Relevant *in vitro* and *ex vivo* assessments for small molecules and biologics

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I have no conflicts of interest to disclose

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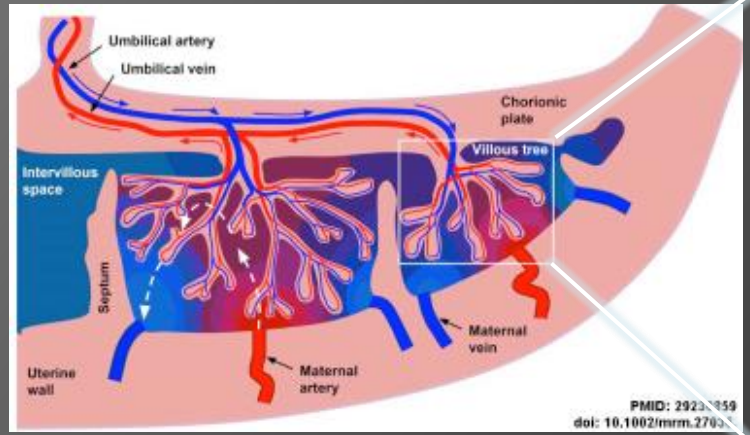
FDA Workshop: Evaluating Immunosuppressive Effects of In Utero Exposure to Drug and Biologic Products

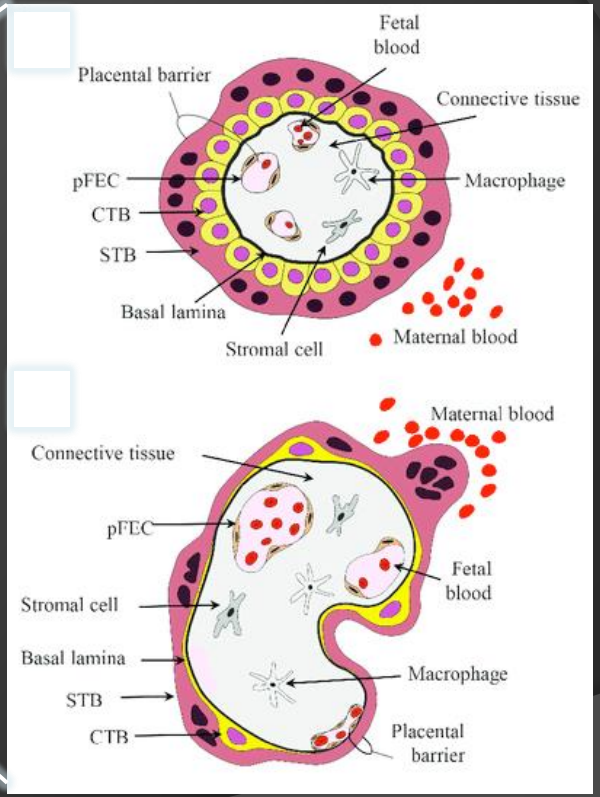
Today's talk

- Take a look at the barrier structure and transport systems
- Describe the current, standard assessment models
- Discuss the newer models which have, or are being developed

Barrier structure



Slator et al (2017)



First trimester

Third trimester

Chatuphonprasert et al (2018)

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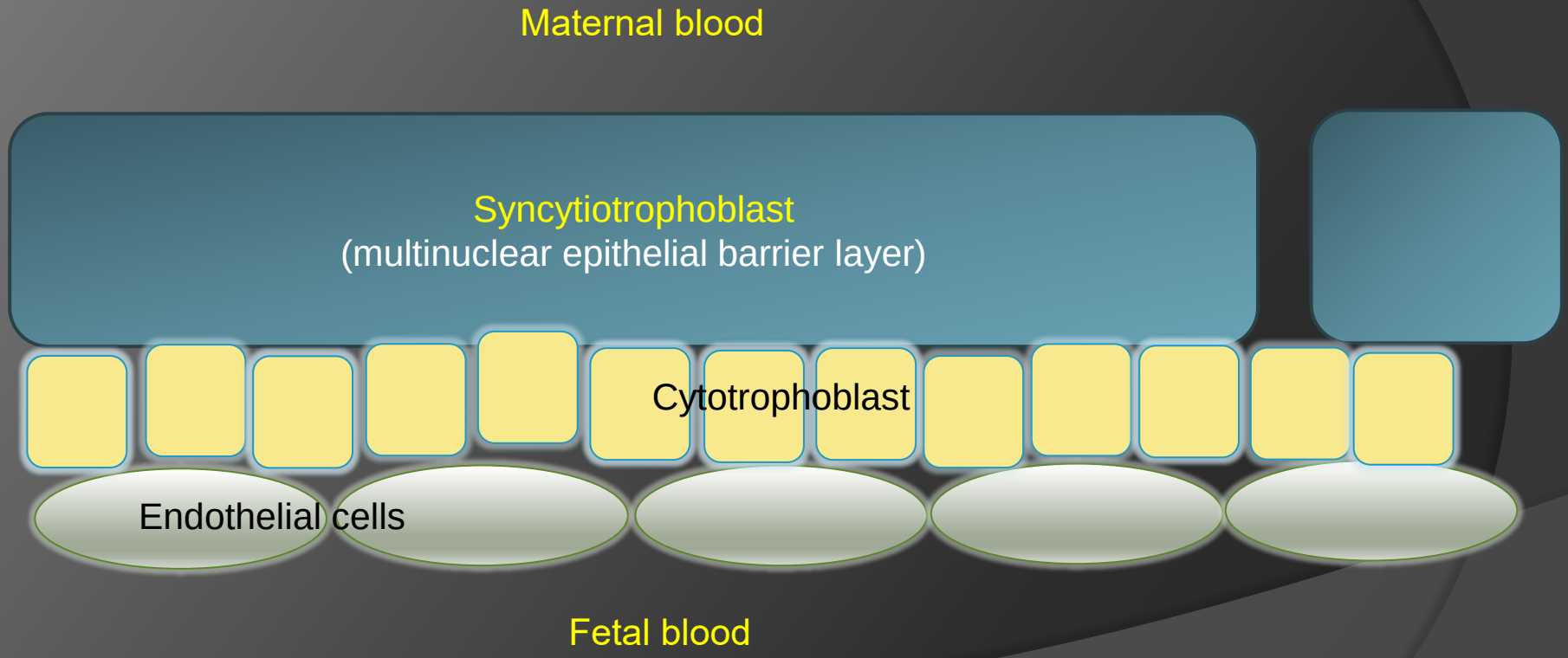
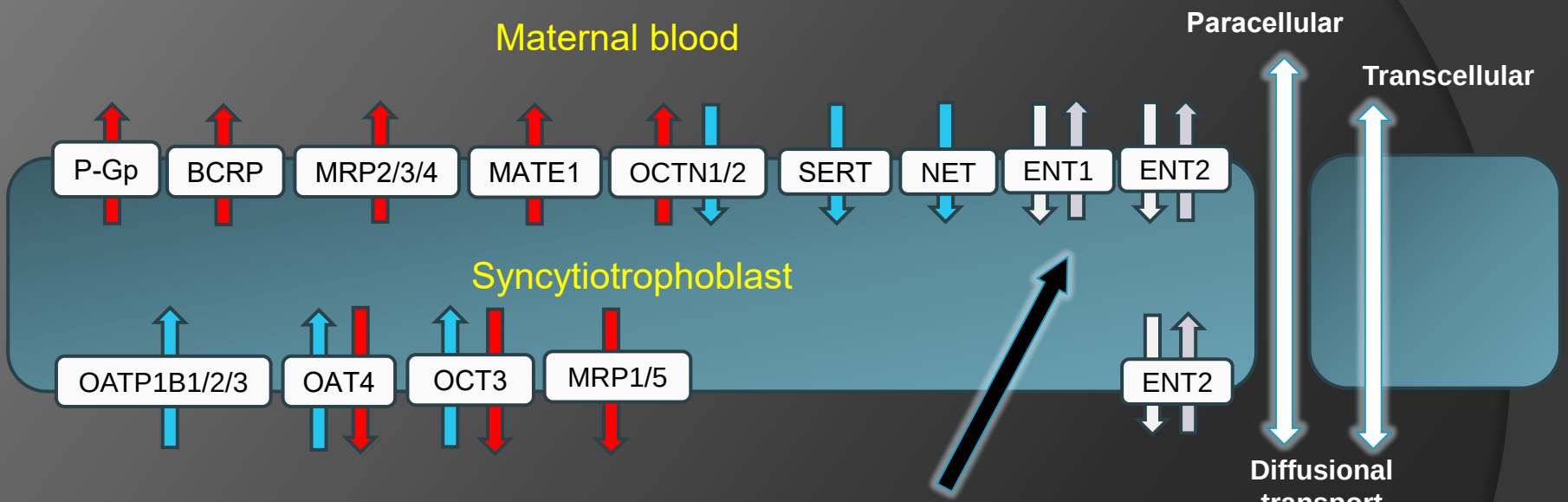
Barrier structure



Transport systems



SLC29A1/2/3 (ENT1/2/3) - equilibrative nucleoside transporters – JAK inhibitors

SLC28A2 (CNT2) – concentrative nucleoside transporter – JAK inhibitors

SLC19A1/2/3 (THTR-1/2) – thiamine/folate transporters – calcineurin inhibitors

Fetal blood

Current techniques – *in vitro*

In vitro

- Cell lines, e.g. JAR, JEG3, BeWo (choriocarcinoma),
  - Pros – immortal, proliferative, some morphologic similarities to primary cells
  - Cons – mostly triploid or tetraploid, substantial differences with primary cells in expression, epigenetics
- Transwell - permeable membrane separating fluid reservoirs
  - Pros – useful as a barrier transport model, can include extracellular matrix layer and other cell layers
  - Cons – only as good as the barrier cell utilized, potential for overgrowth when cells are grown to provide a confluent monolayer, possibly limited degree of syncytialization

Current techniques – *ex vivo*

Ex vivo

- Syncytiotrophoblast membrane vesicles (microvillous, basal)
  - Pros – easily prepared and stored, faithful membrane representation
  - Cons – single membrane only, absence of regulation, no metabolism or interaction with intracellular components
- Primary cells, cytotrophoblast (CTB)
  - Pros – diploid, gene / protein expression representative of the *in vivo* environment, can be differentiated to multinuclear syncytiotrophoblast cells (STB)
  - Cons - non-proliferatve (term), genetically dissimilar, poor barrier models in 2D culture, limited duration of culture

Current techniques – *ex vivo*

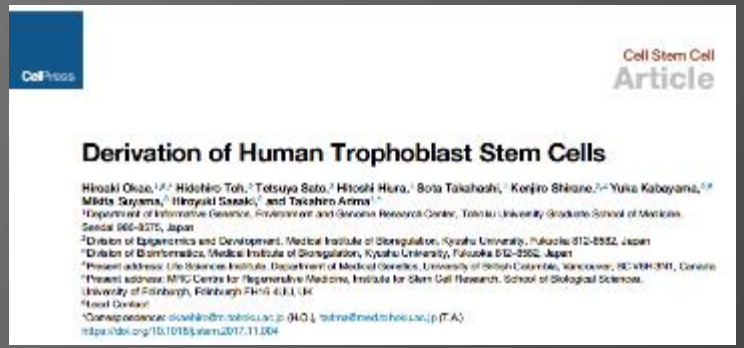
Ex vivo/contd

- Explants
  - Pros – full structural and compositional representation, potential for superfusion,
  - Cons – no fetal circulation, cannot be manipulated, rapid degeneration / limited duration
- Lobule perfusion
  - Pros – full structural and compositional representation, maternal and fetal circulations, possible to use samples from pathological pregnancies
  - Cons – black box system, limited to analysis of fetal and maternal circulation outputs, , cannot be manipulated, limited duration, limited to 3rd trimester

More recent technologies

- Trophoblast stem cells (TSC)
- Trophoblast organoids (TB-O)
- Placenta-on-a-chip (POC)

Trophoblast stem cells (TSCs)


Cell Stem Cell
Article

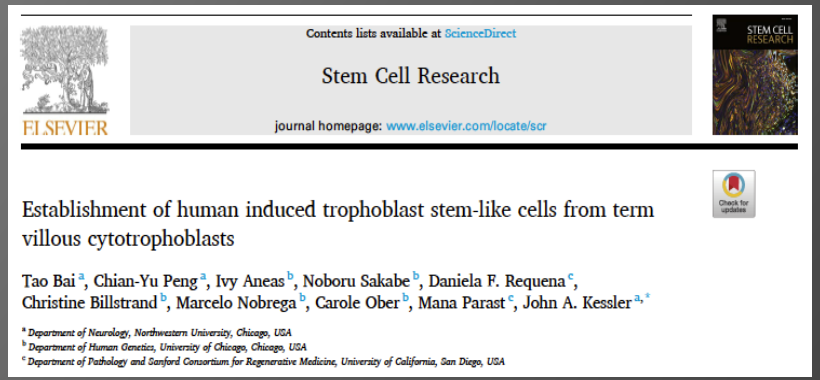
Derivation of Human Trophoblast Stem Cells

Okae et al (2017)

Derived from first trimester placental tissue, which is not always available.

- First trimester villous cytotrophoblast cells (vCTB) are used to generate “cytotrophoblast-like” stem cells (TS^{CT}), a proliferative line with characteristics similar to primary CTB
- TS^{CT} can be differentiated into extravillous trophoblast cells (TS^{EVT}) and syncytiotrophoblast cells (TS^{ST}) using specific cytokines and pathway inhibitors
- TS^{CT}, TS^{EVT} and TS^{ST} have transcriptomes/methylomes similar to primary equivalents
- TSCs can be stably manipulated using siRNA, CRISPR, etc.
- TS^{CT} can be stored frozen and can be utilized for > 50 passages

Trophoblast stem cells (TSCs)



Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Establishment of human induced trophoblast stem-like cells from term villous cytotrophoblasts

Tao Bai [a], Chian-Yu Peng [a], Ivy Aneas [b], Noboru Sakabe [b], Daniela F. Requena [c], Christine Billstrand [b], Marcelo Nobrega [b], Carole Ober [b], Mana Parast [c], John A. Kessler [a,*]

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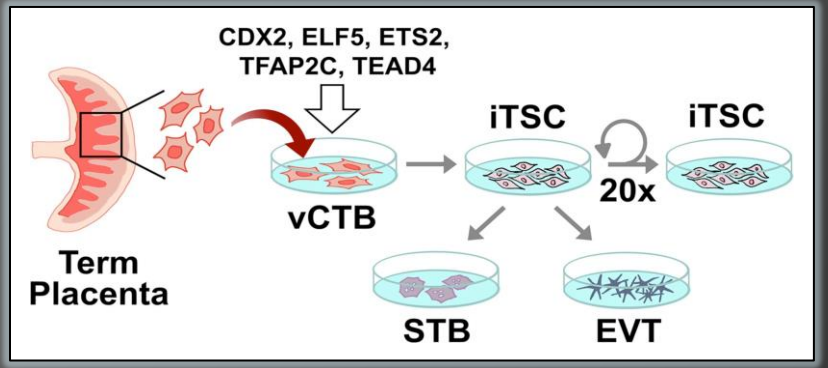


Bai et al (2021)

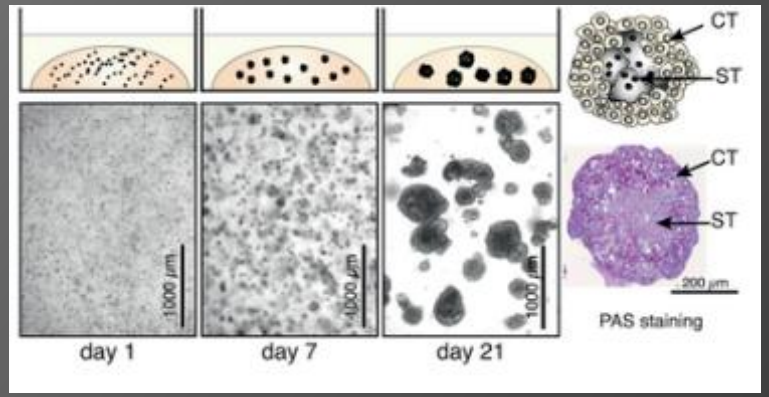
- Starting material readily available
- Proliferative cultures
- High degree of similarity to *in vivo* primary cell
- Possibility of using cells from pregnancy pathologies

TSCs are maintained within a specific culture system only by employing a specific range of cytokines and pathway inhibitors.

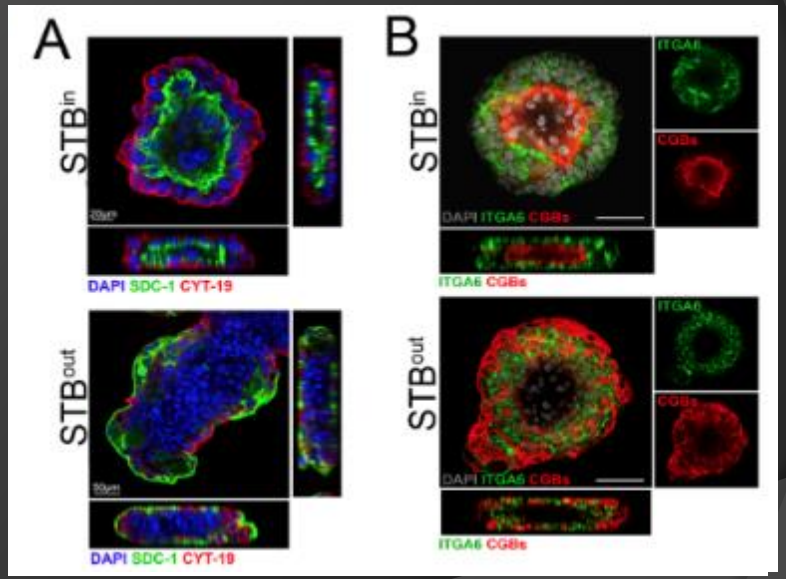
Nevertheless, these are much better models than the choriocarcinoma and transformed cell lines currently in use

Placental organoids

Human trophoblast organoids (TB-ORGs) are a three-dimensional *ex vivo* culture model that can be used to study various aspects of placental development, physiology and pathology.

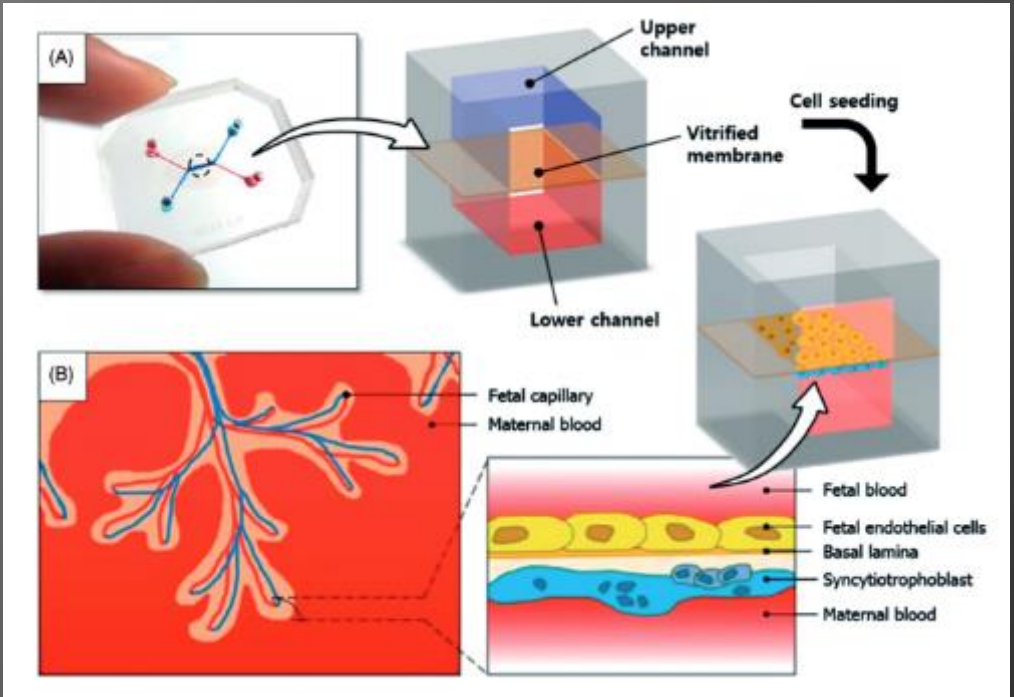


Haider et al (2018)



Shannon et al (2024), Haig et al (2024)

Placenta-on-a-chip (POC) – basic structure



Lee et al (2015)

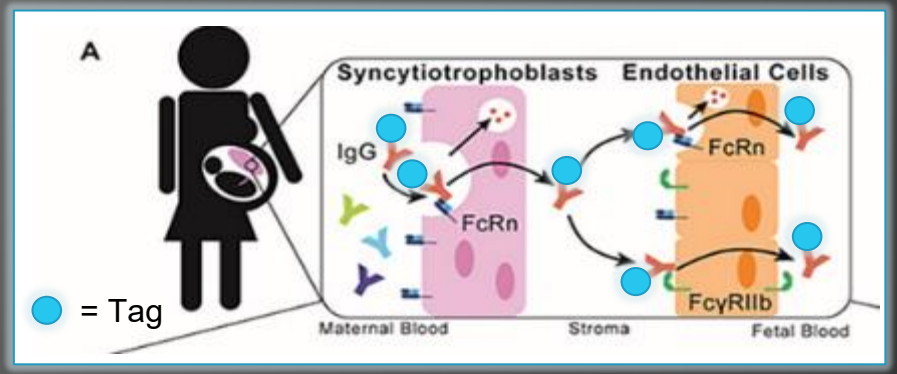
While the original POC models employed cell lines such as BeWo to mimic the syncytial layer barrier, la models are now using various TSC cells which can be differentiated in multi-nuclear STB.

In addition, these models now incorporate other layers including extracellular matrix elements, endothelial cells

- Multiple cell layers ✓
- Maternal and fetal flow ✓
- Stable ✓
- Useful duration ✓
- Capable of manipulation ✓
- Use of carriers ✓

Placenta-on-a-chip – biologics

The placenta-on-a-chip model lends itself to useful modeling of the transfer of biologics via the FcRn system. While tagging the biologic, it is also possible to manipulate the components of the POC system to examine the role of individual components, interactions with plasma proteins and other aspects controlling transfer of biologics.

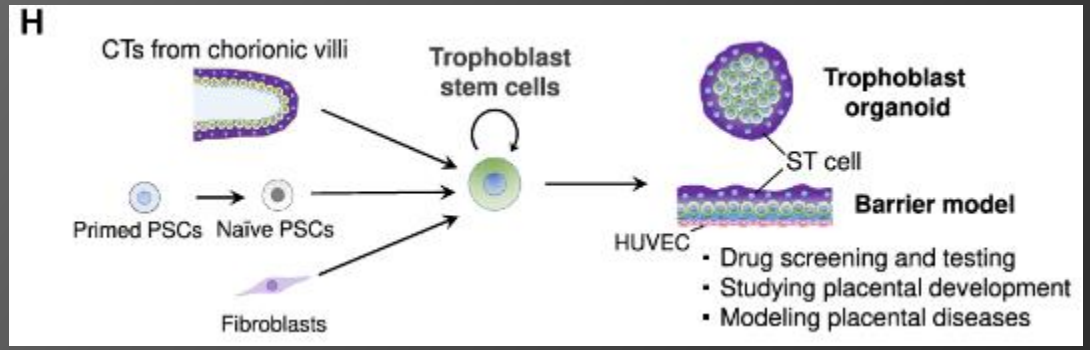


Adapted from Wessel & Dohlatshahi et al (2023)

- Endocytosis ☑
- Exocytosis ☑
- Degradation ☑
- Endothelial interaction ☑

Placental barrier models

It is now possible to to use TSC to create barrier models from a variety of sources containing STB and endothelial cells in the appropriate orientation for measuring uptake and reflux of drugs and biologics across a barrier layer



Hori et al (2024)

The challenges now are to improve these models with more components of the villous structure (e.g. CTB, immune cells) and to develop culture conditions which ultimately do not rely on specific cytokines and inhibitors.

The future

- New models have or are being developed that more faithfully reproduce the complex features of the human placental barrier
- Rather than a black box which is incapable of being manipulated, the elements in these models are defined, controlled and can be manipulated to test specific characteristics
- The combination of these models, with new methods of *in vitro, ex vivo* and *in vivo* assessment, will provide the means to determine the transfer and metabolism of drugs and biologics.

Supported by

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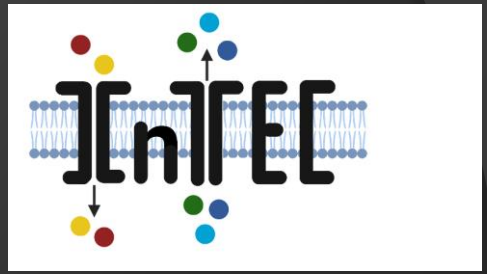

NIH Integrated Transporter Elucidation Center

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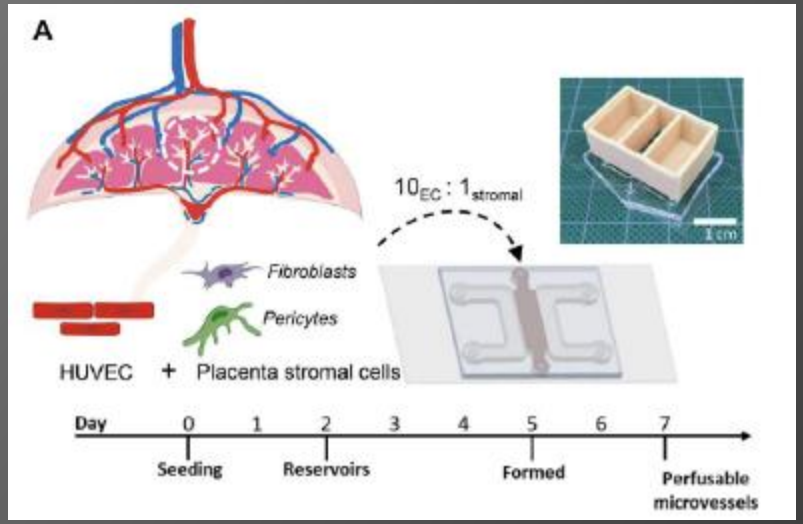
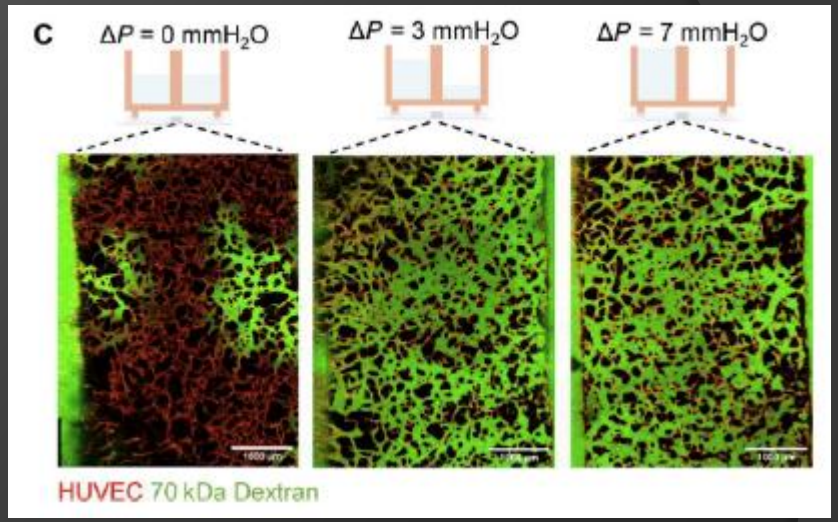
Placental microvessel perfusion





Cherubini et al (2023)

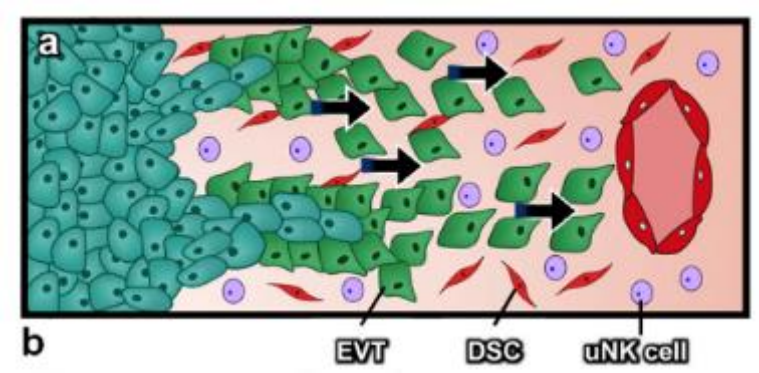
Flow over a base of human umbilical vein endothelial cells (HUVEC), combined with pericytes, fibroblast and stromal cells produces a network of microvessels which can be perfused. This provides a model for another component of the placental barrier, the endothelial layer of the microvessels. The potential exists to combine this with other models to more accurately simulate the placental barrier.

Means of assessment

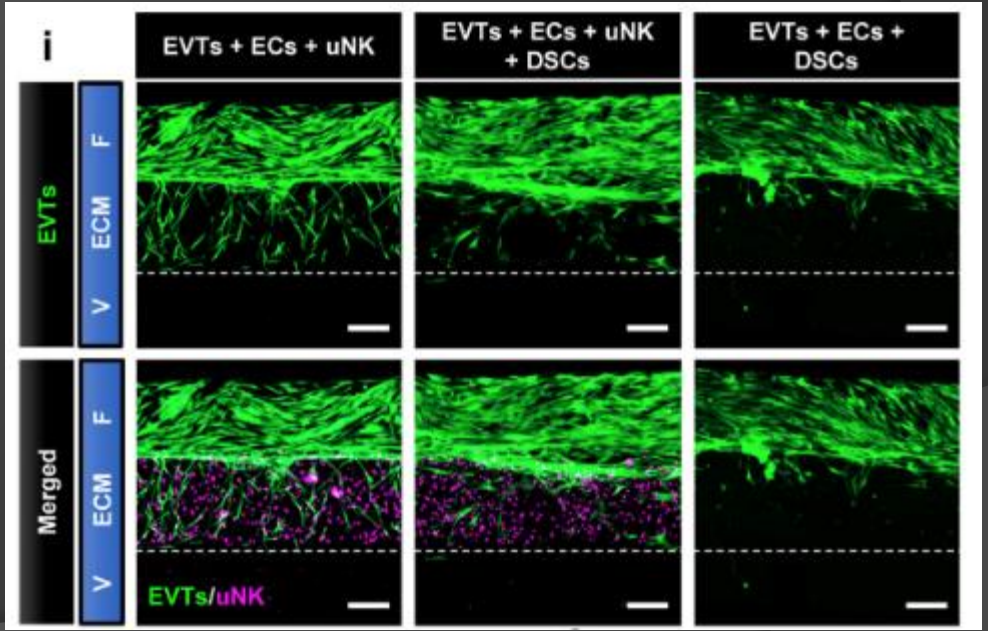
These apply to assessment of effects on the placenta

- TRIC (Trophoblast retrieval and isolation from the cervix)
- Extracellular vesicles (EV) in the maternal circulation
- Cell-free fetal DNA in the maternal circulation (cffDNA)
- Fetal (nucleated) red blood cells in the maternal circulation
- (Placental stromal) Hofbauer cells in the maternal circulation

Placenta-on-a-chip – multicellular

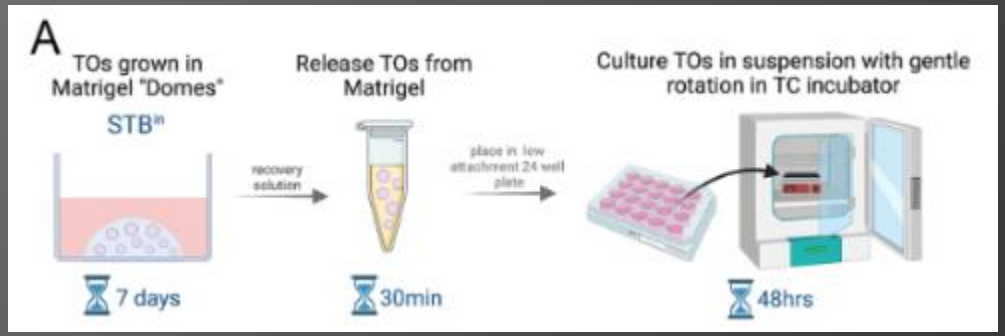


Park et al (2022)



Placental organoids

But recently, methods have been devised to generate right side out organoids, i.e. a syncytial shell over CTB



Yang et al (2024)

Comparison of TB-ORGs with inside-out orientation (STB^{in}) and outside-out orientation (STB^{out})