A Human 3D Thyroid Microtissue Model for the Identification of Endocrine Disrupting Compounds

Abstract #762
P30-46

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ABSTRACT

Purpose: Thyroid hormone dysregulation affects organ growth, metabolism, and neurodevelopmental processes. Disruptions in thyroxine (T4) synthesis, particularly during fetal and neonatal development, can lead to severe cognitive and skeletal abnormalities. With rising chemical exposure, regulatory agencies seek better models to identify endocrine-disrupting chemicals (EDCs) that affect thyroid function. Traditional in vitro models often fail to replicate the physiological conditions of the human thyroid. This study evaluates primary human thyrocytes in a 3D thyroid microtissue model as a new approach methodology (NAM) for assessing thyroid disruption.

Methods: Cryopreserved primary human thyrocytes were cultured on Matrigel-coated 96-well plates to form 3D thyroid microtissues. Cells were stimulated with bovine thyroid-stimulating hormone on day 2 of culture, and media were exchanged every other day thereafter. Microtissues were maintained in culture until day 9, at which point they were exposed to known thyroid peroxidase (TPO) inhibitors (methimazole, catechin, kaempferol, resorcinol, triclosan, and others), sodium/iodide symporter (NIS) inhibitors (potassium hexafluorophosphate), and thyroid-stimulating hormone receptor (TSHR) inhibitors (K1-70 recombinant antibody) across a range of concentrations. T4 levels were quantified via ELISA, while cell viability was assessed using CellTiter-Glo (ATP). The half-maximal inhibitory concentration (IC50) for each compound was calculated using GraphPad Prism software.

Results: Human thyroid microtissues formed uniform follicular structures and exhibited increased T4 production in response to bTSH, as compared to untreated controls. Eight TPO inhibitors reduced T4 synthesis dose-dependently, with IC50 values ranging from 0.08 µM (methimazole) to 42.08 µM (epigallocatechin gallate). The NIS inhibitor potassium hexafluorophosphate had an IC50 of 2.15 µM, inhibiting T4 synthesis by more than 85%. The TSHR inhibitor K1-70 recombinant antibody reduced T4 levels by 70% at the highest tested concentrations, though complete inhibition curves were not obtained. High K1-70 doses also decreased cell viability. Triclosan exhibited significant ATP reduction, highlighting the importance in distinguishing direct thyroid disruption from cytotoxicity.

Conclusion: The 3D thyroid microtissue model successfully mimics native follicular structure, sustains T4 synthesis, and responds to both small molecule and antibody-based inhibitors. This NAM provides a valuable tool for toxicology, environmental screening, and thyroid-targeted drug development, enhancing next-generation risk assessment for thyroid-disrupting chemicals.

INTRODUCTION

Chemicals that disrupt thyroid function can cause pronounced effects on thyroid hormone (TH) homeostasis resulting in significant adverse effects in humans, including neurodevelopmental impairment and preterm birth. In recent years, different government agencies across the world have increased efforts to develop programs to identify, screen, and assess 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. Thyroid hormone production in vivo is controlled through the hypothalamus-pituitary-thyroid axis (HPT). Perturbations in systemic TH levels can occur when one or more pathways involved in synthesis or degradation are impacted. Currently, many of the high-throughput in-vitro models used for this purpose do not possess all phases of TH synthesis, and the use of animals for toxicity testing is time consuming and expensive. Here, we describe a fully human in vitro 3D microtissue system that retains the capacity to synthesize and secrete T4 through activation of the TSH receptor, with sufficient dynamic range and stability to enable detection of perturbations over a reasonable chemical exposure window. The model was subsequently challenged with thyroid disrupting compounds (TDCs) known to have adverse impacts on the production of T4.

MATERIALS AND METHODS

Primary human thyrocytes were isolated from donated human tissue, made possible by the generous gift of an individual or their family in the LifeNet Health tissue procurement network. The cryopreserved thyrocytes were thawed (p1) and plated in 3D cell culture format using Matrigel-coated 96-well plates. After 48h in culture, media was exchanged with fresh media containing 1.0 mIU/mL bovine thyroid stimulating hormone (bTSH) allowing microtissue formation until day 8 and media was exchanged every other day thereafter. On day 8, cell cultures were dosed with either known TPO inhibitors, an NIS inhibitor (potassium hexafluorophosphate KPF6), or anti-TSHR (thyroid-stimulating hormone receptor) recombinant human antibody K1-70. The exposures (with TSH) were refreshed on day 1, day 3, and day 5 post-initial exposure. After 6 days (144 hours) of bTSH stimulation and compound exposure (14 total days in culture), T4 production and cell viability was assessed. Compounds selected were thyroid disrupters, known to inhibit T4 production via TPO or NIS inhibition. Stock concentrations were prepared in dimethyl sulfoxide (DMSO; Sigma) at 1000x concentrations and diluted in thyrocyte plating media, resulting in 0.1% final DMSO concentration in all exposures. The anti-TSHR (thyroid-stimulating hormone receptor) recombinant human antibody K1-70 was prepared in culture media.

Thyroxine (T4) production was analyzed in culture media using the Thyroxine (T4) Competitive ELISA Kit (ThermoFisher; EIAT4C). Cell viability was determined by assessing intracellular ATP using CellTiter-Glo® Cell Viability Assay (Promega; G7570).

RESULTS

Figure 1: Schematic of the Work-Flow Used for Compound and Antibody Treatment of 3D Thyrocyte Cultures.

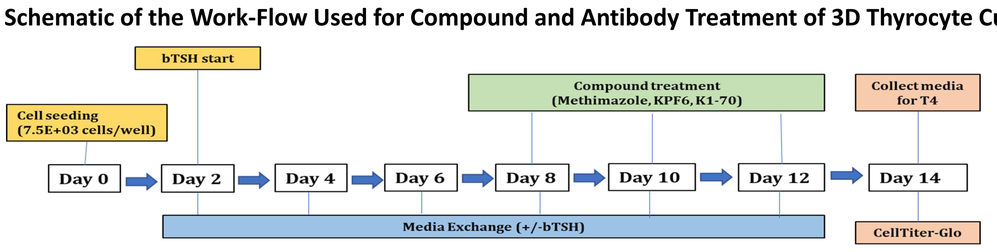
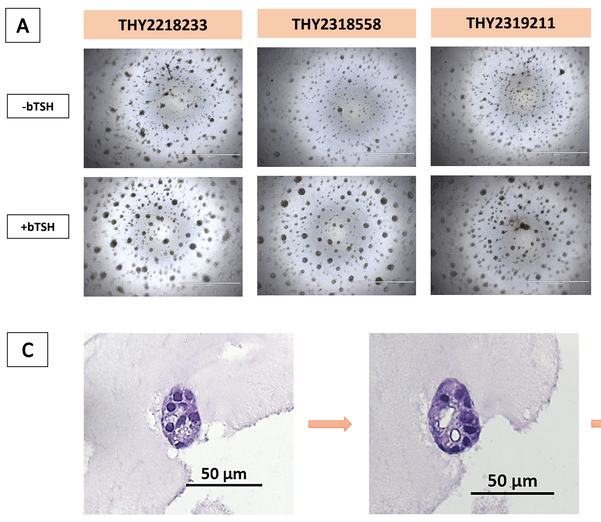


Figure 2: Formation of 3D Thyroid Microtissues and Subsequent Follicular Structure.



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

Fig. 2. (A) Representative images of microtissues formed without bTSH (- bTSH) and with 1.0 mIU/mL bTSH (+bTSH). (B) Average normalized T4 levels (ng/106 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. (C) Representative images of a H&E stained microtissue showing the formation of the follicular structure at different layers.

Figure 3: T4 Inhibition In Response 6 Day Exposures to a TPO Inhibitor, NIS Inhibitor, and Anti-TSHR Antibody

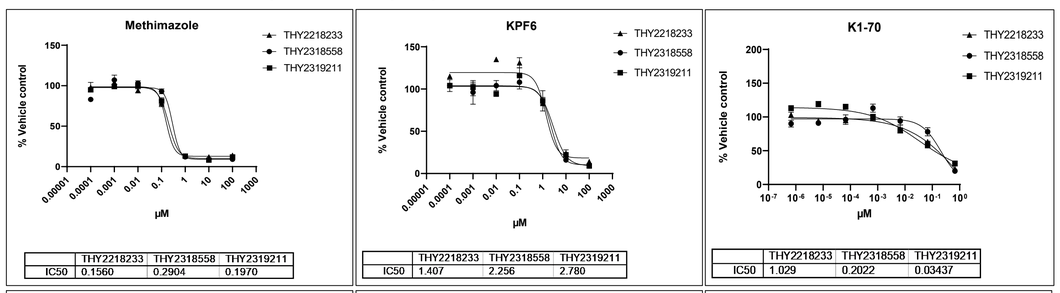


Fig. 3. T4 synthesis as dose response curves along with the respective IC50 values for each of the compound treatment with small compound methimazole, KPF6, and the anti-TSHR antibody K1-70. Corresponding cell viability data is also shown. Data was normalized to vehicle controls.

Figure 4: T4 Inhibition In Response 6 Day Exposures to a Series of Known TPO Inhibitors

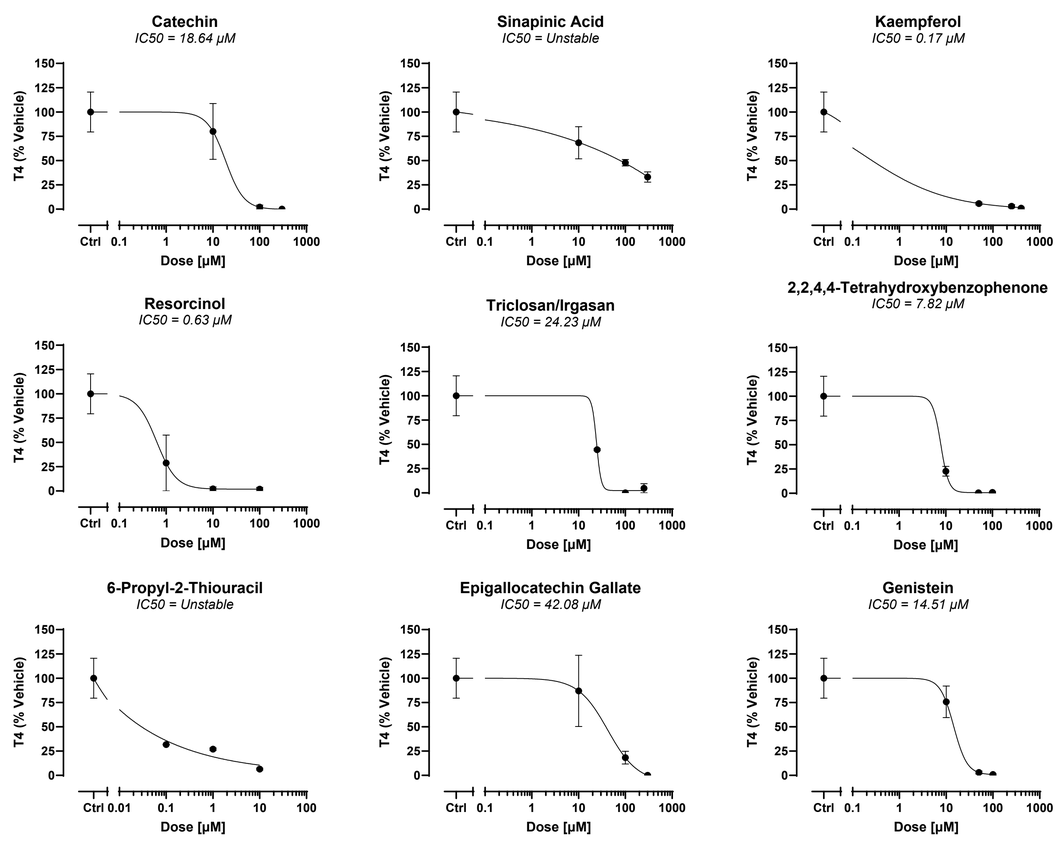


Fig. 4. Human thyroid microtissues were induced with bovine TSH to produce T4 and challenged by an additional 9 known TPO inhibitors for 6 days. Culture media was assessed for inhibition of T4 production versus the vehicle control (+ bTSH).

Table 1: T4 Inhibition In Response 6 Day Exposures to a Series of Known TPO Inhibitors

Compound Name	CASRN	Concentrations Tested (µM)	IC50 in Literature (µM)	IC50 Obtained (µM)
Catechin	225937-10-0	10, 100, 300	102.5 [1]	18.64
Kaempferol	520-18-3	50, 250, 400	215.5 [1]	0.17
Methimazole	60-56-0	0.0001, 0.001, 0.01, 0.1, 1, 10, 100	0.07 - 3.91 [2,3]	0.16 – 0.29
Resorcinol	108-46-3	1, 10, 100	0.36 - 17.9 [3]	0.63
Triclosan	3380-34-5	25, 100, 250	38.4 - 197.0 [3]	24.23
2,2,4,4-Tetrahydroxybenzophenone	131-55-5	10, 50, 100	0.56 - 38.5 [3]	7.82
Epigallocatechin Gallate	989-51-5	10, 100, 300	2.2 – 87.3 [4 †]	42.08
Genistein	446-72-0	10, 50, 100	0.45 - 3.5 [5]	14.51
Sinapinic Acid	530-59-6	10, 100, 300	113.4 [1]	ND
6-Propyl-2-Thiouracil	55-52-5	0.1, 1, 10	0.04 - 18.9 [2,3]	ND

Table 1. Table 2. The IC50s for inhibition of T4 production of each EDC was determined using GraphPad prism and compared to values identified in the literature. ND = no data. Superscript numbers indicate the referenced literature where the IC50 values were identified. † = IC50s of receptor tyrosine kinases potentially related to TPO inhibition as ECGC appears to be an indirect inhibitor of TPO.

CONCLUSIONS

- **When plated on Matrigel, primary human thyroid cells form a stable 3D human thyroid microtissues with robust T4 production upon TSH stimulation.**
- **The response profiles of thyroid hormone T4 to both small molecule and antibody-based reference TDCs were achieved in a dose response manner.**
- **The Human Thyroid Microtissues are a promising NAM for identifying new chemical entities with potential for thyroid disruption, as well as a potential promising approach for thyroid antibody-based therapeutic research.**

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