Perturbation of thyroid hormone synthesis by pesticides and other environmental agents are known to cause numerous developmental, metabolic and cognitive disorders in humans. Cells derived from animal studies may not reflect the phenotypic and functional changes of human thyrocytes (THCs). Therefore, a new in vitro model is needed to address this gap. We recently developed an in vitro human thyroid model (HTPM) with an average yield per vial of 70-80% and an average yield per plate of 85%. THCs were cryopreserved in a serum-free bio-preservation medium. Thyroid cells from multiple batches were thawed in human thyrocyte plating medium and seeded in 96-well plates at a seeding density of 7500-2500 cells/well and maintained in human thyrocyte culture medium containing 1 mIU/mL bovine thyroid stimulating hormone (TSH). The aim of this study was to identify characteristics and evaluate the potential TDCs on various T4-synthetic pathways. The focus on effects of TDCs has been increasing over the past 20 years due to species differences in thyroid stimulating hormone receptor (TSHR), sodium/iodide symporter (NIS) and thyroid peroxidase (TPO) sensitivity and cognitive disorders in humans. Data collected from animal studies may or may not reflect the potency and duration of compound-induced effects due to species differences in thyroid stimulating hormone receptor (TSHR), sodium/iodide symporter (NIS) and thyroid peroxidase (TPO) sensitivity. Discovered TDCs may occur through various modes of action (Toxicol. Sci. 2020;174:63-78). The purpose of this study was to validate cryopreserved primary human thyrocytes (PHT) which are isolated from normal thyroids for TDC screening. The focus on effects of TDCs has been increasing over the past 20 years due to species differences in thyroid stimulating hormone receptor (TSHR), sodium/iodide symporter (NIS) and thyroid peroxidase (TPO) sensitivity and cognitive disorders in humans. Data collected from animal studies may or may not reflect the potency and duration of compound-induced effects due to species differences in thyroid stimulating hormone receptor (TSHR), sodium/iodide symporter (NIS) and thyroid peroxidase (TPO) sensitivity. Discovered TDCs may occur through various modes of action (Toxicol. Sci. 2020;174:63-78). The purpose of this study was to validate cryopreserved primary human thyrocytes (PHT) which are isolated from normal thyroids for TDC screening.

**MATERIALS & METHODS**

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**Isolation of Human Thyroid Follicular Epithelial Cells**

THCs were isolated from healthy thyroid tissues as previously described and cryopreserved using CryoMed (Cryomeds, LLC). Dispersed thyrocyte monolayers and 3D microtissues were prepared with cryopreserved PHT on TC-treated and Matrigel® (Corning) coated 96-well plates respectively. The 3D microtissues were treated with 0 or 1 mIU/mL TSH. TG secretion and T4 synthesis were measured at select time points in collected media samples using ELISA kits (Thyrogen Diagnostics, Inc.) T4 synthesis was measured using an automated cell counting system (TC-20, BioRad) and 0.4% trypan blue staining.

**RESULTS**

**3. INTRODUCTION**

The purpose of this study was to validate cryopreserved primary human thyrocytes (PHT) which are isolated from normal adult donor thyroids at LifeNet Health in a 2D and 3D microtissue format as an in vitro model for determining effects of potential TDCs on various T4-synthetic pathways. The focus on effects of TDCs has increased over the past 20 years and EPA urges the development of reliable and relevant non-animal, non-rodent approaches. Due to extensive passage in culture and tumor specific origin, the current immortal thyroid cells typically do not retain the essential characteristics of native thyroid function and signaling pathways. Therefore, there is a need to develop an in vitro primary human thyroid model isolated from normal thyroids for TDC screening.

**CONCLUSIONS**

- ≥80% of the recovered PHT were viable at post-thaw.
- KRT7 and TGF expression confirmed that cryopreserved human thyrocytes retain thyroid follicular epithelial cell markers.
- Cryopreserved thyrocytes formed 3D thyroid microtissues (diam: 5mm-15mm) at 14 days of culture.
- TSH promoted TG secretion and T4 synthesis.
- All qualified kits maintained native thyroid functions: TG secretion and T4 synthesis in 3D cultures.
- TPO inhibitors, methimazole and 6-propyl-2-thiouracil inhibited T4 synthesis of thyroid microtissues in a dose-dependent manner.
- Cryopreserved thyrocytes represent a useful in vitro tool for the testing of TDCs.

**REFERENCES/Acknowledgements**


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**TABLE 1.** Donor specifications.