HUMAN-BASED PLACENTA-EMBRYO CHIP

FOR DEVELOPMENTAL TOXICITY ASSESSMENT OF NANOPARTICLES AND DRUGS

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INTRODUCTION

The general lack of **developmental and reproductive toxicity** (DART) studies led to a significant gap in understanding effects of **drugs, nanoparticles (NPs) and environmental pollutants** on the developing embryo creating significant safety concerns. Existing *in vitro* approaches to study embryotoxicity often neglect maternal-foetal interactions and placenta functions which can indirectly influence embryo-foetal development. This highlights the need for advanced models that can accurately reproduce the maternal-embryonic axis to allow the evaluation of both, direct and indirect effects of xenobiotics on the developing embryo. Thus, we established a **human-based co-culture on chip** integrating a **placental barrier model and an embryoid body** (EB) within a single microfluidic platform.

THE MODEL

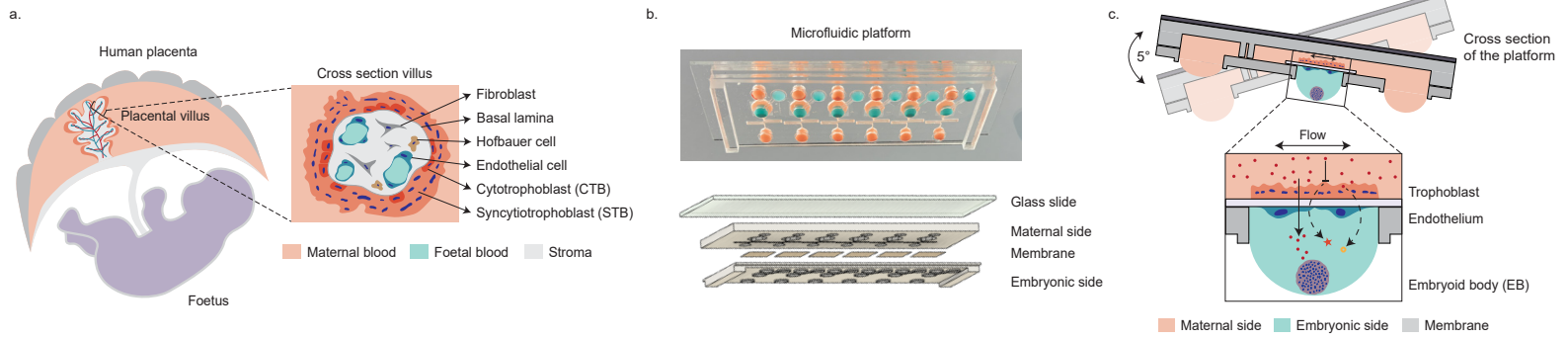


Figure 1. Placental structure and cellular composition. b) Partially open microfluidic chip in a **hanging drop configuration** enabling the co-culture of EBs in close vicinity to the placental barrier, allowing **no loss of short-lived intermediates**. The **pump-free device** (flow achieved by tilting) features two PDMS layers representing the maternal and embryonic side, separated by a porous membrane [1]. c) Cross section of the platform and biological model.

PLACENTAL BARRIER CHARACTERIZATION

Successfully achieved confluent co-culture barrier of **primary trophoblast cells**, isolated from human term placenta, and human placental vascular endothelial cells (HPVECs) for 9 days on chip. The placental barrier featured a spontaneously syncitialized trophoblast layer resemnling the in vivo characteristics: e.g. apical microvilli and hormone secretion (hCG).

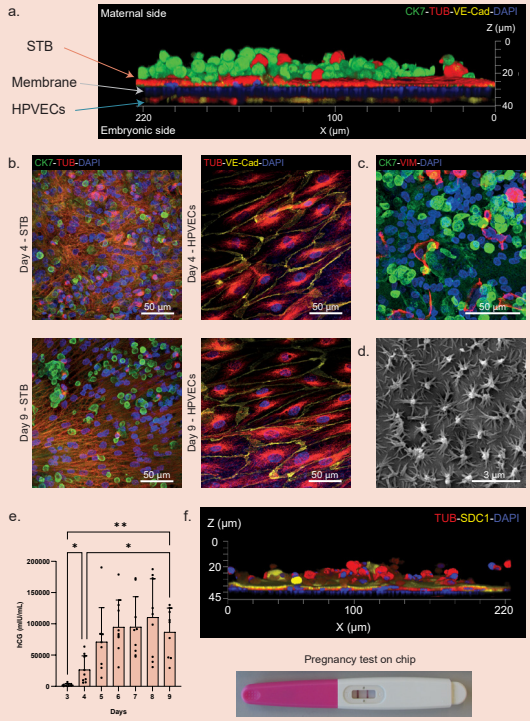


Figure 2. Confocal images of a) 3D rendering of the co-culture on both sides of the membrane; b) STB on the maternal side and endothelial cells (HPVECs) on the embryonic side on day 4 and 9 of culture. Staining: cytokeratin 7 (green, trophoblast marker), VE-cadherin (yellow, adherens junctions), tubulin (red, microtubules), DAPI (blue, nuclei). c) High purity of trophoblast layer, with only few mesenchymal cells (vimentin,red). d) Polarization of STB demonstrated by apical microvilli. e) Spontaneous syncytialization was confirmed by increase in hCG hormone production and staining for syndecan-1 (yellow, STB). Data represent mean and SD of n=8-9 biological replicates, Two-way mixed effects ANOVA with Greenhouse-Geisser correction and Tukey's post hoc multiple comparison test; ns= non significant, * p≤0.05, ** p≤ 0.01.

PLACENTAL TRANSPORT STUDIES

Barrier tightness was verified by size-dependent exclusion of passive diffusion markers.
Suitability for drug and NP transport studies is shown by similar ranking and translocation data compared to *ex-vivo* human placenta perfusion.

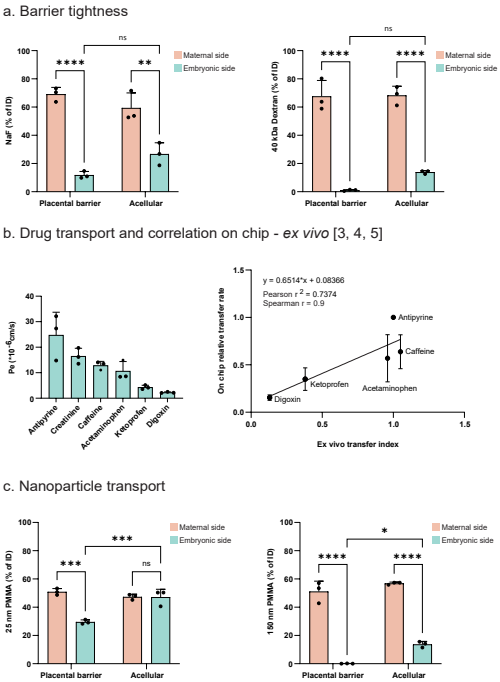


Figure 3. Placental barrier assessment for transport studies: a) Permeability studies with sodium fluorescein (NaF, 5µM 3h) and 40kDa FITC-Dextran (200µg/ml 3h). b) Right side: transport studies of 5 different compounds (antipyrine, caffeine, acetaminophen, ketoprofen, digoxin 100µM, 1h; creatinine 0.5 mg/ml, 1h), apparent permeability on chip; right side: comparison transfer on chip vs placenta perfusion data [3, 4, 5, 6]. c) 25nm (500µg/ml, 24h) and 150nm (50µg/ml, 24h) PMMA NP transport studies showing size-dependent transport and comparable data with *ex-vivo* placenta perfusion studies [2]. Data represent mean and SD of n=3 biological replicates. Two-way ANOVA with Tukey's post hoc test for multiple comparison; ns= non-significant, ** p≤ 0.01, **** p≤ 0.0001

References:
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ON CHIP EXPOSURE

As proof of concept, placenta-EB co-culture and EB monoculture were exposed to valproic acid (VPA), a known teratogenic neurotoxicant. Co-culture: the placental barrier remained functional, showing an active VPA transport, while EBs revealed disrupted ectodermal structures (neural rosettes and early neuronal differentiation). Monoculture: no or slight disruption of ectodermal structures suggesting a contribution of the placenta to the toxicity mechanism (e.g. active transport).

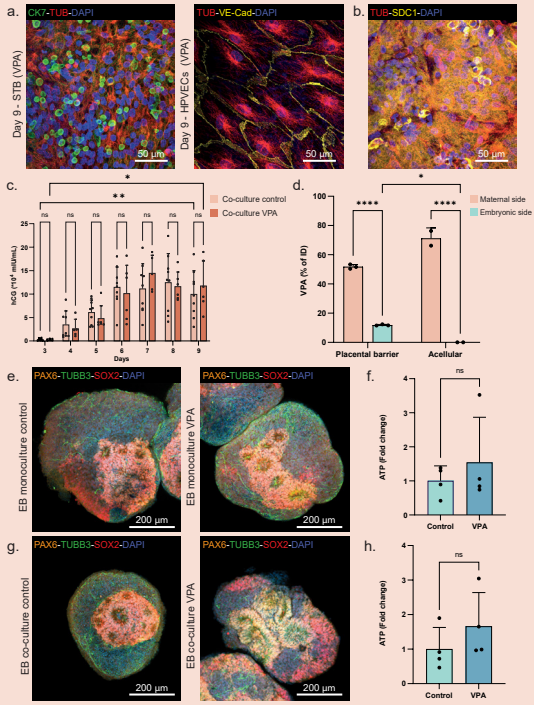


Figure 4. Confocal images of a) STB and HPVECs day 9 of culture after VPA exposure. Staining see fig.2. Syncytialization and endocrine function after exposure confirmed by syndecan-1 staining (yellow, STB) and c) hCG secretion. Data represent mean and SD, n=8-9. Statistics see fig.2. d) Transport of VPA (100µM, 1h): faster transport in presence of the placental barrier. Data represent mean and SD, n=2-3. Statistics: see fig.3. e) EBs day 9 monoculture on chip and f) ATP content. Data represent mean and SD, n=4. g) EBs day 9 co-culture with placental barrier on chip and h) ATP content. Data represent mean and SD, n=4. Stainings EBs: SOX2 (red, pluripotency marker, nuclei), PAX6 (orange, ectodermal marker, nuclei), βIII Tubulin (green, neuronal marker), DAPI (blue, nuclei). ATP: t-test with Welch's corrections, ns= non-significant.

OUTLOOK

These results highlight the promising potential of the human-based placenta-EB chip for the assessment of embryotoxicity and demonstrate a high predictive value for placental drug delivery and NP transport. The platform will help to gain early-stage data for drug and NP hazard assessment, contributing to the progression of the nanomedicine field while addressing industry gaps in developmental toxicity risk evaluation and the protection of sensitive popu-lations such as pregnant women and the developing foetus.

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