Evaluation of Thyroxine Synthesis in Cryopreserved Primary Human Thyrocytes for Screening Thyroid-Disrupting Chemicals Using ELISA and LC-MS/MS

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INTRODUCTION

To streamline efforts in identifying and evaluating chemicals that may interfere with the human endocrine system while chemical production and use continue to rise in the 21st century, the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) has established a tiered testing strategy to screen the extensive list of chemicals. Tier 1 tests focus on screening to identify chemicals that potentially interact with the endocrine system while Tier 2 involves long-term studies of the adverse effects. Cell lysates, microsomes, cell lines, or engineered cells are often employed as *in vitro* methods for chemical screening. However, these approaches lack human physiological relevance as the native thyroid phenotype and ability to synthesize thyroid hormones are absent. Emerging organoid models and organ-on-a-chip technologies often offer better recapitulation of biological processes. This study evaluated the use of cryopreserved primary human thyrocytes (P1) as an *in vitro* model for testing thyroid-disrupting chemicals (TDCs). Both ELISAs and LC-MS/MS were employed as quantitative methods to determine the levels of T4 and T3 synthesized. Results indicated that all donor lots formed microtissues and T4 levels met the quality benchmark for TDC applications (>1.0 ng/mL)[1]. Dose response curves and IC50 values were obtained for all donor lots.

MATERIALS & METHODS

Primary Human Thyrocyte Cell Cultures

Primary thyroid epithelial cells from male and female donors (18-52 years of age, BMI ≤ 35, n=3) were isolated and cryopreserved as previously described[2]. The cryopreserved cells were thawed and plated in Matrigel-coated Revvity 96 well plate at 7,500 viable cells/well. Cells were stimulated with 1.0 mIU/mL bovine thyroid stimulating hormone (bTSH) starting on day 2 of culture, followed by dosing of methimazole and hexafluorophosphate (KPF6), at the concentrations of 100 – 0.0001 µM, from day 8 to 14.

Assay analysis

On day 14, medium samples were collected for thyroxine hormone quantification by LifeNet Health using the Invitrogen™ Thyrocyte T4 Competitive ELISA kit (EIAT4C) and Corteva Agriscience's analytical laboratory using LC-MS/MS (T4 and T3). The sensitivity of Invitrogen T4 ELISA kit was 0.33 ng/mL while the detection limit of the LC-MS/MS was 0.1 ng/mL and 0.05 ng/mL for T4 and T3, respectively.

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

Data analysis

Microsoft Excel and GraphPad Prism software were used for data analysis and IC50 value calculation.

IC50 values were obtained using non-linear regression four parameter algorithm with the following equation:

Y=Bottom + (Top-Bottom)/(1+(IC50/X)^HillSlope)

X: Compound concentration (µM)
Y: % response compared to vehicle control (DMSO)
Top: Maximum value of the curve
Bottom: Minimum value of the curve

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.

RESULTS

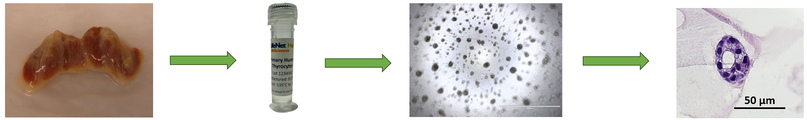
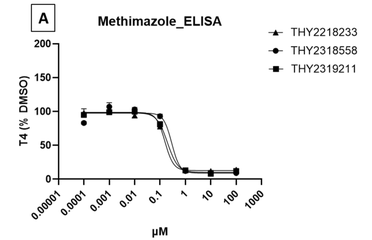


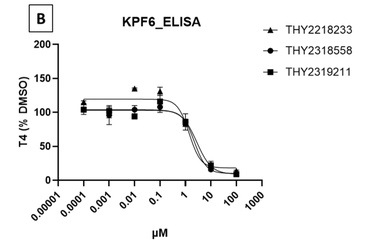
Figure 1: Schematic of thyrocyte isolation, cryopreservation, and cell culturing in 3D format.

Table 2: Average T4 (ng/mL/48 hrs) quantified by ELISA and LC-MS/MS in bTSH-stimulated vs non-stimulated cultures.

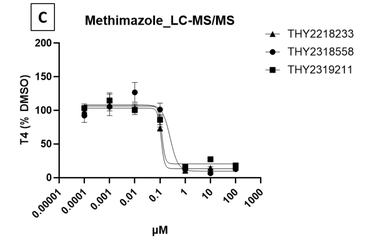
	Average T4 (ng/ mL/48 hrs)					
	ELISA			LC-MS		
	THY2318233	THY2318558	THY2319211	THY2318233	THY2318558	THY2319211
0 mIU/mL bTSH	0.29 ± 0.00	0.85 ± 0.22	0.36 ± 0.00	0.64 ± 0.35	0.70 ± 0.26	0.69 ± 0.03
1 mIU/mL bTSH	2.97 ± 0.50	14.62 ± 1.46	2.45 ± 0.10	5.69 ± 1.60	10.53 ± 1.78	5.21 ± 1.04



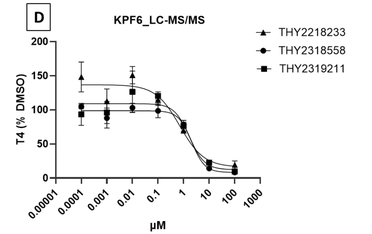
	THY2218233	THY2318558	THY2319211
IC50 (µM)	0.1560	0.2904	0.1970



	THY2218233	THY2318558	THY2319211
IC50 (µM)	1.407	2.256	2.780

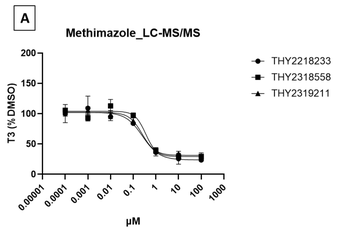


	THY2218233	THY2318558	THY2319211
IC50 (µM)	0.1108	0.2549	0.1195

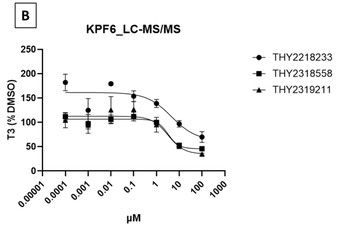


	THY2218233	THY2318558	THY2319211
IC50 (µM)	0.7134	2.060	1.691

Figure 2: T4 dose-response curves and IC50 values generated using ELISA (A,B) and LC-MS/MS (C,D) from methimazole and KPF6 treated cultures.



	THY2218233	THY2318558	THY2319211
IC50 (µM)	0.2572	0.3576	0.2477



	THY2218233	THY2318558	THY2319211
IC50 (µM)	4.590	3.206	3.247

Figure 3: T3 dose-response curves and IC50 values generated using LC-MS/MS from methimazole (A) and KPF6 (B) treated cultures.

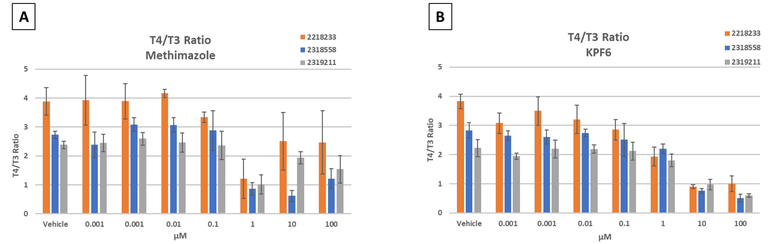


Figure 4: T4/T3 ratio calculated from LC-MS/MS data for all donors treated with methimazole (A) and KPF6 (B) across all tested concentrations.

CONCLUSIONS

- Microtissue formation were achieved in all 3 donor lots with measured T4 levels that met the current TDC applications criteria on day 14 (>1.0 ng/mL) as defined in Foley *et al*. publication.
- Dose-response curves and IC50 values for T4 were consistent across all donors as well as quantifying methods (ELISA and LC-MS/MS) indicating assay reliability.
- Vehicle controls' T4/T3 ratios for two out of the three tested donor lots were > 2.5 (2218233 and 2318558) and the ratio for the third donor lot was >2.0 (2319211).
- Cryopreserved primary human thyrocytes offer a promising approach for evaluating potential thyroid disrupting chemicals and molecules.

REFERENCES / ACKNOWLEDGEMENTS

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

Reference:

1. Foley B, Hopperstad K, *et. al.* (2024). Technical evaluation and standardization of the human thyroid microtissue assay. Toxicol Sci., 199(1), 89-107. doi: 10.1093/toxsci/kfae014. PMID: 38310358; PMCID: PMC11784494.
2. Deisenroth, C., Soldatow, V. Y., *et. al.* (2019). Development of an in vitro human thyroid microtissue model for chemical screening. Toxicol Sci, 174(1), 63-78. doi:10.1093/toxsci/kfz238.