ASSESSMENT OF A 3D PRIMARY HUMAN THYROID MICROTISSUE ASSAY FOR IDENTIFYING THYROID-DISRUPTING CHEMICALS

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ABSTRACT

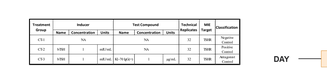
Background and Purpose: The European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) have jointly published guidance for identifying endocrine disruptors. Together with the U.S. EPA's Endocrine Disruptor Screening Program (EDSP), these initiatives emphasize the need for high-throughput screening (HTS) approaches to efficiently evaluate potential thyroid-disrupting chemicals (TDCs) and minimize reliance on traditional, low-throughput, animal-based methods. Deisenroth et al. previously developed a novel 3D human thyrocyte microtissue platform to identify TDCs that act through various mechanisms relevant to thyroid hormone synthesis. This study was designed to evaluate the within-laboratory reproducibility and performance of the assay using primary human thyrocytes from one donor lot, and to assess the applicability of the 3D model for characterizing responses to known TDCs using thyrocytes from a different single donor. **Methods:** Thyrocytes were derived from healthy donors (≤55 years, BMI ≤35) and cultured in a 96-well 3D format. Control groups included baseline (CT-1), TSHR agonism (CT-2), and TSHR antagonism (CT-3) conditions to assess model sensitivity and specificity. Cells were maintained for 14 days with medium changes every 2 days. Culture media were collected on day 14 for T3 and T4 quantification by ELISA, and microtissue viability was determined using the CellTiter-Glo® assay. Model performance was evaluated by dynamic range, precision, and screening quality metrics. For evaluation of the model with known TDCs, medium samples were collected on day 14 after a 6-day exposure (0.001-100µM) for T4 quantification by ELISA. The half-maximal inhibitory concentration (IC_{50}) for each compound was calculated using GraphPad Prism. **Results:** The thyrocyte lots met all specification criteria for screening applications. Microtissues were formed within 4 days and evenly distributed. Microtissues produced T4 concentrations exceeding 1.0 ng/mL at day 14, with robust dynamic ranges (rS/B >2.5-fold), coefficients of variation <30%, and Z'-factors >0.6 for T3 and T4. Dose-dependent inhibition of T4 synthesis was observed with 6-day exposures (Days 8–14) to three reference TDCs, methimazole (MMI), propylthiouracil (PTU), and sodium perchlorate ($NaClO_4$), yielding average IC_{50} values of 0.50 (SE 0.01) µM, 0.96 (SE 0.18) µM, and 32.89 (SE 5.46) µM, respectively, while the negative control (methomyl) showed no effect. No cytotoxicity was observed for any of the reference compounds. **Conclusions:** In summary, the primary human thyrocyte 3D microtissue assay using ELISA represents a promising strategy for evaluating and prioritizing potential TDCs based on human hazard-related bioactivity. Moreover, the model enables the identification of compounds acting directly on the thyroid and supports simultaneous evaluation of multiple MIEs (e.g., TPO, NIS, TSHR) for small-molecule screening in both agriscience and therapeutic research.

INTRODUCTION

Exposure to chemicals that disrupt thyroid function can alter thyroid hormone (TH) homeostasis, causing adverse effects such as neurodevelopmental impairment and a higher risk of sudden cardiac death. The increasing use of chemicals across pharmaceuticals, pesticides, and other industries has elevated human exposure, prompting agencies like U.S. EPA, ECHA and EFSA to screen for thyroid-disrupting activity (1).

Existing *in vitro* and *in vivo* models are limited. Current cell-based models fail to capture essential features of thyroid physiology, including follicular organization and hormone synthesis, whereas animal testing is time-consuming and costly. To overcome these limitations, a human 3D thyroid microtissue model was developed capable of synthesizing and secreting T4 via thyroid-stimulating hormone (TSH) receptor activation, exhibiting sufficient dynamic range and stability for evaluating TH disruption. The objective of this study was to assess within-laboratory reproducibility and assay performance and evaluate the model using known TDCs.

MATERIALS & METHODS



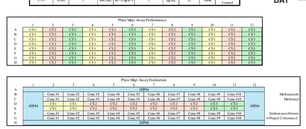


Table 1. Control groups and plate layouts.

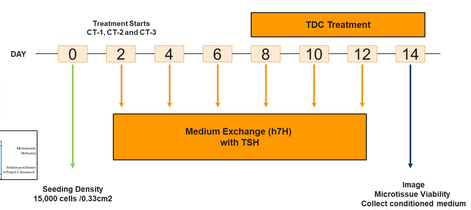


Figure 1. Workflow for thyroid microtissue development and TDC screening.

Isolation of Human Thyroid Follicular Epithelial Cells

Thyrocytes were isolated from healthy thyroid tissues as previously described (2). Cryopreserved primary human thyrocytes from a single donor were thawed, resuspended in h7H medium and plated onto Matrigel® (Corning) coated 96-well plates to form 3D thyroid microtissues.

Treatments and Assays

The thyroid microtissues were treated with 0 or 1 mIU/mL bovine thyroid stimulating hormone (bTSH) from days 2-14. T3 and T4 levels in conditioned media samples were measured on Day 14 using ELISA kits from Invitrogen. Cells were exposed to known TDCs between days 8-14 in the presence of 1 mIU/mL bTSH. The half-maximal inhibitory concentration (IC_{50}) for each compound was calculated using GraphPad Prism.

RESULTS

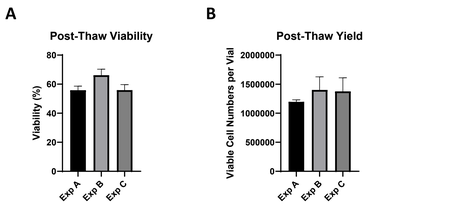


Figure 2. Post-thaw outcomes of cryopreserved thyrocytes (THY2118180). Cell viability **(A)** and yield **(B)** were determined with AO/PI staining and an automated cell counting system (Cellometer Vision, Nexcelom) ($p > 0.05$).

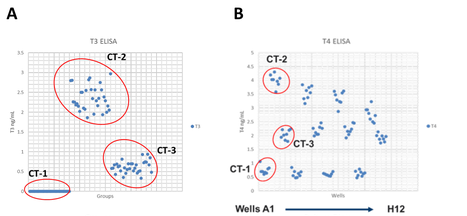


Figure 3. T3 **(A)** and T4 **(B)** levels of CT-1, CT-2 and CT-3 groups (THY2118180).

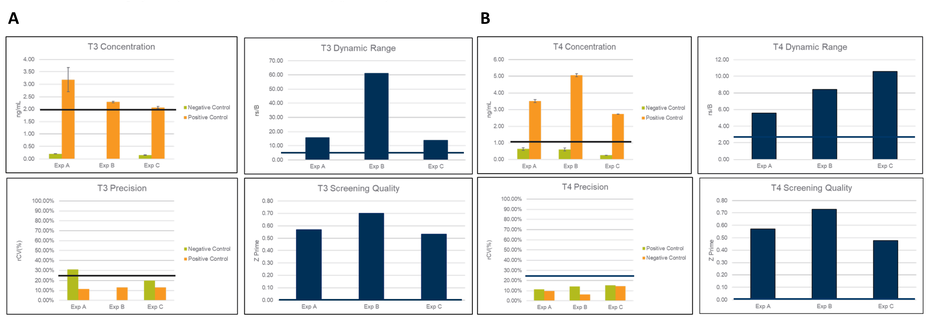


Figure 4. Model performance for T3 **(A)** and T4 **(B)** was evaluated using dynamic range, precision, and screening quality metrics. bTSH-induced T3 and T4 secretion at day 14 from 3D microtissues derived from donor THY2118180. The microtissues were treated with 0 mIU/mL (CT-1) or 1 mIU/mL bTSH (CT-2). Dark blue horizontal lines represent the minimum requirements.

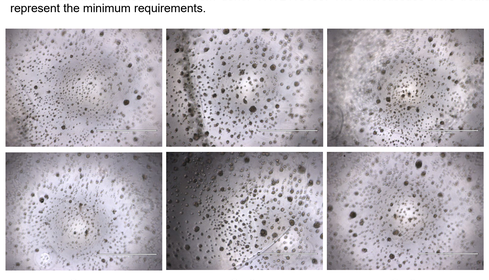


Figure 5. Representative images of 3D thyroid microtissues from donor THY2118180 on day 14 of culture. Scale bar is 1000 µm. (Magnification: 40X)

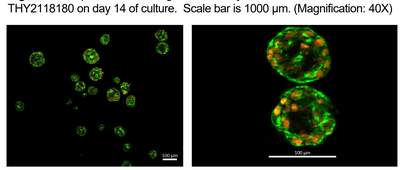


Figure 6. Morphology of 3D microtissues. Follicle-like morphology of 3D microtissues at day 9 of culture. Microtissues were stained for nuclei (orange; DAPI) and actin (green; Phalloidin conjugated to Alexa Fluor 488). Scale bar is 100 µm.

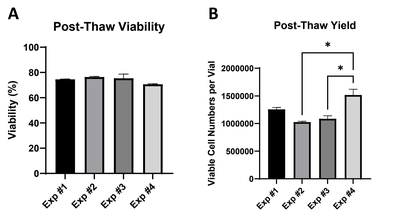


Figure 7. Post-thaw outcomes of cryopreserved thyrocytes (1911550). Cell viability **(A)** and yield **(B)** were determined with AO/PI staining and an automated cell counting system (Cellometer Vision, Nexcelom) (* p value < 0.05).

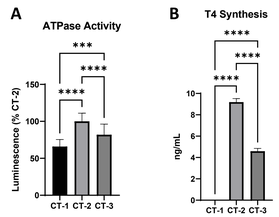


Figure 8. Evaluation of microtissue biomass **(A)** and T4 synthesis **(B)**. Data for normalized ATPase activity (% CT-2) and the absolute T4 hormone concentrations (ng/ml) (***p value of 0.005 and ****p value <0.0001).

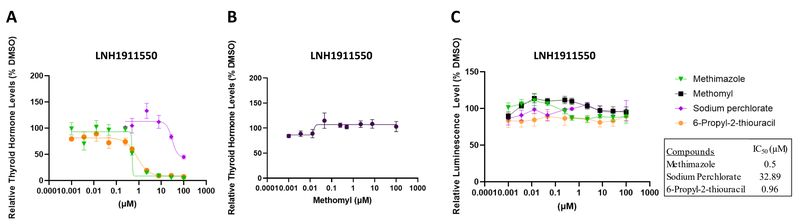


Figure 9. Evaluation of the reference chemical responses for donor LNH1911550. Inhibition of T4 synthesis by known TDCs; sodium perchlorate, a sodium iodide symporter (NIS) inhibitor; methimazole, a thyroid peroxidase (TPO) inhibitor; and 6-Propyl-2-thiouracil, a TPO/iodothyronine deiodinase type 1 (DIO-1) inhibitor **(A)**. Methomyl served as the negative control **(B)**. Microtissue biomass measured by CellTiter-Glo ATPase activity assay **(C).** Mature thyroid microtissues from a healthy donor (LNH1911550) were exposed to TDCs or negative control (0.001- 100µM) from day 8-14 (n=4 independent experiments).

CONCLUSIONS

- LifeNet Health's 3D thyroid microtissue model met U.S. EPA specification criteria for TDCs screening applications as defined by Foley *et al* (3).
  - Thyrocytes formed evenly distributed microtissues and displayed the 3D follicular structure required for T4 production.
  - Microtissues produced T4 (>1.0 ng/mL on day 14).
  - Average robust dynamic ranges (rS/B) of T3 and T4 were >2.6-fold change.
  - The robust coefficient of variation for positive and negative groups (T4) were ≤25%.
  - Average Z'-factors were >0.5 for T3 and T4.
- The 3D thyroid microtissue model represents multiple mechanisms of thyroid hormone synthesis.
- 3D thyroid microtissue model can evaluate TDCs for human risk assessment.

REFERENCES / ACKNOWLEDGEMENTS

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4. Deisenroth C *et al.* (2025) Interlaboratory Validation of the Human Thyroid Microtissue Assay. *Toxicol Sci.* doi: 10.1093/toxsci/kfaf166. Epub ahead of print. PMID: 41344865.

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