REVIEW

Advances in the Construction and Application of Thyroid Organoids

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Summary

Organoids are complex multicellular structures that stem cells self-organize in three-dimensional (3D) cultures into anatomical structures and functional units similar to those seen in the organs from which they originate. This review describes the construction of thyroid organoids and the research progress that has occurred in models of thyroid-related disease. As a novel tool for modeling in a 3D multicellular environment, organoids help provide some useful references for the study of the pathogenesis of thyroid disease.

Key words

Organoids • Stem cells • Thyroid • Thyroid cancer

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Introduction

Organoids are three-dimensional aggregates of embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or adult stem cells (AdSCs). These aggregates are formed *in vitro* by chimeric matrix gels, which have the ability to encapsulate the complexity of physiological and pathological structures in an organism, with the ability to function in place of a patient's organs (Fig. 1). At present, studies on organoids mainly use Matrigel matrix glue derived from the basement membrane matrix of EHS mouse sarcoma that contains adhesin, collagen, and various growth factors that facilitate the targeted differentiation of stem cells into thyroid cells [1]. It is well known that stem cells have the potential to differentiate into different types of tissue cells. Organoid technology is a new *in vitro* model based on this characteristic of stem cells. For example, since 2009, Hans Clevers, the progenitor of organoids, has used Lgr5+ intestinal stem cells to grow mouse intestinal organoids *in vitro* [2]. This technique is now beginning to flourish with organoid models of a wide range of tissues having been created, including the pancreas, liver, endometrium, retina, stomach, and esophagus. In 2019, an organoid was evaluated by The New England Journal of Medicine as an excellent model for studying preclinical human disease [3]. In this regard, organoid technology has emerged as the best currently available biotechnology for establishing *in vitro* models.

Organoid technology is coming to the fore in the field of thyroid disease research. The thyroid gland is essential for the maintenance of growth and development, lipid metabolism, and many other functions in the human body. Over the past few years, thyroid dysfunction disorders such as hyperthyroidism and hypothyroidism caused by disruption of thyroid hormone homeostasis have become increasingly prevalent [4], and have greatly reduced the quality-of-life of patients, thereby contributing to a serious burden on health care services. At present, *in vitro* studies of thyroid disease in China and other countries are limited to animal models and two dimensional (2D) cell culture systems, which cannot reproduce the complex physiology of the thyroid gland. This makes it particularly difficult to further clarify the pathogenesis of thyroid disease and develop novel

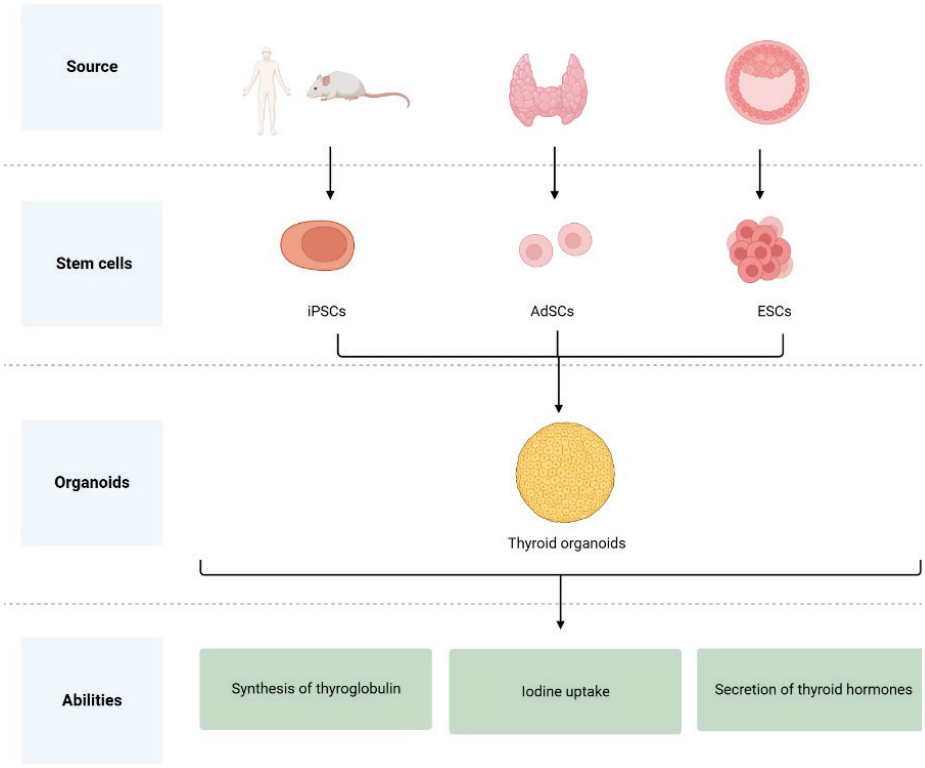

Fig. 1. Sources and functions of an organoid.

alternative therapies.

The advent of organoid technology has not only made it possible to simulate the microenvironment of thyroid tissue [5] but has also solved the problems of inter-species variation in animal models and experimental ethics. Therefore, the successful development of organoid models of normal thyroid tissue and its associated diseases is urgently needed, not only to replace impaired thyroid cells and restore the function of thyroid hormone secretion for use in regenerative medicine but also for research on the pathogenesis and treatment of diseases. Therefore the development of these models will undoubtedly be a great boon to patients with thyroid diseases.

Construction of thyroid organoids

In 2012, Antonica *et al.* [6] were the first to use mouse embryonic stem cells (ESCs) to construct a thyroid organoid *in vitro*. This study demonstrated that ESCs-derived thyroid follicular cells (TFCs) had the ability to self-form thyroid follicular tissue and iodide organization *in vitro*. Initial progress included the induction of mesenchymal and hematopoietic stem cells to become thyroid cells by adding thyrotropin and other factors to the *in vitro* culture medium, and also the establishment of a thyroid organoid model of thyroid cells in an *in vitro* 3D culture system. This model has thyroid functions such as thyroglobulin synthesis, thyroid hormone production and release, and iodine uptake and can be used to identify potential applications for organoids in thyroid morphology, physiology, pathology, or thyroid reconstruction [7]. During human embryonic development, fertilized eggs form three embryonic layers; endoderm, mesoderm, and ectoderm. Under certain conditions, these layers form a specific cell lineage, with subsequent fetal tissue progenitor cells guiding the development and maturation of functional organs. Considering the ability of progenitor cells to give rise to a variety of mature cell types in specific tissues, derivatives of the foregut endoderm (AFE), such as thyroid cells, are of great clinical interest. In 2017, researchers at Boston University School of Medicine achieved the transformation of AFE cells into thyroid epithelial cells using transient overexpression of the transcription factor NKX2-1. This transformation was highly developmental stage specific, dependent on FOXA2 expression levels, and required precise regulation of the bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) signaling pathways [8]. Notably, the successful construction of organoids was heavily dependent on the components of the culture medium in which they were embedded, with these components constituting key signaling pathways that simulate *in vivo*

stem cells ecology, resulting in the cells differentiating into specific cell types of organs. This suggests that the regulation of key signaling pathways is important for guiding the directed differentiation of stem cells. Previous studies [1] have demonstrated that BMP4 and FGF2 activate key pathways driving thyroid development *in vivo* and *in vitro*, and also provided information on the regulatory mechanisms underlying early thyroid organ development. Other studies have shown that embryonic mouse stem cells (mES) and human embryonic stem cells (hES) are transfected with lentiviral vectors to stably express Nkx2.1 (NK2 homologue 1) and Pax8 (pairing box 8). It has been confirmed that transfected cells stimulated with Activin A and TSH differentiate into thyroid cells and contribute to the formation of functional three-dimensional follicular structures [9,10] Therefore, these findings will help us to develop the right media components to help ESCs activate the signaling pathways that regulate stem cell differentiation into thyroid cell structures and produce targeted cell types in large numbers. This will also allow us to conduct clinically relevant types of research to improve the health problems of patients with thyroid and related diseases.

As a result of the continuous development of technology and the increasing maturity of organoid technology, in 2021, Dutch academics [11] successfully isolated and characterized cells from mouse and human thyroid tissue and developed an *in vitro* 3D culture system using a subpopulation of cells with potential stem cell characteristics to produce organoids resembling the thyroid gland. They also demonstrated the ability of these thyroid organoids to develop into thyroid tissue under renal tegument in a mouse model of hypothyroidism. Recently, Liang *et al.* [12] at Fudan University reported the development of the human embryonic thyroid gland using single-cell technology and organoid models and established the first human fetal thyroid organoid (hFTO), which preserved the genealogical and molecular characteristics of thyroid tissue at the embryonic stage. Interestingly, these researchers also found that adding forskolin to the *in vitro* culture system to activate the cAMP signaling pathway induced hFTO to mature into a human maturation thyroid organoid (hMTO) that had the ability to secret thyroid hormones. This organoid also has the potential to help understand the critical period of thyroid embryonic development and tap into the signaling mechanisms at a particular stage. Given the paucity of current knowledge on human thyroid embryonic development, this human embryonic-derived tissue construct of a functionally mature thyroid organoid can recapitulate key events in fetal thyroid development and is of great clinical importance to better understanding congenital thyroid disorders in children.

In summary, techniques for the construction of thyroid organoids are gradually being refined, with single-cell profiling identifying the involvement of multiple signaling pathways in the development of thyroid organoids. Several studies in human and mouse-derived thyroid organoids have also shown that cAMP activation is essential for thyroid proliferation and differentiation [1,13,14]. At present, preliminary progress has been made on the types and concentrations of cytokines that activate or inhibit the corresponding signaling pathways in the directed differentiation of stem cells. If the organoid model can be used to investigate the mechanism for developmental regulation of thyroid organs, this will undoubtably help to promote related research in the field of thyroid function.

Applications of thyroid organoids

Models of thyroid disease

Hyperthyroidism

Thyroid hormone maintains the homeostasis of the system through negative feedback regulation of the hypothalamus-pituitary-thyroid axis. Hyperthyroidism is caused by an abnormal increase in thyroid hormone secretion in the body. In 2019, scholars [15] conducted a retrospective study and showed that patients with Grave's disease had only half the chance of avoiding radioactive I131 treatment after receiving treatment with antithyroid drug (ATD). Therefore, investigating new treatment methods for hyperthyroidism is a major issue facing the field of endocrinology. In 2021, American scientists [16] raised the possibility of deriving thyroid organoids from human adult stem cells, and explored the clinically relevant response of serum from patients with Grave's disease to human thyroid follicular cell organoids (TFCOs). It was finally confirmed that in the presence of a patient's serum, TFCOs contained the complete mechanism for producing thyroid hormones, a finding which greatly promoted the use of organoids in hyperthyroid disease models. At the same time, it also implied that in the future the complex dynamic microenvironment could be reproduced by adding cytokines or activated antibodies to the organoid culture system, or even by developing more advanced technologies, which contributed to the understanding of

autoimmune diseases. It is well known that hyperthyroidism is not only a thyroid disease, but can also develop in the long-term into hyperthyroidism-related eye disease (GO). For a long time, accurate biological models to simulate the pathological state of GO have been lacking in china and other countries. However, organoids may provide a new way of solve this problem. For example, Japanese researchers [17] made use of human orbital fibroblasts of GO patients to prepare a 3D organoid model, and simulated the influence of drugs on the tissues around the eyes of patients. This experimental approach played a particular role in the selection of drugs for GO patients. In view of the development of hyperthyroidism and hyperthyroid-related eye diseases involving a variety of genetic and immune factors, the culture of *in vitro* organoids remains challenging.

Hypothyroidism

The treatment of patients with hypothyroidism is dominated by alternative regimens, namely, exogenous supplementation of thyroid hormones. However, research data show that after replacement therapy, more than one-third of patients still have no improvement in symptoms [18]. Moreover, the quality-of-life of a large number of patients is seriously affected, with some patients even developing mental health problems [19]. At present, the application of organoids in hypothyroidism is based mostly on normal thyroid organoids. Scientists have used adult stem cells derived from normal mouse tissue to construct thyroid organoids and then transplanted them into mice with hypothyroidism. They found that the organoids could regenerate into mature thyroid follicular structures in vivo, producing free T4 [11]. This tissue-derived organoid model, although an important tool for studying the mechanisms of adult tissue disease, has relatively limited differentiation ability and is limited to mechanistic studies of early thyroid maturation or development. Therefore, other stem cell-derived organoid models have been developed to overcome this difficulty. Romitti *et al.* [20] derived human thyroid organoids from ESCs and detected a significant increase in plasma T4 levels after transplantation into a mouse model of hypothyroidism. That study also showed that organoids were positively programmed to efficiently synthesize thyroid hormones *in vitro* around day 58 by regulating signaling pathways and over-expressing the transcription factors, NKX2-1 and PAX8. This system demonstrated that organoids had the ability to rescue low thyroid hormones both *in vivo* and *in vitro*. Taken together, these results indicated that the thyroid organoid model based on stem cell culture has the ability to restore thyroid function because of its regenerative properties.

Thyroid carcinoma

Papillary thyroid carcinoma (PTC) is one of the most common malignancies of the endocrine system, and despite its favorable prognosis, thyroid cancer patients with advanced metastatic, iodine-refractory disease have a higher mortality rate. Previous research models were mostly patient-derived tumor xenografts (PDXs), in which human thyroid tumor cells were transplanted into immunodeficient mice [21]. This method is not only time-consuming and prone to new unknown mutations, but also does not truly reflect the tumor microenvironment. In the past decade, there has been a significant trend towards patient-derived organoid (PDO) culture models that reproduce *in vivo* structures, parental tissue characteristics, and genetic function, whilst preserving tumor heterogeneity between individuals [22]. Some researchers [23] have used canine thyroid follicular cell carcinoma (FTC) tissues to construct FTC organoids, and compared them with the original tumor tissues. This showed that both the organoid and original tissue significantly expressed the sodium iodide transporter (NIS), which is very important for iodine uptake in thyroid follicular cells. This indicates that canine FTC-derived organoids can be used as an appropriate *in vitro* model for studying iodine uptake and opens up a new research approach for treating canine thyroid cancer. In the same year, Chen *et al.* [24] obtained primary tissues surgically removed from patients with papillary thyroid carcinoma (PTC) for use in an *in vitro* 3D culture. Following histological characterization, DNA sequencing, drug screening, and cell proliferation experiments it was shown that the derived PTC organs were similar to the corresponding parental tumor tissues in terms of tissue structure, expression profile, and genome landscape. At the same time, it was also demonstrated that the presence of the estrogen receptor α (ERα) in estradiol promoted the proliferation of PTC organoids, indicating that ERα specific antagonists could be a potential treatment for thyroid cancer. This was the first application of a thyroid cancer organoid culture system for use as anti-tumor drug and hormone drug testing in the world, breaking the bottleneck in the field of thyroid cancer drug treatment. In 2023, a research team [25] established

an *in vitro* BRAF V600E mutant and wild-type PTC organoid model to evaluate the therapeutic effect of different anticancer drugs, and finally found that TKI (a small molecule) had a good response to treatment among more than a dozen drug combination strategies. The team also tested resistance to BRAF inhibitors and showed that activating the HER family could overcome resistance, thereby providing an innovative application in this field that will offer new hope for more cancer patients.

The establishment of organoids provides imaginative and important treatment for thyroid cancer patients, by not only creating a 3D co-culture environment for a variety of cells, but also identifying abnormal gene expression in patients using high-throughput sequencing. At the same time, the culture medium for thyroid cancer organoids is constantly being optimized. Pecce *et al.* [26] have developed a new culture medium whose main component is natural growth factors produced by thyroid cancer cells. Organoids can grow in this thyroid-specific culture medium for about 10 months. This cost-effective alternative culture method is a major step forward in the field of thyroid cancer organoids. However, the current culture system for thyroid carcinoma organoids still has some deficiencies. For example, some immune cells may be lost during the culture process, and are therefore not conducive to the realization of precision medicine for thyroid cancer. Therefore, it is necessary in the future to continue to optimize the culture program and develop a more accurate co-culture system for immune cells and thyroid carcinoma organoids.

Hashimoto's thyroiditis

Hashimoto's thyroiditis (HT) is a specific autoimmune disease, with an increasing number of studies having shown that HT patients are at higher risk of thyroid cancer than normal people [27]. At present, the pathogenesis of HT remains unclear, and the method of creating a mouse model of HT by injecting excessive iodine [28] or immunoglobulin [29] is still controversial. Moreover, even if the model is successful, the physiological changes in mice do not correspond to the physiological changes observed in HT patients. Therefore, researchers have devoted themselves to the development of HT organoid models to enrich the understanding of the pathogenesis of the disease. In recent years, great progress has been made in constructing Hashimoto's thyroiditis organoids using stem cells derived from patient tissue. In 2020, Vilgelm *et al.* [30] proposed an efficient, minimally invasive organoid culture technology based on fine needle aspiration (FNA). This led to development of an organoid culture that used the least amount of tissue that was conducive to high-throughput drug screening experiments. Subsequently, in 2021, a research team at Fudan University [31] used fine needle puncture to develop a tissue derived HT organoid culture in HT patients, and compared it with mass spectrometry analysis of thyroid tissues of healthy people with simple thyroid nodules. This showed that HT organoids had significant characteristics for up-regulation of chemokines, including CCL2 and CCL3, that have a key role in the formation of HT. Patient-derived HT organoids can be combined with proteomics and other omics to provide a highly creative research platform for more accurate understanding of the physiological and pathological changes of thyroid tissue in HT patients and drug screening.

Organoid chip

Organoids are a type of biological model in a static environment, and therefore do not provide a sufficiently precise simulation of a local microenvironment. Moreover, a traditional 3D organoid culture is limited in terms of nutrient transport and can only absorb nutrients by passive diffusion. With the growth of organoids, oxygen and nutrients from passive diffusion cannot meet their metabolic needs, eventually resulting in limited organoid growth and maturation [32]. With the continuous innovation and development of organoid biological models, the emergence of organoid chip technology has solved these limitations of traditional culture programs. This approach uses microfluidic chip technology as the core, combines cell biology and biomedical engineering technology, and makes organoids more bionic when simulating natural human tissues and organs by using the characteristics of shear force. In recent years, there has also been developments in 3D bioprinting technology. Serex *et al.* [33] developed a print head based on microfluidic technology. This platform can adjust the concentration of print units in real time through concentration and distribution, which solves the difficulty that traditional organoid models cannot realize adequate information exchange between cells and cytokines. In addition, the development of this bioengineering technology provides greater opportunities to construct more accurate reproduction of complex

organizational structures.

Due to the enormous complexity of thyroid diseases in terms of cell distribution, vascular network, and immune microenvironment, the development of highly bionic thyroid organoids is still a long way off. A microfluidic platform has the ability to evaluate the sensitivity of thyroid tissue to radioiodine therapy in real time, which would be of great significance for thyroid cancer patients who do not respond to radioiodine therapy. In addition, biopsies of both a benign and malignant human thyroid were placed in a microfluidic culture apparatus, that showed thyroid tissue slices could survive for 4 days *in vitro* [34], making it possible to cryopreserve and resuscitate the organoids. Current studies that involved cutting up surgically resected tissue of thyroid cancer patients, followed by cryopreservation using a gradient cooling method, and preservation in liquid nitrogen for 15-18 months, demonstrated that resuscitation and primary cell extraction could still be carried out *in vitro* organoid cultures. This confirmed that cryopreservation had no significant effect on cell activity and proliferation rate [35]. In 2023, Kühnlenz *et al.* [36] improved the previous 3D culture method and built a microfluidic thyroid-liver platform to investigate the influence of liver enzymes on thyroid hormone catabolism. The two-organ model chip model perfused in that study was the first step towards a complex multi-module human platform. Subsequently, Spaletta *et al.* [37] used a combination of human biological artificial organs and chip organs to develop a scaffold bioreactor unit with the aid of a computer, which maximally restored the complex structure of the thyroid lobes and the arterial structure of the thyroid. The combination of this multi-model technique was formed preliminarily in the field of thyroid research, which is of great value for the study of the pathological effects that thyroid diseases may have on other organs.

Summary and Prospect

Organoid technology is showing unique advantages in thyroid research. This technology is often used to study the physiological structure of human organs and tissues *in vitro*, and has the ability to restore hormone production in functional thyroid tissues. This has provided more opportunities for the advancement of regenerative medicine and tumor precision medicine. However, at the same time, organoid model also has many limitations, such as a lack of vascularization structure, and therefore cannot accurately control the internal microenvironment. The method for establishing a model has also not been standardized. For clinical application in the field of transplantation, it is necessary to further clarify the location of transplantation, the number of transplanted cells, and whether graft rejection will occur.

In recent years, organoids have been increasingly closely combined with other real-time imaging, microfluidic, and 3D bioprinting technologies. New aggressive biomarkers of thyroid cancer can be identified by combining organoids with microfluidic technology [38], which improves the efficiency and precision of tumor medicine. The combination of organoid and *in vivo* real-time imaging technologies is expected to enable the first observation of the development of early human organ tissues. In the future, the integration of multiple research models is the best plan to further promote medical research. Continuous optimization of the culture conditions of *in vitro* models is expected to build a more reliable platform for thyroid disease research and drug screening, and lead to a broader clinical application of this novel technology.

Conflict of Interest

There is no conflict of interest.

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