Recapitulating the human placental barrier with trophoblast stem cells and a microfluidic device

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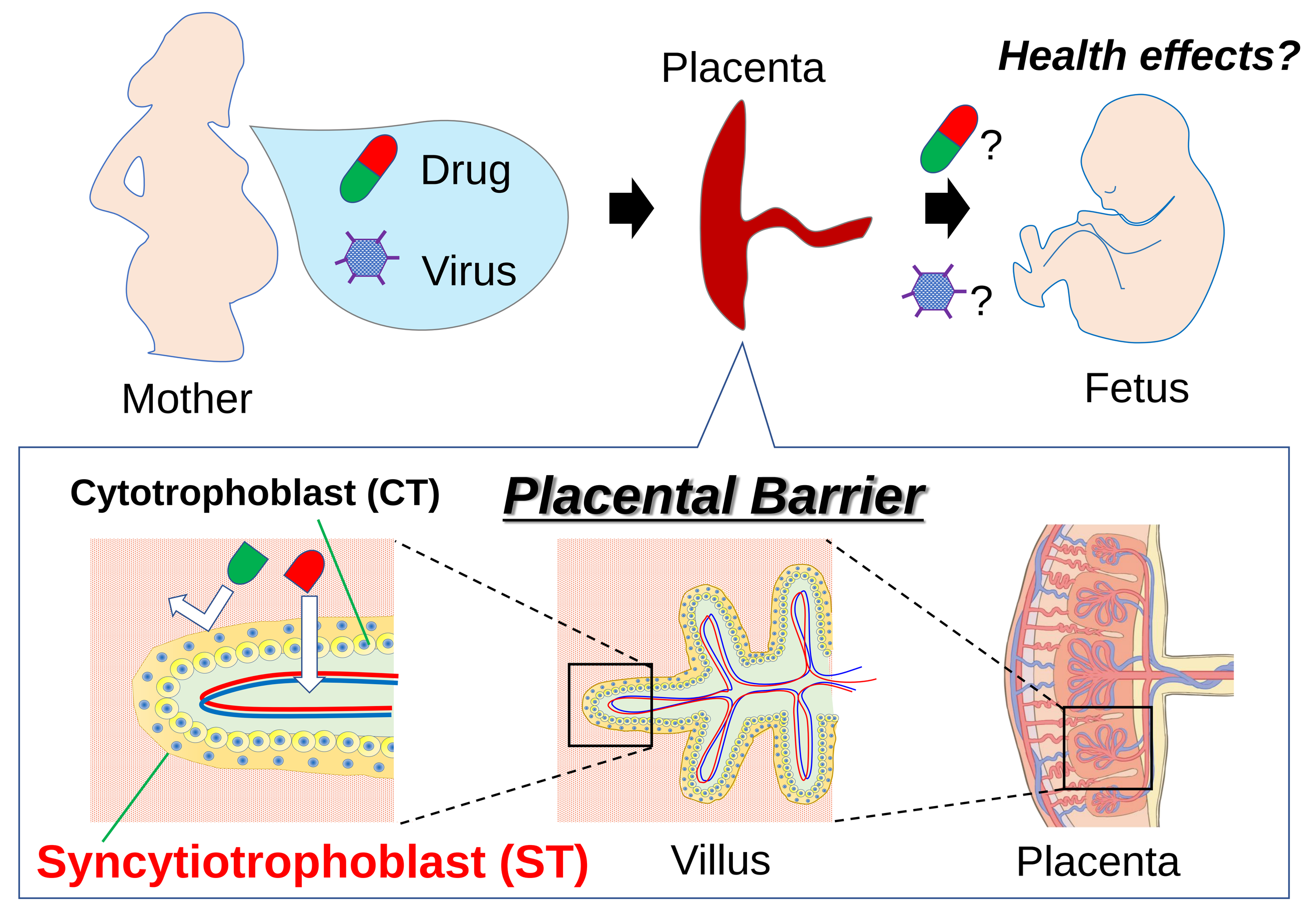
INTRODUCTION

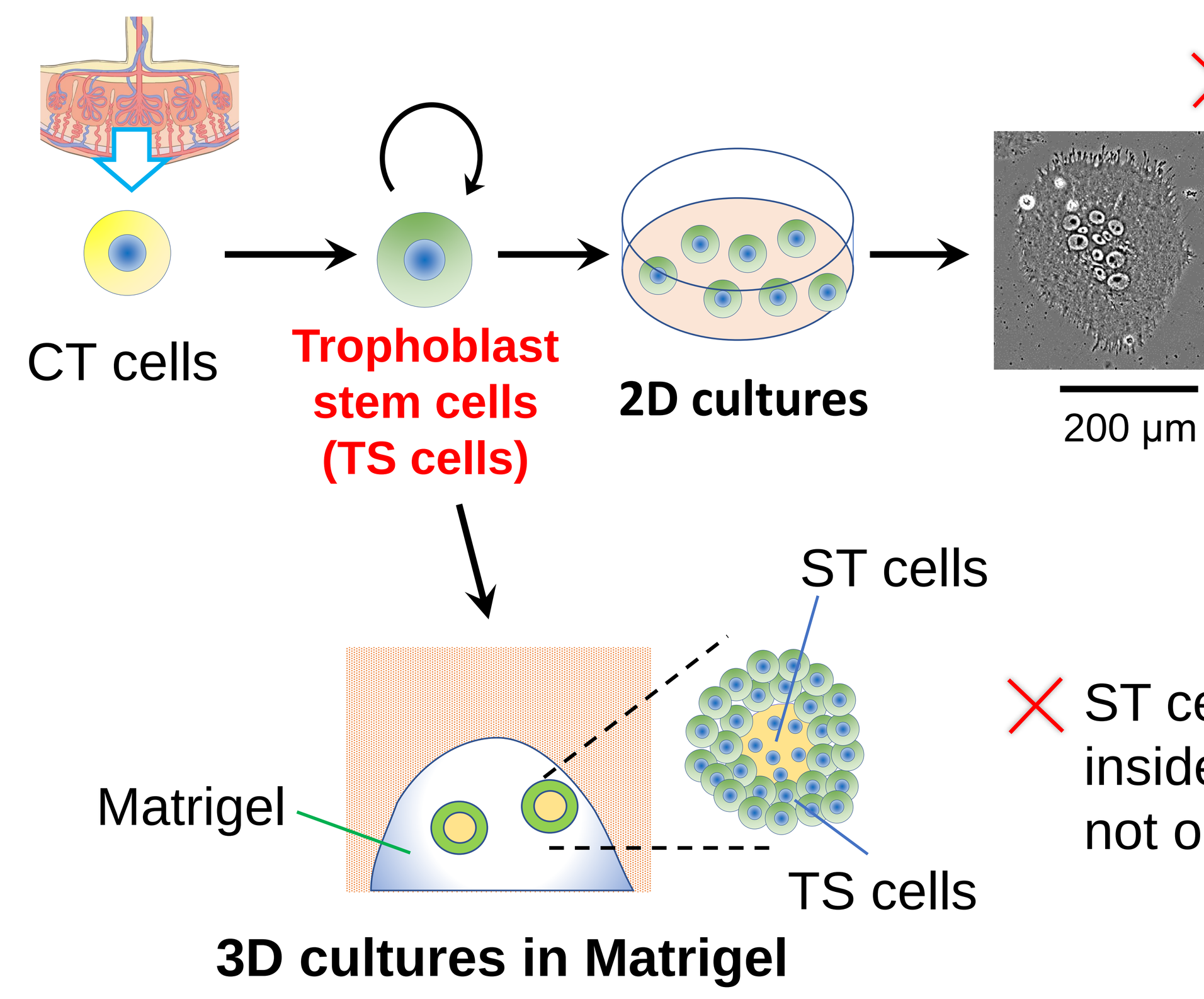


[The placenta and its structure]
The placental villus plays a critical role in the barrier function against xenobiotics including drugs. The surface of the villus is directly interacting with maternal blood. The villus consists of two kinds of trophoblast linage cells: syncytiotrophoblast (ST) and cytotrophoblast (CT) cells. CT cells can fuse and give rise to ST cells, which form the outer layer of the villi and serve as the barrier.

It is difficult to predict accurately what chemicals or viruses can pass the human placental barrier.

The placenta is structurally different between humans and experimental animals.

How to make the placental barrier like the human placenta?

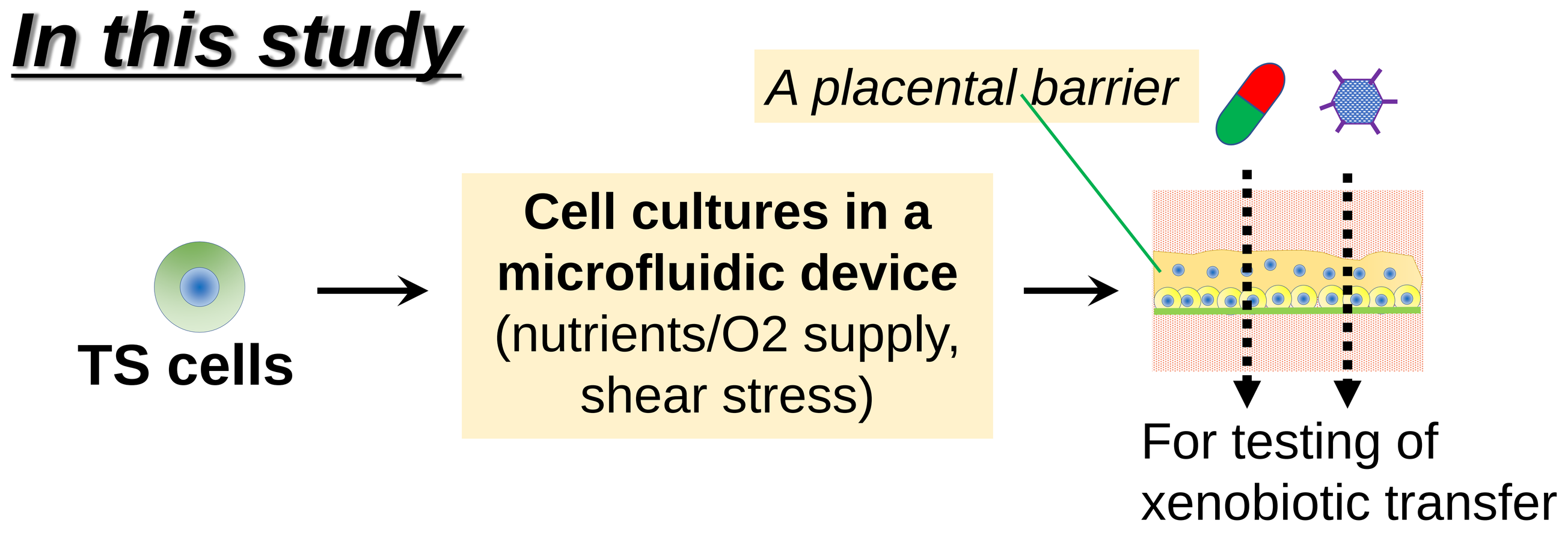


In 2D cultures, TS cells can differentiate into ST cells, but they cannot form the villi structure.

ST cells were formed inside organoids but not outside.

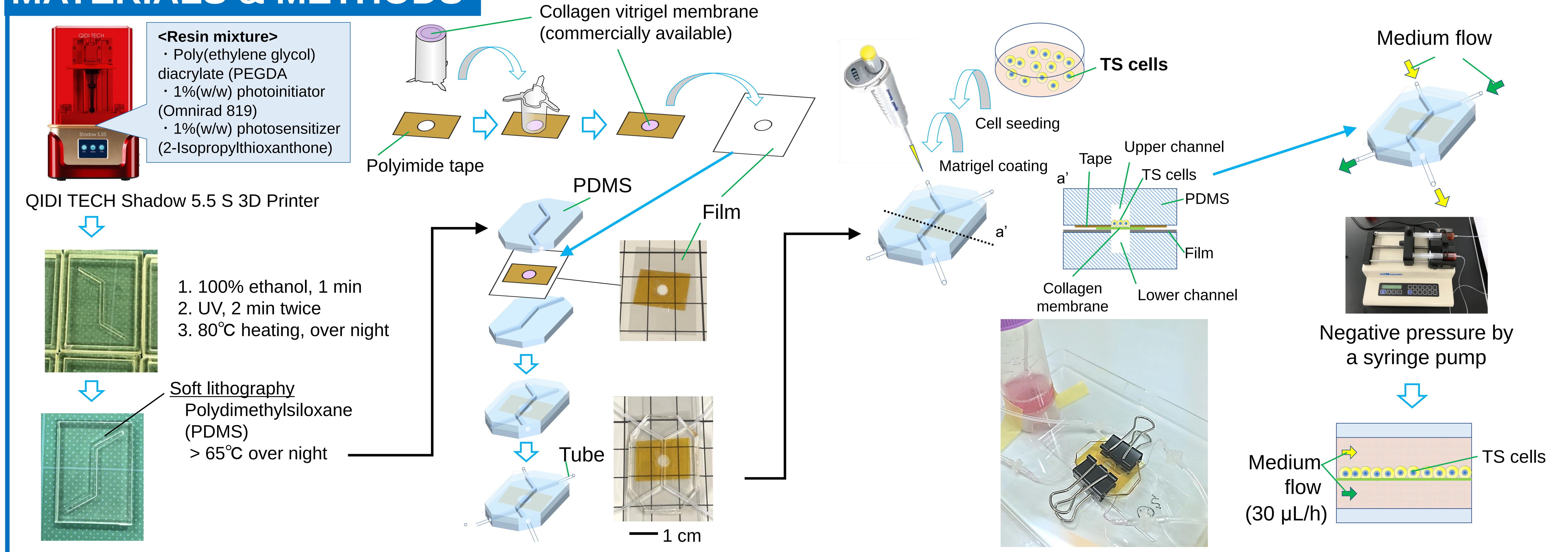
[Backgrounds]
The development of human ST barrier models has been desired to predict the transfer of xenobiotics to the fetus through the barrier. Recently, human trophoblast stem (TS) cells that have comparable functions to CT cells and can be maintained for a long time were established from our group [1]. In 2D cultures with conventional plates, TS cells can differentiate into ST cells. However, it has not been easy to construct a trophoblastic bilayer like the villi's surface and use it to assess drug permeability.

In this study



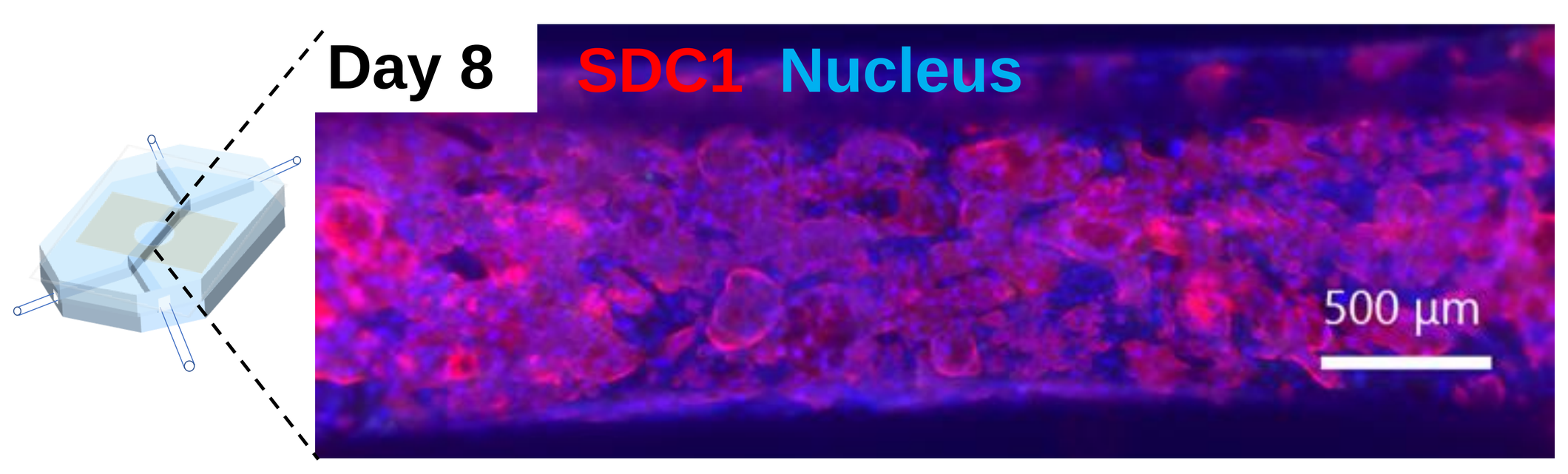
This study presents a microfluidic device with TS cells, which can provide cell constructs with a structure like the human placental villi.

MATERIALS & METHODS

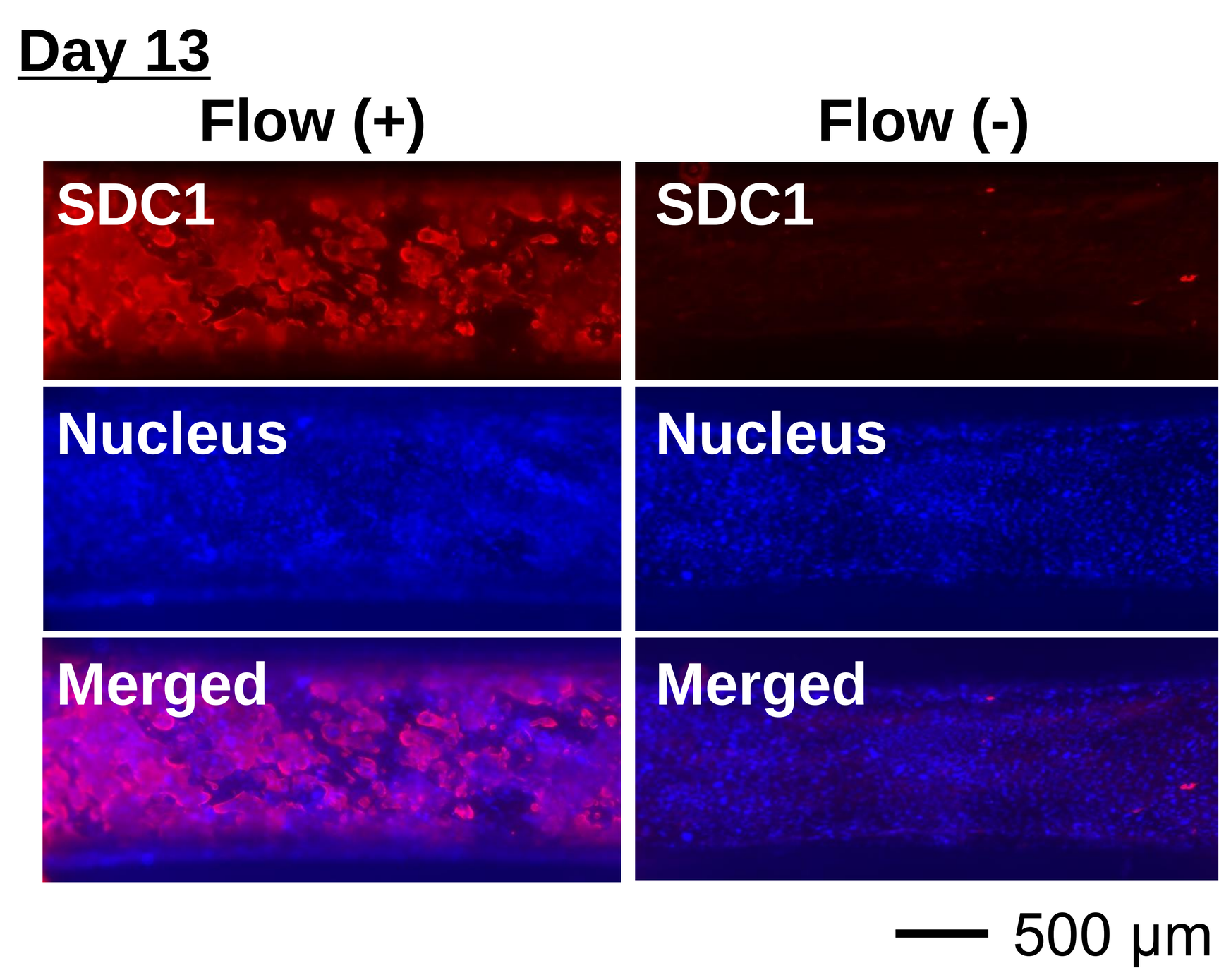


[Methods]
A microfluidic device was fabricated by soft lithography with polydimethylsiloxane (PDMS). A 3D-printed mold was made with a printer (QIDI TECH Shadow 5.5 S printer), poly(ethylene glycol) diacrylate (PEGDA, Mn: 250) as a resin, 1%(w/w) photoinitiator (Omnirad 819), and 1%(w/w) photosensitizer (2-Isopropylthioxanthone). The mold was used to cast the PDMS device. This PDMS device has the upper and lower channels to perfuse culture media. A vitrified collagen membrane, which serves as a permeable basement membrane, was placed between the upper and lower channels. To perform perfusion cultures, silicone tubes were connected to the device. The upper channel was coated with Matrigel to help cells to attach the upper channel. TS cells were seeded into the microfluidic device through an inlet of a silicone tube, and culture media were flowed at 30 μL/h using a syringe pump. After cell cultures, cells were fixed with paraformaldehyde, stained with antibodies, and then analyzed by fluorescence microscopy.

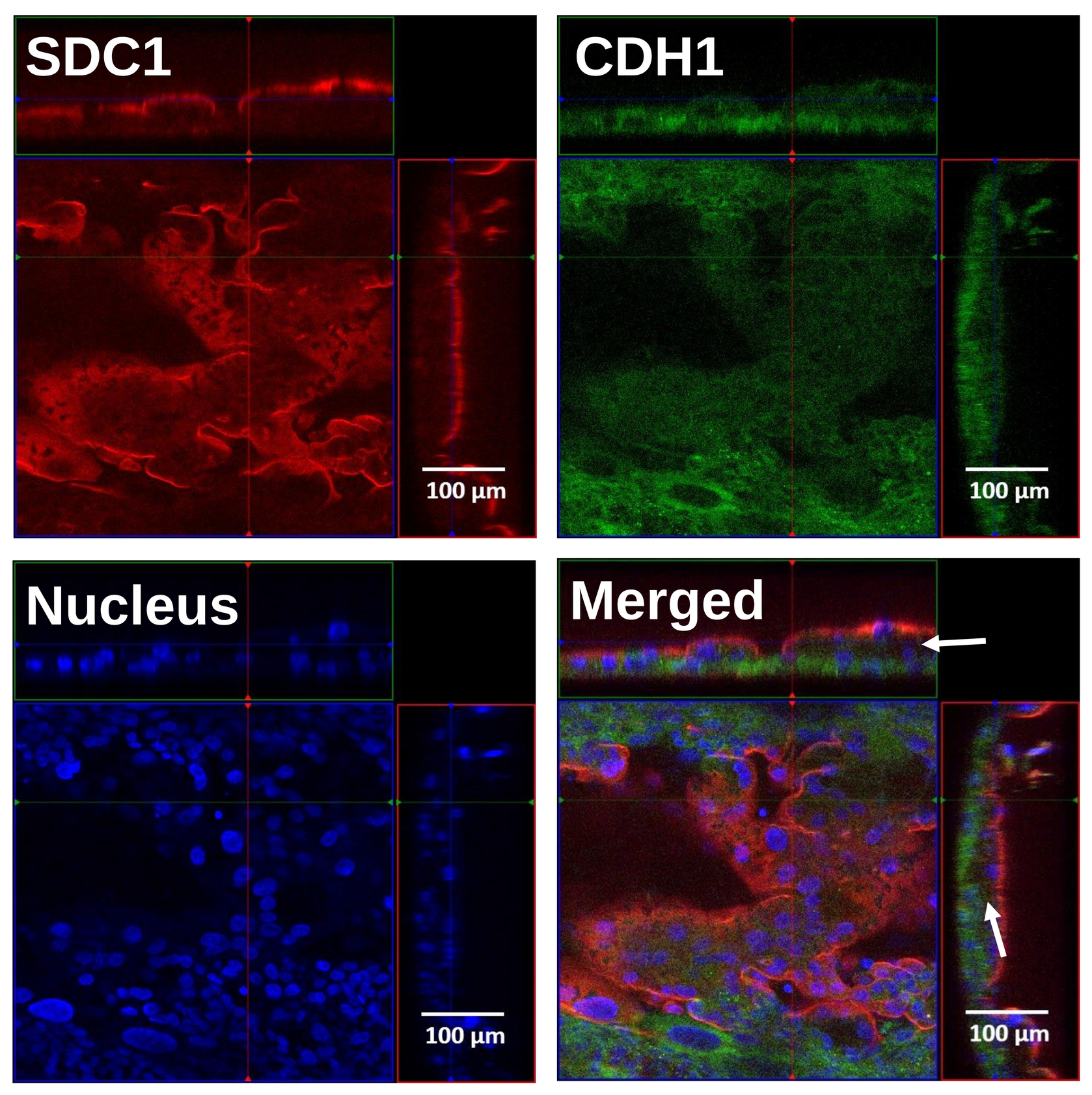
RESULTS



Medium perfusion enhanced differentiation of TS cells into ST cells, which was confirmed by immunostaining of syndecan-1 (SDC-1) (**left figure**). ST cells were hardly observed in a microfluidic device without the perfusion (**center figures**). The ST cells formed a single layer on undifferentiated cells, resembling the structure of the human placental villi (**right figures**).



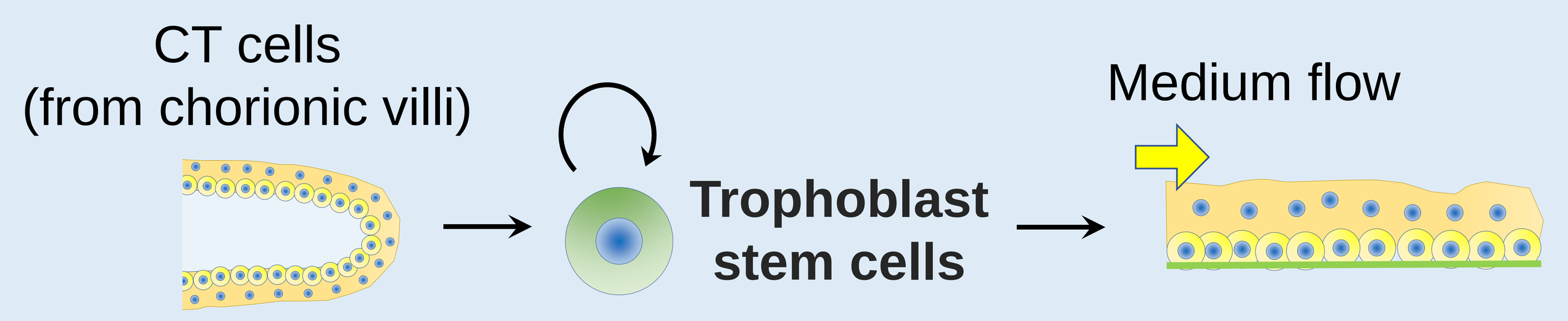
<Protein marker>
SDC1(Syndecan-1) for ST cells .
CDH1 (E-cadherin) for undifferentiated cells.



Arrows indicate a single layer of ST cells.

Summary

- **TS cells differentiated and formed a trophoblastic barrier in a microfluidic device.**



Applications
- **Drug screening**
- **Studying placental development**
- **Disease models**

Reference:
1. Okae H, et al. Cell Stem Cell. 2018 Jan 4;22(1):50-63