

ARTICLE IN REVIEW:

Successful Evaluation of Endocrine Disruption Potential of Cosmetics using Cryopreserved Human Thyrocytes as part of the Skin-Liver-Thyroid Chip3 model

PUBLICATION: Frontiers in Pharmacology, 2023

TITLE: Development of a microphysiological skin-liver-thyroid Chip3 model and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions¹

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STUDY DESIGN: Preclinical (*in vitro*)

SUMMARY: The TissUse HUMIMIC Chip3 model, is a non-animal model composed of Phenion® full thickness skin, liver spheroids, and thyroid follicles [either fresh [Provio] or cryopreserved [LifeNet Health or TissUse] human thyrocytes]. Due to the limited availability of fresh thyrocytes, researchers switched to using cryopreserved thyrocytes to generate thyroid follicle-like organoids. Despite their smaller size, the cryopreserved thyrocyte-derived thyroid follicles formed aggregates of cells with clear presence of lumen, typical of thyroid follicles *in vivo*. This Chip3 model was used to evaluate exposure to cosmetic ingredients, daidzein and genistein, known to inhibit thyroid hormone production, whereby changes in thyroid hormones, thyroxine (T_4) and 3,3',5-triiodothyronine (T_3), were indicative of endocrine disruption. Prior to comparing the dose effects, the researchers confirmed the inhibitory activity of these compounds. Results showed that metabolism occurred via a detoxification pathway whereby both inhibitory activities were decreased after a 24-hour preincubation with liver spheroids. When evaluating dose effects, the highest concentration of daidzein that maintained T_4 and T_3 levels was 0.235 $\mu\text{g}/\text{cm}^2$, a value consistent with similar products considered safe. Additionally, despite different hormone levels measured, the ratios of $T_4:T_3$ were similar between cryopreserved and fresh human thyrocytes and resembled that of healthy human serum. Therefore, the Chip3 model, through “the incorporation of the relevant exposure route (dermal), metabolism in the skin and liver, and the bioactivity endpoint” (use of cryopreserved or fresh human thyrocytes), represents a viable alternative to animal models to provide a relevant assessment of toxicodynamic effects over time, similar to those *in vivo*.

Cryopreserved human thyrocytes preserved hormone production with $T_4:T_3$ ratio similar to freshly isolated human thyrocytes

Cryopreserved and fresh human thyrocytes both preserved hormone production. Despite lower hormone levels with thyrocyte-derived follicles, the level was still high enough to evaluate the necessary endpoints. Additionally, the ratios of $T_4:T_3$ for both were similar and resembled the unbound ratio in the serum of healthy humans.

Cryopreserved human thyrocytes can address various limitations observed with fresh human thyrocytes

The limited supply of fresh thyroid follicles in this study was overcome with the use of thyrocyte-derived thyroid follicles which “formed aggregates of cells with the clear presence of lumen.” This switch “...allowed easy scheduling of experiments and the prequalification of thyrocytes from healthy donors before using them in the Chip3, which is not possible using freshly isolated thyroid follicles.”

A hormone-competent model that serves as a viable alternative to animal models

This human cell-based model has the advantage of mimicking a relevant safety profile and conditions “closer to those *in vivo* than 2D cell/tissue assays lacking metabolic function.” Therefore, it presents a viable alternative to animal models for evaluating cosmetic ingredients and other endocrine disrupting compounds, especially in Europe whereby the use of animal models for evaluating the safety of cosmetics ingredients is banned.

Skin-Liver-Thyroid Chip3 Model

Fig 1. The set up of the skin-liver-thyroid Chip3 model.

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Reference

1. Tao TP, Maschmeyer I, LeCluyse EL, Rogers E, Brandmair K, Gerlach S, Przibilla J, Kern F, Genies C, Jacques C, Najjar A, Schepky A, Marx U, Kühnl J, Hewitt NJ. Development of a microphysiological skin-liver-thyroid Chip3 model and its application to evaluate the effects on thyroid hormones of topically applied cosmetic ingredients under consumer-relevant conditions. *Front Pharmacol*. 2023 Feb 8;14:1076254. doi: 10.3389/fphar.2023.1076254.

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