Constructing an *In Vitro* 3D Model of the Human Placenta

Michael Zheng, Seerat Bhatia, Chidambra D. Halari, & Peeyush K. Lala

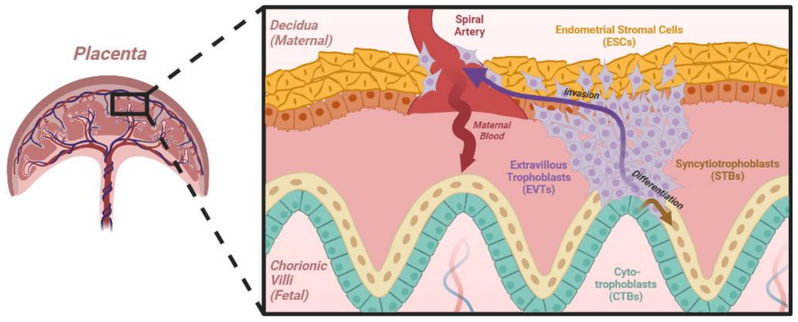
Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

Placental Structure

A healthy pregnancy is dependent on the **placenta**, a temporary organ that facilitates the exchange of key molecules between the maternal and fetal circulation systems.

The main fetal cellular constituents are **trophoblasts**. **Cytotrophoblasts (CTBs)**, differentiate into (a) **syncytiotrophoblasts (STBs)** that provide exchange and endocrine functions, and (b) **extravillous trophoblasts (EVTs),** which invade the uterus. EVTs remodel the spiral arteries to become wider, allowing increased maternal blood flow to the fetus.

The uterine endometrium undergoes remodelling to form the decidua, which involves the differentiation of **endometrial stromal cells (ESCs)** into decidual cells.



Previous *In Vitro* 3D Placental Models

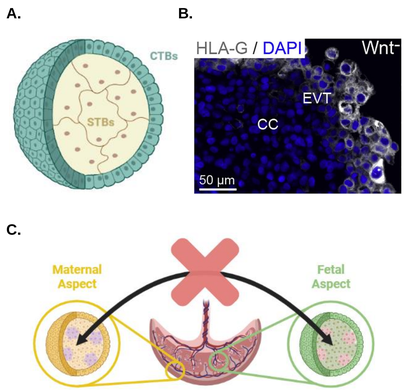


Figure 1. Drawbacks of previous *in vitro* 3D placental models. A. Reverse order of CTB and STB layers in hTSC organoids. **B.** Immunostaining of human leukocyte antigen G (HLA-G), an EVT marker, in hTSC organoid grown in medium lacking a Wnt stimulator (Wnt⁻ condition). This was reproduced from Haider et al. (CC BY-NC-ND license). **C.** Absence of in vitro placental models combining fetal and maternal aspects in 3D form.

Since investigating human placentas with non-invasive methods is challenging, comprehensive *in vitro* placental models are needed for in-depth studies.

Human trophoblast stem cells (hTSCs) are derived from first trimester CTBs, are self-renewing, and can differentiate into STBs and EVTs due to their naïve state.[1]

hTSC organoids displayed an inverse order of CTBs and STBs. To induce EVTs, hTSC organoids were grown in a specialized medium.[2,3]

While previously established *in vitro* 3D placental models are promising, **they do not adequately represent and combine fetal and maternal components.**

Objectives and Expectations

1. To promote cellular interactions between trophoblasts and endometrial stromal cells by apposing fetal and maternal spheroids
2. To characterize the distribution of trophoblast subpopulations and matrix markers in hTSC-derived spheroids

- **Our *in vitro* 3D placental models would partially recapitulate the morphology of the placenta *in situ,* but further progress can be achieved**

Spheroid Formation Technique

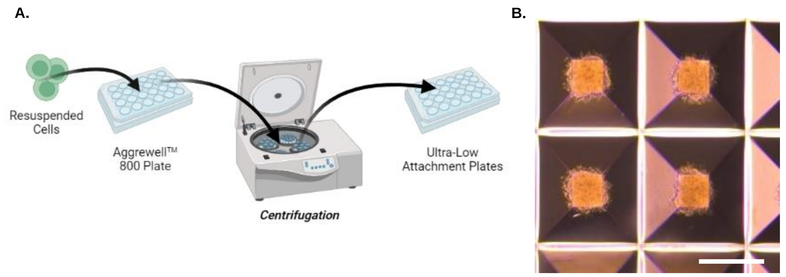


Figure 2. Methodology for forming spheroids. A. Resuspended cells were added to an Aggrewell™ 800 plate and centrifuged. Afterwards, the Aggrewell™ 800 plate was placed in the incubator for one to four days. The resulting spheroids were transferred into an ultra-low attachment plate to grow for an additional two to six days. **B.** HTR spheroids in an Aggrewell™ 800 plate 24 hours after centrifugation. Scale bar equals 200 µm.

- This technique allowed for relatively rapid production of spheroids for experimentation

Simulating the Fetal-Maternal Interface

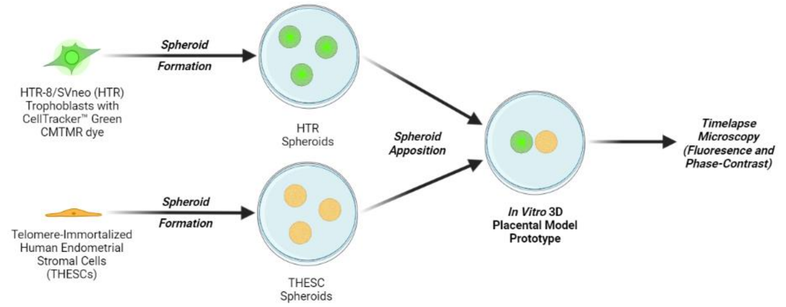


Figure 2. Approach for modelling interactions at the fetal-maternal interface. Spheroids made from HTRs and THESCs were apposed to develop the *in vitro* placental model prototype.

- HTR-8/SVneo (HTR) is a first-trimester trophoblast cell line[4]
- Detecting CellTracker™ Green CMTMR dye distinguished HTR spheroids from telomere-immortalized human ESC (THESC) spheroids in the timelapse microscope

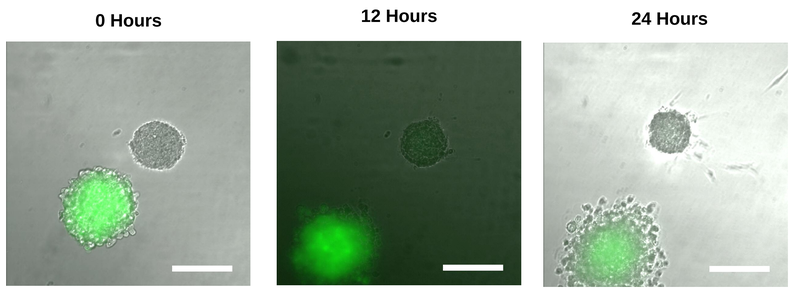


Figure 3. Interactions between HTR and THESC spheroids. Spheroids containing HTRs (previously incubated with CellTracker™ Green CMTMR dye) and THESCs (unstained) were plated together and monitored over the next 24 hours. Phase-contrast images were merged with ones detecting the fluorescent CellTracker™ Green CMTMR dye. Scale bar equals 200 µm.

- Cell migration (sprouting) from the HTR spheroid suggests EVT outgrowth
- **Cellular interactions between the HTR and THESC spheroids were not observed, which could be attributed to an inadequate juxtaposition**

Human Trophoblast Stem Cell Spheroids

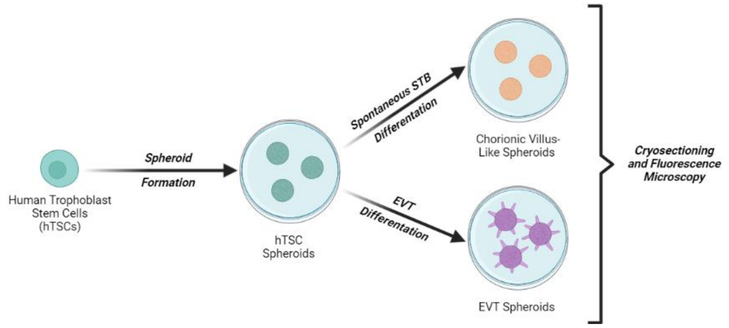


Figure 4. Approach for investigating the distribution of trophoblast subpopulations. Spheroids made from hTSCs were grown into chorionic villus-like spheroids and EVT spheroids.

- With this method, our lab has previously created chorionic villus-like hTSC spheroids with CTBs and STBs on the interior and exterior, respectively
- Culturing in EVT differentiation medium resulted in EVT-enriched hTSC spheroids
- **Ongoing efforts to replicate the above results and then examine matrix markers, whose presence would qualify them as organoids**

Discussion

- Our initial attempt of apposing HTR and THESC spheroids did not sufficiently replicate trophoblast invasion of the endometrial stroma
- Our model limitations include the absence of certain placental cellular constituents (e.g., maternal and fetal endothelia)
- **Our findings lay the fundamental groundwork for developing an *in vitro* 3D placental model that includes fetal and maternal aspects**

Future Directions

1. **To compare HTR and hTSC spheroids and determine which cell line would be more suitable for representing trophoblasts**
2. To incorporate all three trophoblast subpopulations within a single spheroid
3. To establish interactions between trophoblast and decidualized THESC spheroids
4. To create *in vitro* 3D models that simulate pathological placentas for novel therapeutic insights by altering the expression of decorin (DCN) in THESCs
   - DCN restrains trophoblast invasiveness[5] and its over-expression in decidual cells is associated with preeclampsia (PE) with or without fetal growth restriction (FGR),[6,7] conditions that both involve a hypo-invasive placenta

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All illustrated figures were made on BioRender.com.

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