Research on nanoparticles in human perfused placenta: State of the art and perspectives

Leonie Aengenheister\textsuperscript{a,b}, Rodolfo R. Favaro\textsuperscript{b}, Diana M. Morales-Prieto\textsuperscript{b}, Lea A. Furer\textsuperscript{a}, Michael Gruber\textsuperscript{c}, Christian Wadsack\textsuperscript{c}, Udo R. Markert\textsuperscript{b}, Tina Buerki-Thurnherr\textsuperscript{c,d}\footnote{Corresponding author. E-mail address: tina.buerki@empa.ch (T. Buerki-Thurnherr).}

\textsuperscript{a} Laboratory for Particles-Biology Interactions, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014, St. Gallen, Switzerland
\textsuperscript{b} Placenta Lab, Department of Obstetrics, Jena University Hospital, Am Klinikum 1, 07747, Jena, Germany
\textsuperscript{c} Department of Obstetrics and Gynecology, Medical University of Graz, Auenbruggerplatz 14, 8036, Graz, Austria

\textbf{ARTICLE INFO}

\textbf{Abstract}

Increasing human exposure to nanoparticles (NPs) from various sources raises concerns for public health, especially for vulnerable risk groups like pregnant women and their developing fetuses. However, nanomedicine and the prospect of creating safe and effective NP-based formulations of drugs hold great promise to revolutionize treatment during pregnancy. With maternal and fetal health at stake, risks and opportunities of NPs in pregnancy need to be carefully investigated. Importantly, a comprehensive understanding of NP transport and effects at the placenta is urgently needed considering the central position of the placenta at the maternal-fetal interface and its many essential functions to enable successful pregnancy. The perfusion of human placental tissue provides a great opportunity to achieve predictive human relevant insights, circumventing uncertainties due to considerable differences in placental structure and function across species. Here, we have reviewed the current literature on the \textit{ex vivo} human placenta perfusion of NPs. From 16 available studies, it was evident that placental uptake and transfer of NPs are highly dependent on their characteristics like size and surface modifications, which is in line with previous observations from \textit{in vitro} and animal transport studies. These studies further revealed that special considerations apply for the perfusion of NPs and we identified relevant controls that should be implemented in future perfusion studies. While current studies mostly focused on placental transfer of NPs to conclude on potential fetal exposure, the \textit{ex vivo} placental perfusion model has considerable potential to reveal novel insights on NP effects on placental tissue functionality and signaling that could indirectly affect maternal-fetal health.

\textbf{1. Introduction}

NPs are small nano-sized materials (at least one dimension < 100 nm)\cite{1} with a high surface-to-volume ratio, which are omnipresent in our environment. NPs occur naturally (e.g. during forest fires and volcanic eruptions or as endogenous fatty acid liposomes or micelles) or can form incidentally or intentionally from anthropogenic sources (e.g. pollution particles from combustion processes or industrially produced NPs). Engineered NPs that are designed for a specific purpose can offer manifold advantageous characteristics compared to their bulk form such as a superior optical, electrical, magnetic, mechanical, and thermal properties, e.g. due to their higher surface-to-volume ratio. Advances in synthesis processes, nowadays, enable that NPs can be produced in almost every desired size, shape and material composition and, thus, are frequently used in industrial, medical, and consumer applications\cite{2-6}.

The rapid increase in NP manufacturing and the concomitant potential release of particles into the environment raises public concerns on human health risks. This is particularly relevant for high-risk groups including pregnant women and the developing fetus. Indeed, epidemiological studies linked higher prenatal exposure to fine particulate matter (PM\textsubscript{2.5} < 2.5 \textmu m) from ambient air pollution, to increased incidences of preterm birth, low birth weight, or autism spectrum disorders\cite{7-10}.

Beside the safety assessment of unintentional NP exposure, recent research is exploring the potential of engineered NPs to revolutionize therapy during pregnancy\cite{11-13}. The use of prescription medications...
during gestation involves some complex decision-making, balancing treatment efficacy with protection of mother and fetus. Specific administration of medications to either the mother, the placenta or the fetus is thus, of utmost relevance [14]. NPs designed to target specifically maternally, placental or fetal tissues could deliver drugs preferentially to the side of action, thereby maximizing efficacy and minimizing adverse side effects of the therapeutic drug [11–13]. Especially, particle systems that mimick naturally occurring NPs such as liposomes or extracellular vesicles (EVs) hold great promises for the application as drug carrier during pregnancy. For instance, liposomes have highly promising characteristics such as low toxicity, biodegradaibility and flexibility for functionalization with targeting ligands, and they were among the first approved nano-based drug formulations (Doxil® [15]).

In any case, the safe design and use of NPs in future applications requires a fundamental understanding of their effects on human health. In pregnancy, a transient organ, the placenta, is formed at the maternal-fetal interface, which performs a plethora of indispensable functions to serve the changing needs of the growing fetus and to adapt the maternal organism. Some of the main functions of the placenta are the exchange of nutrients and oxygen for carbon dioxide and other waste products, the production of pregnancy supporting hormones and the protection of the fetus against infections and xenobiotics. Hence, normal placental development and function are crucial for maternal and fetal health. It is, therefore, of paramount importance to understand if NPs are translocated to the fetal side, potentially causing direct fetotoxic effects, but also if NPs interfere with placental functions and signaling, thereby eliciting indirect placenta-mediated developmental toxicity [16].

The majority of studies investigating developmental toxicity of NPs originate from pregnant rodents [17,18]. However, direct extrapolation of these data to human pregnancy is questionable, especially regarding placental translocation, uptake and underlying mechanisms since the development, structure and function of the placenta differs greatly between species [19,20]. To circumvent species-specific differences, several in vitro and ex vivo placental models were developed using human cell lines, primary cells or placental tissue [21–27]. In comparison to the ex vivo placental perfusion, these models offer different advantages such as a higher screening throughput and longer exposure times. They are seen as valuable complementary models (for comprehensive review on advantages/limitations of different placental models see Refs. [28–30]). However, for investigations on placental translocation of substances and NPs, the ex vivo placenta perfusion model is still considered as the gold standard. This model elegantly allows to assess the potential effects of NP interactions and underlying mechanisms under dynamic near-physiological conditions using the full complexity of human term placental tissue. Therefore, the possibility to access human placenta after delivery, provides a unique opportunity to obtain human-relevant data on NP translocation and their effects on placental function. With this review, we compile the current knowledge from human ex vivo placental perfusion studies of NPs. Further on, we discuss potential future applications of the ex vivo placenta model as well as specific considerations in the design of future placental perfusion studies with NPs.

1.1. Ex vivo placental perfusion of NPs

The ex vivo placental perfusion model has been developed by Panigel et al., in 1967 [31] and continuously modified by Schneider et al., in 1972 [32] (for a review on the history see Schneider/Albrecht et al. manuscript submitted for this special issue). Initially, the model has been applied to study the placental transfer of endogenous compounds and pharmaceutical drugs. From 1990 onwards, perfusion studies were extended to environmental chemicals (Mathiesen et al. manuscript submitted for this special issue) followed by the first perfusion of a liposomal NP formulation in 1996 [33].

To get an overview on the current state of the art, a literature search was done on different scientific publication platforms (pubmed, google scholar, scopus) using keywords such as “placental perfusion”, “human placenta” combined with “nanoparticle”, “nanomaterial”, “soot”, “diesel exhaust”, “nanomedicine”, “liposomes” or “extracellular vesicles”. Only studies which employed the ex vivo human placental perfusion system to study NP interactions were considered (Table 1, Table S1). A total of 17 studies were published between 1996 and 2020, including five studies on liposomal NP, three on polystyrene (PS) NPs, two on gold NPs, and one each on TiO₂ NPs, silica NPs, dendrimers and a block copolymer NPs. The main focus was to determine maternal-fetal translocation rates of the NPs while some studies provided further information on accumulation and localization of the particles in the placental tissue.

Overall, translocation was observed for most of the investigated NPs in the ex vivo placental perfusion model after 1–6 h of perfusion, but the transfer was mostly low (lower percentage or even sub-percentage range) with the exception of plain 50–80 nm PS NPs (ranging from 13.7 ± 8.4% to 35.6 ± 7.2% of the initial dose) [39–41]. Importantly, even though that applied NP doses were relatively high likely over-estimating realistic unintentional exposure scenarios, the cited studies demonstrated no influence on barrier integrity as controlled by appropriate transfer of passive diffusion markers (antipyrine or creatinine) and/or absence of fetal to maternal leakage of perfusate (Table S1). For nanomedicines reported concentrations might be feasible for intravenous bolus administrations.

However, most NPs showed a propensity to accumulate in the placental tissue, in particular in the syncytiotrophoblast (ST) layer. Since the ST exerts important immunological, endocrine, metabolic and protective functions, future research should more closely address the short- and long-term consequences of NP accumulation on placental tissue viability, functionality and signaling (reviewed in Ref. [16]). In some of the studies, different particle functionalization or sizes were included in order to unveil if different physicochemical properties could affect placental accumulation and transfer. Such structure-activity relationships (SAR) have been previously established for NPs [50] and are essential for the implementation of safe-by-design approaches for NPs. For example, Wick et al. have demonstrated that the translocation of fluorescent PS NPs across the placenta is clearly size-dependent, and that PS beads larger than 240 nm hardly cross this tissue barrier [39]. Size-dependent translocation was also demonstrated for liposomes, with small liposomes (74 nm) crossing the placental barrier in higher amounts compared to large (147 nm) or multilamellar (296 nm) liposomes [47]. However, material composition and surface modifications play a crucial role as well. Even if the size suggests that NPs below ~ 296 nm should be able to cross the placental barrier, polyethylene glycol-coated gold (Au) particles between 15 and 30 nm did not show any transfer across the placental barrier [34]. Moreover, Graflmuller et al. reported a higher transport of plain or carboxylated PS beads compared to amine-modified PS NPs of similar sizes [41]. Thus, the findings from placental perfusion studies indicate that the capability of the NPs to cross the placental barrier does not only depend on particle size, but also on other characteristics such as the material composition, surface charge, surface modifications, agglomeration, biological surrounding and dissolution behavior. Accordingly, instead of a single characteristic, combinations of NP properties should be considered as recently highlighted in a multi-hierarchical SAR assessment visualizing the contributions of seven basic properties of Fe₂O₃ to its diverse bio-effects [51].

Identification of SARs for placental translocation of NPs could also guide the selection of a drug carrier to support passive targeting of drugs to maternal, fetal or placental tissues in addition to active targeting strategies with surface ligands [11–13].

For instance, nanocarriers that could limit placental translocation of fetotoxic anti-epileptic drugs or antiocoagulants such as valproic acid or warfarin would be needed for maternal therapies with improved safety. Here, placental perfusion studies showed that fetal drug concentrations could be reduced by 30–60% and placental uptake was minimized when these drugs were administrated within cationic liposomes [33,48]. On
| NP (functionalization, size, applied dose) | Perfusion parameters (mode*, duration, main medium contents) | Placental transfer | Placental uptake | Ref |
|---|---|---|---|---|
| PEGylated AuNPs (10–30 nm); 2.0 × 10^{-7.9} × 10^{11} NPs/mL | Open (18 min) and closed (6 h) | No placental transfer | AuNPs detected in placental tissue; mainly ST and CT layer, not in endothelium of fetal capillaries | [34] |
| PEGylated AuNPs (3 nm), carboxylated AuNPs (4 nm); 25 μg/mL | Open (1 h); Krebs-Ringer phosphate-bicarbonate buffer without serum | 4.7 μg/g tissue for PEGylated AuNPs vs. 2–14 μg/g tissue for carboxylated AuNPs; AuNPs mostly found attached to/in the outer ST layer; PEGylated AuNPs penetrated deeper into the tissue | Mass concentration of Ag fraction that accumulated in the placenta (% of applied dose); AgPEC: > 25 nm = 0.75%; ≤ 25 nm = 15% AgCOONa: > 25 nm = 4.2%; ≤ 25 nm = 7.5% | [35] |
| Fluorescently labeled non-PEGylated PS beads; 50 nm; 25 μg/mL | Closed (6 h); M199 medium/Earl’s buffer with BSA (10 g/L) | Ag fraction > 25 nm that crossed the placental barrier (mass percentage): 0.0148 ± 0.0192% for AgPEG and 0.0062 ± 0.0020% for AgCOONa | Ag fraction > 25 nm: 0.1578 ± 0.1032% for AgPEG and 0.0151 ± 0.0077% for AgCOONa | [36] |
| Fluorescently labeled PS beads, 50 nm; 25 μg/mL | Closed (6 h); NCTC-135 medium/Earl’s buffer with BSA (8 g/L) | Fetal particle concentrations after 3 h: 50 nm ~ 8.9 ± 1.8 μg/mL, 80 nm ~ 7.47 ± 1.77 μg/mL, 240 nm ~ 2.03 ± 0.29 μg/mL, and 500 nm ~ 0.31 ± 0.21 μg/mL | Fetal Ti concentrations < limit of detection (6 h) | [37] |
| Fluorescently labeled non-functionalized (50 & 240 nm) and carboxylate-modified (50 & 300 nm) PS beads; 25 μg/mL | Closed (6 h); M = F and reverse (F = M); M199 medium/Earl’s buffer with BSA (10 g/L) | Plain 50 nm: M = F = 13.7 ± 8.4%; F = M = 23.7 ± 5.8%; COOH 50 nm: M = F = 1.4 ± 0.5%; F = M = 7.2 ± 1.3%; Plain 240 nm: F = M = 2.4 ± 0.7%; F = M = 6.1 ± 4.1%; COOH 300 nm: F = M = 1.2 ± 0.7%; F = M = 5.3 ± 0.5% | Placental uptake of PS beads up to a diameter of 240 nm | [39] |
| Fluorescently labeled non-functionalized, carboxylated or amine-modified polystyrene beads (60–534 nm); 25 μg/mL | Closed (6 h); M199 medi-um/Earl’s buffer with BSA (10 g/L) | Transfer of plain (87 nm = −8%; 504 nm = −2.2%) and carboxylated (101 nm = −6%; 534 nm = 1%) PS beads but not of all amine-modified beads (Transfer values are rough read-outs from published figures) | Fluorescent amine-modified particles found in the ST and the villous mesenchyme (no results shown for plain and carboxylated PS) | [41] |
| Fluorescently labeled plain PS beads, 80 nm; 40 μg/mL | Closed (6 h); DMEM/Earl’s buffer with BSA 5 g/L or HSA 40 g/L and 8.6% human plasma or HSA 40 g/L or IgG 10 g/L | BSA (6 h): 6.7 ± 1.1 μg/mL; HSA/Plasma (6 h): 9.8 ± 2.8 μg/mL; HSA (6 h): 11.6 ± 0.5 μg/mL; IgG (6 h): 5.3 ± 0.8 μg/mL | No measurable transport | [42] |
| Fluorescently labeled silica NPs (25 & 50 nm); 100 μg/mL | Closed (6 h); DMEM/F12 media with human serum albumin (maternal 30 g/L, fetal 40 g/L) | M = F = 2.26 ± 0.12 μg/mL; F/M-ratio: 0.07 ± 0.02 | No detectable transport | [43] |
| Texas-red conjugated HPMA118-DEMAEMA13 particles containing a PLAC1-hFGF plasmid; size and dose not indicated | Closed (1 h); Earle’s bicarbonate buffer with 0.017 mM BSA | M = M = 4.2 ± 4.9%; 50 nm: M = F = 4.6 ± 2.4% | NPs localized in the ST layer of the villi | [45] |
| Liposome encapsulated carboxyfluorescein; small (74 nm), large (147 nm), multilamellar (296 nm); 20 nM | Closed (2 h); Krebs solution without serum but different dextran concentrations (maternal 7.5 g/L, fetal 30 g/L) | No detectable transport | Not investigated | [46] |
| Liposome encapsulated carboxyfluorescein; small (74 nm), large (147 nm), multilamellar (296 nm); 20 nM | Closed (2 h); autologous maternal and cord blood diluted with Te-199 medium (mean haematocrit: maternal 6, fetal 14) | Small (M = F = 3.7 ± 0.4%; FAUC = 186 ± 27% dose min^{-1}; F/M-ratio = 0.05 ± 0.005) | Large (M = F = 0.8 ± 0.1%; FAUC = 43.5 ± 6.8% dose min^{-1}; F/M-ratio = 0.009 ± 0.002) | [47] |
| Multilamellar (M = F = 0.4 ± 0.2%; FAUC = 19.0 ± 7.0% dose min^{-1}; F/M-ratio = 0.003 ± 0.005) | Warfarin (M = F = 14.9 ± 1.1%; FAUC = 890.7 ± 95.4% dose min^{-1}; F/M-ratio = 0.04) | Small (15.2 ± 1.6%) | Large (3.0 ± 0.4%) | [48] |

(continued on next page)
the other hand, anionic liposomes containing the thyroid hormone thyroxin T4 significantly increased the transfer of T4 from the maternal to the fetal circulation, which may be useful as an approach to fetal thyroid hormone replacement [49].

Of note, the majority of the cited studies used placentae from uncomplicated pregnancies (Table S1) and only one study included placentae obtained from pregnancies exhibiting gestational diabetes mellitus or intra uterine growth restriction [46]. In this particular study, liposomes did not penetrate the placental barrier independent from the pregnancy condition [46]. However, an additional adverse condition could, for example, enhance or reduce NP translocation to the fetal side. Moreover, the exact role of maternal particulate exposure in the pathogenesis of pregnancy complications is not completely understood so far.

1.2. Future perspectives

Previous studies employing the ex vivo placental perfusion model mainly focused on NP translocation or uptake to estimate fetal exposure. However, studies on indirect developmental toxicity effects of NPs on the fetus or the mother, which could be mediated by NP induced placental dysfunction, are underrepresented in literature so far [16, 52].

With recent technological advancements, perfusion studies could be exploited to tackle further questions on the impact of NPs on the human placenta beyond maternal-fetal transfer. For instance, omics-based technologies like proteomics, metabolomics or transcriptomics, can reveal detailed insights into molecular and functional mechanisms underlying or succeeding NP-tissue interactions [42, 51].

Another emerging research field relates to the exposome paradigm and the fact that pregnant women are exposed to multiple environmental factors, including but not limited to NPs. In addition, exposure that occurs before pregnancy could result in an accumulation in the female body before pregnancy leading to an in utero exposure later on. While an individual stressor alone might not be sufficient to induce a sustained response, they could do so if they act synergistically to induce developmental toxicity. Therefore, future research should investigate the impact of combined exposures (e.g. mixture of different kinds of NPs or a combination of environmental chemical pollutants and NPs) to elucidate potential additive effects. Here, the ex vivo placental perfusion could provide a reliable model to achieve first mechanistic insights on placenta-specific responses from a complex tissue under near-physiological conditions.

Extracellular vesicle (EV) signaling is another emerging research line where placenta perfusion studies may be explored to assess the effects of NPs on placentamediated communication. EVs are cell-derived particles in the micro- or nano-range (mostly microvesicles or exosomes), delimited by a lipid bilayer and packed with molecular information, including RNAs and proteins [53]. Placenta-derived EVs are indicated to be involved in crucial processes such as the formation of the maternal-placental vascularization and the maintenance of maternal-fetal immune tolerance [54, 55]. There is first evidence that NPs can interfere with EV communication [56, 57] and thus, it is conceivable that NP accumulation in placental tissue might interfere with EV signaling processes relevant to successful pregnancy outcome. Here, the ex vivo placenta perfusion model could be used to elucidate potential underlying toxicity mechanisms such as: (I) expulsion of NPs internalized by placental cells via EVs to the fetal and/or maternal side, (II) interference of NPs with the release of placental EVs, and (III) modification of EV cargo (other than NPs) leading to adverse effects in target cells. In case of adverse effects on placental EV signaling, a modification in placenta-derived EVs could be used as an early biomarker for adverse NP exposure.

On the other hand, endogenous or engineered EVs (“EV mimetics”) are discussed as vehicles for delivering therapeutic molecules [58, 59]. They might allow the design of novel safe therapies to treat placental complications or deliver drugs across the placental barrier as it is currently studied in the brain [60]. Here, the ex vivo placental perfusion may be used to collect placental-derived EVs [61], but also to study the translocation of EVs from non-placental and artificial origins. For example, the translocation of maternally derived EVs to fetal tissues has been shown in mice [62], but this concept has not been confirmed in humans yet. Overall, further studies on EVs in human placenta ex vivo perfusion have the potential not only to elucidate crucial safety aspects for pregnant women and the fetus, but also to support the development of safe NP-based therapeutic approaches during pregnancy.

2. Methodological considerations for ex vivo perfusion of NPs

The placenta perfusion model has been developed, optimized and verified for the perfusion of biomolecules and pharmaceutical drugs. For these, good correlation between transfer rates from perfusion studies with physiological data has been obtained [63]. However, NPs have unique properties and often behave differently from soluble small molecules, which might require small adaptations to the perfusion...
system or the inclusion of additional controls (Fig. 1).

A first aspect relates to the choice of the perfusion medium. Ex vivo placental perfusion is not yet standardized in regards of the used perfusion medium. Perfusion media are mostly based on cell culture media containing specific additives like plasma expanders (dextranes) and/or proteins like bovine or human serum albumin [32,36,44]. Proteins and components of the medium will adhere to the NP surface to form a so-called protein- or bio-corona [64,65]. This corona forms de novo in the medium depending on the NP material and the preincubation time of the particles in the medium [66-68]. Its composition has a decisive impact on the NP-bio interactions such as on particle uptake and toxicity [69,70]. Obvious approaches to achieve a more physiological bio-corona in placental perfusion studies are the usage of human plasma or serum to pre-coat the NPs or to complement the perfusion medium [65]. However, the addition of blood plasma requires the use of anticoagulants to prevent the formation of thrombi, which again can change the composition of the protein corona [71]. Recently, it has been investigated whether a preincubation with human plasma alters the transfer of 80 nm PS NPs across the human placenta. The combination of placental perfusion and proteomics analysis revealed human albumin as the predominant plasma protein on the corona in media containing physiological human albumin concentrations. This precoating led to a significantly enhanced placental transfer of the NPs compared to a precoating media containing bovine serum albumin or human immunoglobulin G [42].

Further modifications of the perfusion protocol and quality measures depending on the investigated type of NPs, might be required. For instance, the use of a suitable control to verify a sufficient overlap between the maternal and fetal circulations and to normalize the translocation data is mandatory. Antipyrine is frequently used as a passive diffusion control that equilibrates in maternal and fetal circulation within about 60 min of perfusion. However, since placental transport of NPs is considerably slower than for small molecules and drugs, the implementation of a diffusion marker with slower diffusion kinetics in addition to/instead of antipyrine should be considered. Creatinine was proposed as a suitable candidate [72] since it is not produced in significant amounts by the placenta and is transferred by simple diffusion.

The results from perfusion studies, it is crucial to understand the colloidal stability of NP suspensions in perfusion medium. Many NPs have a strong propensity to agglomerate in biological media, which can increase non-specific adherence and loss of agglomerates to tubing and to other components of the perfusion system [35,37]. This will ultimately result in a considerable discrepancy between applied dose and concentration of the compound delivered to the placental barrier. Therefore, stability of NP suspensions should be carefully characterized in the respective perfusion medium for the same incubation time and under similar conditions as applied for perfusion studies. The exact analysis of non-stable polydisperse particle suspensions is currently a major challenge. However, a combination of analytical techniques for particle size distribution such as Nanoparticle Tracking Analysis (NTA), Dynamic Light Scattering (DLS) or Small Angle X-ray Scattering (SAXS) can provide valuable information on particle agglomeration/sedimentation characteristics [35,77]. To assess absorption of NPs to the perfusion system, control perfusions in the absence of placental tissue should be included to every study protocol. However, the obtained absorption values only provide an estimate on particle binding to the device, but do not directly correlate to the placenta perfusion experiments since proteins released from the placental tissue and/or slightly different pressure conditions can partially stabilize the NP suspension [35].

Solubility of NPs is another critical parameter in NP perfusion studies, in particular for proper interpretation of placental translocation. For instance, for Ag NPs, dissolution and reprecipitation processes have been observed in maternal and fetal perfusates as well as in placental tissue [36]. Consequently, the mere presence of NPs in the fetal circulation does not necessarily imply that they have crossed the placenta in the particulate form. Characterization of NP suspensions by inductively-coupled plasma-mass spectroscopy (ICP-MS) may provide insights on particle dissolution in perfusion medium over time while ICP-MS analysis in single-particle mode (spICP-MS) of perfusates and placental tissue samples allows distinguishing between particulate and ionic forms of soluble metal-containing NPs [36].

The translocation of NPs to the fetal side can also be falsely determined by the release of previously accumulated NPs in the placental tissue with a similar material profile. For example, recent studies demonstrated that placenta exhibit basal levels of black carbon or TiO₂ particles [38,78]. For black carbon particles, placental load was 0.95 × 10⁴ ± 0.66 × 10⁴ and 2.09 × 10⁴ ± 0.96 × 10⁴ particles per mm² tissue after low and high exposure to air pollution during pregnancy, respectively [78]. In another recent study, Guillard et al. demonstrated a basal placental Ti level of 0.10 mg/kg with some placenta even exhibiting much higher Ti levels of up to 0.5 mg/kg tissue [38].

These NPs can be released to the fetal side during perfusion and potentially disturb the NP measurement in the fetal compartment. Further characterization of the NPs via Scanning Electron microscopy-

---

Fig. 1. Graphical summary of specific considerations that needs to be taken into account in ex vivo placental perfusion studies of NPs and endpoints that can be affected by these NP behaviors.
energy dispersive Xray (STEM-EDX) could help to identify different types of NPs as applied in Ref. [38]. Special care and appropriate control measures need to be applied additionally when labeled NPs are used to track placental uptake, tissue distribution and translocation. These labels, most commonly fluorescent dyes or radioactive labels, might alter NP properties and their interaction with biological materials [79] and loss of these labels can lead to wrong conclusions [41]. Therefore, the label intensity should be monitored over time and filtration/dialysis experiments should be performed with suitable approaches to understand signal intensity, label stability and leakage of labels from NPs during experiments. Similar considerations should be applied for functionalized NPs, where the presence and stability of the functional groups highly determines the characteristics of the particles. For a previous ex vivo placental perfusion study, a re-characterization of different commercial polystyrene NPs has shown that the majority of NPs were not appropriately functionalized (e.g. lack of positive/negative charge) or were contaminated with a second fraction of smaller NPs [41]. In summary, a comprehensive NP characterization is indispensable for generating reliable study protocols, for interpreting placental perfusion results and determining SARs for the safe use of NPs.

Further, working with NPs can result in altered analytical outcome depending on interactions of the NPs intrinsic optical, fluorescent or reactive properties with the specific assays [80–82]. Therefore, appropriate interference controls must be implemented to exclude false positive or negative results. This is currently only a minor topic in the context of ex vivo perfusion studies since predominantly the particles themselves are detected to understand placental translocation but might become more relevant if additional functional endpoints will be included (e.g. impact of NPs on placental tissue viability or functionality).

3. Conclusions

The ex vivo placental perfusion approach enables us to undertake studies on the placental barrier close to physiological conditions and on intact tissue.

So far, the uptake and translocation has been investigated only for a few NP types. Nevertheless, these studies indicate that NP properties (single or combined) may have a considerable effect on NP-placenta interactions. Therefore, future research efforts should continue to elucidate these SARs to guide the design of sustainable NP-based industrial and nanomedical applications.

In summary, the ex vivo human placenta perfusion system provides a highly valuable tool to estimate not only the fetal exposure to NPs but also their direct impact on placental tissue, supporting the establishment of a realistic safety assessment and the development of future nanomedical therapies during pregnancy.

Declaration of interests

None.

Sources of funding

This research was supported by funding from the Swiss National Science Foundation (TBT; grant no 31003A_179337; LA: grant no IZSEZ0_193940). MG was supported by the Medical University of Graz through the PhD MolMed. The Placenta Lab Jena has been supported by the German Research Foundation (DFG, Ma1550/12-1 to URM and RRF; Mo2017/3-2 to DMMP) and the Interdisciplinary Centre for Clinical Research of the University Hospital Jena (IZKF, FP05 to DMMP).

Table S1
Additional information to perfusion studies (cited in Table 1)

| Ref  | Number of perfusions (n) | Further information on patient group | Placental tissue viability/NP toxicity on BeWo trophoblasts |
|------|--------------------------|-------------------------------------|-----------------------------------------------------------|
| [34] | FEGylated AuNPs: 10 nm: 1 (closed) | Uncomplicated pregnancies, non-smokers | F. > M leakage (<2 mL/1), antipyrine transfer |
|      | 15 nm: 1 (closed), 1 (open)       |                                     |                                                            |
|      | 30 nm: 1 (open)                  |                                     |                                                            |
| [35] | FEGylated AuNPs: 2 (360 min) + 1 (330 min) | Uncomplicated term pregnancies, placenta obtained after caesarean section | F. > M leakage (<4 mL/A)/no decrease in viability of BeWo trophoblast cells up to 50 μg/mL and 48 h of exposure (MTS assay) |
|      | Carboxylated AuNPs: 2 (360 min) + 1 (300 min) |                                     |                                                            |
| [36] | 3 per NP                        | Uncomplicated term pregnancies, placenta obtained after caesarean section | F. > M leakage (<4 mL/A)/no decrease in viability of BeWo trophoblast cells up to 100 μg/mL and 6 h of exposure but reduced viability after 24 h exposure (MTS assay) |
| [37] | 3 per NP                        | Uncomplicated term pregnancies, placenta obtained after caesarean section | F. > M leakage (<4 mL/A)/no decrease in viability of BeWo trophoblast cells up to 25 μg/mL and 48 h of exposure (MTS assay) |
| [38] | 6 with NP, 1 without NP          | Uncomplicated term pregnancies, placenta obtained after vaginal or caesarean section | F. > M leakage (<20% transfer) |
| [39] | at least 4 per NP               | Uncomplicated term pregnancies, placenta obtained after vaginal or caesarean section | F. > M leakage (<4 mL/A), antipyrine transfer (equilibrium after 4–6 h) |
| [40] | at least 3 per NP               | Uncomplicated term pregnancies, placenta obtained after caesarean section | F. > M leakage (<4 mL/A)/no decrease in viability of BeWo trophoblast cells up to 100 μg/mL and 24 h of exposure (MTS assay) |
| [41] | between 1 and 4 perfusions per NP | Uncomplicated term pregnancies, placenta obtained after caesarean section | F. > M leakage (<4 mL/A) |
| [42] | 5 per condition (except for IgG-Medium 3) | Uncomplicated term pregnancies (>38 weeks of gestation), placenta obtained after vaginal delivery or caesarean section | F. > M leakage (<24 mL at end of perfusion), glucose consumption and lactate production, antipyrine transfer (<0.3 within initial 30 min of perfusion) antipyrine transfer |
| [43] | 4                               | Placenta from normal term deliveries | F. > M leakage (<3 mL/A), glucose consumption and lactate production/ no decrease in viability of BeWo trophoblast cells up to 500 μg/mL and 24 h of exposure (MTT assay) |
| [44] | 3 per NP                        | Uncomplicated term pregnancies, placenta obtained after vaginal delivery or caesarean section | F. > M leakage (<4 mL/A)/no decrease in viability of BeWo |
| [45] | 1 control without NP, 6 with NPs | Gender of offspring given (5 females, 2 males), term (>39) | (continued on next page)
updated systematic review and meta-analysis, Environ. Pollut. 227 (2017) 596–605, https://doi.org/10.1016/j.envpol.2017.03.055.

[8] H.E. Voll, F. Lurmann, B. Penfold, I. Hertz-Picciotto, R. McConnell, Traffic-related air pollution, particulate matter, and autism, Arch. Gen. Psychiatr. 70 (2013) 71–77, https://doi.org/10.1001/jamapsychiatry.2013.266.

[9] R. Raz, A.L. Roberts, K. Lyall, J.E. Hart, A.C. Junt, F. Laden, M.G. Weisskopf, Autism spectrum disorder and prenatal air pollution before, during, and after pregnancy: a nested case–control analysis within the nurses’ health study II cohort, Environ. Health Perspect. 123 (2015) 264–270, https://doi.org/10.1289/ehp.1406151.

[10] E.O. Talbott, V.C. Arena, J.R. Rager, J.E. Clougherty, D.R. Michanowicz, R. Raz, A.L. Roberts, K. Lyall, J.E. Hart, A.C. Junt, F. Laden, M.G. Weisskopf, Autism spectrum disorder and prenatal air pollution before, during, and after pregnancy: a nested case–control analysis within the nurses’ health study II cohort, Environ. Health Perspect. 123 (2015) 264–270, https://doi.org/10.1289/ehp.1406151.

[11] B. Zhang, R. Liang, M. Zheng, L. Cai, X. Fan, Surface-functionalized nanoparticles as efficient tools in targeted therapy of pregnancy complications, Int. J. Mol. Sci. 20 (2019) 3642, https://doi.org/10.3390/ijms20153642.

[12] J.A. Keelan, J.W. Leong, D. Ho, K.S. Iyer, Therapeutic and safety considerations of nanoparticle-mediated drug delivery in pregnancy, Nanomedicine 10 (2015) 2229–2247, https://doi.org/10.2217/nnm-2015.14.58.

[13] N.S. Irvin-Choy, K.M. Nelson, J.P. Giegli, E.S. Day, Design of nanomaterials for applications in maternal/fetal medicine, J. Mater. Chem. B 8 (2020) 6548–6561, https://doi.org/10.1039/d0tb00612h.

[14] M.D. Joshi, Drug delivery during pregnancy: how can nanomedicine be used? Ther. Deliv. 8 (2017) 1023–1025, https://doi.org/10.4155/tde-2017-0084.

[15] Y. Barenholz, Doxil® – the first FDA-approved nano-drug: lessons learned, J. Contr. Release 160 (2012) 117–134, https://doi.org/10.1016/j.jconrel.2012.03.020.

[16] B.B. Dugershaw, L. Aengenheister, S.S.K. Hansen, K.S. Hougaard, T. Buerki-Thurnherr, Recent insights on indirect mechanisms in developmental toxicity of nanomaterials, Part. Fibre Toxicol 17 (2020), https://doi.org/10.1186/s41269-020-00359-x.

[17] C. Muoth, L. Aengenheister, M. Kucki, P. Wick, T. Buerki-Thurnherr, Nanoparticle transport across the placental barrier: pushing the field forward, Nanomedicine 11 (2015) 941–957, https://doi.org/10.2217/nmm-2014-0138.

[18] K.S. Hougaard, L. Campagnolo, P. Chavatte-Palmer, A. Tardre, D. Rousseau, Ralliard, S. Valentino, M.V.D.Z. Park, W.H. de Jong, G. Woltrek, A.H. Piensma, B. Lons, G.R. Hitchison, J.S. Hansen, U. Vogel, P. Jackson, R. Slama, A. Pietroiusti, F.R. Castron, A perspective on the developmental toxicity of inhaled nanoparticles, Reprod. Toxicol. 56 (2015) 118–140, https://doi.org/10.1016/j.reprotox.2015.05.015.

[19] E. Bune, U.R. Markert, The immunology of the macaque placenta: a detailed analysis and critical comparison with the human placenta, Crit. Rev. Clin. Lab Sci. 56 (2019) 118–145, https://doi.org/10.1080/08939641.2018.1538200.

[20] A. Schmidt, D.M. Morales-Prieto, J. Pastuschek, K. Frohlich, U.R. Markert, Only humans have human placentae: molecular differences between mice and humans, J. Reprod. Immunol. 108 (2015) 65–71, https://doi.org/10.1016/j.jri.2015.03.001.

[21] C. Muoth, A. Wichter, M. Monopoli, M. Correia, N. Ehrlich, K. Loschechner, A. Gallud, M. Kucki, L. Diener, P. Manzer, W. jochum, F. Wick, T. Buerki-Thurnherr, A 3D co-culture microtissue model of the human placenta for nanotoxicology assessment, Nanoscale 8 (2016) 17322–17332, https://doi.org/10.1039/C6NR0749B.

[22] L. Aengenheister, K. Viehe, C. Muoth, R. Schonenberger, L. Diener, P. Wick, T. Buerki-Thurnherr, An advanced human in vitro co-culture model for translocation studies across the placental barrier, Sci. Rep. 8 (2018) 1–12, https://doi.org/10.1038/s41598-018-23416-0.

[23] C. Blundell, E.R. Hess, A.S. Schanzer, C. Coutifaris, E.J. Su, S. Parry, D. Huh, A microphysiological model of the human placental barrier, Lab Chip 16 (2016) 3065–3073, https://doi.org/10.1039/c6lc00259e.

[24] X. Huang, M. Liu, E.C. Ontsouka, S. Kallol, M.U. Baumann, D.V. Surbek, C. Albrecht, Establishment of a confluent monolayer model with human primary trophoblasts: novel insights into placental glucose transport, Mol. Hum. Reprod. 22 (2016) 442–456, https://doi.org/10.1093/molehr/gaw018.

[25] R.K. Miller, O. Gembacher, M.A. Turner, J.D. Aplin, I. Canigaglia, B. Huppmann, Human placental explants in culture: approaches and assessments, Placenta 26 (2005) 439–448, https://doi.org/10.1016/j.placenta.2004.10.002.

[26] A. Nishiguchi, C. Gilmore, A. Sood, M. Matsuaki, G. Collett, D. Tannista, I. L. Sargent, J. McGarvey, N.D. Haleman, J. Hanley, P. Day, S. Grant, C. Murdoch-Davies, H. Kemp, P. Verkade, J.D. Aplin, M. Akashi, C.P. Case, In vitro placenta–placental explants in culture: approaches and assessments, Placenta 26 (2005) 439–448, https://doi.org/10.1016/j.placenta.2004.10.002.

[27] S. Shibata, E.H. Kobayashi, N. Kohayashi, A. Iwase, T. Arima, Unique features and emerging in vitro models of human placental development, Reprod. Med. Biol. 19 (2020) 301–313, https://doi.org/10.1046/j.2011-010-0237.0.
and E: the anti-atherogenic impact of the placenta, Sci. Rep. 9 (2019) 6225, https://doi.org/10.1038/s41598-019-42522-1.

[75] B. Warth, K. Preindl, P. Manser, P. Wick, D. Marko, T. Buerki-Thurnherr, Transfer and metabolism of the xenoestrogen zearalenone in human perfused placenta, Environ. Health Perspect. 127 (2019) 107004, https://doi.org/10.1289/EHP4860.

[76] M. Gruber, B. Hirschmugl, S. Kopp, U. Lang, G. Desoye, C. Wadsack, Abstracts. https://www.scitechnol.com/peer-review/7th-international-bioNanoMed-2016-congress-JCWR.pdf, 2016. (Accessed 10 September 2020).

[77] S. Mourdikoudis, R.M. Pallares, N.T.K. Thanh, Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties, Nanoscale 10 (2018) 12871–12934, https://doi.org/10.1039/c8nr02278j.

[78] H. Boeve, E. Bongaerts, E.M. Bijnen, N.D. Saenen, W. Gysemans, M. Van Eyken, M. Pluizyn, M.B.J. Roefs, M. Ameloot, T.S. Nawrot, Ambient black carbon particles reach the fetal side of human placenta, Nat. Commun. 10 (2019) 3866, https://doi.org/10.1038/s41467-019-11654-3.

[79] S. Snipstad, S. Hak, H. Bagehiro, E. Salheim, Œ. Merch, S. Leu, E. von Haartman, M. Back, K.P.R. Nilsson, A.S. Klymenchenko, C. de Lange Davies, A.K.O. Åslund, Labeling nanoparticles: dye leakage and altered cellular uptake, Cytometry 91 (2017) 760–766, https://doi.org/10.1002/cyto.a.22853.

[80] E.J. Petersen, C. Hirsch, J.T. Elliott, H.F. Krug, L. Aengenheister, A.T. Arif, A. Boggi, A. Kinner-Ovaskainen, S. May, T. Walser, P. Wick, M. Roesslein, Cause-and-effect analysis as a tool to improve the reproducibility of nanobioassays: four case studies, Chem. Res. Toxicol (2019), https://doi.org/10.1021/acs.chemrestox.9b00165.

[81] N. Bohmer, A. Rippl, S. May, A. Walter, M.B. Heo, M. Kwak, M. Roesslein, N. W. Song, P. Wick, C. Hirsch, Interference of engineered nanomaterials in flow cytometry: a case study, Colloids Surf. B Biointerfaces 172 (2018) 635–645, https://doi.org/10.1016/j.colsurfb.2018.09.021.

[82] K.J. Ong, T.I. MacCormack, R.J. Clark, J.D. Ede, V.A. Ortega, L.C. Felix, M.K. M. Dang, G. Ma, H. Fenniri, J.G.C. Veinot, G.G. Goss, Widespread nanoparticle-assay interference: implications for nanotoxicity testing, PloS One 9 (2014), e90650, https://doi.org/10.1371/journal.pone.0090650.