Development of an In Vitro Human Thyroid Microtissue Model for Chemical Screening

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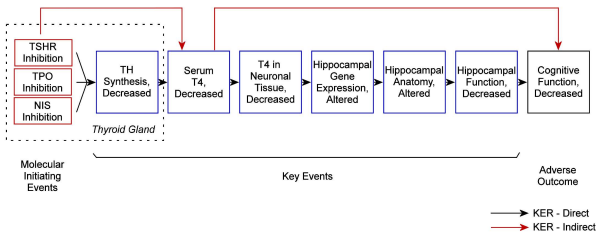
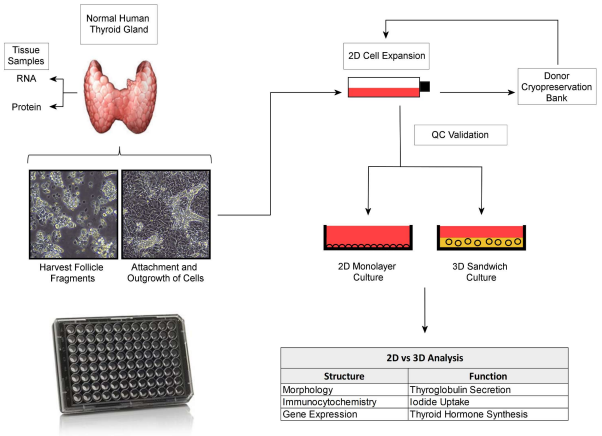
Objective



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



2D vs 3D Analysis	
Structure	**Function**
Morphology	Thyroglobulin Secretion
Immunocytochemistry	Iodide Uptake
Gene Expression	Thyroid Hormone Synthesis

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.

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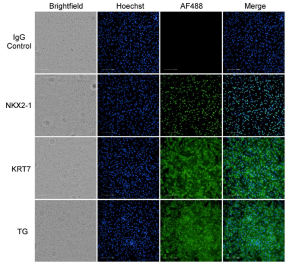
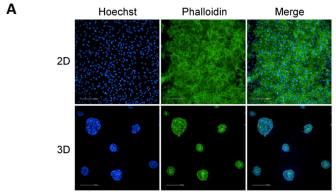
Thyroid Tissue-derived Cell Characterization

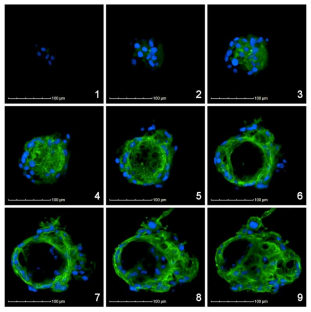


Biomarker Analysis			
	% POS	SEM	N
NKX2-1	95.2	1.7	6
KRT7	90.5	2.5	6
TG	53.4	16.1	6

Biomarker profiling of thyroid tissue-derived follicular epithelial cells. Post-isolation immunocytochemistry of thyroid-derived cells in a 2D monolayer. The cell-level frequency of IgG control (α-Mouse IgG), NKX2-1 (Thyroid Transcription Factor 1), KRT7 (Cytokeratin 7), and TG (Thyroglobulin) staining were quantitatively evaluated by high-content imaging across 6 independent human donors for verification of thyroid follicular epithelial cell enrichment. AlexaFluor 488 (green) and Hoechst 33342 (blue). All micrographs are at 200X magnification.

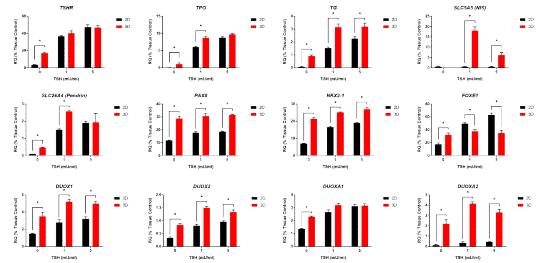
2D vs 3D: Morphological Characterization





Morphological assessment of 2D monolayers vs 3D self-assembled microtissues. (A) Day 10 cultures stained with Hoechst 33342 (blue) and Phalloidin conjugated to AlexaFluor 488 (green) were used to evaluate the nuclei and actin cytoskeleton, respectively. All micrographs are at 200X magnification. (B) Follicular-like morphology of a 3D microtissue at day 10 of culture visualized with confocal microscopy.

2D vs 3D: Gene Expression



Gene expression profiling of 2D monolayers and 3D microtissues. Quantitative RT-PCR was used to profile markers for differentiation (*PAX8, NKX2-1, FOXE1*) and thyroid hormone biosynthesis (*TSHR, TPO, TG, SLC5A5, SLC26A4, DUOX1, DUOX2, DUOXA1, DUOXA2*) at day 8 of culture. Medium was supplemented with different concentrations (0, 1, 5 mU/ml) of thyroid stimulating hormone (TSH) for the duration of culture. 2D (black) and 3D (red) samples (n=4) were normalized and expressed as the relative quantity (RQ) percentage to donor-matched tissue.

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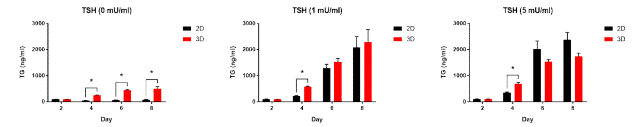
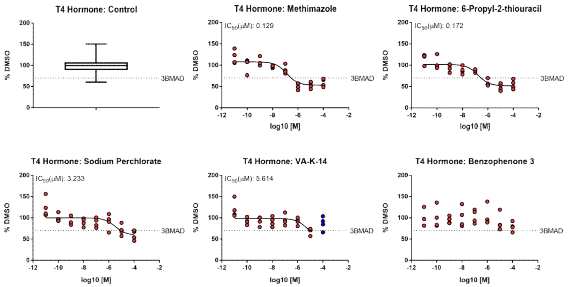
2D vs 3D: Thyroglobulin Secretion



TSH-induced thyroglobulin secretion in 2D monolayers and 3D microtissues. Thyroglobulin protein secretion was measured from conditioned medium every 2 days for a total of 8 days. 2D (black) and 3D (red) samples (n=4) were exposed to different thyroid stimulating hormone concentrations (0, 1, 5 mU/ml) for the duration of culture. Thyroglobulin concentrations (ng/ml) were assayed using a chromogenic ELISA and interpolated from the standard curve.

Thyroid Hormone Disruption: Reference Chemical Evaluation



Evaluation of reference chemical inhibition of thyroid hormone synthesis in a 3D microtissue culture model. Human thyroid microtissues were matured in a 96-well 3D culture model for 10 days. Reference chemicals targeting TPO (Methimazole, 6-Propyl-2-thiouracil), NIS (Sodium Perchlorate), TSHR (VA-K-14), or negative control (Benzophenone 3) were administered in concentration response (10 pM – 100 μM) for a total duration of 4 days. Conditioned medium was collected to assay thyroxine (T4) concentrations by chromogenic ELISA. Values were normalized to plate-based solvent controls (DMSO) and plotted across experiments (n=4). DMSO values were used to define the baseline median absolute deviation (BMAD) and cutoff (3BMAD). Cytotoxicity was concurrently evaluated by measuring ATP levels at day 14. A 3-parameter Hill model was fit to chemicals exceeding the T4 cutoff, up to the highest viable concentration tested, and used to derive the inhibition concentration at 50% (IC50). Plate-based mean T4 levels (red) and cytotoxic concentrations (blue) are shown.

Summary: Clear Advantages to a 3D Human Thyroid Model for Identification of Thyroid Disrupting Chemicals

- **Phenotypic Relevance:** Follicular-like morphology, TSHR activation, thyroglobulin synthesis, iodide uptake, thyroid hormone synthesis and secretion
- **Molecular Initiating Events:** All molecular components of the thyroid system are present in a physiological balance
- **Key Event**: Beyond MIEs, enables empirical evaluation of thyroid hormone synthesis perturbation in a long-term human culture model
- **Screening Throughput**: Amenable to medium-throughput (10s-100s), concentration-response testing of HTS prioritized chemicals
- **Automation Accessible**: Microtiter plates suitable for automated liquid handling, acoustic dosing, and high-content imaging
- **Sampling Design**: Conditioned cell culture supernatant sampling for thyroglobulin and thyroid hormones enables kinetic testing and chronic-dosing paradigms
- **Regulatory Relevance**: Interpretation of chemical mediated effects on hormone output in the human thyroid gland