Review Article

Culture Models for Studying Thyroid Biology and Disorders

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The thyroid is composed of thyroid follicles supported by extracellular matrix, capillary network, and stromal cell types such as fibroblasts. The follicles consist of thyrocytes and C cells. In this microenvironment, thyrocytes are highly integrated in their specific structural and functional polarization, but monolayer and floating cultures cannot allow thyrocytes to organize the follicles with such polarity. In contrast, three-dimensional (3-D) collagen gel culture enables thyrocytes to form 3-D follicles with normal polarity. However, these systems never reconstruct the follicles consisting of both thyrocytes and C cells. Thyroid tissue-organotypic culture retains 3-D follicles with both thyrocytes and C cells. To create more appropriate experimental models, we here characterize four culture systems above and then introduce the models for studying thyroid biology and disorders. Finally, we propose a new approach to the cell type-specific culture systems on the basis of in vivo microenvironments of various cell types.

1. Introduction

Thyroid gland is composed of spheroid structures called thyroid follicles (Figure 1(a)), which consist of both many thyrocytes and a few C cells. Each follicle, which is an essential structural and functional unit of the thyroid, is supported by the stroma that contains interfollicular extracellular matrix (ECM), a capillary network and a few stromal cell types such as fibroblasts and inflammatory cells (Figure 1(a)). Thyrocytes have specific structural polarity: their apical pole with numerous microvilli faces the follicle lumen, and their basal side with basal lamina faces the stroma (Figure 1(b)). This is a specialized structure, compared to other endocrine organs, and results in thyroid hormone biosynthesis and release in a basal-apical (follicle lumen)-basal direction by thyrocytes (Figure 1(b)) [1].

To investigate both thyroid biology and diseases, monolayer and floating culture systems have been developed and widely used [2–5]. These methods have certainly facilitated the above-mentioned issues of the thyroid. However, the conventional methods, in which thyrocytes are unable to organize follicle structures, cannot satisfactorily provide thyrocytes with normal cellular integration [2–5]. In contrast, three-dimensional (3-D) collagen gel culture system allows thyrocytes to achieve follicle structures with their physiological polarity. This method is, thus, suitable for studying the normal and pathologic behavior of thyrocytes in a microenvironment which more closely simulates physiological conditions [4, 5]. These culture systems can not, however, reconstruct follicle structures that consist of both thyrocytes and C cells. To overcome this issue, we developed a new organotypic culture system of thyroid tissue fragments that contain the two cell types, using a concept of 3-D air-liquid interface (ALI) [6–8]. Our thyroid tissue-organotypic culture system retains 3-D follicle structures with both thyrocytes and C cells for a long term [6–8]. Given that highly integrated thyrocytes function to maintain body homeostasis through their intercommunication with neighboring thyrocytes, C cells, the other cell types, ECM molecules, and cytokines, the highly integrated thyrocyte-based experimental system seems critical for investigating both thyroid biology and disorders.
First, we review the characteristics of four culture systems above in order to create more appropriate experimental models. Second, we introduce some experimental culture models regarding the studies of the biology, regeneration, and diseases of the thyroid. Finally, we propose a new approach to the cell type-specific culture systems on the basis of in vivo microenvironments of various cell types.


Thyrocyte monolayer culture initiated by Pulvertaft et al. in 1959 [2] has been used for studying the proliferation and differentiation of thyrocytes. However, monolayer culture cannot satisfactorily enable thyrocytes to achieve normal structural and functional polarities. In this culture system, thyrocytes organize a continuous epithelial pavement, adhering to the surface of the plastic dish (Figure 1(c)), and they show apical-basal polarity, with their apical side with microvilli facing the culture medium, and the basal (attached) side without basal lamina facing the plastic surface of the culture dish. In the epithelial sheet, some thyrocytes organize dome-like structures. The elevation of the cells from the plastic surface results in the formation of these structures, although the exact mechanism by which this occurs remains unclear. Thyrocytes covering these structures show microvilli on the side which contacts the culture medium, and they form foot processes on the luminal side. The plastic surface just under these structures is comprised of an acellular area.

In floating culture, thyrocytes organize themselves into inside-out follicles (vesicles), but not epithelial sheets, although they do form a continuous monolayer pavement on the plastic dish after long-term culture through their production of ECM molecules. The component cells of the vesicles have microvilli on the side that contacts the culture medium, and they have foot processes at the luminal side. In addition, the differentiating factor thyrotropin (TSH)
transiently induces polarity inversion in the cells. In contrast, inside-out follicles undergo polarity inversion in a TSH-independent manner when vesicles are embedded and cultured within 3-D collagen gel. This suggests that a 3-D environment of ECM molecules is more important for the stability of thyrocyte integration than the addition of soluble TSH.

In these conventional systems, thyrocytes do not independently undergo thyroid folliculogenesis as is observed in 3-D collagen gel culture. After isolated thyrocytes are seeded in the conventional systems, the cells easily regain apical-basal polarity, as described above. In these conditions, thyrocytes do not require further polarization. In contrast, thyrocytes embedded in collagen gel are completely deprived of cellular polarity as described below. To regain normal cellular polarity, the cells must reconstruct 3-D follicle structures, in which the cells can undergo physiological polarization. Therefore, the essential reasons that there is no organization of thyroid folliculogenesis in conventional culture systems are considered to be: (1) that thyrocytes have apical-basal polarity as an epithelial phenotype even under an environment without follicle structures and (2) thyrocytes have no 3-D ECM environment. The monolayer and floating culture systems are certainly considered to be useful for simply investigating both the proliferation and differentiation of thyrocytes, but these systems seem inappropriate for studying these issues in a more physiological way.

3. 3-D Collagen Gel Culture System [4, 5]

Using the major component of ECM, type I collagen, Elsdale and Bard initiated collagen gel culture in 1972 [9]. Thyrocytes embedded in a collagen gel (Figure 2) reconstruct follicle structures, in which they show physiological cellular polarity (Figure 1(b)). They have numerous microvilli at the apical surface of the follicle lumen, and they form basal lamina at the contact side with collagen gel (Figure 3). This structural polarity allows thyrocytes to undergo thyroid hormone biosynthesis and release in a basal-apical (follicle lumen)-basal direction by the component thyrocytes of reorganized follicles (Figure 1(b)). The organization of this structural and functional cellular polarity is important for the homeostasis of normal thyrocytes. Thyrocytes cultured in a monolayer on a layer of collagen gel cannot form thyroid follicles. The 3-D collagen gel culture system enables thyrocytes to simulate in vivo cellular integration and behavior. This method could be successfully applied to the following experiments: (1) thyroid folliculogenesis; (2) functional actions of thyrocytes with stimulation of various factors such as TSH, iodide, methimazole, propylthiouracil, and cytokines; (3) interaction between thyrocytes and C cells, or mesenchymal cell types such as endothelial cells, fibroblasts, and inflammatory cells, involving experimental models for autoimmune thyroid diseases and cancer; (4) thyrocyte-ECM interaction; (5) cell transplantation. This method could also be applied to the investigation of other endocrine cell types, including pancreatic islet, adrenocortical, parathyroid, and pituitary cells, and adipocytes.

4. Thyroid Tissue-Organotypic Culture System [6–8]

Thyroid follicles have two specialized cell types: principal thyrocytes and a few C cells (parafollicular cells). However, such viable follicles consisting of the two cell types cannot be organized and retained for a long term in any conventional monolayer, floating, 3-D collagen gel, and organ cultures. The follicles in vivo are embedded by interfollicular ECM, supported by a dense network of capillaries. This suggests that both 3-D ECM and sufficient oxygen supply are critical for the maintenance of follicular structure and function. By
undergo terminal di" concentration of free calcium. This suggests that C cells may

4 and 5(a)). These follicles consist of both thyrocytes and C
cells with their specific differentiation (Figure 5(b)). In the
tissue periphery, thyrocytes actively undergo the growth and

5.1. A Model for Thyroid Tissue Regeneration. We previously

established thyroid tissue-organotypic culture system as described above [6–8]. In our system, thyroid follicles actively

regenerate at the peripheral part of the fragments, while the
gel culture of thyroid tissue fragments with im-

proved oxygenation through air exposure-induced air-liquid

interface (ALI) (Figure 4).

In this system, viable 3-D follicles within thyroid tissue

fragments are maintained for more than 6 months (Figures

4 and 5(a)). These follicles consist of both thyrocytes and C
cells with their specific differentiation (Figure 5(b)). In the
tissue periphery, thyrocytes actively undergo the growth and

mother (primary) follicle-derived thyroid folliculogenesis.
Likewise, isolated or clustered thyrocytes, which are located

in the tissue periphery at the starting time of the culture,
organize follicles. C cells show no proliferative ability and
cannot grow even with the stimulation of various con-
centrations of free calcium. This suggests that C cells may

undergo terminal differentiation. A capillary network gradually

disappears within the tissue fragments. Given that thy-
roid tissue-organotypic culture system retains the functional
thyroid follicles with both thyrocytes and C cells for more

than 6 months, this method is useful for analyzing the
roles of thyroid gland in the biological behaviors of various
functional cell types.

5. Experimental Culture Systems

As described above, thyrocytes are highly integrated within

the follicles of thyroid gland. Thus, the highly integrated
thyrocyte-based experimental system seems critical for inves-
tigating both thyroid biology and disorders in a more
physiological way [1]. To facilitate the studies of thyroid
biology and disorders, we here introduce such experimental
models for analyzing thyroid tissue regeneration, thyrocyte-
other cell type interaction, and thyroid tissue-other cell type
interaction.
established. Thus, various tissues other than these tissues above should be applied to organotypic culture system. In addition, the injection of various stem cell types, including embryonic stem cells [19] and iPS cells [20, 21], into tissue fragments may allow us to study in vitro organogenesis with their proliferation and differentiation in a tissue microenvironment-dependent way. Since these issues are critical for regenerative medicine, further extensive studies are inevitably needed.

5.2. Models for Thyrocyte-Other Cell Type Interaction. A better understanding of the interactions between thyrocytes and other cell types such as C cells, fibroblasts, endothelial cells, and inflammatory cells seems critical for addressing the mechanisms of thyroid homeostasis and disorders, including autoimmune diseases and cancer. We here introduce some experimental models for challenging these issues.

5.2.1. Thyrocyte-C Cell Interaction. For analyzing thyrocyte-C cell interaction, the above-mentioned thyroid tissue-organotypic system (Figure 4) is useful, because these cell types are localized within thyroid follicles in vivo and are difficult to culture C cells primary-isolated from the thyroid. Furthermore, the organotypic system can retain the viable two cell types within the follicles for a long term (Figures 4 and 5). In this system, we have neither detected the proliferative ability of C cells even in the stimulation of free calcium nor have addressed the interactions in detail [6–8]. This system will probably disclose many critical unresolved issues regarding the growth and differentiation of C cells, and thyrocyte-C cell interaction.

5.2.2. Thyrocyte-Other Cell Type Interaction. In general, thyrocytes contact with thyroglobulin (Tg)-containing colloid and ECM at the apical surface and the basal side, respectively.
In addition, thyrocytes within thyroid follicles initially interact with the various stromal cell types at the basal side. For analyzing the interactions between thyrocyte and other cell types such as fibroblasts, endothelial cells, and inflammatory cells, the following model (Figure 7(a)) is useful. This model is organized as follows. First, each of the stromal cell types is embedded in collagen gel in an inner dish with a permeable nitrocellulose membrane in its bottom. After thyrocytes are seeded and monolayer-cultured on the gel layer, Tg solution is overlaid on the surface of thyrocytes cultured on the other cell type-embedded gel layer. The inner dish is placed in a larger outer dish, and medium is then added to the outer dish. This culture assembly simulates the in vivo integrated thyrocytes. To easily analyze the protein, mRNA and DNA expression of both thyrocytes, and other cell types under their interactions, the inner dish, in which Tg solution-exposing thyrocytes are cultured on acellular collagen gel layer, is placed in the outer dish where other cell types are cultured in a monolayer or 3-D collagen gel (Figure 7(b)). Given that the inner dish has a permeable nitrocellulose membrane in its bottom, various molecules can freely cross between the inner and outer dishes. For estimating the mechanisms of autoimmune diseases of the thyroid, analyses of the interactions between thyrocytes and macrophages or T and B lymphocytes will probably disclose the basic mechanisms of the initial immune crosstalk among these cell types.

Instead of normal thyrocytes, the utility of neoplastic thyrocytes in these models with or without Tg solution will allow us to investigate the mechanisms of the survival,
growth, invasion, and metastasis of the cancer cells and cancer stem cells. In fact, we have demonstrated the apoptosis, growth, differentiation and invasion of various cancer cell types under the concept of cancer-stromal interaction, using these models [22–24]. Finally, this model is also useful for studies regarding the radiation biology of the thyroid, including radiation bystander effects [23].

Given that Tg concentration within thyroid follicles varies from 0.1 mg/mL up to 250 mg/mL [25, 26], this range of Tg concentration may be used in the experiment. In addition, $10 \times 10^5$ of thyrocytes or the other cell types are suitable in these systems above (**). The culture duration from 1 to 2 weeks would be enough to investigate the cell-cell interaction (**).

5.3. Thyroid Tissue-Other Cell Type Interaction. For estimating the roles of thyroid tissue in biological behaviors of various cell types, for example, lymphocytes, cardiomyocytes, hepatocytes, adipocytes, osteoblasts, and nerve cells, thyroid tissue-based culture model is useful, because thyroid tissue-organotypic culture system can retain functional thyroid follicles with both thyrocytes and C cells for a long term.

This model (Figure 8) is organized similarly to the model for studying thyrocyte-other cell type interaction. Thyroid tissue fragments are embedded in ALI-treated collagen gel in an inner dish with a nitrocellulose membrane. The inner dish is placed in a larger outer dish in which each of other cell types is already cultured. Using the similar model without ALI, we have demonstrated the active interactions between adipose tissue and renal tubular cells [27], osteoblasts [12], or cardiomyocytes [28]. In adipose tissue-tubular cell interaction, adipose tissue promotes the hypertrophy, polarization, and differentiation of tubular cells by attenuating their growth and apoptosis through opposing endocrine or paracrine effects of leptin and adiponectin, while tubular cells inhibit the regeneration of preadipocytes and mesenchymal stem cells [27]. In adipose tissue-osteoblast interaction, adipose tissue inhibits the proliferation and differentiation of osteoblasts, while osteoblasts prohibit the regeneration of preadipocytes and mesenchymal stem cells [12]. In adipose tissue-cardiomyocyte interaction [28], adipose tissue induces the lipotoxicity in cardiomyocytes, promoting the lipid accumulation and apoptosis of the cells together with the inhibition of their growth and differentiation. In turn, cardiomyocytes inhibit the regeneration of both MSC-like cells and preadipocytes from ATFs. Cardiomyocytes also promote the production of adiponectin from ATFs, while they inhibit that of leptin. Finally, an interesting study regarding adipose tissue-thyrocyte interaction is now going in our laboratory.

6. A New ALI-Based Classification of Culture System

ALI is a microenvironment of the skin, cornea, and respiratory and digestive tracts that are in continuity with the external environment atmosphere. The surface-lining cell types are situated at two-dimensional (2-D) ALI microenvironment. In solid organs, for example, thyroid, adipose tissue, liver, and kidney, various cell types are localized in the extravascular space that consists of ECM and tissue fluid percolated from blood vessels. The tissue fluid blended by
Figure 9: Scheme of a new classification of culture systems. (a) Liquid-rich monolayer culture system. The usual monolayer culture under a submerged condition with enough medium is suitable for culturing the surface-lining cell types of endothelial cells, ependymocytes, renal tubular cells, and so on. (b) 2-D ALI culture system is useful for culturing the surface-lining cell types of epidermis, cornea, and respiratory and digestive tracts. (c) 3-D ALI culture is suitable for culturing parenchymal and stromal cell types of solid organs.

On the basis of our new concept of ALI, the microenvironments of many cell types of the body are subdivided mainly into the following three types. The first is the liquid-rich microenvironment of surface-lining cell types on which enough fluid is overlayed, for example, those of the cardiovascular system and cerebral ventricle. The second is the 2-D ALI microenvironment of surface-lining cell types on which sufficient liquid is not overlayed, for example, those of the skin, cornea, and respiratory and digestive tracts. The third is the 3-D microenvironment of parenchymal and stromal cell types of solid organs, for example, thyroid, adrenal, adipose tissue, liver, and kidney. We propose a new classification of culture systems (Figure 9).

7. Conclusions

For studying thyroid biology and diseases, we characterized monolayer, floating, 3-D collagen gel, and organotypic culture systems. Since these methods have their particular advantages and drawbacks, one can use each system in its specific way. We hope that these culture methods facilitate the studies of thyroid biology and disorders. Especially, experimental culture models described here will be probably a promising tool for disclosing many unresolved issues regarding normal and pathologic thyroid.

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