat spontaneously and found that cardiomyocytes in the organoids can exhibit higher maturity after *in vivo* transplantation [35]. At present, there have been many studies that can demonstrate that organoid technology has great potential for application in the field of regenerative medicine.

5. Conclusions

Organoids, as an emerging technology, have great potential in scientific research areas including developmental biology, disease pathology, cell biology, regenerative mechanisms, precision medicine, and drug toxicity and efficacy testing. Organoid culture enables the study of human development to provide a platform free from ethical constraints and a new stage for drug screening, it also complements existing 2D culture methods and animal model systems in a highly informative way.

As a new generation of CVD research models, cardiac organoids have been developed rapidly in the past decade, greatly facilitating CVD-related research. However, compared with other tissue organoids, cardiac organoid research is still in the initial stage, the current cardiac organoids are not as perfect as the human heart, and there are still some obstacles to overcome for the construction of disease models. First, the culture of cardiac organoids generally requires adding extrinsic growth factors or molecular inhibitors, which might have a certain impact on the results of drug experiments; second, due to the lack of process control and the gap in the industry standard, the human factor is too involved in the cardiac organoid culture process, and the low degree of automation leads to a large error caused by systematic chance; third, the existing cardiac organoids may be functionally different from the adult heart, and may be as functionally incomplete as the newborn, and the immature phenotype of cardiomyocytes is an important obstacle to their use in transmedicine, *in vitro* drug toxicity, and pharmacological analysis [36]; in addition, organoids cannot fully mimic the state that cells are *in vivo* because the culture process of organoids lacks interactions with microenvironmental components such as immune cells, thyroid hormones, adrenal

hormones, and so on, as compared to the living body [37]. Therefore, in future research, we should consider how to achieve the reproducibility and consistency of cardiac organoid culture, so as to generate cardiac organoids with high standard, high quality and high efficiency; how to make the culture conditions and environmental stimuli more realistically simulate the human microenvironment, so as to construct cardiac organoids that can better reproduce the physiological responses in the human body; and how to induce the developmental maturation is another factor to consider in the improvement of the model.

In general, the continuous development of organoid technology will further bridge the gap between basic research and translational medicine, bringing unlimited possibilities for human development and disease research. In the future, the combination of organoid culture with smart materials, biomimetic materials, 3D printing technology, microfluidic systems and other advanced technologies is expected to break the existing research bottlenecks and provide the possibility of constructing sensitive, accurate, and sustainable cardiac organoids.

References

[1] Mensah, G. A., Roth, G. A., and Fuster, V. (2019) The Global Burden of Cardiovascular Diseases and Risk Factors. Journal of the American College of Cardiology, 74, 2529–2532.

[2] Roth, G. A., Mensah, G. A., Johnson, C. O., and Addolorato, G. (2020) Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology, 76, 2982–3021.

[3] Writing committee of the report on cardiovascular health and diseases in china. (2022) Report on Cardiovascular Health and Diseases in China 2021: An Updated Summary. Biomedical and environmental sciences: BES, 35, 573–603.

[4] Gorzalczany, S. B., and Rodriguez Basso, A. G. (2021) Strategies to apply 3Rs in preclinical testing. Pharmacology Research & Perspectives, 9, e00863.

[5] Kim, J., Koo, B.-K., and Knoblich, J. A. (2020) Human organoids: model systems for human biology and medicine. Nature Reviews Molecular Cell Biology, 21, 571–584.

[6] Rossi, G., Manfrin, A., and Lutolf, M. P. (2018) Progress and potential in organoid research. Nature Reviews Genetics, 19, 671–687.

[7] Lancaster, M. A., and Knoblich, J. A. (2014) Organogenesis in a dish: Modeling development and disease using organoid technologies. Science, 345, 1247125–1247125.

[8] Clevers, H. (2016) Modeling Development and Disease with Organoids. Cell, 165, 1586–1597.

[9] Schutgens, F., and Clevers, H. (2020) Human Organoids: Tools for Understanding Biology and Treating Diseases. Annual Review of Pathology: Mechanisms of Disease, 15, 211–234.

[10] Xu, H., Jiao, Y., Qin, S., Zhao, W., Chu, Q., and Wu, K. (2018) Organoid technology in disease modelling, drug development, personalized treatment and regeneration medicine. Experimental Hematology & Oncology, 7, 30.

[11] Hofbauer, P., Jahnel, S. M., Papai, N., Giesshammer, M., Deyett, A., Schmidt, C., et al. (2021) Cardioids reveal self-organizing principles of human cardiogenesis. Cell, 184, 3299–3317.e22.

[12] Chen, V. C., Ye, J., Shukla, P., Hua, G., Chen, D., Lin, Z., et al. (2015) Development of a scalable suspension culture for cardiac differentiation from human pluripotent stem cells. Stem Cell Research, 15, 365–375.

[13] Guyette, J. P., Charest, J. M., Mills, R. W., Jank, B. J., Moser, P. T., Gilpin, S. E., et al. (2016) Bioengineering Human Myocardium on Native Extracellular Matrix. Circulation Research, 118, 56–72.

[14] Cho, J., Lee, H., Rah, W., Chang, H. J., and Yoon, Y. (2022) From engineered heart tissue to cardiac organoid. Theranostics, 12, 2758–2772.

[15] Kupfer, M. E., Lin, W.-H., Ravikumar, V., Qiu, K., Wang, L., Gao, L., et al. (2020) In Situ Expansion, Differentiation, and Electromechanical Coupling of Human Cardiac Muscle in a 3D Bioprinted, Chambered Organoid. Circulation Research, 127, 207–224.

[16] Zhang, Y. S., Arneri, A., Bersini, S., Shin, S.-R., Zhu, K., Goli-Malekabadi, Z., et al. (2016) Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials, 110, 45–59.

[17] Lindsey, M. L., de Castro Brás, L. E., DeLeon-Pennell, K. Y., Frangogiannis, N. G., Halade, G. V., O'Meara, C. C., et al. (2021) Reperfused vs. nonreperfused myocardial infarction: when to use which model. American Journal of Physiology-Heart and Circulatory Physiology, 321, H208–H213.

[18] Richards, D. J., Li, Y., Kerr, C. M., Yao, J., Beeson, G. C., Coyle, R. C., et al. (2020) Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity. Nature Biomedical Engineering, 4, 446–462.

[19] Tzatzalos, E., Abilez, O. J., Shukla, P., and Wu, J. C. (2016) Engineered heart tissues and induced pluripotent stem

cells: macro- and microstructures for disease modeling, drug screening, and translational studies. Advanced drug delivery reviews, 96, 234–244.
[20] Shinnawi, R., Shaheen, N., Huber, I., Shiti, A., Arbel, G., Gepstein, A., et al. (2019) Modeling Reentry in the Short QT Syndrome With Human-Induced Pluripotent Stem Cell–Derived Cardiac Cell Sheets. Journal of the American College of Cardiology, 73, 2310–2324.
[21] Zhang, Y., Zhang, J., Butler, J., Yang, X., Xie, P., Guo, D., et al. (2017) Contemporary Epidemiology, Management, and Outcomes of Patients Hospitalized for Heart Failure in China: Results from the China Heart Failure (China-HF) Registry. Journal of cardiac failure, 23, 868–875.
[22] Tiburcy, M., Hudson, J. E., Balfanz, P., Schlick, S., Meyer, T., Chang Liao, M.-L., et al. (2017) Defined Engineered Human Myocardium with Advanced Maturation for Applications in Heart Failure Modeling and Repair. Circulation, 135, 1832–1847.
[23] Majumdar, U., Yasuhara, J., and Garg, V. (2019) In Vivo and In Vitro Genetic Models of Congenital Heart Disease. Cold Spring Harbor Perspectives in Biology, 13, a036764.
[24] Nees, S. N., and Chung, W. K. (2019) Genetic Basis of Human Congenital Heart Disease. Cold Spring Harbor Perspectives in Biology, 12, a036749.
[25] Drakhlis, L., Biswanath, S., Farr, C.-M., Lupanow, V., Teske, J., Ritzenhoff, K., et al. (2021) Human heart-forming organoids recapitulate early heart and foregut development. Nature Biotechnology, 39, 737–746.
[26] Mokry, L. E., Ahmad, O., Forgetta, V., Thanassoulis, G., and Richards, J. B. (2014) Mendelian randomisation applied to drug development in cardiovascular disease: a review. Journal of Medical Genetics, 52, 71–79.
[27] Lal-Nag, M., McGee, L., Guha, R., Lengyel, E., Kenny, H. A., and Ferrer, M. (2017) A High-Throughput Screening Model of the Tumor Microenvironment for Ovarian Cancer Cell Growth. SLAS Discovery, 22, 494–506.
[28] Van de Wetering, M., Francies, Hayley E., Francis, Joshua M., Bounova, G., Iorio, F., Pronk, A., et al. (2015) Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients. Cell, 161, 933–945.
[29] Mills, R. J., Parker, B. L., Quaife-Ryan, G. A., Voges, H. K., Needham, E. J., Bornot, A., et al. (2019) Drug Screening in Human PSC-Cardiac Organoids Identifies Pro-proliferative Compounds Acting via the Mevalonate Pathway. Cell stem cell, 24, 895–907.e6.
[30] Xuan, W., Tipparaju, S. M., and Ashraf, M. (2022) Transformational Applications of Human Cardiac Organoids in Cardiovascular Diseases. Frontiers in Cell and Developmental Biology, 10, 936084.
[31] Sallam, K., Thomas, D., Gaddam, S., Lopez, N. E., Beck, A., Beach, L. Y., et al. (2022) Modeling Effects of Immunosuppressive Drugs on Human Hearts Using Induced Pluripotent Stem Cell–Derived Cardiac Organoids and Single-Cell RNA Sequencing. Circulation, 145, 1367–1369.
[32] Takeda, M., Miyagawa, S., Fukushima, S., Saito, A., Ito, E., Harada, A., et al. (2018) Development of In Vitro Drug-Induced Cardiotoxicity Assay by Using Three-Dimensional Cardiac Tissues Derived from Human Induced Pluripotent Stem Cells. Tissue Engineering Part C: Methods, 24, 56–67.
[33] Cossu, G., Birchall, M., Brown, T., De Coppi, P., Culme-Seymour, E., Gibbon, S., et al. (2018) Lancet Commission: Stem cells and regenerative medicine. The Lancet, 391, 883–910.
[34] Gao, L., Gregorich, Z. R., Zhu, W., Mattapally, S., Oduk, Y., Lou, X., et al. (2018) Large Cardiac Muscle Patches Engineered From Human Induced-Pluripotent Stem Cell–Derived Cardiac Cells Improve Recovery From Myocardial Infarction in Swine. Circulation, 137, 1712–1730.
[35] Varzideh, F., Pahlavan, S., Ansari, H., Halvaei, M., Kostin, S., Feiz, M.-S., et al. (2019) Human cardiomyocytes undergo enhanced maturation in embryonic stem cell-derived organoid transplants. Biomaterials, 192, 537–550.
[36] Sakaguchi, H., Kadoshima, T., Soen, M., Narii, N., Ishida, Y., Ohgushi, M., et al. (2015) Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nature Communications, 6, 8896.
[37] Jabs, J., Zickgraf, F. M., Park, J., Wagner, S., Jiang, X., Jechow, K., et al. (2017) Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. Molecular Systems Biology, 13, 955.