



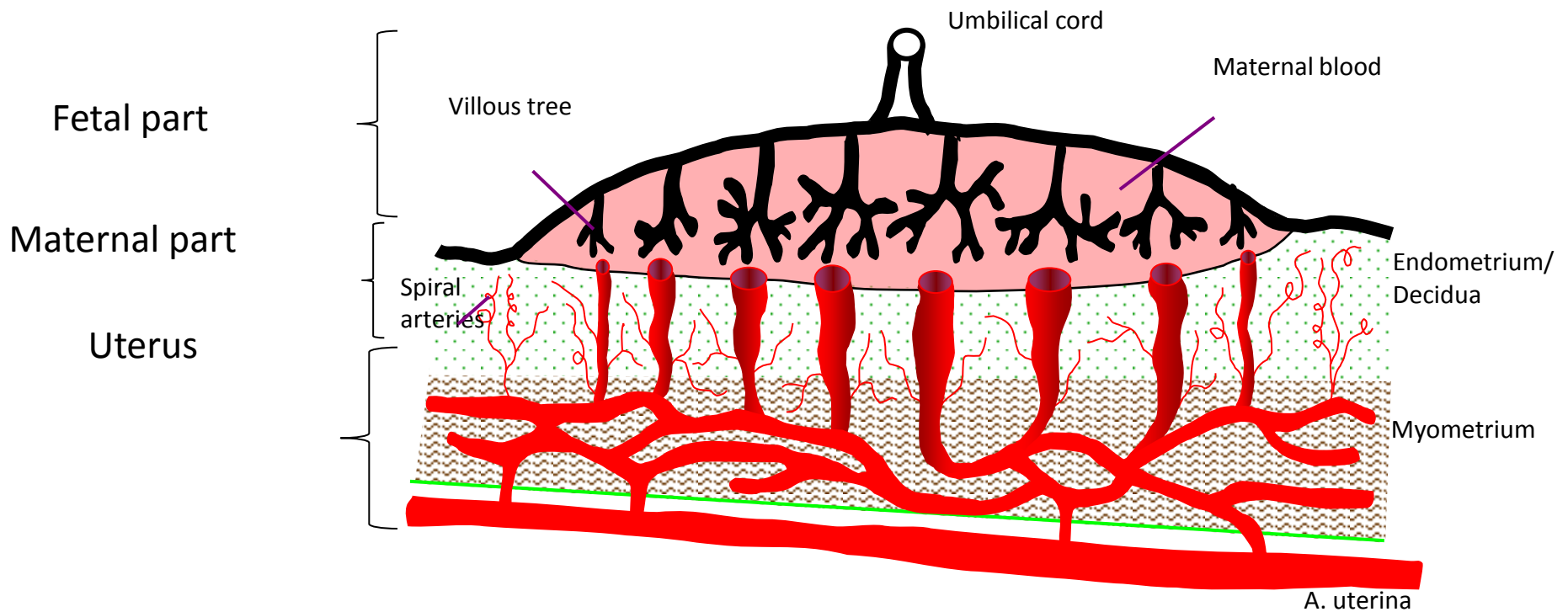
Nanotoxicology of human placenta: evaluation of suitable model for environmental toxicity.

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Medical University of Graz*

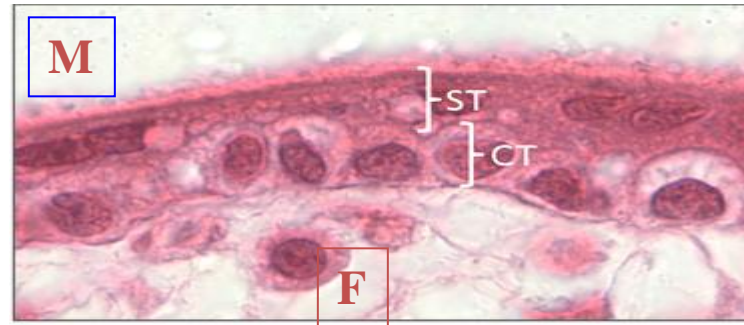
21 June 2017

Structure of human placenta



Structure of placental barrier

1st trimester haemo-dichorial



12 week- establishment of circulation

3d trimester haemo-monochorial



Huppertz B. 2011 with modifications

M- maternal circulation

ST - syncytium (one-cytoplasm layer with apoptotic nuclei)

CT - cytotrophoblasts

F –endothelial cells, fetal vessel

Placenta is a species specific organ



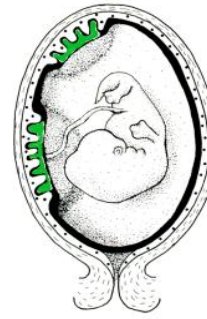
placenta diffusa



placenta cotyledonaria



placenta zonaria

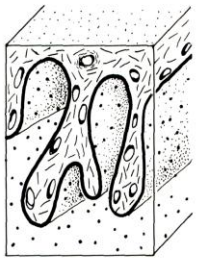


placenta bidiscoidalis

rat



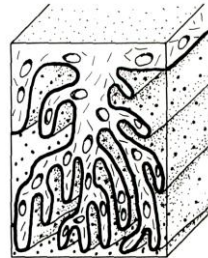
placenta discoidalis



folded type



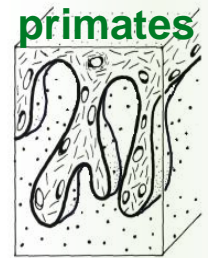
trabecular type



lamellar type

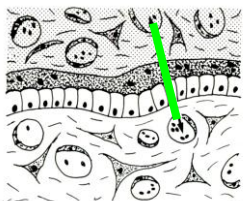


labyrinthine type

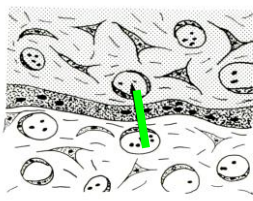


villous type

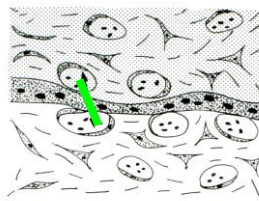
Human,
primates



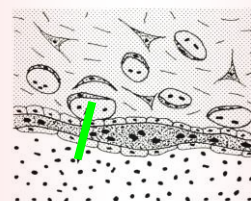
epithelio-chorial



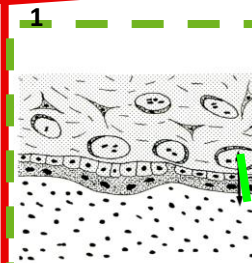
syndesmo-chorial



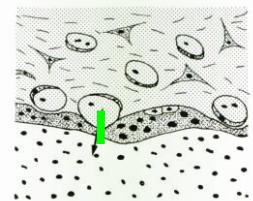
endothelio-chorial



hemo-trichorial

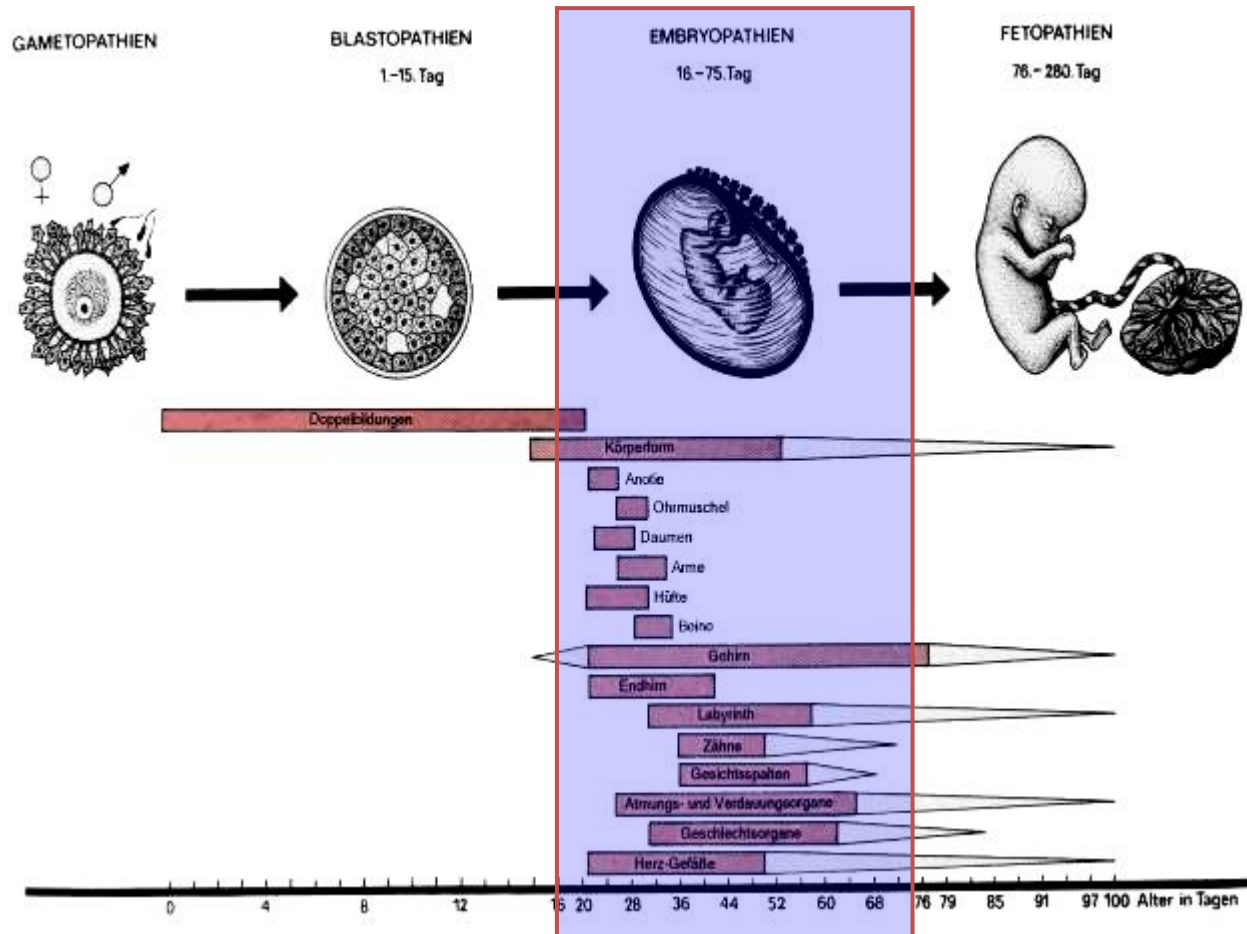


hemo-dichorial
1st trimester



hemo-mono-chorial
3d trimester

Most sensitive period “embryonic” period



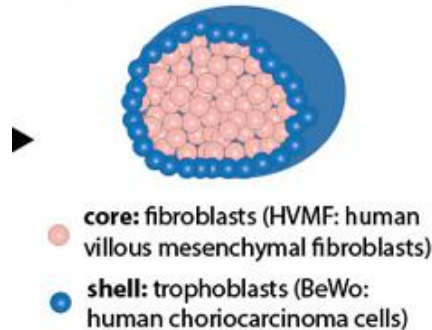
Nanotoxicology of placenta: models of research

in vitro

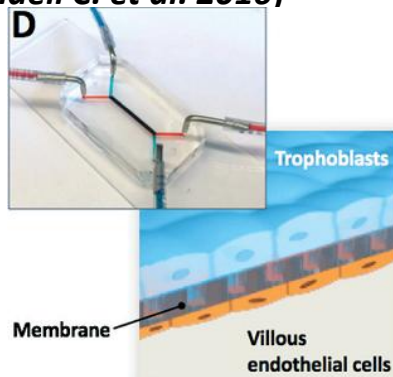
- BeWo cell monolayer (*Cartwright L. et al. 2012*),
- primary cells,
- Microtissue 3D model

of human placenta (*Muoth C. et al. 2016*)

3D placental co-culture MT model



- Microphysiological model of placental barrier (*Blundell C. et al. 2016*)



in vivo

- rodents
- rabbits

ex vivo

- Placental explants (early, term)
- Placental perfusion model (term)

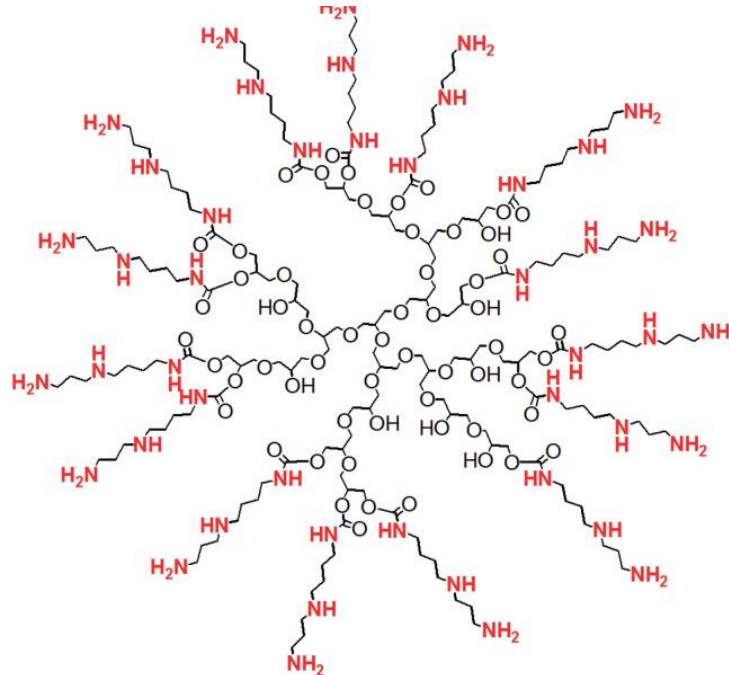
No standardised model for studying of nanotoxicity of placenta

Charged polyglycerol dendrimers, 5nM

Neutral
(hydroxyl- group)

negative
(sulfate group)

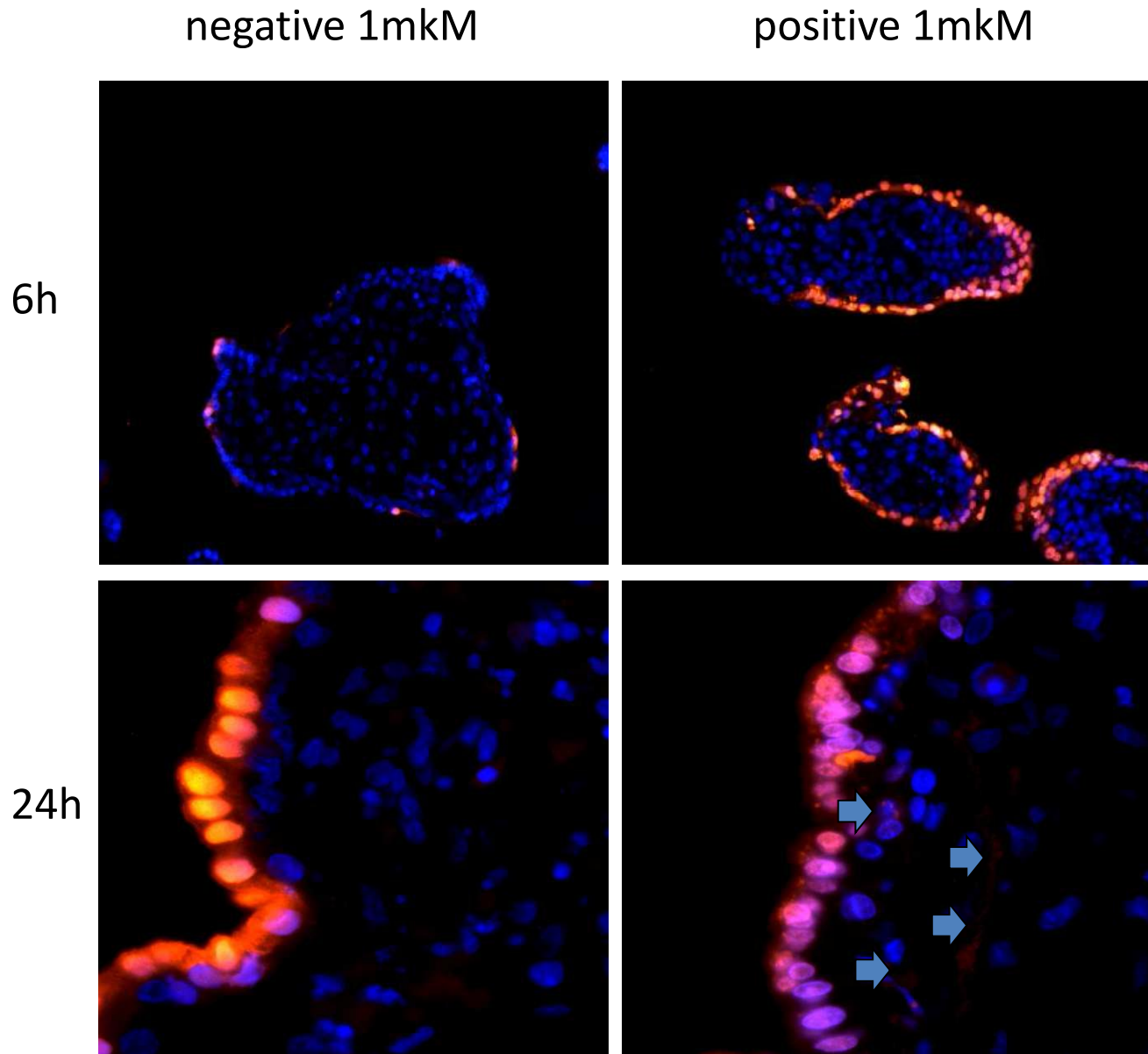
positive
(amine group)



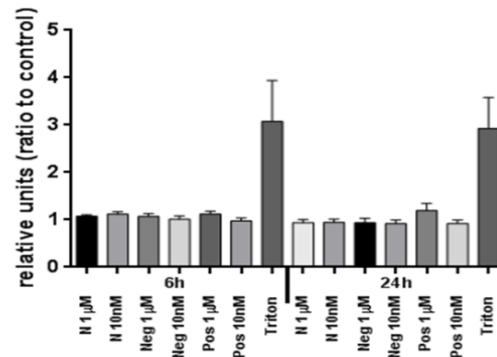
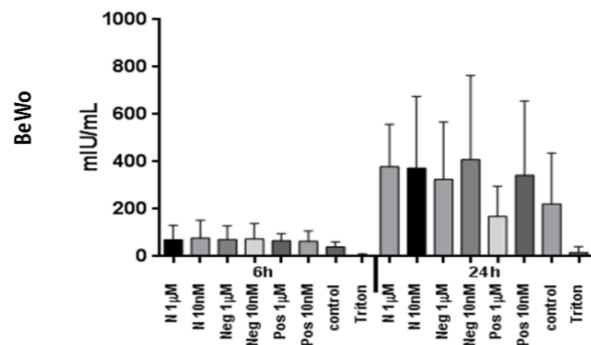
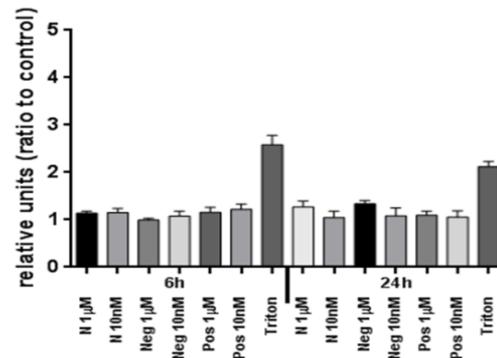
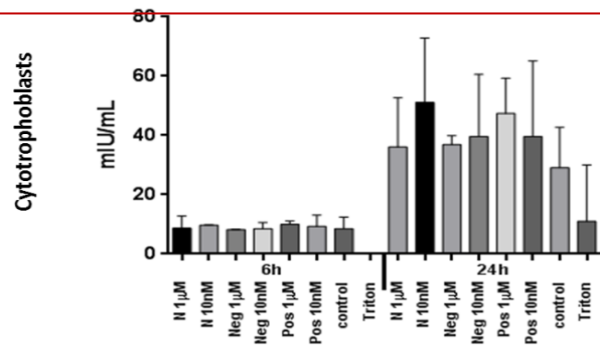
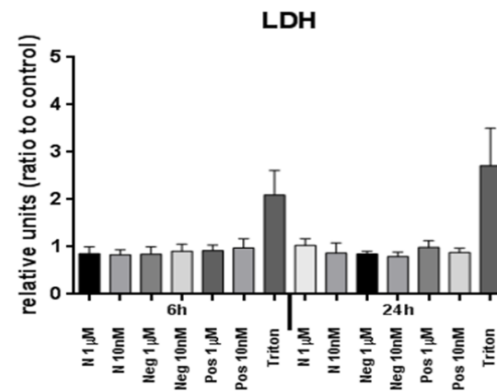
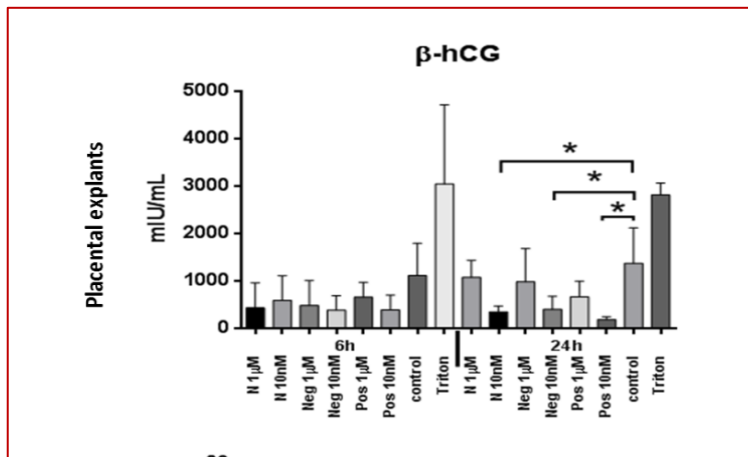
Cadleron et al. 2010

- TRITC –labeled
- Biotinylated

Localisation of polyglycerol NP in early placenta explants



Toxicity



Pollutants interaction with human placenta

- **Maternal exposure to diluted diesel engine exhaust alters placental function and induces intergenerational effects in rabbits.**

Valentino SA, Tarrade A, Aioun J, Mourier E, Richard C, Dahirel M, Rousseau-Ralliard D, Fournier N, Aubrière MC, Lallemand MS, Camous S, Guinot M, Charlier M, Aujean E, Al Adhami H, Fokkens PH, Agier L, Boere JA, Cassee FR, Slama R, Chavatte-Palmer P. Part Fibre Toxicol. 2016 Jul 26;13(1):39. doi: 10.1186/s12989-016-0151-7.

- **Association of serum concentrations of persistent organic pollutants (POPs) and risk of pre-eclampsia: a case-control study.**

Eslami B, Malekafzali H, Rastkari N, Rashidi BH, Djazayeri A, Naddafi K. J Environ Health Sci Eng. 2016 Nov 24;14:17.

- **In Utero Fine Particle Air Pollution and Placental Expression of Genes in the Brain-Derived Neurotrophic Factor Signaling Pathway: An ENVIRONAGE Birth Cohort Study.**

Saenen ND, Plusquin M, Bijnen E, Janssen BG, Gyselaers W, Cox B, Fierens F, Molenberghs G, Penders J, Vrijens K, De Boever P, Nawrot TS. Environ Health Perspect. 2015 Aug;123(8):834-40. doi: 10.1289/ehp.1408549.

- **Pulmonary exposure to metallic nanomaterials during pregnancy irreversibly impairs lung development of the offspring.**

Paul E, Franco-Montoya ML, Paineau E, Angeletti B, Vibhushan S, Ridoux A, Tiendrebeogo A, Salome M, Hesse B, Vantelon D, Rose J, Canouï-Poitaine F, Boczkowski J, Lanone S, Delacourt C, Pairon JC. Nanotoxicology. 2017 May;11(4):484-495.

Biomarkers Used in Studying Air Pollution Exposure During Pregnancy and Perinatal Outcomes: A Review.

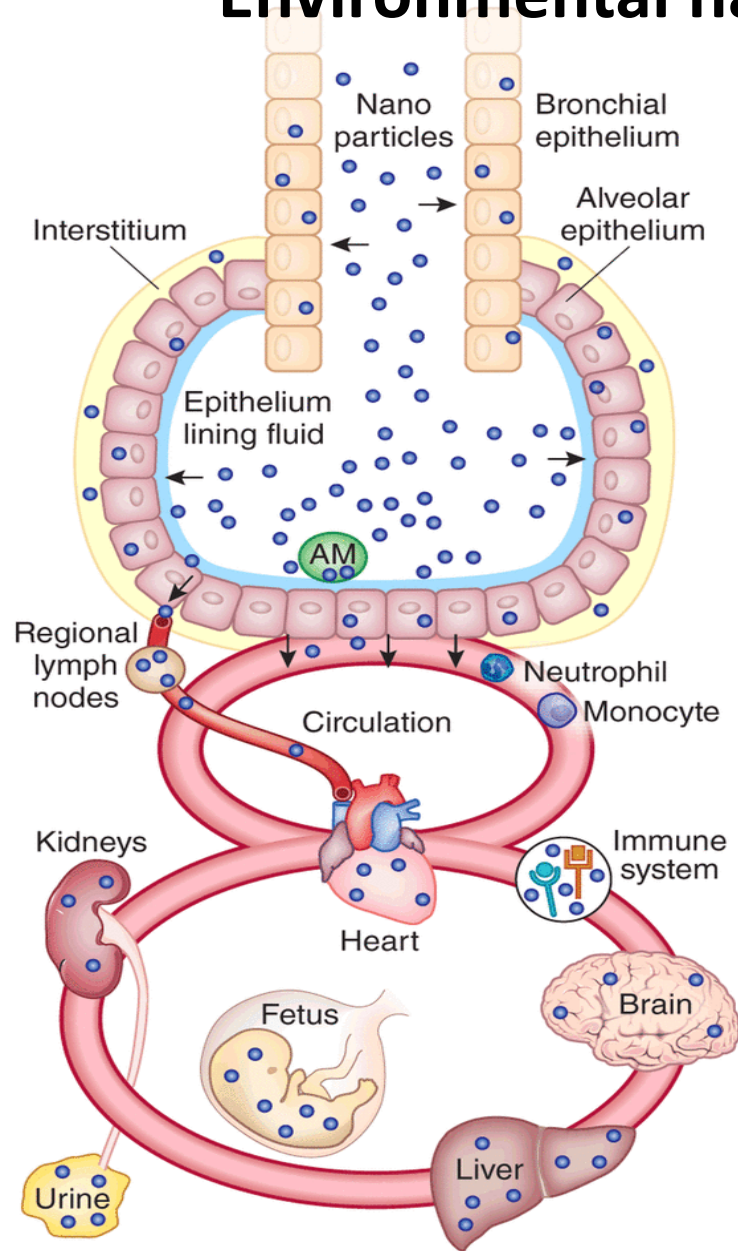
Desai G, Chu L, Guo Y, Myneni AA, Mu L.

Biomarkers. 2017 Jun 5:1-34.

- The most consistent positive association was between polycyclic aromatic hydrocarbon (PAH) exposure during entire pregnancy and cord blood PAH DNA adducts.
- Exposure to particulate matter (PM) and nitrogen dioxide (NO₂) showed consistent inverse associations with **mitochondrial DNA (mtDNA) content**, particularly in the third trimester of pregnancy.
- No single pollutant showed strong associations with all the biomarkers included in this review. C-reactive proteins (CRPs) and oxidative stress markers increased
- **telomere length** decreased with increasing air pollution exposure.
- **Placental global DNA methylation and mtDNA methylation** showed contrasting results with air pollution exposure, the mechanism behind which is unclear.
- Most studies except those on PAH DNA adducts and mtDNA content provided insufficient evidence for characterizing a critical exposure window.

Further research using biomarkers is warranted to understand the relationship between air pollution and perinatal outcomes.

Environmental nanoparticles exposure



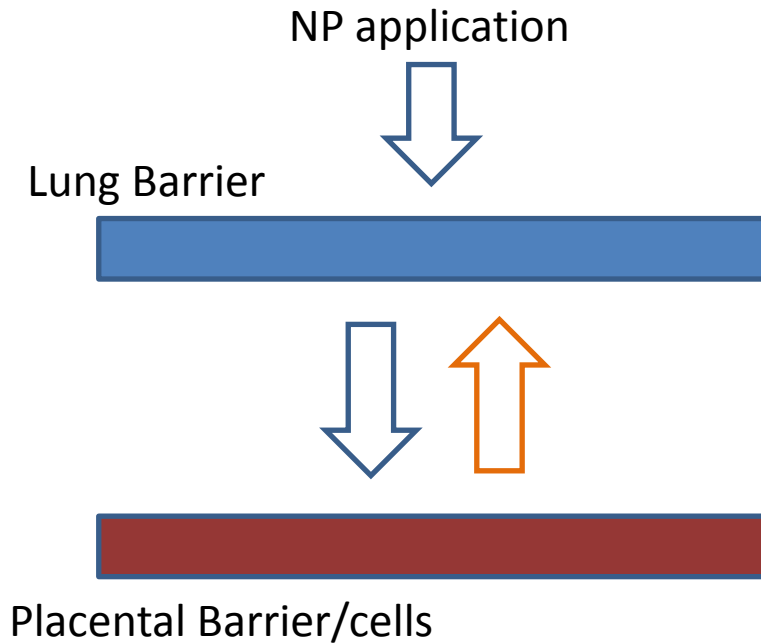
Katie Vicari

Possible routes of toxicity to placenta

- Toxicity of eNP
- Proinflammatory response in lung barrier
- Proinflammatory response in blood cells

Nature Biotechnology 28, 1275–1276 (2010)

Pollutants interaction with human placenta. Possible solutions for in vitro models



- Toxicity of PM on placental cells/tissues
- Influence of placental hormones on lung barrier (change of Macrophages phenotype)

Model of lung barrier

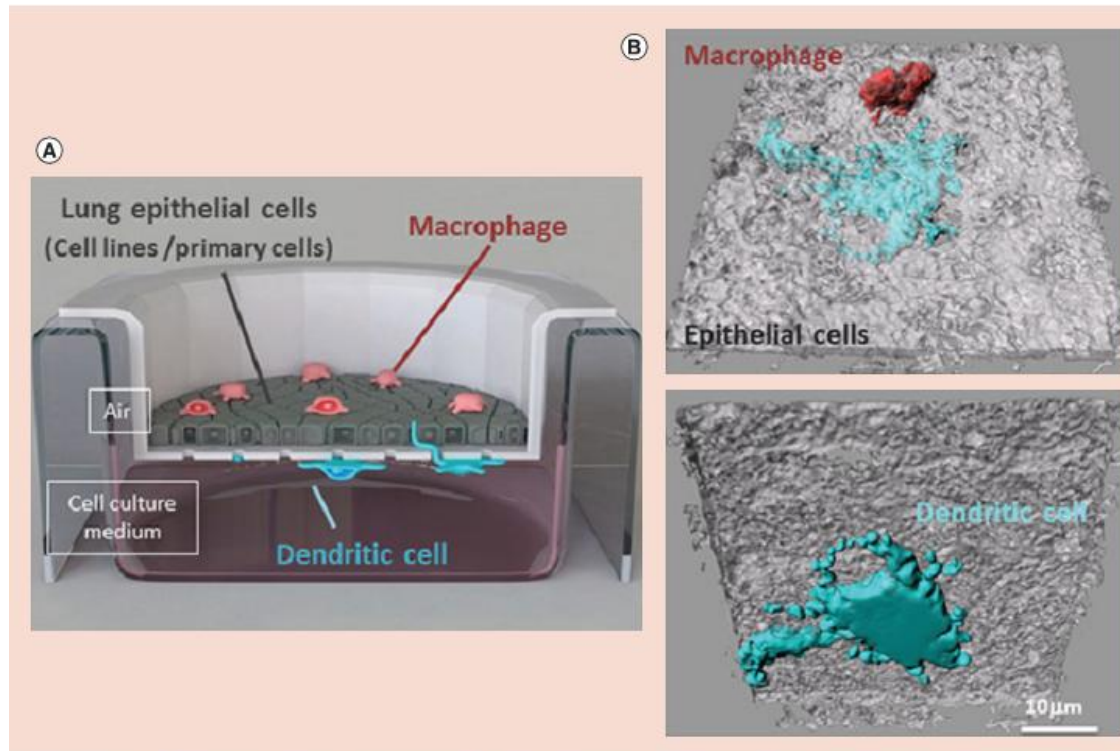


Figure 3. (A) Schematic drawing of the triple-cell co-culture model composed of epithelial cells, monocyte-derived macrophages and dendritic cells. The cells are exposed to air in the upper chamber and to cell culture medium in the lower part. (B) Laser scanning images of the model. Epithelial cells (white, volume rendering), macrophages (red, surface rendering), and dendritic cells (light blue, surface rendering) are shown. The same data set is shown from above (upper image) and from below (lower image). Adapted with permission from [90].

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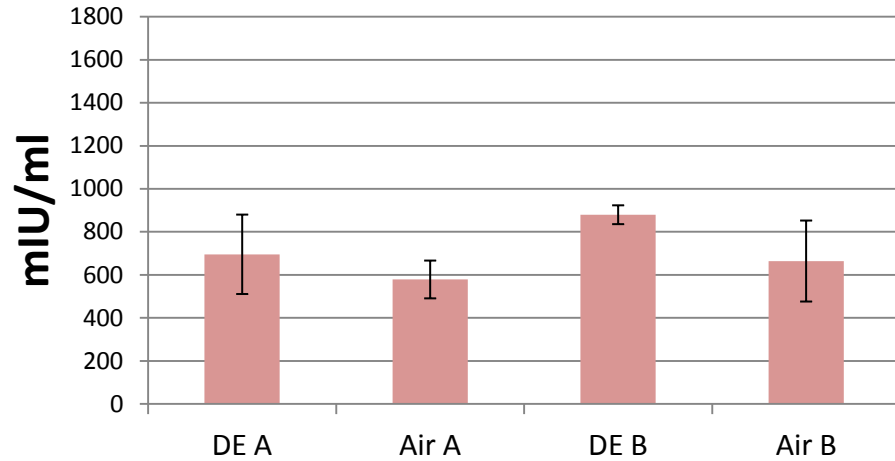
*Authors contributed equally

Pilot experiments

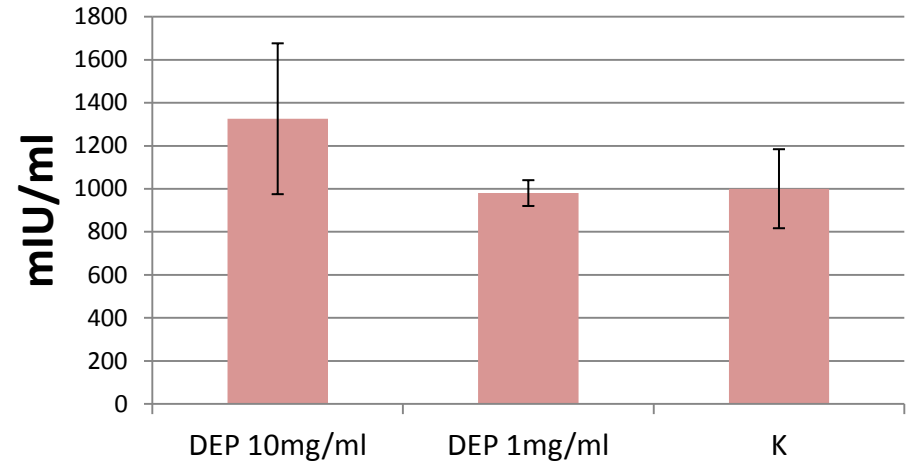
Exposure of first trimester placental explants to:

- Conditioned medium - collected after exposure of lung barrier to diesel exhaust for 6 h: consists of lung (A-A549 cells) and bronchial (B-16HBE14o-) epithelial cells
- DEP diluted in cultured medium

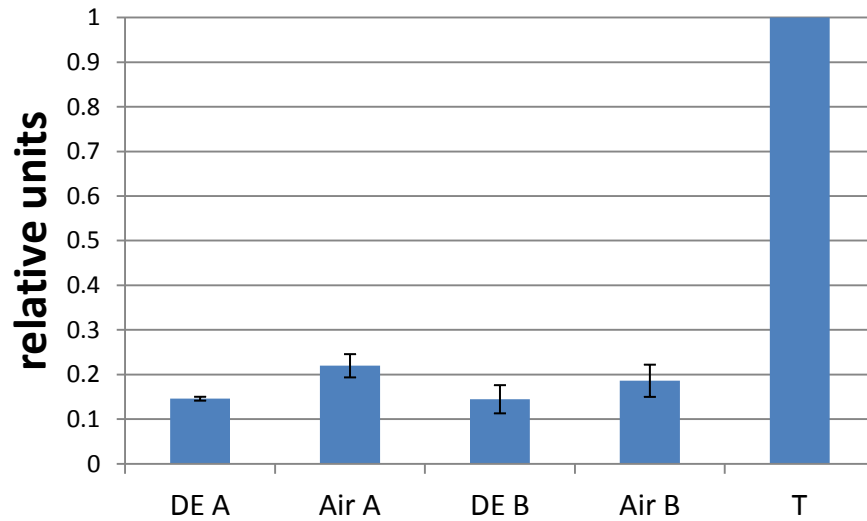
β -hCG, conditioned media, 12h



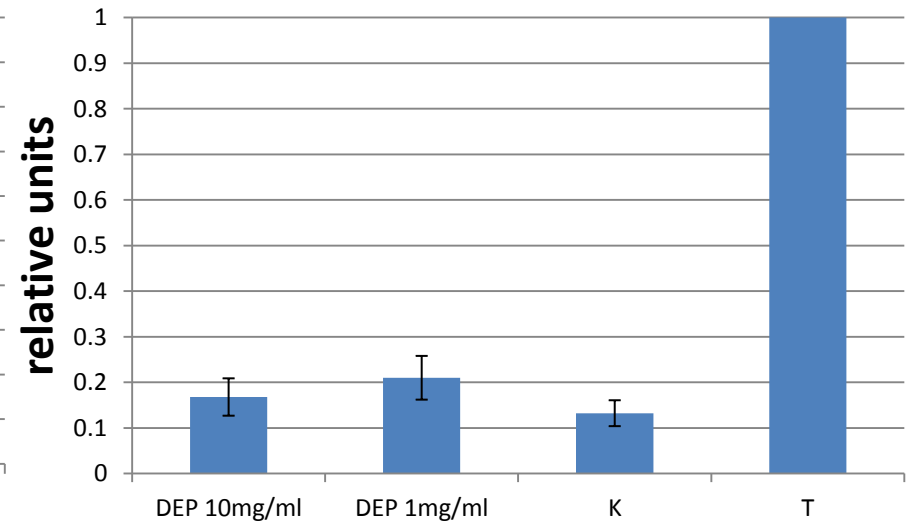
β -hCG, DEP, 12h



LDH, conditioned media, 12h

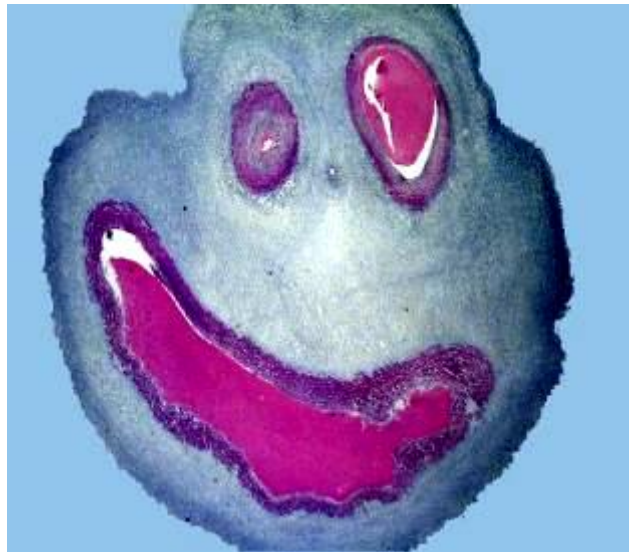


LDH, DEP 12h



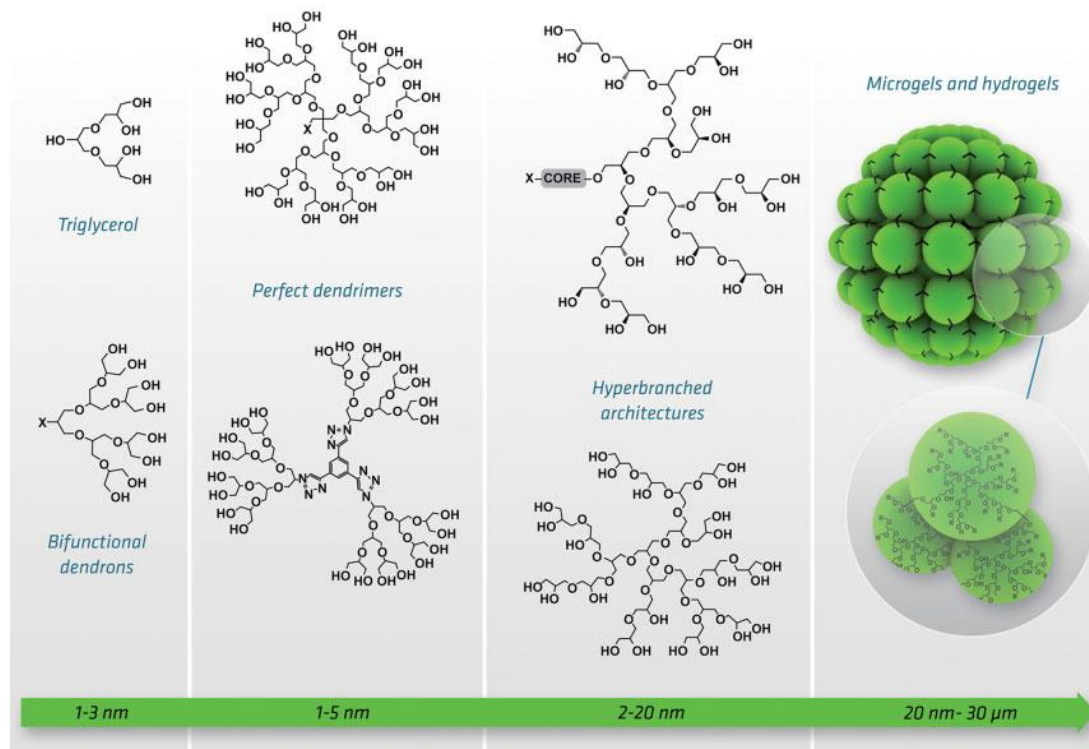
Conclusions

- Preliminary experimental setup for study of environmental toxicity for human placenta was adjusted
- Placental early explant model shows no sign of toxicity according to LDH measurements
- Diesel particles may have an effect on production of hCG in early human placenta



Thank you for your attention!

Nanoparticles: dendritic polyglycerols (cooperation with Prof R. Haag, Free University, Berlin)



Cadleron et al. 2010

- Poliglycerol-based NPs – potential drug carriers/contrast agents/biosensors
- Dendritic structures-> synergy between the nanosized dimensions combined with the high density of functional groups
- Cytotoxicity studies show the polymeric systems to be non-toxic over a wide concentration range (Kumari M. et al 2015)

Methods of study:

Exposure to NP:

- placental explants of 1 trimester placenta
- BeWo cells (trophoblast cell line)
- Primary cytotrophoblasts isolated from term placentas

Concentrations: 1 μ M and 10nM

Time points: 6 and 24 hours

Cultivation in medium and human serum

Toxicity:

- LDH (cytotoxicity)
- hCG level in culture medium (in collaboration with Prof. Obermayer-Pietsch, institute of Endocrinology, Graz) (metabolism changes)

Localisation of Nanoparticles:

- Fluorescence Microscopy