3D Primary Human Thyroid Microtissues: An In Vitro Model for Studying Thyroid Hormone Disruption

T. Nguyen-Jones[1], E. Rogers[1], K.K. Wolf[2], T. Stone[2], S. Presnell[1], E.L. LeCluyse[2], J. Chen[1]

[1]LifeNet Health LifeSciences, Virginia Beach, VA, [2]LifeNet Health LifeSciences, Research Triangle Park

Abstract #5183/LB284

INTRODUCTION

The rise of chemical production and usage during the 21st century in a wide range of areas such as pharmaceuticals, pesticides, and other industries have come with an increased risk of human exposure to toxic substances which could lead to serious health hazards. Disruption of thyroid hormone synthesis is one of the potential threats that could result in neurodevelopmental impairment and preterm birth. In recent years, different government agencies across the world have increased efforts to develop programs to identify, screen, and determine the potential effects of chemicals found in the environment on thyroid hormone synthesis. These efforts include United States Environmental Protection Agency (US-EPA)'s Endocrine Disruptor Screening Program (EDSP) and the European Chemical Agency (ECHA)'s guidance on endocrine disruptors. Due to the need of screening an extensive list of available chemicals, the demand for a high-throughput approach to replace the conventional low-throughput animal dependent method has become critical. Currently, many of the high-throughput *in vitro* models used for this purpose often employ microsomes or cell lines which do not always accurately represent the human thyroid physiological conditions or native properties especially the follicular structure that is responsible for thyroid hormone synthesis. Therefore, the development of more physiologically relevant human models has become an emergent need. The goal of this study was to evaluate the use of P1 cryopreserved human thyrocytes from healthy donors in the 3D thyroid microtissue assay for determining the response profiles of different thyroid-disrupting chemicals (TDCs).

MATERIALS & METHODS

Cryopreserved thyrocyte cell cultures

Thyrocytes from healthy male and female donors (18-52 years of age, n = 3) were isolated using previously described procedure[1]. The cryopreserved thyrocytes were thawed (p1) and plated in 3D cell culture format using Matrigel-coated 96-well plates. On day 2 of cultures, cells were stimulated with 1.0 mIU/mL bovine thyroid stimulating hormone (bTSH) allowing microtissue formation until day 8. On day 8, cell cultures were dosed with either known TPO inhibitor (methimazole), or NIS inhibitor (potassium hexafluorophosphate KPF6) at 100 – 0.0001 µM, or anti-TSHR (thyroid-stimulating hormone receptor) recombinant human antibody K1-70 at 0.667 – 6.67x10^{-7} µM.

Lot # (P1)	Sex	Age	Race	BMI
2218233	M	52	Hispanic	27.9
2318558	M	18	African-American	22.07
2319211	F	36	Caucasian	26

Table 1: Donors' demographic information.

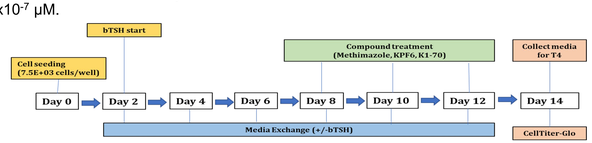


Figure 1: Schematic of the work-flow used for compound treatment of thyrocyte cultures.

Assay analysis

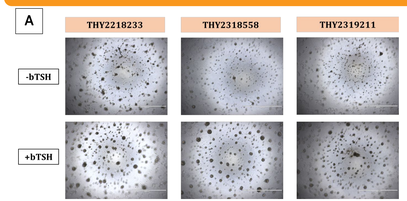
On day 14, media samples from cell cultures were collected for T4 level quantification using Invitrogen™ Thyrocyte T4 Competitive ELISA kit (EIAT4C). Cells' viability were determined using Promega CellTiter-Glo® Luminescent Cell Viability Assay (G7570).

For histology, microtissues were fixed using formalin and proceeded with conventional processing and H&E staining procedure.

Data analysis

Data analysis was performed using Microsoft Excel and IC50 values were determined using GraphPad Prism software.

RESULTS



B

	Average T4 (ng)/ 10^6 cells/48 hrs		
	THY2318233	THY2318558	THY2319211
0 mIU/mL bTSH	3.96 ± 0.00	11.40 ± 2.98	4.82 ± 0.00
1 mIU/mL bTSH	42.31 ± 6.58	196.28 ± 14.02	32.53 ± 1.06

Figure 2: (A) Representative images of microtissues formed without bTSH (- bTSH) and with 1.0 mIU/mL bTSH (+bTSH). (B) Average normalized T4 levels (ng/10^6 cells/48 hrs) recorded via ELISA for each of the thyrocyte donor lot with and without bTSH stimulation. For THY2318233 and THY2319211, T4 background of –bTSH was only detected once out of three replications.

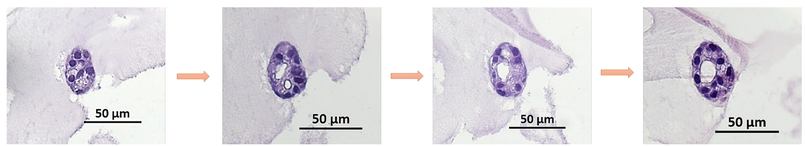


Figure 3: Representative images of a H&E stained microtissue showing the formation of the follicular structure at different layers.

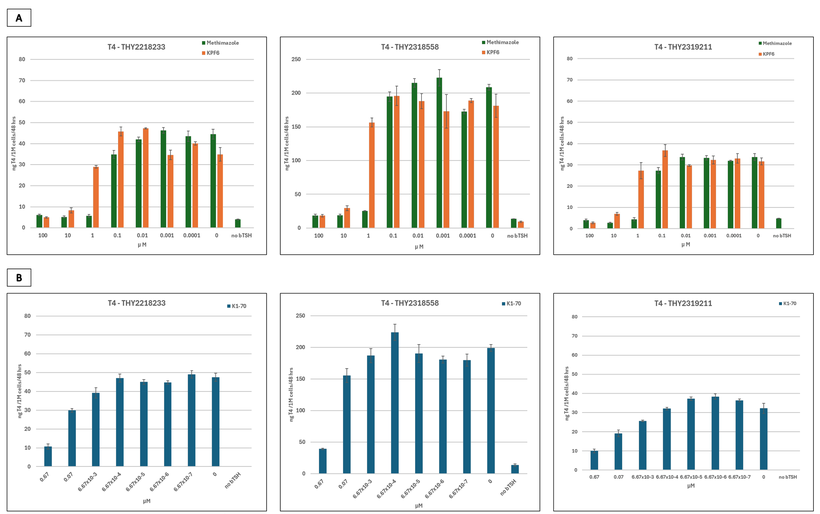
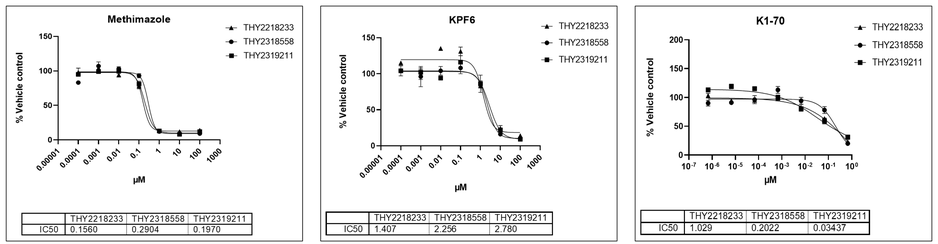


Figure 4: Normalized T4 levels (ng/10^6 cells/48 hrs) obtained for each thyrocyte donor lot when treated with (A) 100 – 0.0001 µM of small compounds methimazole and KFP6, and (B) 0.667 – 6.67x10^{-7} µM TSHR antibody K1-70 along with vehicle controls (0 µM) and no bTSH controls.



	THY2218233	THY2318558	THY2319211
IC50	0.1560	0.2904	0.1970

	THY2218233	THY2318558	THY2319211
IC50	1.407	2.256	2.780

	THY2218233	THY2318558	THY2319211
IC50	1.029	0.2022	0.03437

Figure 5: T4 synthesis as dose response curves along with the respective IC50 values for each of the compound treatment with small compound methimazole and KPF6 (100 – 0.0001 µM) and TSHR antibody K1-70 (0.67 – 6.67x10^{-7} µM). Data was normalized to vehicle controls (0 µM).

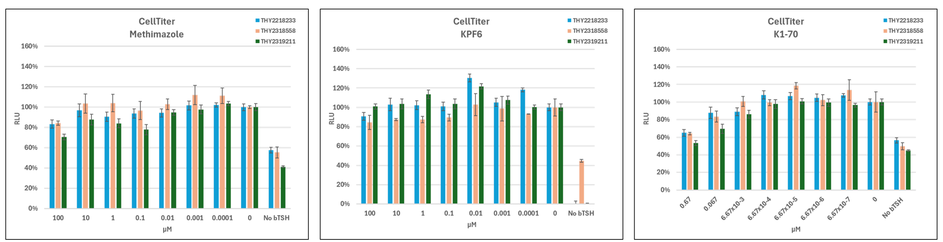


Figure 6: Viability measured on day 14 for cells treated with small compound Methimazole and KPF6 (100 – 0.0001 µM) and TSHR antibody K1-70 (0.67 – 6.67x10^{-7} µM). Data was normalized to vehicle controls (0 µM).

CONCLUSIONS

- Follicular structures of the native thyroid were successfully reconstructed for T4 synthesis via culturing of cryopreserved thyrocytes (P1) in a 3D cell culture format using Matrigel-coated 96-well plates.
- The response profiles of thyroid hormone T4 to both small molecule and antibody-based reference compounds were achieved in a dose response manner.
- 3D thyrocyte microtissue model offers a platform for environmental chemical screening as well as a potential promising approach for thyroid antibody-based therapeutic research.

REFERENCES / ACKNOWLEDGEMENTS

We would like to thank Davorka Softic for her contribution to the H&E microtissue staining work.

Reference:

1. Deisenroth, C., Soldatow, V. Y., et. al. (2020). Development of an in vitro human thyroid microtissue model for chemical screening. Toxicol Sci, 174(1), 63-78. doi:10.1093/toxsci/kfz238.