Development of a Human Thyroid Microtissue Model for Evaluation of Thyroid Hormone Synthesis

Chad Deisenroth [1], Valerie Soldatow [2], Cassandra Brinkman [1], Edward LeCluyse [2], Russell Thomas [1]

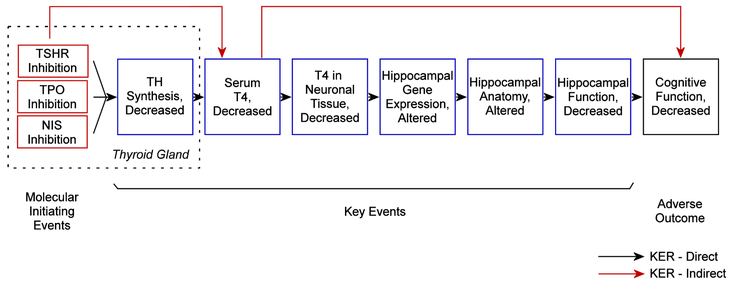
[1] National Center for Computational Toxicology, US EPA, RTP, NC, USA

[2] LifeNet Health, Virginia Beach, VA, USA

Chad Deisenroth | deisenroth.chad@epa.gov | 919-541-3927

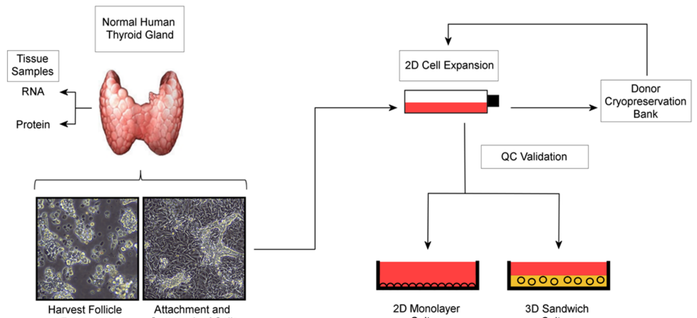
www.epa.gov

Objective



Develop an *in vitro* organotypic culture model for evaluating disruption of thyroid hormone synthesis in the human thyroid gland. Inhibition of key molecular initiating events in the thyroid gland such as Thyroid Stimulating Hormone Receptor (TSHR), Thyroperoxidase (TPO), and the Sodium Iodide Symporter (NIS) lead to decreased serum thyroxine (T4) levels, resulting in adverse neurodevelopmental outcomes in mammals. An assay that evaluates the function of these targets in an integrated functional model is required to evaluate chemical hazards identified in high-throughput screening platforms.

Study Design

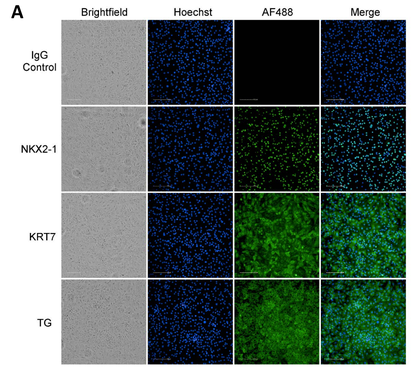


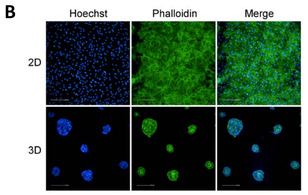
Structure Analysis: 2D vs 3D		
Endpoints	**Markers**	**Method**
Morphology	Phalloidin AF488 and Hoechst 33342	Confocal Fluorescence Microscopy
Immunocytochemistry	NIS, TG, NKX2-1, CK7	Confocal Fluorescence Microscopy
Gene Expression	TSHR, TG, NIS, TPO, PDS, PAX8, NKX2-1, FOXE1, DUOX1/2, DUOXA1/2	qRT-PCR
Protein Expression	TSHR, TG, NIS, TPO, NKX2-1	Immunoblotting

Function Analysis: 2D vs 3D		
Key Event	**Markers**	**Method**
TSHR	Thyroglobulin Secretion	TG ELISA
NIS	Iodide Uptake	RAIU Assay
TPO	TPO Activity	AUR Assay
TH Synthesis	T3 and T4 Secretion	TH ELISA

Overview of model characterization and assay development. Intact thyroid glands derived from primary human donors are processed for cell isolation, limited expansion, and initial quality control assessment. Early passage donor cells are plated in 2D and 3D culture formats for structural and functional analysis of key phenotypic features.

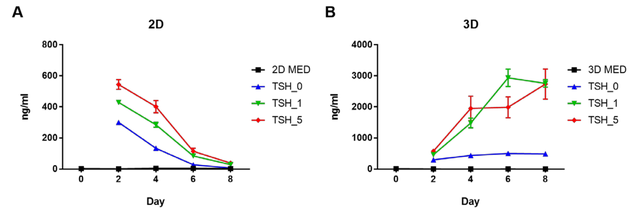
Thyroid-derived Cell Characterization





Biomarker profiling and evaluation of 2D vs 3D models. Immunocytochemistry of thyroid-derived cells in a 2D monolayer from donor LNH1722161 at passage 1 on day 2 of culture. IgG control (α-Mouse IgG), NKX2-1 (Thyroid Transcription Factor 1), KRT7 (Cytokeratin 7), and TG (Thyroglobulin) are shown. Morphological assessment of 2D monolayers vs 3D self-assembly of microtissues at day 10 of culture (B). All micrographs are at 200X magnification.

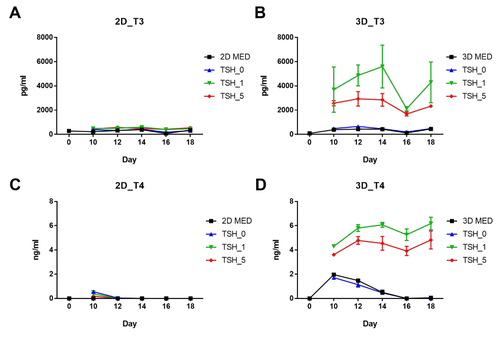
2D vs 3D: Thyroglobulin Secretion



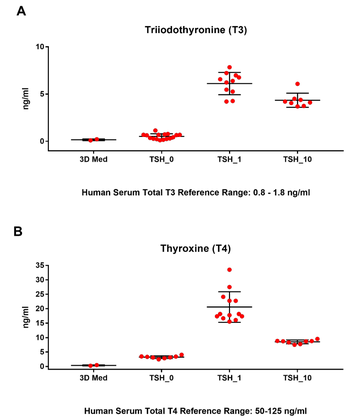
Thyroglobulin (TG) secretion is enhanced in 3D culture model. Donor LNH1722161 was monitored in 2D (A) and 3D (B) cultures for 8 days. TG (ng/ml) was measured from conditioned h7H culture medium containing 0, 1, or 5 mU/ml Thyroid Stimulating Hormone (TSH). Data are mean +/- SD of technical replicates from a single donor at Passage 1.

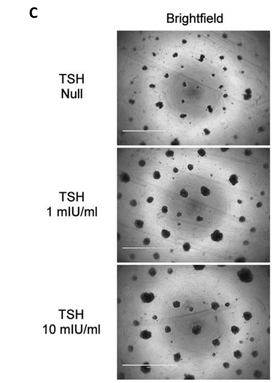
2D vs 3D: Thyroid Hormone Synthesis

Thyroid hormone is secreted and sustained over time in the 3D culture model. Donor LNH1722161 was monitored for Triiodothyronine (T3) and Thyroxine (T4) accumulation from days 10-18 of culture. T3 (pg/ml) and T4 (ng/ml) were measured from conditioned h7H culture medium containing 0, 1, or 5 mU/ml Thyroid Stimulating Hormone (TSH). Two dimensional monolayer cultures did not produce detectable hormone levels (A and C), in contrast to three dimensional cultures that produced hormone over the duration of testing (B and D). Data are mean +/- SD of technical replicates from a single donor at Passage 1.



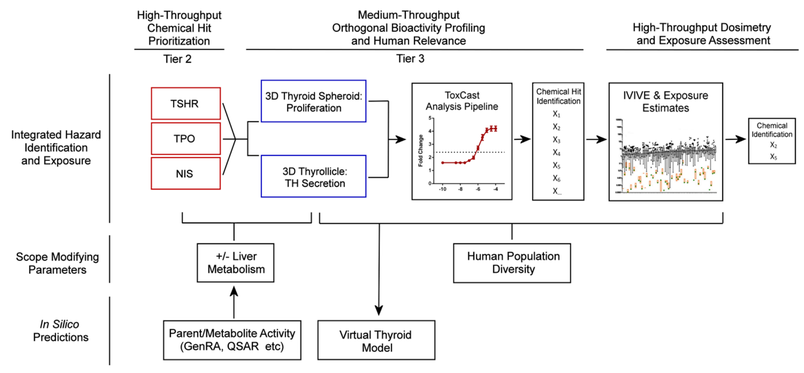
TSH Modulation of Microtissue Size and Hormone Synthesis





TSH modulates the size of microtissues and level of thyroid hormone secretion. Donor LNH1811621 was evaluated for Triiodothyronine (T3) and Thyroxine (T4) accumulation on day 13 of culture. T3 (ng/ml) and T4 (ng/ml) were measured from conditioned h7H culture medium containing 0, 1, or 10 mU/ml Thyroid Stimulating Hormone (TSH). Peak hormone levels were detected at an intermediate TSH concentration of 1 mU/ml, despite larger microtissues observed at TSH 10 mU/ml. Data are mean +/- SD of technical replicates from a single donor at Passage 1.

Proposed Integration of 3D Thyroid Microtissue Assays for Thyroid Disruption Screening



- **Hazard Identification**: Refinement of bioactivity hit calls and evaluation of variability in human populations
- **Toxicodynamics**: Increased comprehension of apical endpoint dosimetry and bioactivity/exposure margins
- ***In Silico* Predictions**: Data generation to enhance prediction of parent/metabolite activity and construct virtual simulations of thyroid perturbation

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