Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2016

Electronic supplementary information (ESI)

# A 3D co-culture microtissue model of the human placenta for nanotoxicity assessment

Carina Muoth[a], Adrian Wichser[a], Marco Monopoli[b,#], Manuel Correia[c], Nicky Ehrlich[c,§], Katrin Loeschner[c], Audrey Gallud[d], Melanie Kucki[a], Liliane Diener[a], Pius Manser[a], Wolfram Jochum[e], Peter Wick[a] and Tina Buerki-Thurnherr[a]

[a] Particles-Biology Interactions, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland.

[b] Centre for BioNano Interactions, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland.

[c] Research Group for Nano-Bio Science, Division for Food Technology, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark.

[d] Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Nobels väg 13, 171 77 Stockholm, Sweden.

[e] Institute of Pathology, Cantonal Hospital St.Gallen, Rorschacherstrasse 95, 9007 St. Gallen, Switzerland.

[§] current address: CAPEC-PROCESS, DTU, Technical University of Denmark, Søltofts Plads, 2800 Kgs. Lyngby, Denmark.

[#] current address: School of Pharmaceutical & Medicinal Chemistry, Royal College of Surgeons in Ireland, 123 Stephen's Green, Dublin 2, Ireland.

## 1. Experimental section

### 1.1. *Nanoparticle characterization*

The CuO NPs were characterized by transmission electron microscopy (TEM) by applying 10 µl of diluted CuO NP suspensions (10 µg/ml) in deionized water to a 200 mesh Formvar/carbon-coated copper grid (FCF-200-Cu, FORMVAR CARBON FILM, Electron Microscopy Science, USA). TEM micrographs were obtained using a Tecnai G2 T20 instrument (FEI, Eindhoven, The Netherlands) operated at 200 kV accelerating voltage. Surface area was determined by nitrogen adsorption/desorption on a Belsorp Mini II instrument (BEL, Japan), after pre-treating the samples under vacuum at 140°C for 0.5 h. The specific surface area of $TiO_2$ and CuO NPs was obtained from nitrogen adsorption isotherms, according to the Brunauer−Emmett−Teller (BET) equation. UV-Vis spectra of CdTe-COOH NP stock suspensions diluted in ultra-pure water or supplemented FGM to a final concentration of 100 or 250 µg/ml were recorded in the range of 400 - 800 nm using a Genesis 10S UV-Vis spectrophotometer (Thermo Scientific, Reinach, Switzerland). Hydrodynamic diameters of the NP suspensions were determined using a Zetasizer Nano ZS (Malvern Instruments, UK). Stock suspensions (1 mg/ml) were prepared as described in Materials & Methods and were further diluted to 100 µg/ml in $H_2O$ or FGM. Measurements (three consecutive measurements with a minimum of 15 runs of 10 s each) were performed at 25 °C at a detection angle of 90° using a He-Ne laser operating at a wavelength of 633 nm. The release of ions from CuO or $TiO_2$ NPs was determined by ICP-MS. Therefore, suspensions of 50 µg/ml CuO or $TiO_2$ NPs in FGM were prepared as described in Materials and Methods part and incubated at 37 °C/ 5 % $CO_2$ for 10 min or 24 h. Ions were separated from NPs either by ultrafiltration (only CuO NPs; Amicon Ultra-4 Centrifugal Filter Unit, 3kDa, Milipore, USA) or by centrifugation at 10,000xg for 10 min (Heraeus Multifuge 3S-R, Thermo Fisher Scientific, Reinach, Switzerland). Supernatants were transferred into a test tube containing concentrated nitric acid. The solution was allowed to stand for 24 h and then subjected to ICP-MS analysis (sector field inductively coupled plasma mass spectrometer (SF-ICP-MS); Element 2, Thermo Finnigan, Bremen, Germany) with external calibration. Values were corrected for the background levels of Ti (0.7 %) or Cu (0.03 %) in the medium and expressed as percentage of total mass. Endotoxin content was evaluated using the QCL-1000™ Endpoint Chromogenic LAL Assay (Lonza) protocol. The enzymatic reaction was per-

formed on NPs alone, or NPs in presence of lipopolysaccharide (LPS). Briefly, the stock suspensions of NPs were diluted at 100 µg/ml in endotoxin-free culture medium and 50 µL of each suspension were dispensed into a 96-well plate. LPS was added at 1 EU/ml, followed by 1 h incubation at RT. The reaction was initiated by adding first the proenzyme and then the substrate. After 16 min, a stop solution of acetic acid 25 % v/v in $dH_2O$ was added and the absorbance was read at 405 nm, using an Infinite 200 Tecan microplate reader operating with Magellan v7.2 software.

### 1.2. *ZO-1 staining*

MTs were incubated with FGM either in the presence or absence of 20 µM forskolin for 24 h. Prior to the whole mount tissue staining, MTs were washed with PBS, fixed using 4 % PFA for 60 min and treated with 0.2 % Triton X-100 for 30 min. Subsequently, MTs were washed with PBS, blocked in 5 % goat serum for 60 min and incubated with the primary antibody mouse anti-ZO-1 (1:100, 339100, Invitrogen, Basel, Switzerland) over night at 4 ºC. After washing in PBS, MTs were exposed to the secondary antibody Alexa Fluor 488 goat anti-mouse IgG (1:400, A11029, Molecular Probes, USA) and to Dapi (4′,6-Diamidin-2-phenylindol) (Sigma-Aldrich, Buchs, Switzerland) for 5 h at room temperature. Following another washing step with PBS, MTs were analyzed by confocal microscopy.

### 1.3. *Ex vivo human placenta perfusion*

The placentas were obtained from uncomplicated term pregnancies after caesarean section at the Kantonsspital and the Klinik Stephanshorn in St. Gallen. Written informed consent was obtained prior to delivery. The project was approved by the local ethics committee and performed in accordance with the principles of the Declaration of Helsinki. A dually perfused, closed placenta perfusion system was used as described previously [12, 75]. The perfusions were performed with M199 tissue culture medium (Sigma-Aldrich, Buchs, Switzerland) diluted with Earl's buffer (dilution 1: 2), 1 g/l glucose (Sigma-Aldrich, Buchs, Switzerland), 10 g/l bovine serum albumin (Sigma-Aldrich, Buchs, Switzerland), 10 g/l dextran 40 (Sigma-Aldrich, Buchs, Switzerland), 2500 IU/l sodium heparin (Sigma-Aldrich, Buchs, Switzerland), 250 mg/l amoxicillin (Sigma-Aldrich, Buchs, Switzerland) and 2.2 g/l sodium bicarbonate (Sigma-Aldrich, Buchs, Switzerland). 25 µg/ml $TiO_2$ NPs were added to the maternal and particle concentration in the fetal and maternal circuit was determined after 0, 15, 30, 60,

120, 180, 240, 300 and 360 min by ICP-MS analysis (SF-ICP-MS; Element 2, Thermo Finnigan, Bremen, Germany) with external calibration. Particle concentrations were corrected for the perfusion medium volume in the tubes and volume loss due to sampling before placental transfer was calculated as percentage of transferred PS beads compared to the initially added particle amount.

## 2. Results

### 2.1. *Nanoparticle characterization*

Table S1: NP characterization

| | $TiO_2$ | CuO | CdTe-COOH |
|---|---|---|---|
| Primary Particle Size (nm) | 4[1] | 20[1] | 3.2[2] |
| Hydrodynamic diameter (nm) in DD water/PDI | 17/0.375 | 1165/0.335 | N/A |
| Hydrodynamic diameter (nm) in medium/ PDI | 8284/0.541 | 1852/0.442 | N/A |
| Specific surface Area (BET) ($m^2/g$) | 98 ± 10 | 42 ± 2 | N/A |
| UV-vis peak (nm) | N/A | N/A | 549 |
| Dissolution in medium (%) after 10 min/ 24 h[3] | 0.5/1.5 | 13/59 | N/A |

Abbreviations: DD, double distilled; PDI, polydispersity index; TEM, transmission electron microscopy; N/A, not applicable
[1] calculated from TEM micrographs; [2] calculated from the position of the exciton band; [3] Separation of ions from NPs by centrifugation

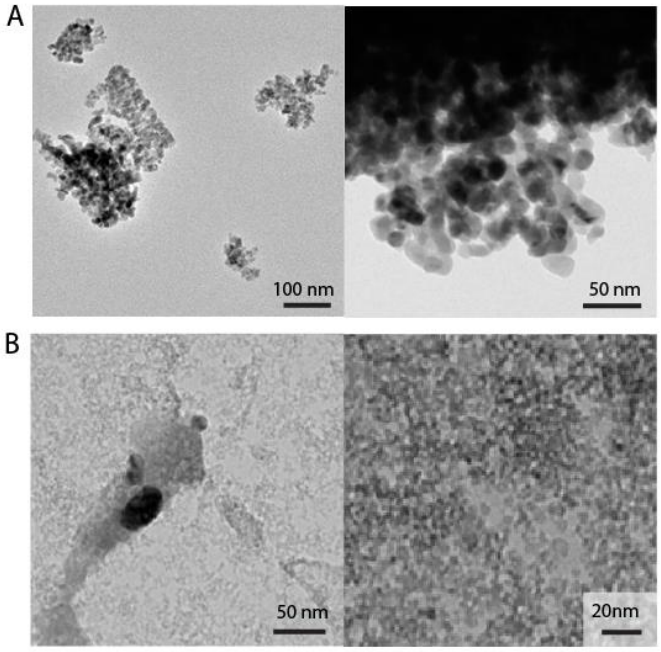


**Figure S1. TEM of CuO and $TiO_2$ NPs.** Representative micrographs of CuO NPs (A) and $TiO_2$ NPs (B).

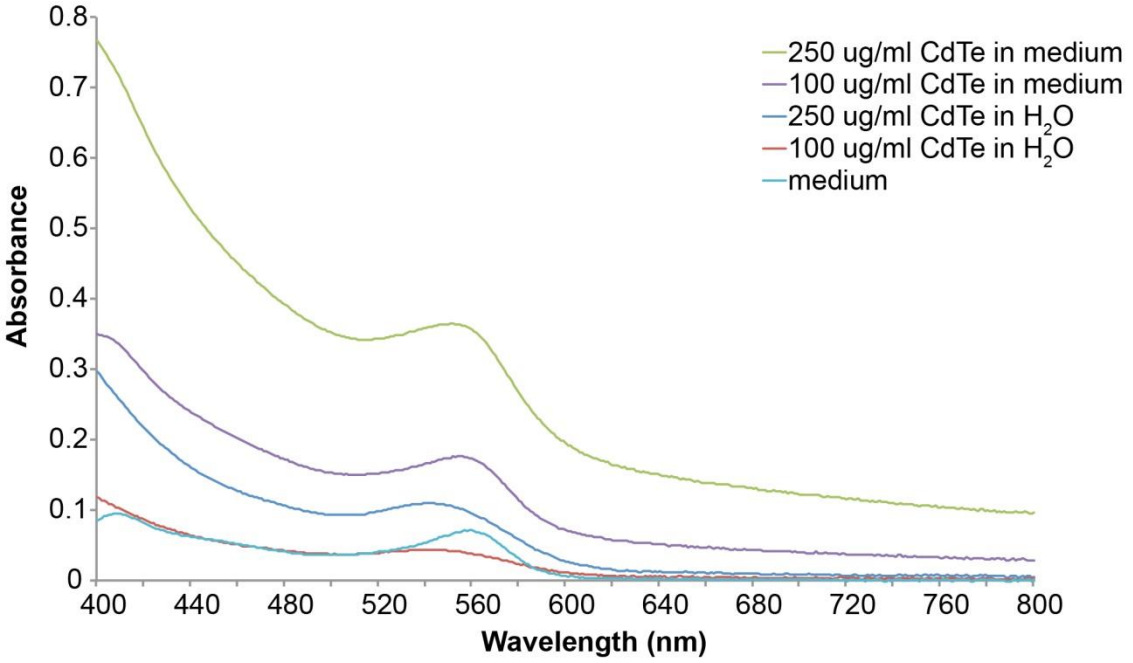


**Figure S2. UV-Vis measurements of CdTe-COOH NPs in FGM and water.** Absorbance was measured for 100 and 250 µg/ml CdTe-COOH NPs either in cell culture medium or in water.

## 2.2. *Characterization of placental co-culture MTs*

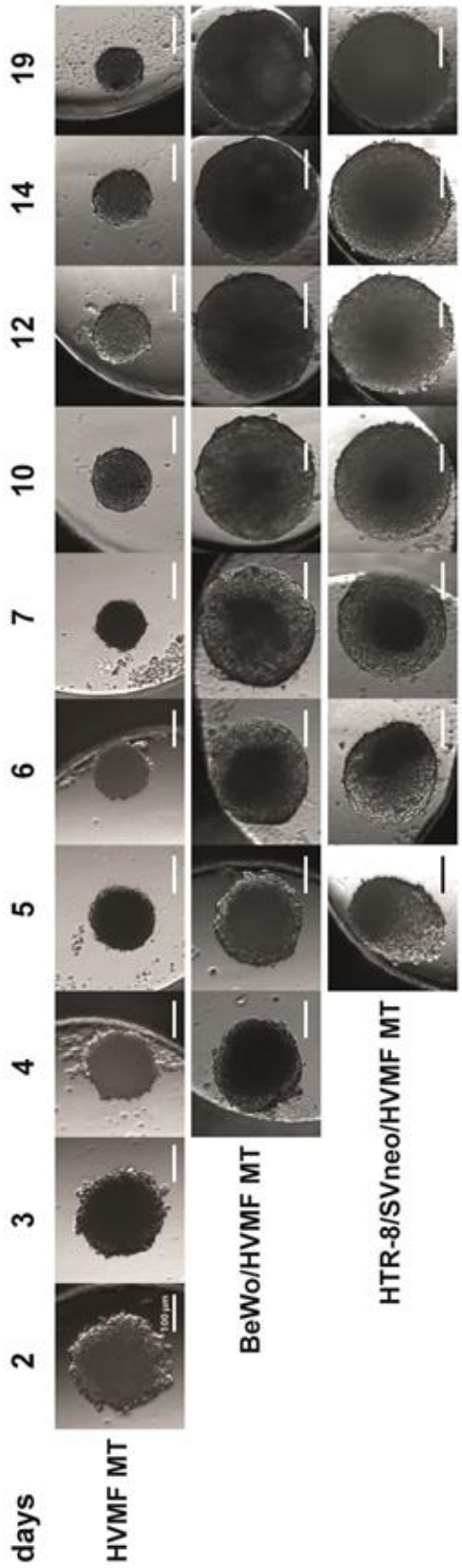


**Figure S3. Bright-field microscopic analysis of core and co-culture MTs.** Representative images of HVMF MTs (upper row), BeWo/HVMF MTs (middle row) and HTR-8/SVneo/HVMF MTs (lower row) at different days of cultivation. Scale bar is 100 µm.

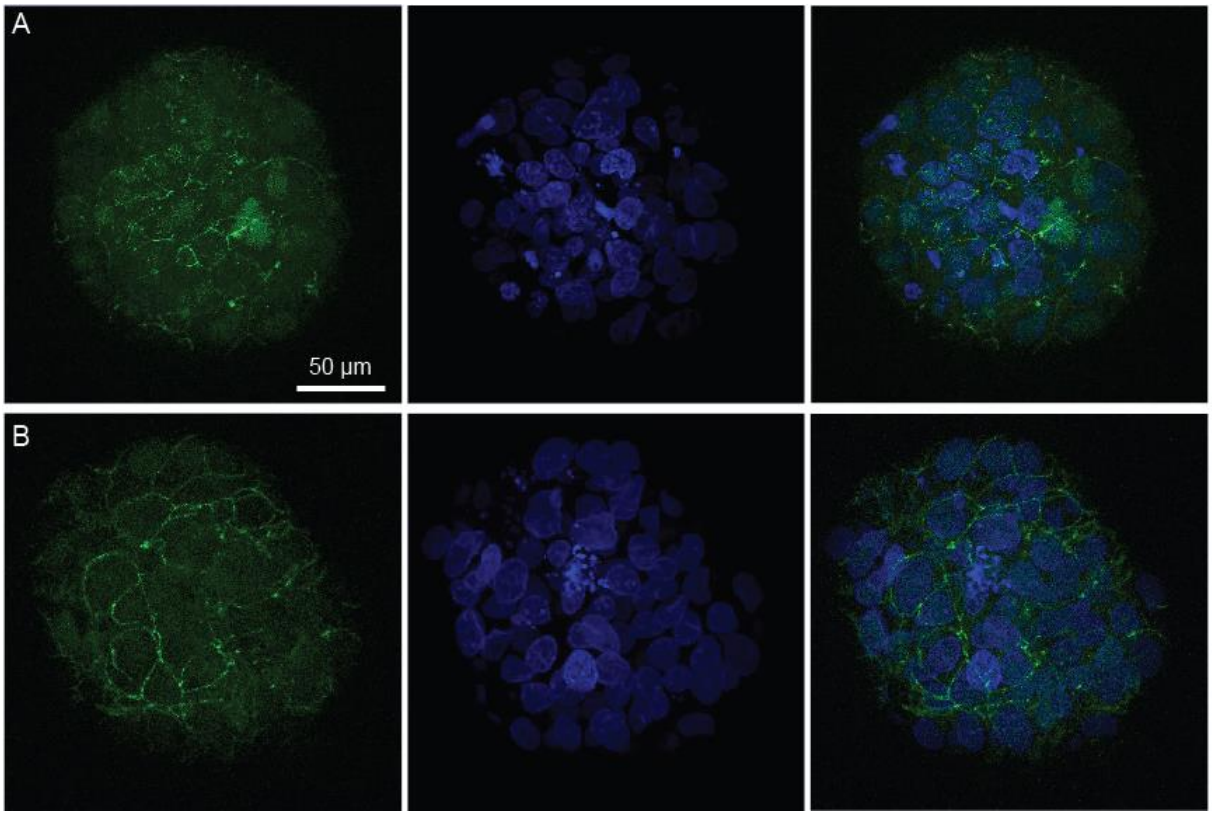


**Figure S4. Confocal microscopic analysis of tight junctions in co-culture MTs**. MTs were cultivated in the absence (A) or presence of 20 µM forskolin for 24 h (B) followed by a fluorescent staining using anti-ZO-1 antibody (green) to visualize tight junctions and Dapi (blue) to visualize nuclei.

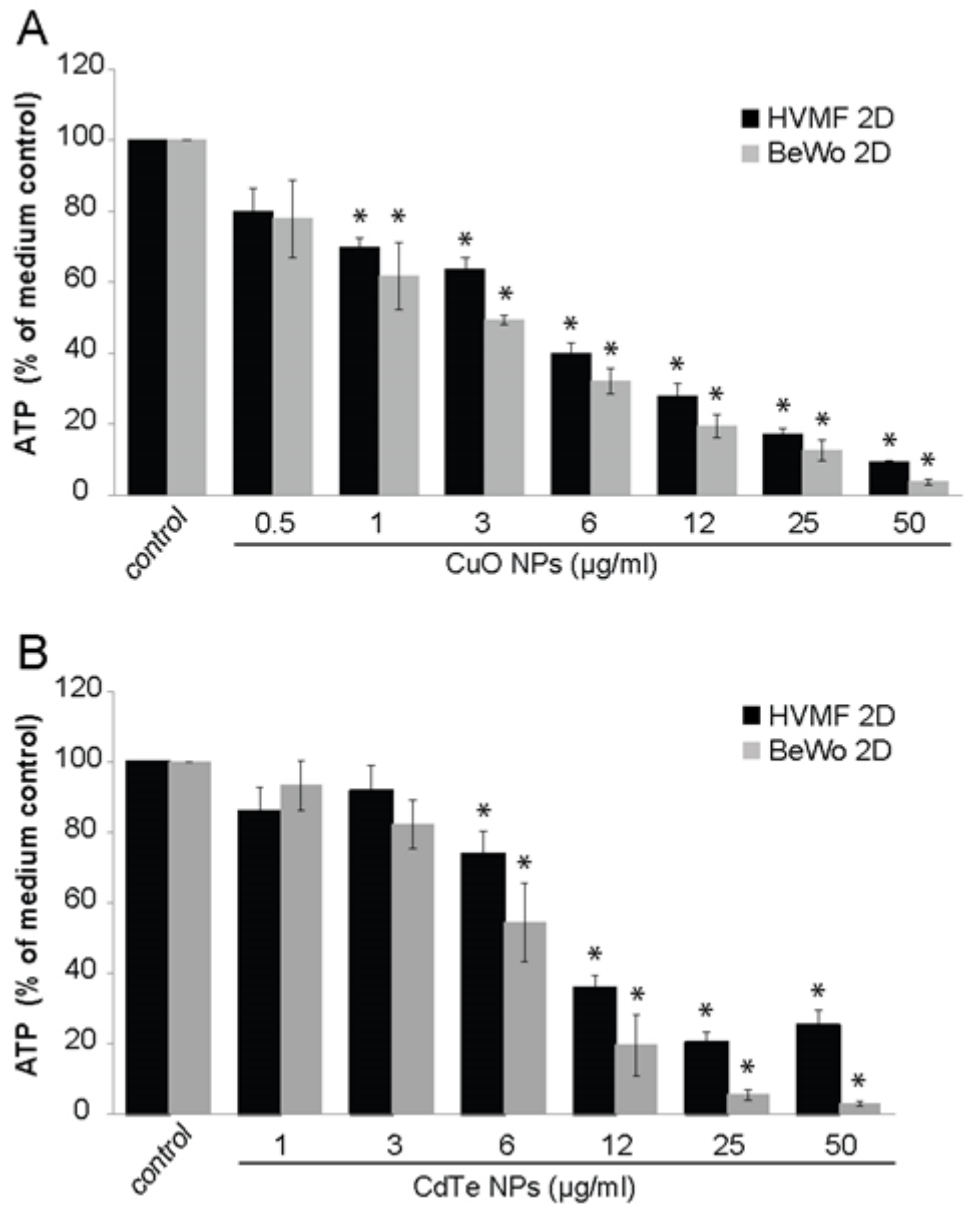


**Figure S5. Effects of CuO NPs and CdTe-COOH NPs on 2D BeWo and HVMF cell viability.** 2D BeWo or 2D HVMF monocultures were exposed to CuO (A) or CdTe-COOH NPs (B) at concentrations ranging from 0.5 to 50 µg/ml for 24 h in order to evaluate NP cytotoxicity using the ATP assay. Data represent the mean percentages of duplicates compared to the untreated medium control ± SEM of 3 - 5 independent experiments. Note that the data for 2D BeWo were reproduced from Figure 6 to highlight differences to 2D HVMFs.

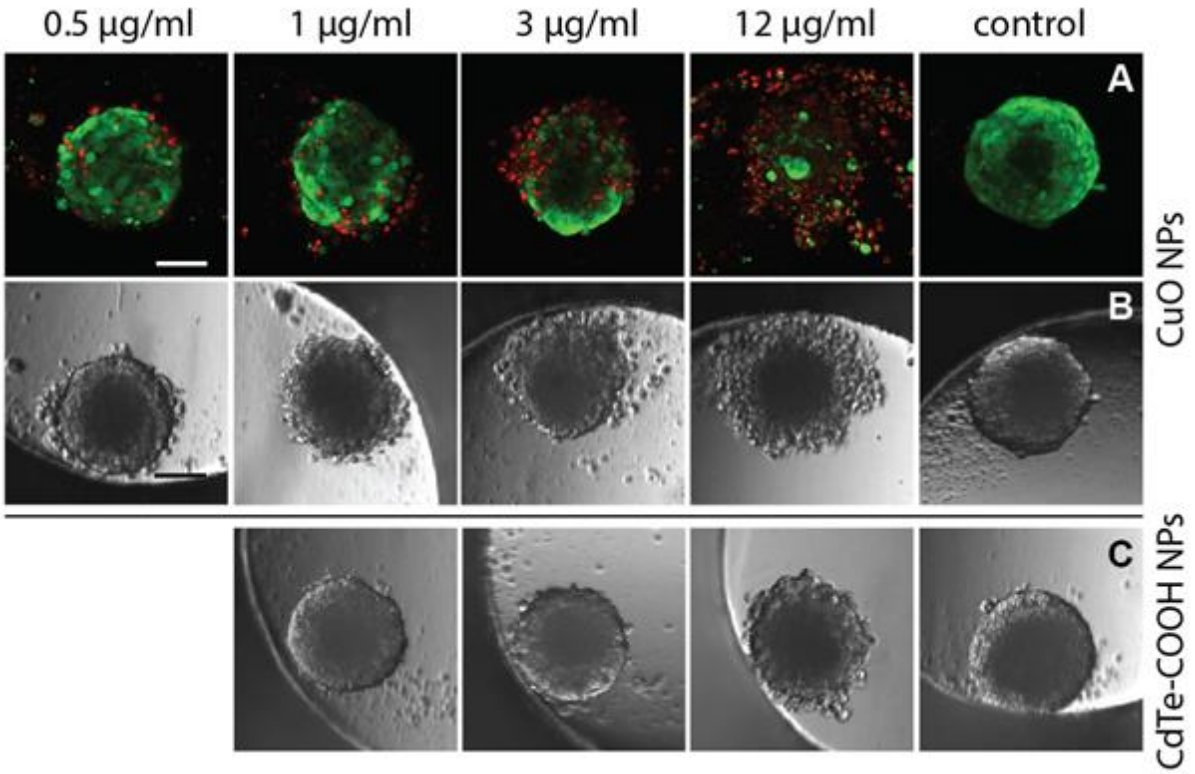


**Figure S6. Live/dead staining and bright-field microscopy of co-culture MTs after the exposure to CuO NPs or CdTe-COOH NPs.** BeWo/HVMF MTs were exposed to 0.5, 1, 3 or 12 µg/ml of CuO (A and B) or to 1, 3 or 12 µg/ml of CdTe-COOH NPs (C) for 24 h. Representative images show viable cells in green and dead cells in red as well as tissue morphology. Note that live/dead staining was not performed for CdTe-COOH NPs as they are highly fluorescent. Scale bar is 100 µm.

## 2.4. *$TiO_2$ NP uptake in co-culture MTs and translocation in the ex vivo human placenta perfusion model*

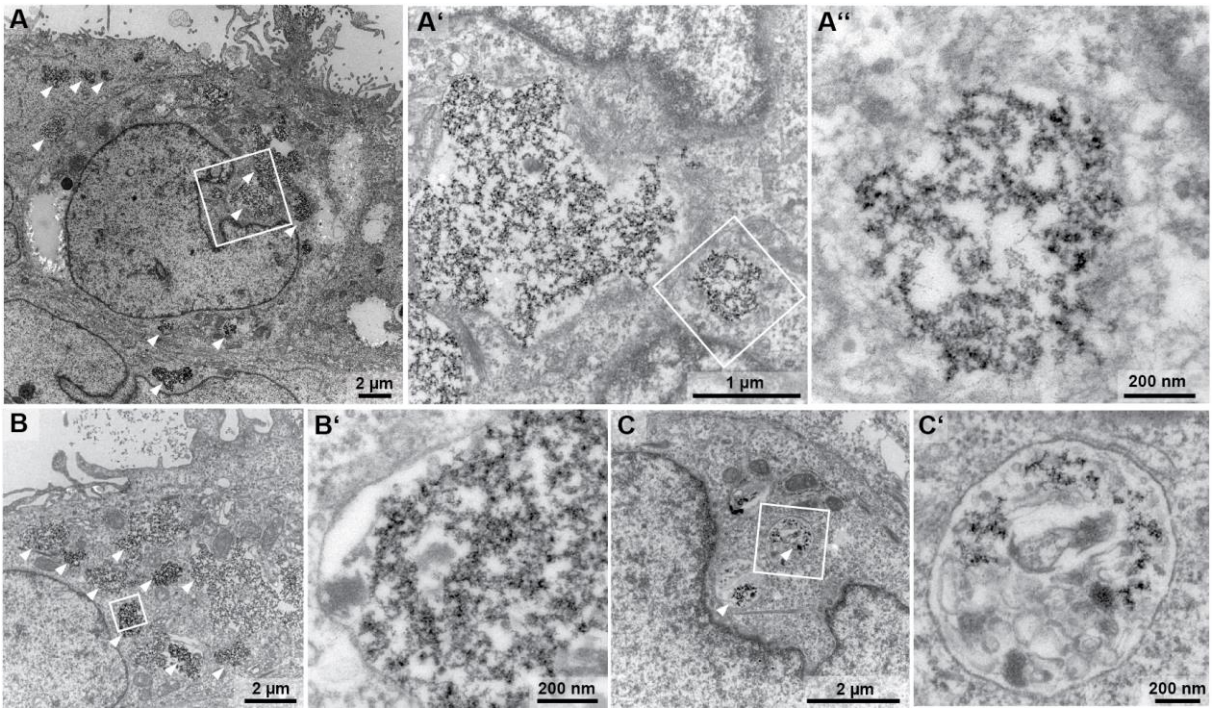


**Figure S7. TEM micrographs of a co-culture MT exposed to $TiO_2$ NPs.** After co-culture MT formation was complete, MTs were exposed to 100 µg/ml $TiO_2$ NPs for 24 h and subsequently processed for TEM analysis. $TiO_2$ NP agglomerates (arrowheads) were found freely in the cytoplasm (A-A'') or in membrane-bound vesicles (B,B'; putative endosome; C,C' putative lysosome). X' and X'' represent enlargements of the quadrants in X or X', respectively.

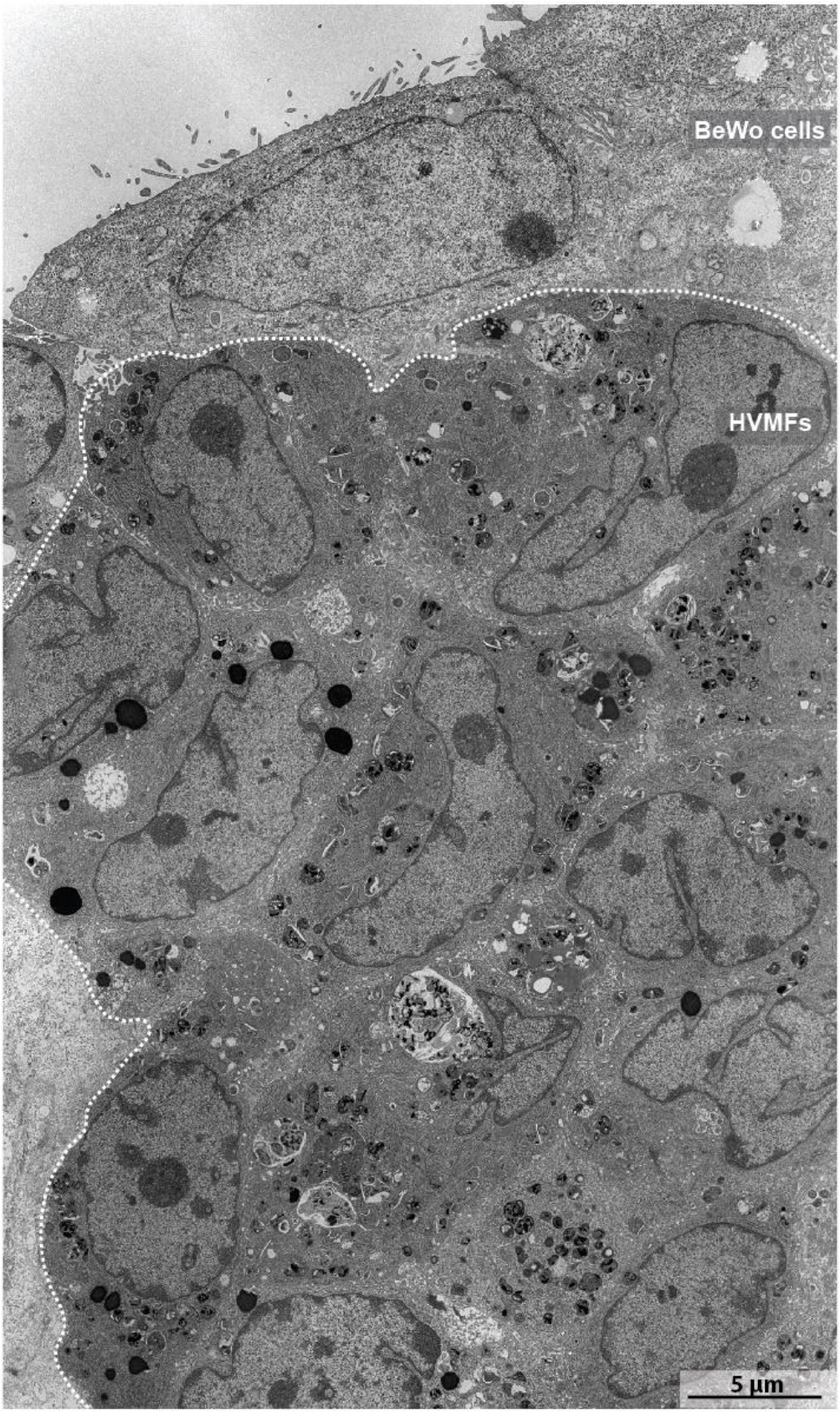


**Figure S8. TEM micrograph of a representative control co-culture MT.** After co-culture MT formation was complete, MTs were cultivated in medium for 24 h and subsequently processed for TEM analysis.

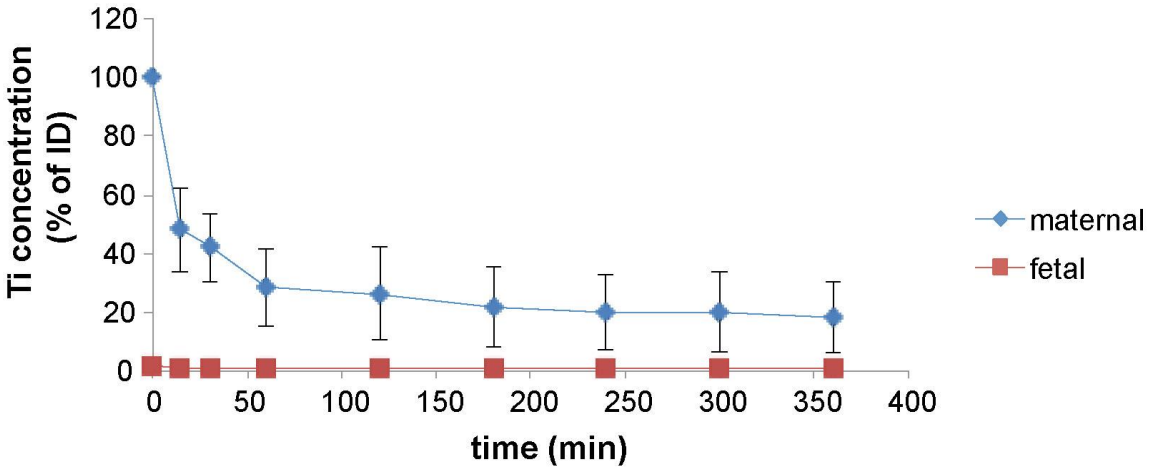


**Figure S9. *Ex vivo* placenta perfusion studies with $TiO_2$ NPs.** The initial dose (ID) of $TiO_2$ NPs added to the maternal circuit was 25 µg/ml. The amount of particles was measured in the maternal and fetal circuits after the indicated time points by ICP-MS.