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Review

# Experimental human placental models for studying uptake, transport and toxicity of micro- and nanoplastics

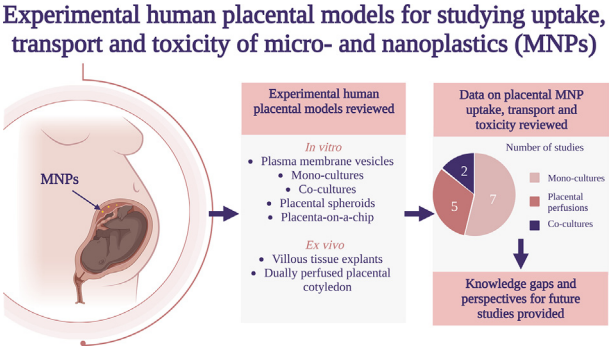
Hanna M. Dusza [a,*,1], Jeske van Boxel [b,1], Majorie B.M. van Duursen [b], Markus M. Forsberg [c], Juliette Legler [a], Kirsi H. Vähäkangas [c]

[a] Division of Toxicology, Institute for Risk Assessment Sciences, Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[b] Amsterdam Institute for Life and Environment, Faculty of Science, Vrije Universiteit Amsterdam, the Netherlands
[c] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland

## HIGHLIGHTS

- Placenta is crucial for fetal development and pregnancy.
- First evidence of microplastics in human placentas emerge, but more data is needed using relevant methodology.
- Human *in vitro* and *ex vivo* placental models are recapitulated and comprehensively reviewed.
- The current understanding of transplacental transport and toxicity of microplastics is summarized.
- The most pressing knowledge gaps are identified and perspectives for future research provided.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Micro- and nanoplastics (MNPs) are ubiquitous in the environment and have recently been found in human lungs, blood and placenta. However, data on the possible effects of MNPs on human health is extremely scarce. The potential toxicity of MNPs during pregnancy, a period of increased susceptibility to environmental insults, is of particular concern. The placenta provides a unique interface between maternal and fetal circulation which is essential for *in utero* survival and healthy pregnancy. Placental toxicokinetics and toxicity of MNPs are still largely unexplored and the limited studies performed up to now focus mainly on polystyrene particles. Practical and ethical considerations limit research options in humans, and extrapolation from animal studies is challenging due to marked differences between species. Nevertheless, diverse *in vitro* and *ex vivo* human placental models exist *e.g.*, plasma membrane vesicles, mono-culture and co-culture of placental cells, placenta-on-a-chip, villous tissue explants, and placental perfusion that can be used to advance this research area. The objective of this concise review is to recapitulate different

*Abbreviations:* ABC, ATP binding cassette; ABCB1, P-glycoprotein; ABCG2, breast cancer resistance protein; CdTe-COOH, carboxylic acid functionalized CdTe quantum dots; CRH, corticotrophin-releasing hormone; CTs, cytotrophoblasts; CuO, copper oxide; DDT, dichlorodiphenyltrichloroethane; ECM, extracellular matrix; ER, estrogen receptor; EVCTB, extravillous cytotrophoblast; EVTs, extravillous trophoblasts; Fluo-PS, fluorescent polystyrene; GPMVs, giant plasma membrane vesicles; hCG, human chorionic gonadotropin; hPL, human placental lactogen; HPVEC, human placental vascular endothelial cells; HUVEC, human umbilical vein endothelial cells; IL, interleukin; MNPs, micro- and nanoplastics; MPs, microplastics; NPs, nanoplastics; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyls; PGH, placental growth hormone; PL, placental lactogen; PR, progesterone receptor; PS, polystyrene; ROS, reactive oxygen species; ST, syncytiotrophoblast; $TiO_2$, titanium dioxide; TLR, toll-like receptor; VCTs, villous cytotrophoblasts.

* Corresponding author at: Institute for Risk Assessment Science, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80177, NL-3508 TD Utrecht, the Netherlands.
*E-mail address:* h.m.dusza@uu.nl (H.M. Dusza).

[1] Authors contributed equally.

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human placental models, summarize the current understanding of placental uptake, transport and toxicity of MNPs and define knowledge gaps. Moreover, we provide perspectives for future research urgently needed to assess the potential hazards and risks of MNP exposure to maternal and fetal health.

## Contents



## 1. Introduction

The environment is widely polluted with microplastics (MPs, <5 mm) and nanoplastics (NPs, <1 μm), collectively called micro- and nanoB122plastics (MNPs) (Gigault et al., 2018; Rodríguez-Seijo and Pereira, 2017). MNPs are synthetic polymers that are formed by weathering, mechanical abrasion, and photodegradation of plastic products and waste (Prata et al., 2020; Wu et al., 2019). MNPs are also intentionally produced for various purposes such as drug delivery systems and cosmetics (*e.g.*, sunscreens) (Barnes et al., 2009; Wu et al., 2019). Thus, environmental exposures are typically to a mixture of MNPs of various sizes, polymer types and chemical compositions. Exposure to MNPs in humans occurs mainly through ingestion and inhalation (Cox et al., 2019; Prata et al., 2020). Recently, MNPs have been found in lung tissue and blood confirming internal human exposure (Amato-Lourenço et al., 2021; Jenner et al., 2022; Leslie et al., 2022) and raising concerns about their effects on human health (Vethaak and Legler, 2021). Of special concern is the potential toxicity of MNPs during pregnancy. The fetus is highly susceptible to toxicity due to intense and strictly regulated cell proliferation, apoptosis, cell differentiation and cell migration during organogenesis. The so-called developmental windows of susceptibility define periods of heightened vulnerability to environmental toxicants, during which disturbance of pre- or postnatal development could contribute to adult-onset disease (Ho et al., 2016; Segal and Giudice, 2019). In the case of MNPs, direct developmental toxicity may arise from particles in maternal blood that cross the placental barrier and damage fetal tissues (Dugershaw et al., 2020). However, developmental toxicity may also arise from the damage to the placenta itself, which may affect not only fetal but also maternal health during pregnancy.

The placenta is a multifaceted temporary organ at the interface of maternal and fetal circulation, crucial for fetal growth and development. Contrary to the old belief that the placenta forms an impermeable layer that protects the fetus from harmful substances present in maternal blood, it is now known that a large variety of environmental pollutants with diverse molecular structures can cross the placenta. Transplacental transport of licit and illicit drugs, xenobiotics, as well as engineered metallic and carbonaceous nanoparticles is now well documented (Aengenheister et al., 2021; Bongaerts et al., 2020; Myllynen and Vähäkangas, 2013; Syme et al., 2004). More recently, (ultra)fine particles from air pollution have been detected in placental tissue, notably ambient black carbon particles (Bové et al., 2019) and nanoparticles from diesel exhausts (Bongaerts et al., 2021). Carbon and metal-bearing nanoparticles were also recently found in placental phagocytes (Liu et al., 2021). Epidemiological data indicate that exposure to (ultra)fine particles during pregnancy is associated with various complications such as pre-eclampsia (Malmqvist et al., 2013; Su et al., 2020), preterm birth (Wang et al., 2018b), low birth weight (Wojtyla et al., 2020) and stillbirth (DeFranco et al., 2015). They may also predispose the fetus to malformations such as congenital heart defects (Lavigne et al., 2019) and diseases later in life such as respiratory diseases (Hehua et al., 2017). Recently, MNPs have been detected for the first time in human placental samples and meconium (Braun et al., 2021; Ragusa et al., 2021). This demonstrates that environmental (ultra)fine particles including MNPs reach placental cells and that a human fetus may be exposed *in utero*. Considering the central role of the placenta in fetal and maternal wellbeing during pregnancy, there is an urgent need to understand the potential placental toxicokinetics and toxicity of MNPs.

Due to practical and ethical considerations, *in vivo* studies with rodents are the most commonly used to study placental function and development.

So far, only a few studies have investigated the transplacental transport of MNPs. *In vivo* rodent studied have shown that MNPs can cross the placental barrier and accumulate in the internal organs of the offspring (Bongaerts et al., 2020; Fournier et al., 2020; Ho et al., 2017; Huang et al., 2015). It is hypothesized that consequent toxicity, such as metabolic disturbance, alterations of behaviour, neurotoxicity and increased mortality, are mediated through changes in gene expression, oxidative stress, inflammation and cell death (González-Acedo et al., 2021). The placenta is, however, the most species-specific organ with outstanding evolutionary diversity. The functional (*e.g.*, expression of transporter proteins), as well as anatomical (*e.g.*, the number of trophoblast cell layers) differences between species are important factors determining placental permeability to nutrients, chemicals and particles, making extrapolation from rodent models to humans challenging (Furukawa et al., 2014; Schröder, 1995; Soncin et al., 2015). The use of models relevant to humans, derived from human placental tissue, such as primary cells or cell lines of placental origin, are therefore considered superior to using other mammalian models when analysing various aspects of placental biology and toxicity, including uptake and toxicity of MNPs. So far, a limited number of studies employing human *in vitro* placental cell models and *ex vivo* placental perfusion have investigated transplacental transport of MNPs, and MNP toxicity and the mechanisms underlying toxicity have rarely been addressed. The objective of this concise review is to summarize the current state of the science of placental MNP research, with a focus on the available *in vitro* and *ex vivo* placental models relevant to humans. First, we summarize the development, physiology and morphology of the human placenta. Next, we critically evaluate different models of the human placenta and recapitulate the current understanding of placental toxicokinetics and toxicodynamics of MNPs. Lastly, we highlight areas that require further scrutiny including potential toxicity endpoints and key components to consider when investigating the placental MNP exposure.

## 2. Human placenta

### 2.1. Placental development and physiology

The placenta is the first organ that forms during human embryogenesis. It develops from the trophectoderm (*i.e.*, early trophoblast cells surrounding the blastocyst) in the first weeks of pregnancy (Benirschke et al., 2012). Human placentation differs from that of other mammals and is orchestrated by highly regulated temporal and spatial interactions between extraembryonic mesenchymal cells and trophoblastic cells (Carter, 2021). The trophoblastic cells engage in diverse functions throughout gestation, ranging from attachment, invasion, and vascular remodelling to hormone production and nutrient transport. Implantation and placental development have been extensively described elsewhere (Benirschke et al., 2022; Knöfler et al., 2019; Maltepe and Fisher, 2015; Soares et al., 2018; Turco and Moffett, 2019). Trophoblastic cell lineage has two distinctive cell populations, the undifferentiated cytotrophoblasts (CTs) and the fully differentiated syncytiotrophoblast (ST). The undifferentiated CTs either differentiate into villous cytotrophoblasts (VCTs) and further fuse to form multinucleated ST (with a lost capacity to divide) or gain a migratory phenotype to become extravillous trophoblasts (EVTs). Molecular details of the regulation of the differentiation processes are very complex and still being studied (Knöfler et al., 2019). At two weeks after implantation, EVTs begin an aggressive invasion into maternal stroma and remodelling of the resident blood vessels, a process unique to human placentation (Carter and Mess, 2014). At the same time, VCTs start to form primary chorionic villi and proteolytic enzymes continue to break down the extracellular matrix (ECM) to generate intervillous space. By approximately 8 weeks postconception, the blueprint of the placenta is established and maternal blood starts to flow through the placental villi (Jauniaux et al., 2003; Knöfler et al., 2019). The placenta and placental villi continue to change and grow to ensure adequate nutritional supply throughout the developmental stages of the fetus (Knöfler et al., 2019).

At full-term, the human placenta is a large discoid organ that is 2–2.5 cm thick, approximately 15–20 cm in diameter and weighs up to 600 g (Burton et al., 2006; Burton and Fowden, 2015). On one side, there is a chorionic plate with an umbilical cord that connects to the fetus. On the other side, there is a basal plate that is attached to the maternal endometrium (Fig. 1). Villous trees emerging from the chorionic plate begin with stem villi that branch into 3–5 intermediate villi and then further into 10–12 terminal villi (<20 μm diameter), richly vascularized by a fetal capillary network (Haeussner et al., 2014; Mayo et al., 2019). These villous trees float in an intervillous space filled with maternal blood and are separated laterally by incomplete placental septa. At full-term, maternal blood (about 30 % of the maternal cardiac output) from remodelled spiral arteries flows through the intervillous space at a flow rate of 600–700 mL/min, bathing villous trees with a total surface area of 12–14 $m^2$ (Gude et al., 2004; Poston, 1997; Syme et al., 2004). It is here, at the cellular barrier of the villous tree, that the exchange of nutrients, gases and waste products between maternal and fetal blood takes place, and where the exogenous particles such as MNPs present in maternal blood can interact with the placental trophoblastic structures.

### 2.2. Placental barrier

The term "placental barrier" refers to the cell layers that separate maternal blood in the intervillous space from the fetal capillaries in the core of the chorionic villi (Fig. 1) and harbour important molecular structures supporting placental functions, such as specialized transporter proteins. The cell layer in direct contact with maternal blood is a layer of ST with multiple nondividing nuclei and without lateral cell membranes forming thus one continuous layer (Carter and Enders, 2004; Gude et al., 2004; Wick et al., 2010). The plasma membrane of the ST is highly polarized and consists of two horizontal membranes: the brush border membrane facing maternal blood and the basal membrane, which can be distinguished by their protein, receptor, and transporter compositions (Vähäkangas and Myllynen, 2009). In the villous stroma, under the ST, are some remaining CTs that retain the capacity to divide, and by cellular fusion give rise to ST (Fig. 1). Behind ST and CTs lies the connective tissue containing fibroblasts and Hofbauer cells, the placental macrophages (Juch et al., 2018; Syme et al., 2004; Wick et al., 2010). The last layer of the placental barrier in the chorionic villi is the endothelium of the fetal capillaries.

This placental barrier in chorionic villi undergoes dynamic morphological changes during pregnancy that may influence placental MNP toxicokinetics. The ST layer becomes thinner, fetal capillaries move towards the periphery of the villi, CTs become flat rather than cubic, and their relative cell mass in relation to ST diminishes (Aengenheister et al., 2018b). The average thickness of the barrier in the first trimester is approximately 20–30 μm and by the end of the third trimester, the thickness is reduced considerably to as little as 5 μm, to allow increased exchange between the mother and the fetus (Benirschke et al., 2022; Carter and Enders, 2004; Enders and Blankenship, 1999; Feneley and Burton, 1991).

### 2.3. Placental functions

One of the most important placental functions is the transport of nutrients and solutes from maternal to the fetal circulation, through the placental barrier. Various mechanisms of transplacental transport exist including passive diffusion, active transport, pinocytosis and phagocytosis (Syme et al., 2004). Most compounds with low molecular mass (<500 Da) simply diffuse across the placental tissue through the lipid membrane or protein channels, driven mainly by a concentration gradient. There are, however, some exceptions, such as immunoglobulin G (IgG, 160 Da) which is transported mainly *via* pinocytosis (Palmeira et al., 2012). IgG is the most common antibody in the human body and its transplacental transfer takes place through binding to a specialized neonatal Fc receptor (FcRn). The FcRn-mediated transcytosis shuttles IgG in both directions across the ST layer (Sand et al., 2022). Pinocytic extracellular vesicles of various sizes (macrovesicles, microvesicles and exosomes) that carry protein, lipids, DNA, and RNA, and originating from ST, provide a means of feto-maternal communication and transmission of macromolecules between

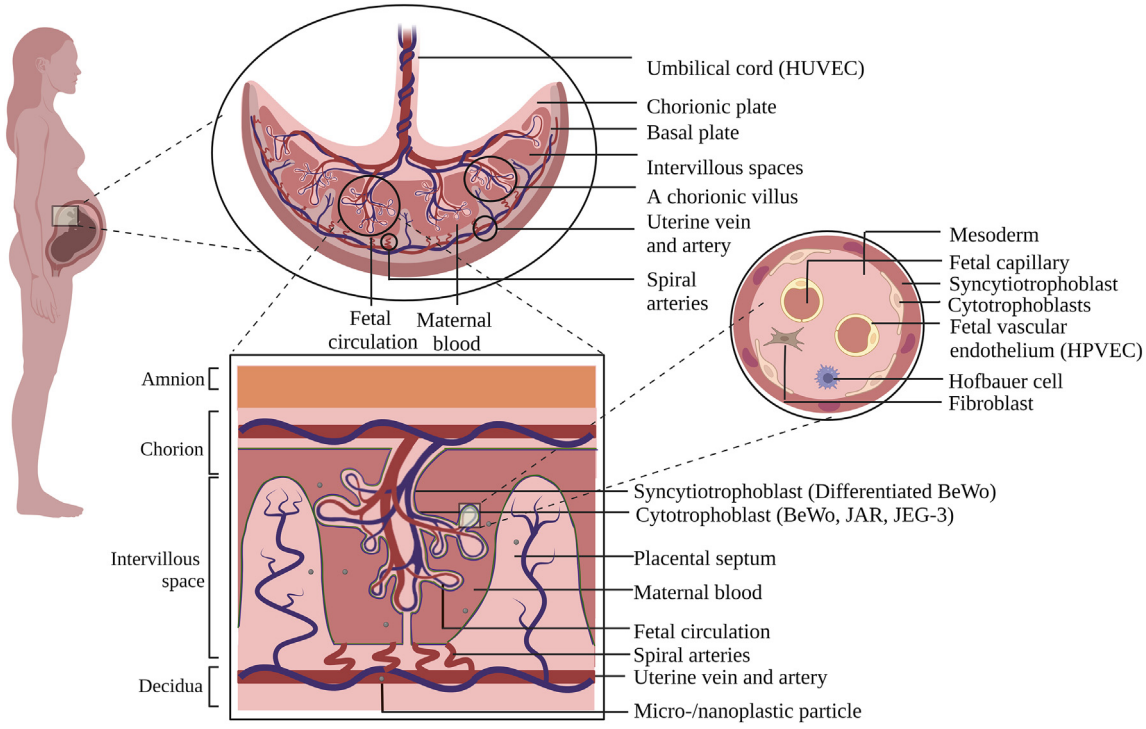


**Fig. 1.** Schematic overview of the morphology of the human placenta and chorionic villi. The different *in vitro* cell models representative of various placental cell types are given in brackets. This figure was created using BioRender.com

cells. Trophoblastic cells are also active phagocytes. They are involved in histotrophic phagocytosis during tissue remodelling throughout implantation and placentation, but also participate in innate immune defense by removing bacteria or other components of the ECM during inflammation (Amarante-Paffaro et al., 2004; Bevilacqua et al., 2010; Choy and Manyonda, 1998). Active placental transport occurs against a concentration gradient and requires protein membrane carriers. The major influx and efflux transporter families in the human placenta are the solute carrier (SLC) and ATP-binding cassette (ABC) transporters (Walker et al., 2017). There are over 30 types of specialized transporter proteins present in placental cells, with varying expressions depending on the stage of pregnancy (Myllynen and Vähäkangas, 2013; Vähäkangas and Myllynen, 2009). The ABC efflux transporters, especially the p-glycoprotein (ABCB1) and the breast cancer resistance protein (BCRP or ABCG2) are abundant in the brush border of syncytiotrophoblast facing maternal blood and are important in protecting the fetus from foreign and potentially teratogenic compounds (Myllynen and Vähäkangas, 2013). In addition, the human placenta contains enzymes for xenobiotic metabolism yielding metabolites with an altered capacity to be transported (Prouillac and Lecoeur, 2010). Overall, the blood flow, surface area, membrane thickness and protein binding are important parameters influencing the uptake and transport of exogenous compounds, and possibly MNPs, at the placental barrier.

Besides transport, the placenta has many other functions including biosynthesis and metabolism of cholesterol, steroid hormones and growth factors essential for the maintenance of pregnancy and fetal development (Gude et al., 2004; Gundacker and Ellinger, 2020). The communication between the mother, placenta and fetus is largely regulated by the placenta through the effects of placental hormones liberated to both maternal and fetal blood, and the exchange of other important molecules between the circulations (Costa, 2016; Knöfler et al., 2019). The numerous bioactive molecules, such as chorionic gonadotropin and placental lactogen, are mainly secreted by the ST (Burton and Fowden, 2015; Costa, 2016; Parrettini et al., 2020). Each placental hormone is tightly regulated in space and time by several factors, including other placental hormones. This delicate hormonal interplay is important for a wide variety of processes that are vital for the establishment and maintenance of pregnancy such as implantation, invasion of trophoblast, placental angiogenesis, hormone synthesis, maternal and fetal metabolic regulation, fetal growth and myometrium contractility, among others (for a comprehensive review see Costa, 2016). The placenta is also an active immunological organ (Ander et al., 2019; Goldstein et al., 2020). Specifically, the interplay between the ST and maternal immune cells actively inhibits infections and protects the fetus from rejection by the maternal immune system (Solano and Arck, 2020). Also, maternal long-chain fatty acids, by inducing cell proliferation, differentiation and angiogenesis, play a crucial role in placental and fetal growth and development (Duttaroy and Basak, 2020). Special placental proteins exist to ensure transcellular and transplacental transfer of fatty acids, including transport proteins in the cell wall (FAT, FATP, p-FABPpm, & FFARs), and the intracellular fatty acid-binding protein FABP. Disruption of these diverse placental functions has been implicated in various adverse pregnancy outcomes such as placental insufficiency, intrauterine growth restriction, preterm birth, pre-eclampsia and loss of pregnancy. However, all in all, placental functions during various stages of pregnancy remain poorly understood and studied (Knöfler et al., 2019). Very little is known about the placental toxicity of environmental factors, such as MNPs, and their potential impact on maternal and fetal health during pregnancy. Nevertheless, diverse *in vitro* and *ex vivo* human placental models exist and can be used to advance this research area.

## 3. Human placental models

Various *in vitro* and *ex vivo* models of the human placenta exist including mono-cultures, co-cultures, 3D models, placenta-on-a-chip, explant cultures and *ex vivo* human placental perfusion models, reflecting different levels of placental organization and complexity (Fig. 2). These models have been successfully used to investigate diverse aspects of placental biology, such as xenobiotic metabolism, steroid metabolism and transfer of drugs, endogenous and exogenous compounds and nanoparticles. The main characteristics of each model are presented in Table 1 and summarized below, moving from less to more complex models based on their

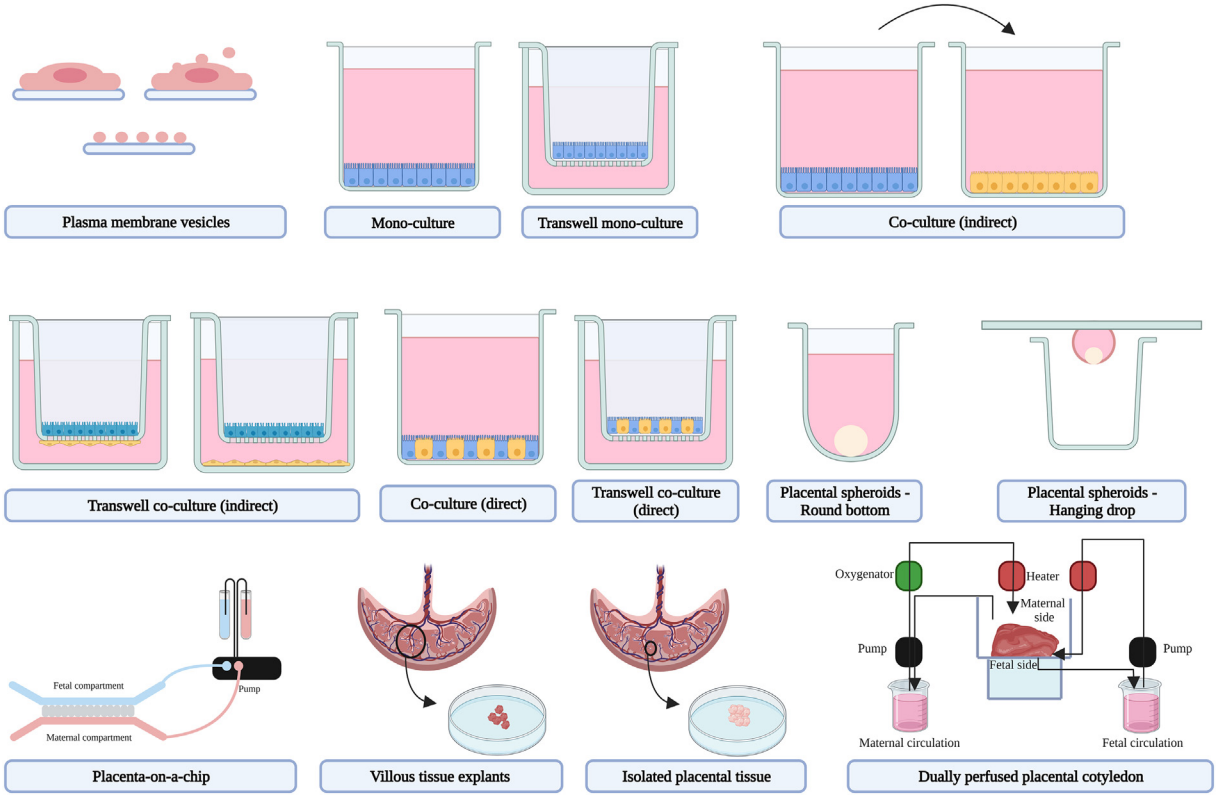


**Fig. 2.** Diverse *in vitro* and *ex vivo* human placental models with increasing order of complexity (from a molecule, cell, tissue to organ) used to study diverse aspects of placental development and functions, including transplacental transport. On a molecular level, plasma membrane vesicles are used. These can be isolated from human placental tissue to study specific transporter proteins. On a cell level, mono- and co-cultures models can be used. Here, diverse placental epithelial and endothelial cells are cultured in a well-plate or a Transwell ® system. Co-cultures can be used in the "indirect" model where no physical contact between different cell types exists, or in the "direct model" where the different cell types are in direct contact. On a tissue level, placental spheroids can be used. Placental spheroids may consist of one or multiple cell types that are cultured in a round bottom well or using a hanging drop technique to recreate a more 'natural-like' microenvironment of the cells. More complex is the placenta-on-a-chip model which combines diverse human placental cells and microfluidics in 3D structures. Moreover, placental tissue explants, such as villous tissue explants can be isolated from the placenta and cultured *in vitro*. On an organ level, placental cotyledon representative of a whole functional unit of the placenta can be dually perfused to study maternal and fetal clearance of various molecules under dynamic, near-physiological conditions.

biological organization. The use of these models in MNP research is discussed in the later sections.

### 3.1. Plasma membrane vesicles

Isolated plasma membrane vesicles from the human placenta have been used to characterize the expression and function of membrane transporter proteins, and to study transport mechanisms of substrates across individual plasma membranes, either the maternal-facing microvillous brush border membrane or the fetal-facing basal membrane (Bissonnette, 1982; Boyd, 1991). Various inhibitors of specialized membrane transporter proteins can be used to study specific transport mechanisms in the apical and basal membranes separately (Ushigome et al., 2003). Nevertheless, the purity of the isolated membrane vesicles has to be considered, as cross-contamination of the contralateral membrane can occur. So far, the microvillous membrane vesicles have not been used in MNP research. Although such cell-free *in vitro* models may lack crucial constituents important for particle uptake (Boyd, 1991). Recently giant plasma membrane vesicles (GPMVs, size range in the lower μm) isolated from various cell lines (HepG2, Caco-2 and HeLa) have been suggested to represent a useful substitute for a cellular model (Zartner et al., 2021). Carboxylated polystyrene (PS) particles of 40 and 100 nm were efficiently transferred to GPMVs (Zartner et al., 2021). Nevertheless, isolated microvillous membrane vesicles are relatively small (about 0.15 μm in diameter) and the size range of MNPs that can be studied using this method is rather limited.

### 3.2. Placental mono-cultures

From all the different placental cell types, the trophoblast is probably the most studied. Different cell lines were developed to study trophoblast functions including gestational choriocarcinoma, immortalized trophoblasts and CT-derived stem cells, however, their use in MNP research so far has been limited. The most commonly used placental cell lines are described in Table 1. Three malignant human choriocarcinoma cell lines, BeWo, JEG-3 and JAR, are commonly used *in vitro* models to investigate diverse aspects of trophoblast function (Drwal et al., 2018). The BeWo and JEG-3 cell lines have been used to study endocrine function since they produce multiple hormones *e.g.*, human chorionic gonadotropin (hCG), progesterone, and androgens (Bode et al., 2006; Jeschke et al., 2007). However, multiple genes are differentially expressed between BeWo and JEG-3 suggesting that they may vary in their function (Drwal et al., 2018). While the JAR cells also produce hormones, they are not frequently used to study endocrine functions. The most frequently used cell model for placental transport studies is the BeWo b30 which is a subclone of the BeWo cell line. This cell line forms a confluent monolayer on a permeable membrane and develops polarized membranes with different expression of growth factors and transporter proteins in apical and basal membranes (Bode et al., 2006). Although BeWo is a carcinoma cell line and there are marked differences in solute carriers and transporter proteins between BeWo cells and trophoblasts *in situ,* transport studies with BeWo b30 cells are usually in agreement with the *ex vivo* results (Correia Carreira et al., 2011; Poulsen et al., 2009). Another trophoblastic cell line, the 3A-sub-E cell line, has

**Table 1**
Most commonly used human placental cell models, their characteristics and experimental considerations.

| Cell line | Origin | Characteristics | Considerations | Used to study | Ref. |
|---|---|---|---|---|---|
| Epithelial | | | | | |
| BeWo | Choriocarcinoma cells | - Repr: cytotrophoblast<br>- Hormones: PGH, P4, E1, E2, hCG, hPL, leptin<br>- Transport: Passive diffusion, carrier systems<br>- Immune: Cytokine and IL excretion | - Do not form monolayer<br>- Used in 3D models<br>- Lower expression of chemokines as compared to JEG-3 | Adhesion, endocrine function and syncytialization | Fujisawa et al., 2000; Gambino et al., 2012; Jeschke et al., 2007; Koh et al., 2014; Magnarin et al., 2008 |
| BeWo b30 | Choriocarcinoma cells | - Repr: cytotrophoblast<br>- Hormones: PGH, P4, E1, E2, hCG, hPL, leptin<br>- Transport: Passive diffusion, carrier systems<br>- Immune: Cytokine and IL excretion | - Form a tight monolayer<br>- Lower expression of chemokines as compared to JEG-3 | Transport, endocrine function and syncytialization | Fujisawa et al., 2000; Gambino et al., 2012; Magnarin et al., 2008 |
| JEG-3 | Choriocarcinoma cells | - Repr: cytotrophoblast; extravillous cells<br>- Hormones: P4, E1, E2, hCG, hPL, leptin<br>- Transport: passive diffusion and active transport<br>- Immune: Cytokine and IL excretion | - Do not form a monolayer<br>- Used in 3D models<br>- A different transport mechanism for glucose than in BeWo, JAR or ACH-3P cells | Proliferation, invasive potential and endocrine function | Fujisawa et al., 2000; Gambino et al., 2012 Koh et al., 2014; Burt et al., 1991; Hackmon et al., 2017 |
| JAR | Choriocarcinoma cells | - Repr: cytotrophoblast<br>- Hormones: P4, E1, E2, hCG, hPL<br>- Transport: passive diffusion and active transport<br>- Immune: Cytokines | - Do not form a monolayer<br>- Do not express HLAG or IGF2(R) | Migration and invasion | Umemura et al., 2013 |
| 3A-Sub-E | Term villous trophoblastic cells | - Repr: term villous trophoblast<br>- Hormones: hCG, able to transform androgens to E1 and E2<br>- Transport: No information<br>- Immune: No information | - Unlimited life span<br>- Rescued from senescence<br>- SV40 transfected<br>- Not often used in research | Apoptosis, cytogenic features | ATCC website |
| ACH-3P | AC1-1 with primary first trimester trophoblasts | - Repr: first trimester trophoblasts<br>- Hormones: hCG<br>- Transport: No information<br>- Immune: HLA-G positive and negative cell subpopulations | - Do not form a monolayer<br>- Polyclonal cell line<br>- Mixed cell populations | Trophoblast invasion and differentiation | Hiden et al., 2007 |
| HTR-8/Svneo | Transfected cells of chorionic villi explants | - Repr: extravillious CTBs<br>- Hormones: hCG<br>- Transport: No information<br>- Immune: No information | - Not immortal: 12–15 passages<br>- SV40 transfected<br>- Mix cell population (epithelial/mesenchymal-like)<br>- Original HRT-8 cells do not produce hCG, suggesting that HRT-8/SVneo are not representative of first trimester placenta *in vivo* | EVCTB invasion, proliferation, regulation, cytokine and hormone production | Abou-Kheir et al., 2017; Verma et al., 2018 |
| Endothelial | | | | | |
| HUVEC | Umbilical vein endothelial cells | - Repr: Macrovascular endothelial cells<br>- Hormones: No hormone production, express ER-β and PR-A<br>- Transport: Passive and active transport<br>- Immune: Cytokines and interleukin | - Used in 3D cultures<br>- Form confluent monolayer<br>- Umbilical cord endothelium<br>- Sex differences in cells<br>- Short life span *in vitro*<br>- Phenotype can change | Regulation of endothelial cell function and angiogenesis | Toth et al., 2008; Wong et al., 2020; Addis et al., 2014; Knyazev et al., 2017; Yang et al., 2018 |
| HPVEC | Placental vascular endothelial cells | - Repr: Microvascular endothelial cells<br>- Hormones: No information<br>- Transport: No information<br>- Immune: No information | - Short life span *in vitro*<br>- Expensive<br>- Poor commercial availability | Vascular defects in placenta, placental barrier, angiogenesis and vasculogenesis | Kreuder et al., 2020 |
| HPEC-A2 | Placental venous endothelial cells | - Repr: Microvascular endothelial cells<br>- Hormones: No information<br>- Transport: Passive and active transport<br>- Immune: No information | - Limited availability | Vascular defects in placenta, placental barrier angiogenesis and vasculogenesis | Scholler et al., 2012; |

Abbreviations: ER, estrogen receptor; EVCTB, extravillous cytotrophoblast; E1, estrogen; E2, estradiol; hCG, human chorionic gonadotropin; HLA-G, human leukocyte antigen-G; hPL, human placental lactogen; IGF2, insulin-like growth factor 2; IL, interleukin; PGH, Placental growth hormone; PR, progesterone receptor; P4, progesterone.

been used to study chemokine receptors and promoter activity, such as the ABCB1 promoter which encodes the placental efflux transporter P-glycoprotein (Mueller et al., 1997; Speidel et al., 2018).

Freshly isolated primary trophoblasts are less frequently used than choriocarcinoma cell lines since they are difficult to isolate and culture, have a short lifespan and are often contaminated by different cell types (Li and Schust, 2015). Moreover, primary CTs can spontaneously differentiate into functional syncytium (followed by a loss of epithelial cell-cell junctions) and therefore a successful establishment of a confluent monolayer from primary CTs is challenging (Bloxam et al., 1997; Bode et al., 2006). Nevertheless, the use of highly viable purified CTs, optimized culture conditions and semipermeable inserts can aid the formation of a tight monolayer (Hemmings et al., 2001; Huang et al., 2016). Two commonly used cell lines derived from primary cells are ACH-3P and HRT-8/Svneo (Table 1). ACH-3P cells may be applied to study autocrine and paracrine regulation of trophoblast formation (Erlandsson et al., 2020; Hiden et al., 2007; Nääv et al., 2020), while HRT-8/Svneo cells may be used to study *e.g.*, extravillous cytotrophoblast (EVCTB) invasion and proliferation. More recently, trophoblast stem cells were derived from human villous CTs and human blastocysts (Okae et al., 2018). These long-term stem cell cultures may give rise to CTs, EVT and ST-like cells that show transcriptomes similar to those of the corresponding primary trophoblasts, providing a new powerful model to study human trophoblast development and function.

The two most common vascular endothelial cell lines applied in placental research are human umbilical vein endothelial cells (HUVEC) and human placental vascular endothelial cells (HPVEC) (Lang et al., 2003; Oettel et al., 2016) (Table 1). HUVECs represent macrovascular endothelial cells while HPVECs represent microvascular endothelial cells involved in the formation of the placental vascular network, and thus marked phenotypic and physiologic differences exist between these cell lines (Lang et al., 2003). The HPEC-A2 cell line is another SV-40-transformed placental venous endothelial cell line, however, it has limited commercial availability and thus is not commonly used in placental research.

Placental mono-cultures allow for low-cost and high-throughput investigation of different aspects of placental biology under controlled and simplified conditions. They can be applied to study transplacental transport (*e.g.*, using the Transwell ® system) or to investigate other functional endpoints (using a standard well plate format). However, as they lack the physiological complexity of the multi-layered cell structures of placental villi (*e.g.*, trophoblasts, mesenchymal, Hofbauer cells and fibroblasts) they do not account for the complex placental cell-cell interactions.

### 3.3. Placental co-cultures

Co-culture models may improve the physiological relevance of *in vitro* models by mimicking the human anatomical architecture of placental tissue and by facilitating cell-cell interactions (Levkovitz et al., 2013). Two different co-culture model types are used, namely direct co-culture and indirect co-culture (Vis et al., 2020). In direct co-culture, distinct cell types are in physical contact with each other which allows direct communication between them. In the indirect co-culture model, the distinct cell types are physically separated from each other, and the communication between them is facilitated through a culture medium (Fig. 2). Co-culture models may show altered protein expression and hormone secretion in comparison with mono-cultures. For example, co-culture of BeWo or JEG-3 cells with the human adrenocortical cell line H295R resulted in higher secretion of progesterone, estradiol, estriol and estrone in comparison with H295R, JEG-3 or BeWo cells cultured alone (Drwal et al., 2018; Thibeault et al., 2014). Both direct and indirect co-culture models may also be applied in a Transwell ® system to determine the transplacental transport of compounds or particles over multiple cell barriers. However, in the co-culture models, the distinct cell types often require different media and supplements to ensure optimal culture conditions. Thus, finding the right medium composition is often challenging. So far co-culture models have rarely been applied to MNP research.

### 3.4. Placental spheroids

Placental spheroids are more comparable to the placenta *in situ* than mono-, or co-cultures since spheroids have multiple cell layers and recreate a 'natural-like'-microenvironment for the cells (Fennema et al., 2013; Shri et al., 2017). The cell-cell interactions in spheroids induce changes in cellular morphology and functions, showing continuous proliferation, differentiation, and changes in gene and protein expression which are more representative of the placenta *in situ* (Edmondson et al., 2014). Placental spheroids can be formed using transfected cell lines, primary or progenitor cells, and by combining different cell types *e.g.*, endothelial precursor cells, cytotrophoblasts and villous stromal cells (Baal et al., 2008; Lin and Chang, 2008). For example, Turco et al. (2018) established trophoblast organoids capable of differentiating to ST and EVTs, that formed complex structures which anatomically and functionally resembled placental villi (Turco et al., 2018). Spheroids can be formed using round-bottom nonadherent well plates or the hanging drop technique (Huang et al., 2020). Both techniques can be used in 96-well plates, which allows for high throughput screening (Rousset et al., 2022). Depending on the cell types used to form spheroids, this model can be used to study cell adhesion, proliferation, placental vascular development and trophoblast invasion (Baal et al., 2008; Fennema et al., 2013). Nevertheless, using spheroids is not without challenges. For instance, it is difficult to exchange culture medium, prevent necrotic damage and control the size and exact composition of the cellular components of spheroids (Lin and Chang, 2008).

### 3.5. Placenta-on-a-chip

The emergence of the 'placenta-on-a-chip' model, combining microfluidics and diverse human placental cells in 3D structures, provides a novel promising tool that exhibits organotypic features of the placenta (Richardson et al., 2020). This model has been already used to study placental translocation, inflammatory responses and complex cellular interactions (Blundell et al., 2016; Lee et al., 2016; Pemathilaka et al., 2019; Yin et al., 2019; Zhu et al., 2018). In the placenta *in situ*, the ECM is an important component of the tissue microenvironment since it regulates cellular adhesion, migration and invasion (O'Connor et al., 2020; Lobo et al., 2016). Therefore, the use of an ECM membrane can be physiologically relevant for creating the placental barrier *in vitro* (Lee et al., 2016). Matrigel®, fibronectin or gelatine, have been used to represent the ECM in the placenta-on-a-chip model (Abbas et al., 2017; Kreuder et al., 2020; Shojaei et al., 2021; Yin et al., 2019). On top of the ECM diverse placental cell types can be grown. Human villous mesenchymal fibroblasts have been used in a combination with the BeWo b30 cells to study the translocation of PS particles across the placental barrier (Muoth et al., 2017). Other cell lines can be included in this model, such as endothelial or immune cells, creating co-culture microtissues and further reducing the gap between *in vitro* and *in vivo* conditions (Muoth et al., 2016). However, similar to co-cultures, the different cell types in the placenta-on-a-chip model may require a specific medium/microenvironment (Richardson et al., 2020). Furthermore, these models are labour-intensive since it is essential to continuously monitor cell-cell communication and their microenvironment for secreted metabolites or hormones, and, cannot be used for high throughput screening. Nevertheless, the potential of the placenta-on-a-chip model has already been demonstrated in the fields of reproductive biology and toxicology (*e.g.*, assessment of engineered nanoparticles for drug delivery), reviewed recently by Young and Huh (2021) and Shojaei et al. (2021).

### 3.6. Villous tissue explants

Placental villous tissue explants from term placentas are most commonly used, although explants from the first trimester (from elective abortions) have also been utilized (Miller et al., 2005). Villous explants from term placentas have been used to evaluate villous metabolism, endocrine function and transport of amino acids and other small molecules

(Avagliano et al., 2012; Fuglsang et al., 2008; Visiedo et al., 2015), whereas early placental tissue explants have been used to study placentation, invasion of EVTs, trophoblast proliferation and differentiation (Genbacev et al., 1992; Vićovac et al., 1995). Villous explants from both early and term placenta have been used to study the effects of xenobiotics on transporter expression (Sieppi et al., 2016) and endoplasmic reticulum (ER) stress (Huovinen et al., 2022). These models maintain the topology of chorionic villi (*i.e.*, contain microvillous membrane of the syncytium, mesenchymal stromal cells, endothelial cells, blood cells and Hofbauer cells) and represent a functional unit of the placenta. However, the tissue has to be carefully dissected from the placenta soon after delivery and assessed for structural integrity and viability after handling and during culture (syncytial detachment, necrosis and other tissue degeneration are often reported) (Sooranna et al., 1999). The production of hormones, growth factors, cytokines and proteins may show significant inter- and intra-placental variation (Crocker et al., 2004a; Miller et al., 2005; Simán et al., 2001). Additionally, different culture conditions (use of diverse supports and inserts, different oxygen levels and culture media composition) can have marked effects on the explant function (Black et al., 2001; Brew and Sullivan, 2017; Crocker et al., 2004b). Lastly, villous explant cultures under static conditions, the most commonly used approach, differ significantly from the *in vivo* situation as they lack the beneficial effects of fluid flow which creates stress on placental villi, specifically on ST (Kupper et al., 2021; Miura et al., 2015). Human placental villous explants under flow culture systems show better structural and biochemical integrity as compared to a static culture (Kupper et al., 2021). To our knowledge, villous explants have so far not been used to study the uptake or toxicity of MNPs in villous structures.

### 3.7. Dually perfused placental cotyledon

Placental cotyledons are functional units of the placenta, containing chorionic villi separated by placental septa. Since its development by Panigel et al., 1967, the *ex vivo* perfusion of a placental cotyledon is still considered the golden standard for the evaluation of placental clearance and transport of various endogenous compounds (Omarini et al., 1992; Sastry, 1999), pharmaceutical drugs (Ala-Kokko et al., 2000; De Sousa Mendes et al., 2016) and environmental pollutants (Mathiesen et al., 2021) under dynamic, near-physiological conditions. This *ex vivo* model takes advantage of the full complexity of the intact human term placental tissue and generally shows a good correlation with *in vivo* data (Hutson et al., 2011). Usually, only a single cotyledon is used and the transfer can be analyzed over time. After perfusion, samples can be taken from placental tissue, which can be processed further for biomarkers (*e.g.*, using genomic or proteomic methods). However, the perfusion time is limited and mainly term placentas are used, because perfusion of the earlier placenta is extremely difficult (Vähäkangas and Myllynen, 2006). Additionally, considering that placental physiology changes significantly during pregnancy, the term placenta does not represent the earlier developmental phases (Hutson et al., 2011; Myllynen and Vähäkangas, 2013). Similar to villous explants, placental perfusion conditions (such as the composition of perfusates and gas mixtures used to oxygenate the tissue, flow rates in maternal and fetal circulations and perfusate volumes) may significantly affect the transport function. Placental viability must be closely monitored during perfusions and interindividual variation in transplacental kinetics, including potential consequences of polymorphisms, must also be considered (Pollex and Hutson, 2011). Human placental perfusion has been used to study the transplacental transfer of various nanoparticles (reviewed by Aengenheister et al. (2021)), but only a limited number of studies have investigated MNPs of which all were PS particles.

## 4. *In vitro* placental PS uptake, transport and toxicity

### 4.1. Uptake

To date, the only MNPs investigated in the *in vitro* placental models were PS particles. Several methods have been used to confirm the uptake of PS particles by placental cells, namely light-, electron- and confocal microscopy (Table 2). So far, only fluorescent PS particles (Fluo-PS), with a size range of 20 nm to 10 μm, have been investigated in placental epithelial and endothelial cells *in vitro* (Table 3). Fluo-PS uptake was investigated in BeWo b30, 3A-sub-E cells, HUVEC and HPEC-A2 cells and all, except the HPEC-A2, took up Fluo-PS particles in a size-dependent manner. However, the exact mechanisms of uptake remain unclear (Table 2). HUVEC and HPEC-A2 are micro- and macrovascular endothelial cells showing phenotypical and physiological heterogeneity and marked differences in gene expression and cell signaling (Lang et al., 2003). Moreover, other microvascular HPVEC cells, have been shown to lack fenestrations and pinocytotic activity (Yazdani et al., 2019). These differences between macro- and microvascular cells may account for the observed difference in PS uptake; however, so far this has not been elucidated.

### 4.2. Transfer

The most studied aspect of *in vitro* placental MNP exposure is the transplacental transport of MNPs across the maternal-fetal interface (Tables 2, 3). For this, the Transwell ® system is commonly used, where cells are separated by a semi-permeable membrane. The Transwell ® model has been used to study the transport of a variety of substrates (*e.g.*, caffeine, silver nanoparticles) over the placental barrier (Abdelkhaliq et al., 2020; Pemathilaka et al., 2019). However, the transport of MNPs has been insufficiently investigated. Only five *in vitro* studies using Transwell ® inserts have examined PS transport across cell layers, of which three have used mono-cultures, one co-culture and one study used both (Table 2). These studies used several methods to determine permeability and monolayer formation (*e.g.*, by measuring transepithelial/endothelial electrical resistance (TEER)), uptake (*e.g.*, by electron microscopy) and transfer (*e.g.*, by microplate reader) (Table 2).

The mono-cultures used BeWo b30, JEG-3, JAR, ACH-3P or HPEC-A2 cell lines, whereas the co-culture consisted of a combination of BeWo b30 and HPEC-A2 cells (Aengenheister et al., 2018b; Hesler et al., 2019; Kloet et al., 2015; Rothbauer et al., 2017). Due to their ability to form a tight monolayer, the BeWo b30 cells are the most frequently used trophoblastic cell line to study the time-dependent transplacental transfer of MNPs using the Transwell set-up (Aengenheister et al., 2018b; Cartwright et al., 2012; Kloet et al., 2015; Rothbauer et al., 2017).

The transplacental transport has been investigated using different sizes (20 nm to 10 μm) and concentrations (0.05 to 1000 μg/mL) of PS particles (Table 3). Additionally, one study investigated negatively and positively charged PS particles (Kloet et al., 2015). All PS particles tested were able to cross the cellular monolayers regardless of the cell type, except for the 70 nm (Aengenheister et al., 2018b) and the negatively charged 50 nm PS particles (Magasphere) (Kloet et al., 2015). Also, neither 50 nor 500 nm negatively charged PS beads were translocated over the BeWo b30/HPEC-A2 co-culture (Hesler et al., 2019). Kloet et al. (2015) argued that the different translocation rates for the negatively charged 50 nm PS particles are possibly due to the different chemical groups on the surface of the PS supplied by the different manufacturers. The particle surface moiety in addition to the surface charge is thus probably an important factor in the placental uptake and transport of MNPs. In the co-culture model developed by Aengenheister and coworkers (2018), fluo-PS beads (70 nm) did not cross the BeWo b30/HPEC-A2 layers, while the smaller beads (49 nm) did (Aengenheister et al., 2018b). Overall, the *in vitro* transcellular transport of MNPs has been shown to be limited, with a maximum of 41 and 2 % of initial concentration measured in the basolateral compartment in mono-culture and co-culture, respectively.

### 4.3. Toxicity

The MNP toxicity, similarly to uptake and transfer, was studied only with PS particles, in a total of 9 *in vitro* studies (Table 3). An overview of the different toxicity endpoints investigated to date is presented in Table 2. Cell viability was most commonly investigated, using the MTT

**Table 2**
Methods used to study uptake, transport and toxicity of PS-MNPs in human *in vitro* placental models.

| | | Model | Cell lines | Ref. |
|---|---|---|---|---|
| **Uptake** | **Detection methods** | | | |
| | Confocal microscopy | Mono-/Co-culture | ACH-3P, BeWo b30, JEG-3, JAR, HPEC-A2, 3A-SubE | Rothbauer et al., 2017, Huang et al., 2015, Zhang et al., 2022, Lu et al., 2022, Hesler et al., 2019 |
| | Light microscopy | Monoculture | HUVEC | Lee et al., 2021 |
| | Electron microscopy | Mono-/Co-culture | BeWo b30, 3A-Sub-E, HUVEC, HPEC-A2 | Cartwright et al., 2012, Kloet et al., 2015, Huang et al., 2015, Zhang et al., 2022, Lu et al., 2022, Aengenheister et al., 2018b, Hesler et al., 2019 |
| | AF4 | Co-culture | BeWo b30, HPEC-A2 | Hesler et al., 2019 |
| | Flow cytometry | Monoculture | 3A-Sub-E, HUVEC | Huang et al., 2015, Lu et al., 2022 |
| | HPLC | Mono-/Co-culture | BeWo b30, HPEC-A2 | Kloet et al., 2015, Aengenheister et al., 2018b |
| **Transport** | **Detection methods** | | | |
| | Spectrophotometer | Mono-/Co-culture | ACH-3P, BeWo b30, JEG-3, JAR, HPEC-A2 | Rothbauer et al., 2017, Cartwright et al., 2012, Aengenheister et al., 2018b, Hesler et al., 2019 |
| | Barrier integrity | | | |
| | TEER | Mono-/Co-culture | ACH-3P, BeWo b30, JEG-3, JAR, HPEC-A2 | Rothbauer et al., 2017, Cartwright et al., 2012, Kloet et al., 2015, Aengenheister et al., 2018b, Hesler et al., 2019 |
| | FITC | Mono-/Co-culture | BeWo b30, HPEC-A2 | Aengenheister et al., 2018b |
| | Na-F | Mono-/Co-culture | ACH-3P, BeWo b30, JEG-3, JAR, HPEC-A2 | Rothbauer et al., 2017, Cartwright et al., 2012, Aengenheister et al., 2018b |
| | Amoxicillin | Mono-/Co-culture | BeWo b30, HPEC-A2 | Kloet et al., 2015, Aengenheister et al., 2018b, Hesler et al., 2019 |
| | Indomethacin | Mono-/Co-culture | BeWo b30, HPEC-A2 | Aengenheister et al., 2018b |
| | Antipyrine | Mono-culture | BeWo b30 | Kloet et al., 2015 |
| **Toxicity** | Cell viability | | | |
| | MTT assay | Mono-culture | ACH-3P, BeWo b30, JEG-3, JAR, HUVEC | Rothbauer et al., 2017, Cartwright et al., 2012, Kloet et al., 2015, Lee et al., 2021, Lu et al., 2022 |
| | MTS assay | Mono-/Co-culture | BeWo b30, HPEC-A2 | Aengenheister et al., 2018b, Hesler et al., 2019 |
| | Annexin-V-FITC | Mono-culture | HUVEC | Lee et al., 2021, Zhang et al., 2022 |
| | CCK8 | Mono-culture | HUVEC | Zhang et al., 2022 |
| | JC1 assay kit | Mono-culture | HUVEC | Zhang et al., 2022 |
| | LDH leakage | Mono-culture | HUVEC | Zhang et al., 2022, Lu et al., 2022 |
| | Protein levels | | | |
| | hCG | Mono-culture | ACH-3P, BeWo b30, JEG-3, JAR | Rothbauer et al., 2017 |
| | IL-6 | Mono-culture | HUVEC | Zhang et al., 2022, Lu et al., 2022 |
| | IL-8 | Mono-culture | HUVEC | Lu et al., 2022 |
| | IL-1β | Mono-culture | HUVEC | Zhang et al., 2022, Lu et al., 2022 |
| | TNFa | Mono-culture | HUVEC | Zhang et al., 2022, Lu et al., 2022 |
| | Other | | | |
| | ROS | Mono-culture | HUVEC | Lee et al., 2021, Lu et al., 2022, Zhang et al., 2022 |
| | TUNEL | Mono-culture | 3A-Sub-E | Huang et al., 2015 |
| | BrDU | Mono-culture | 3A-Sub-E | Huang et al., 2015 |
| | Angiogenesis assay | Mono-culture | HUVEC | Lee et al., 2021 |
| | qPCR | Mono-culture | HUVEC | Zhang et al., 2022, Lu et al., 2022 |
| | Protein corona | Mono-culture | BeWo b30 | Kloet et al., 2015 |
| | ABC transporters | Mono-culture | BeWo b30 | Kloet et al., 2015 |
| | Glucose transport | Mono-culture | ACH-3P, BeWo b30, JEG-3, JAR | Rothbauer et al., 2017 |
| | Transwell migration | Mono-culture | HUVEC | Lee et al., 2021 |
| | Wound healing assay | Mono-culture | HUVEC | Lee et al., 2021 |

Note: The literature search was performed using several databases (PubMed, ScienceDirect, Elsevier) and a combination of the following key words: in vitro, placenta, microplastics, polystyrene, transfer, uptake, toxicity. The literature search was performed between February 2022–May 2022. Abbreviations: ABC; ATP binding cassette; AF4, assymetric flow field flow fractionation; BrdU, bromodeoxyuridine; CCK8, Cell Counting Kit-8; FITC, fluorescein isothiocyanate dextran; hCG, human chorionic gonadotropin; HPLC, high-performance liquid chromatography; IL, interleukin, JC1; tetraethylbenzimidazolylcarbocyanine iodide; LDH, lactate dehydrogenase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; Na-F, sodium fluorescein; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; TEER, transepithelial/endothelial electrical resistance; TNFα, tumor necrosis factor alpha; TUNEL, Terminal deoxynucleotidyl transferase; dUTP nick end labelling.

(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) or MTS (3-(4,5-dimethylthiazol-2-yls)-5-(3-carboxymethoxyphenyl-2-(4-sulfophenyl)-2H-tetrazolim)) assay (Table 2). Only exposure to 50 and 100 nm (1 mg/mL) non-charged PS and positively charged 50 nm PS (20 μg/mL) particles resulted in a reduction of cell viability (Cartwright et al., 2012; Kloet et al., 2015). However, the tested concentration of 1 mg/mL is most probably too high to reflect real human exposure level (the total amount of MNPs measured in human blood was recently estimated at 1.6 μg/mL (Leslie et al., 2022)). Additionally, exposure to 50 nm (>5 μg/mL) and 500 nm (0.01–10 μg/mL) PS particles in a co-culture model with BeWo b30 and HPEC-A2 cells increased mitochondrial activity (Hesler et al., 2019). Nevertheless, PS particles were not transferred over the barrier and did not affect barrier integrity. In 3A-sub-E cells, exposure to 20 nm (500 μg/mL) and 40 nm (200 μg/mL) PS particles resulted in an increased number of TUNEL-positive cells indicating DNA fragmentation upon PS exposure (Huang et al., 2015). Additionally, 20 nm PS particles (200 μg/mL) reduced cell proliferation (BrdU assay) in these cells.

In HUVEC cells, cytotoxicity was observed after exposure to 50 nm PS particles with a concentration of 50 μg/mL (Lee et al., 2021; Lu et al., 2022; Zhang et al., 2022). In addition, all sizes (20–10,000 nm, 1000 μg/mL) resulted in apoptosis, with higher apoptosis rates observed in cells treated with smaller PS particles (Zhang et al., 2022). Furthermore, exposure to 20, 100 and 10,000 nm (1000 μg/mL) PS particles resulted in lactate dehydrogenase (LDH) leakage. Upregulation of reactive oxygen species (ROS) was also seen after exposure to 20, 50 and 100 nm PS particles, at high, most probably far-from-physiological concentrations (1000 μg/mL). In contrast, another study showed that exposure to 500 nm PS particles at lower concentrations (0–80 μg/mL) did not increase ROS levels (Lee et al., 2021). More data is needed to assess whether PS particles cause increased ROS levels in endothelial cells. HUVEC tube formation was

**Table 3**
Human *in vitro* studies on placental uptake, transfer and toxicity of polystyrene particles.

| Cells | Particle size (nm) | Conc. (μg/mL) | Exposure (h) | Assay | Main findings | Ref. |
|---|---|---|---|---|---|---|
| **Epithelial cells** | | | | | | |
| BeWo b30 | 50, 100 | 0.1–1000 | 24 | Transfer | 500 μg/mL tested: all PS transferred (50 nm: 10 %, 100 nm: 0.8 %) | Cartwright et al., 2012 |
| | | | | Uptake (TEM) | Size-dependent uptake | |
| | | | | MTT | Reduction in cell viability - only at 1 mg/mL | |
| BeWo b30 | 50 *(neg & pos charged)* | 0.05–100 | 24 | Transfer | 10 μg/mL tested: No translocation of neg. PS from Megasphere | Kloet et al., 2015 |
| | | | | MTT | pos PS; reduction in cell viability at concentrations >20 μg/mL | |
| BeWo b30, JEG-3, ACH-3P, JAR | 50, 490 | 125–500 | 24 | Transfer | 500 μg/mL tested: Both size of PS transferred (BeWo: 41.74 %, JAR: 49.22 %, ACH-3P: 82.58 %, JEG-3: 79.05 %) | Rothbauer et al., 2017 |
| | | | | MTT | No cytotoxicity | |
| 3A-Sub-E | *20–500* | 200–500 | 4 | Uptake (TEM) | From the highest to the lowest uptake (10 μg/mL): 40, 100, 200, 20 and 500 nm | Huang et al., 2015 |
| | | | | MTT | Cytotoxicity: only at 1 mg/mL | |
| | | | | TUNEL | 120 nm (500 μg/mL) and 40 nm (200 μg/mL) increased number of TUNEL positive cells | |
| | | | | BrdU | Reduced cell proliferation (20 nm, 200 μg/mL) | |
| **Endothelial cells** | | | | | | |
| HUVEC | 500, 1000, 5000 | 20–100 | 72 | MTT | 500, 1000 nm: the higher the conc., the higher the cytotoxicity. The smaller the size, the higher the cytotoxicity. 5000 nm no cytotoxicity | Lee et al., 2021 |
| | | | | Tube formation | Repression of tube formation (500, 1000 nm – max 80 μg/mL) | |
| | | | | DCFH-DA | ROS not detected | |
| | | | | Immunoblotting | Suppression of cell migration- and angiogenesis | |
| | | | | Annexin-V | No apoptotic cells | |
| HUVEC | 20–10,000 | 50–1000 | 24 | Uptake (confocal) | No uptake of particles >500 nm | Zhang et al., 2022 |
| | | | | CCK8/JC1 | Cytotoxicity for 20 nm (50, 250, 1000 μg/mL), 50 nm (250, 1000 μg/mL), 100–10,000 (>1000 μg/mL) | |
| | | | | Annexin-V | Increased apoptosis (all sizes, 1000 μg/mL) | |
| | | | | LDH leakage | 20, 100, 10,000 nm (1000 μg/mL) increased LDH levels | |
| | | | | DCFH-DA | Upregulation of ROS after exposure to 20, 50 and 100 nm PS (1000 μg/mL) | |
| | | | | ELISA | Increased levels of IL-1β, IL-6 and TNF-α (1000 μg/mL) | |
| HUVEC | 100, 500 | 5–100 | 48 | MTT | Cytotoxicity only with PS 500 nm > 50 μg/mL | Lu et al., 2022 |
| | | | | Uptake (FC) | In a size-, time- and concentration-dependent manner (both sizes, 10 and 25 μg/mL) | |
| | | | | LDH | Increased LDH activity (both sizes, 10 and 25 μg/mL) | |
| | | | | DCFH-DA | No ROS induction | |
| **Co-cultures** | | | | | | |
| BeWo b30/ HPEC-A2 | 49, 70 | 6.25–100 | 24 | Transfer | Mono-culture: BeWo (49 nm 1.2 %), HPEC-A2 (49 nm, 2 %), Co-culture: BeWo + HPEC-A2 (49 nm 1.3 %) Exposure conc. 50 μg/mL | Aengenheister et al., 2018b |
| | | | | MTS | No cytotoxicity | |
| BeWo b30/HPEC-A2 | 50, 500 *(neg)* | 0.01–100 | 24 | Transfer | No transport | Hesler et al., 2019 |
| | | | | Uptake (confocal) | Detected in BeWo b30, not in HPEC-A2 layer | |
| | | | | MTS | Increased metabolic activity (50 nm: >5 μg/mL, 500 nm: 0.01–10 μg/mL). | |

Note: The literature search was performed using several databases (PubMed, ScienceDirect, Elsevier) and a combination of the following key words: *in vitro*, placenta, microplastics, polystyrene, transfer, uptake, toxicity. The literature search was performed between February 2022–May 2022. Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; DCFH-DA, 2′-7′dichlorofluorescein diacetate; FC, flow cytometry; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; PS, polystyrene; TEM, transmission electron microscopy; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

repressed in a concentration-dependent manner after exposure to 500 and 1000 nm PS particles (Lee et al., 2021), but not after exposure to 5000 nm PS particles indicating a size-dependent effect.

## 5. *Ex vivo* placental PS uptake, transport and toxicity

To date, five studies have investigated transplacental MNP uptake and transport in the *ex vivo* placental perfusion model, all using term placentas, closed, recirculating perfusion model with separate circulations on fetal and maternal sides, and 3 to 6-hour perfusion duration (Table 4). Fluo-PS particles have been the only MNPs investigated, with perfusate concentrations of 25 or 40 μg/mL, particle sizes ranging from 50 to 534 nm and various PS surface functionalization *i.e.*, plain, carboxylate or amine-modified. Overall, a size-dependent maternal-to-fetal translocation was observed for all PS particles investigated. Smaller particles have been transferred more readily than bigger particles (Table 4). Typically, the lower transfer rate corresponds to a higher accumulation of PS particles in the placental tissue, specifically in the ST layer (Grafmüller et al., 2013, 2015a, 2015b). It seems that PS particles larger than 250 nm accumulate mainly in the ST and do not translocate to the deeper layers of the chorionic villi, at least not after acute exposure (up to 6 h), suggesting that ST may be a major barrier for the transplacental transport of at least larger particles (Wick et al., 2010). This is further supported by data on engineered metallic and carbonaceous nanoparticles where accumulation in placental tissue, specifically in the outer layer of the chorionic villi, has also been observed (Aengenheister et al., 2018a; Bongaerts et al., 2020; Poulsen et al., 2015). Nevertheless, it is unknown whether longer perfusions would result in increased translocation of MNPs to the deeper layers of the chorionic villi. Fluo-PS transport in the reverse direction *i.e.*, fetal-to-maternal, has also been investigated (Grafmüller et al., 2015a). After 6 h of perfusion, a concentration equilibrium between maternal and fetal circulations was not achieved and the PS beads showed a tendency for a higher transfer in the reverse direction. This demonstrates that passive diffusion may not be the main mechanism underlying the placental translocation of PS particles (Grafmüller et al., 2015a). As previously mentioned, the trophoblast is actively involved in pinocytosis and phagocytosis of macromolecules, such as IgG, from the extracellular environment and has importance in the transport of therapeutic monoclonal antibodies such as infliximab (Sand et al., 2022). Such endocytotic pathways were also indicated as potential energy-dependent mechanisms of cellular particle uptake (Foroozandeh and Aziz, 2018). Caveolae-mediated endocytosis of PS nanoparticles up to 100 nm in diameter in endothelial cells (Wang et al., 2009), and, caveolae-mediated endocytosis of pullulan acetate nanoparticles in BeWo b30 cells have been observed (Tang et al., 2018). However, specific mechanisms of MNP uptake by placental trophoblast remain to be identified.

Surface charge and modification of MNPs probably also influence placental transport. Grafmüller et al. (2015b) observed a higher transport of plain or carboxylated PS beads than amine-modified PS of similar sizes. The amine-modified PS particles were mostly found in the ST and the villous mesenchyme. Similarly, amine-modified titanium dioxide nanoparticles showed higher accumulation in placental tissue than carboxylated particles of similar size (Aengenheister et al., 2019). Based on these studies, transplacental transport of particles seems to decrease in order of plain>carboxylated>amine-modified. Furthermore, next to surface modification, serum proteins seem to influence the placental uptake and transport of MNPs. Plasma proteins increased the transplacental transfer of unmodified 80 nm PS-particles and the protein corona composition significantly changed upon crossing the human placenta (Gruber et al., 2020). The key proteins of the corona formed in the plasma medium were albumin, immunoglobulins and apolipoproteins (Gruber et al., 2020). Human plasma-induced corona formation enhanced the transfer of PS particles compared to PS-corona formed by bovine serum albumin and dextran. Furthermore, human albumin significantly increased the transfer of PS-particles compared to immunoglobulin G-corona (Gruber et al., 2020). The presence of

**Table 4**
Human *ex vivo* placental perfusion studies on uptake, transfer and toxicity of polystyrene particles.

| Particle size (nm) and concentration | Perfusion conditions | Assay | Main findings | Ref. |
|---|---|---|---|---|
| 50, 80, 240 and 500 (25 μg/mL) | Closed (3 h); NCTC-135 medium/Earl's buffer with BSA (8 g/L) | Transfer (TEM) | Size-dependent transfer, 8.90 ± 1.80 μg/mL (50 nm) 7.47 ± 1.77 μg/mL (80 nm), 2.03 ± 0.29 μg/mL (240 nm) after 3 h in the fetal perfusion medium | Wick et al., 2010 |
| | | Viability and functionality | Glucose, lactate, human hCG and leptin concentrations not affected | |
| 80 (40 μg/mL) | Closed (6 h); DMEM/Earls buffer with BSA (5 g/L) or HSA (40 g/L) and human plasma (8.6 %) or HSA (40 g/L) or IgG (10 g/L) | Transfer (SP, TEM) | After 6 h of perfusion significantly higher transfer in the plasma medium ((23.2 ± 5.5) μg/mL) than in the buffer medium ((15.5 ± 2.3) μg/mL) | Gruber et al., 2020 |
| | | Shotgun proteomics (LC-MS/MS) | Human plasma-enhanced transfer of Fluo-PS particles compared to PS particles with a corona formed by BSA and dextran. Human albumin induced the transfer of Fluo-PS particles compared to corona formed by IgG | |
| | | Quality and viability | No change in $O_2$ and $CO_2$ tension, glucose and lactate concentration | |
| 50, 240; 50, 300 carboxylate-modified (25 μg/mL) | Closed (6 h); M199 medium/Earl's buffer with BSA (10 g/L), bidirectional transfer | Transfer (TEM) | Transfer from fetal to maternal direction was significantly higher than from the maternal to fetal. Increased translocation of plain Fluo-PS compared with carboxylated Fluo-PS in both directions. Higher transfer rate, lower tissue accumulation. | Grafmüller et al., 2015a, 2015b |
| | | Uptake (FM) | All PS particles accumulated in ST, regardless of the direction of perfusion | |
| | | Viability and functionality | Glucose, lactate, hCG and leptin concentrations not affected | |
| 60–534, plain, carboxylate, amine-modified (25 μg/mL) | Closed (6 h); M199 medium/Earl's buffer with BSA (10 g/L) | Transfer (SP) | Transfer of plain and carboxylated Fluo-PS but not amine-modified particles | Grafmüller et al., 2015a, 2015b |
| | | Uptake (FM) | Amine-modified particles found in the ST and villous mesenchyme | |
| 80 and 500 (25 μg/mL) | Closed (6 h); NCTC-135 medium/Earl's buffer with BSA (10 g/L) | Transfer (SP) | 80 nm Fluo-PS crossed the placental barrier (after 3 h, 20–30 % of the added amount transferred) while 500 nm Fluo-PS were retained in the placental tissue or maternal circuit | Grafmüller et al., 2013 |
| | | Uptake (FM) | Particles accumulated in the villi | |
| | | Viability and functionality | Glucose, lactate, hCG and leptin concentrations not affected | |

Note: The literature search was performed using several databases (PubMed, ScienceDirect, Elsevier) and a combination of the following key words: *in vitro*, placenta, microplastics, polystyrene, transfer, uptake, toxicity. The literature search was performed between February 2022–May 2022. Abbreviations: Fluo-PS, fluorescent polystyrene particles; BSA, bovine serum albumin; HSA, human serum albumin; hCG, human chorionic gonadotropin; ST, syncytiotrophoblast; TEM, transmission electron microscopy; FM, fluorescence microscopy; SP, spectrophotometer.

albumin in perfusion media was also shown to increase the placental transfer rate of hydrophobic compounds such as benzo(*a*)pyrene (Mathiesen et al., 2009). Moreover, it has previously been shown that caveolae-mediated endocytosis of nanoparticles is dependent on the albumin coating of particles (Wang et al., 2009). Therefore, the fact that proteins increase the transplacental transfer of PS particles further indicates the involvement of endocytosis in the transplacental transport of MNPs. Considering that endocytosed nanoparticles tend to accumulate in lysosomes, lysosomal dysfunction and subsequent cell death may be a possible mechanism of MNP toxicity, as previously shown for gold, SiO2 NPs and amine-modified PS nanoparticles (Manshian et al., 2018; Wang et al., 2018a). In summary, the polymer type, size, charge and functional groups on the particle surface can alter the biological reactivity of the particles which can affect the formation of the protein corona, cellular uptake and consequently toxicity of MNPs (Docter et al., 2015; Gruber et al., 2020; Ke et al., 2017).

None of the five studies indicated that the viability and functionality of placental tissue are affected by PS particles during perfusion. No effects on barrier integrity (monitored with passive diffusion markers and/or absence of fetal to maternal leakage of perfusate), on human chorionic gonadotropin, leptin, or lactate production, or on glucose consumption have been observed (Table 4). Perfusion time is usually 6 h or less and thus, no long-term effects can be studied in this model. So far, effects of MNP on other toxicity endpoints such as oxidative stress, inflammation markers or endocrine functions have not been studied in human placental perfusion.

## 6. Future directions

There are numerous knowledge gaps concerning MNPs and human health, specifically potential effects on pregnancy and the developing fetus are largely unexplored (Vethaak and Legler, 2021). Recently, MNPs have been detected in human blood, placental tissue and meconium. Since pregnancy and early life represent the most vulnerable period of human development, toxicity studies on the potential effects of MNPs on placental development and essential metabolic, endocrine and immune functions are urgently needed. Experimental data on the toxicokinetics and toxicodynamics of MNPs in the human placenta is scarce and based solely on PS particles. The results from both *in vitro* and *ex vivo* studies indicate that PS particles are taken up and transported through the placental barrier in a size- and dose-dependent manner, which calls for a comprehensive investigation of placental toxicokinetics and toxicity of other environmentally relevant MNPs, and consequent effects of such exposure on fetal and maternal health during pregnancy.

### 6.1. Choosing the right human placental model

To pursue human developmental toxicity studies with MNPs, human placental models are essential because of the interspecies variation in placental structure, physiology and cell biology. A variety of experimental models using human placental tissue (tissue explant cultures, human placental perfusion), and human placental cells (primary cells, human choriocarcinoma cell lines) either as simple cultures or co-culture models can provide valuable, human-relevant data. As described above, each model has advantages and disadvantages and has to be carefully chosen according to the aim and relevant research question being studied. The use of placental cell lines provides relatively simple, high-throughput and robust tools to investigate cell type-specific processes underlying placental transport and toxicity of MNPs. However, they lack physiological complexity. Tissue explant cultures and human placental perfusions more closely simulate the functional units of term placental tissue and could be useful in studying villous transport, endocrine, metabolic and immune functions (Crocker et al., 2004b; Kupper et al., 2021; Rolfo et al., 2013; Simán et al., 2001) as well as the aetiology of placental diseases such as pre-eclampsia (Crocker et al., 2004a; Orendi et al., 2011). Moreover, villous explants from the first trimester can be used to study EVT proliferation and differentiation (Genbacev et al., 1992; Miller et al., 2005). Recently, villous explants have been used in research on biogenic silver nanoparticles (Costa et al., 2021) and polyglycerol nanoparticles (Juch et al., 2018), and, a combination of villous explants with human placental perfusion has been successfully used to study the transplacental passage of PEGylated liposomes (Valero et al., 2017). In placental perfusion, the transplacental passage of MNPs can be investigated in both directions, maternal-to-fetal and fetal-to-maternal (Grafmüller et al., 2015a). This can elucidate the potential role of placental transporters in the transplacental transfer of MNPs, however, transport processes occurring at specific cell types cannot be differentiated using this method. Also, placental accumulation and potential toxic effects could be investigated by using various markers of toxicity and multi-omics approaches, such as RNA sequencing and metabolomic profiling (Vähäkangas et al., 2019; Zhang et al., 2022). However, taking into account a large number of possible experimental conditions (the multitude of MNP polymer types, shapes and sizes), the most important consideration for tissue explant cultures and placental perfusion studies is the low throughput of these methods: they are laborious, time-consuming, and, morphological and functional characterization of these fragile tissues has to be performed at different time points to ensure robust results. Furthermore, no standardised perfusion protocol for MNP research exists (Mathiesen et al., 2010; Myllynen and Vähäkangas, 2013). Such experiments have to be thus planned and designed carefully to maximize their usefulness. Moreover, due to technical difficulties so far only acute exposures and term placentas have been used. How the morphological changes that occur during placental development may influence the MNP toxicokinetics in the early gestation also need to be considered. More advanced *in vitro* models such as hanging-drop or placenta-on-a-chip provide promising models for microphysiological recapitulation of the human placenta that can be used for high-throughput screening. This has been previously demonstrated in studies with nanoparticles, such as titanium dioxide ($TiO_2$), copper oxide (CuO) and cadmium telluride quantum dots (CdTe-COOH) (Shojaei et al., 2021; Yin et al., 2019). Here, endpoints including oxidative stress, cell apoptosis, barrier integrity and permeability were investigated, as well as the effects on maternal immune cells.

### 6.2. Placental transport and placental toxicity

The ST layer seems to be the main barrier to transplacental PS transport, as demonstrated with the *ex vivo* perfusion studies where bigger particles showed higher retention in ST. The exact mechanisms involved in MNP uptake and transport have not been elucidated, but likely passive diffusion is not the key mechanism underlying placental translocation, at least for the larger spherical PS particles. It is worth noting that ST is actively involved in pinocytosis and phagocytosis of micro- and macrovesicles between maternal and fetal circulations. Although these endocytotic pathways have been considered too slow to have any significant effect on placental drug transport (Syme et al., 2004), their role in MNP transport has not yet been characterized. ST is the first cell layer in contact with the maternal blood and plays a major role in the metabolic, endocrine, and immune functions of the placenta. However, to our knowledge, no *in vitro* studies exist on MNP uptake and toxicity in this cell layer. The possible accumulation of MNPs in ST presents a potential hazard. For example, the trophoblastic cells and structures, specifically ST, contribute to the production of extracellular vesicles. These contain mainly deported multinuclear fragments and sub-cellular components (from 50 nm to 1 μm in size) that are shed by the syncytial surface throughout pregnancy. An increase in placental debris has been associated with the development of maternal pre-eclampsia (Redman et al., 2012). Whether the uptake of MNPs in ST contributes to an increase in the production of extracellular vesicles is not known, but warrants further investigation.

While the body of evidence on the placental transport of MNPs is growing, data on toxicity and the effect of MNPs on placental function is scarce. However, other particles, such as fine particulate matter from ambient air pollution, have been shown to translocate from the respiratory tract to the placenta and contribute to various adverse pregnancy outcomes (Bové et al., 2019; Hehua et al., 2017; Johnson et al., 2021; Malmqvist et al., 2013; Su et al., 2020; Wang et al., 2018b; Wojtyla et al., 2020). MNPs, including fibres and traffic-generated particles, are increasingly found in ambient air pollution further adding to the atmospheric particle burden

(Munyaneza et al., 2022). Yet, it is unknown if atmospheric MNPs pose added or novel risks for the developing child and maternal health during pregnancy. To date, research on MNPs investigated mostly generic acute endpoints such as cell viability, cytotoxicity and barrier integrity with effects observed mainly at high, most probably far-from-physiological concentrations. Whereas, the physiological and pathological placental perturbations, including more subtle endocrine or immune-related effects of particle and chemical toxicity of MNPs at environmentally relevant exposure scenarios have received scant attention. A growing body of literature shows that maternal exposure to other particle types such as engineered nanoparticles may directly or indirectly affect maternal health, placental functions and fetal development, which may subsequently lead to a range of adverse pregnancy outcomes and health effects later in life (Dugershaw et al., 2020). Impairment of placental growth, placental oxidative stress and inflammation, activation of placental toll-like receptors (TLRs), and altered secretion of hormones and vascular factors have been previously identified upon placental exposure to such particles (Dugershaw et al., 2020; Hougaard et al., 2015). Clearly, there are strong indications that MNP could affect placental function, but more studies are urgently needed that address this.

### 6.3. Defining MNP exposure

Experimental placental MNP research has focused mainly on acute exposures (up to 24 h) and tested only one polymer type and shape (commercially available and fluorescently labelled spherical PS particles) under a limited number of conditions. The toxicokinetics and toxicodynamics of MNPs, however, are dependent on many factors such as particle size and shape, surface charge, presence of eco- or bio-corona, as well as various chemicals they may carry, all of which are strongly influenced by environmental weathering processes (Abdelkhaliq et al., 2018; Bhagat et al., 2021; Docter et al., 2015; Jiang et al., 2020; Vethaak and Legler, 2021). It is evident that the environmental MNPs are very complex and differ significantly from the pristine spherical PS particles commonly used in the laboratory setting. For example, the spherical particles differ in their uptake and toxicity from the more environmentally relevant irregular-shaped particles (Choi et al., 2018; Gray and Weinstein, 2017). Weathering and degradation processes increase oxygen-containing functional groups on the particle surface influencing aggregation potential, interaction with cellular membranes, adsorption of co-contaminants, biological activity and likely consequent toxicity of MNPs (Abdelkhaliq et al., 2018; Stock et al., 2022).

Environmental MNPs may also act as vectors and transfer bacterial pathogens and exogenous compounds present inside the particles or on their surface (Jiang et al., 2020). For example, a recent study identified more than ten thousand compounds associated with plastic particles, including monomers, additives and processing aids (Wiesinger et al., 2021). Consequently, in addition to particle toxicity, MNPs may leach plastic additives, such as bisphenol A and phthalates, as well as brominated flame retardants, antioxidants, UV stabilizers and synthetic dyes (Jiang et al., 2020; Wiesinger et al., 2021). Moreover, hazardous environmental compounds *e.g.*, dichlorodiphenyltrichloroethane (DDT) (Bakir et al., 2014), polychlorinated biphenyls (PCBs) (Velzeboer et al., 2014), heavy metals (Guo et al., 2020a; Lee et al., 2019) and polycyclic aromatic hydrocarbons (PAHs) (Conesa, 2022; Guo et al., 2020b) have been shown to adsorb to MNPs and form an eco-corona surrounding the MNPs (Cao et al., 2022). Many of these compounds are suspected endocrine disruptors, carcinogens and developmental toxicants. Animal studies suggest increased toxicity by combined exposure to plastic particles with contaminating chemicals than particles alone (Ma et al., 2016; Pacheco et al., 2018; Rainieri et al., 2018). For example, an increase in the uptake and toxicity of nickel, lead and cadmium in the presence of MNPs has been reported in experimental animal models (Kim et al., 2017; Zhou et al., 2020). In humans, these heavy metals are well-known to cross the placenta, accumulate in fetal tissues and adversely affect placental functions and fetal development (Gundacker and Hengstschläger, 2012). Interestingly, antagonistic interactions between MNPs and co-contaminants (*e.g.*, cadmium, nickel, mercury) have also been observed in diverse animal models (Bhagat et al., 2021). Upon uptake in the body, lipids, proteins, carbohydrates and nucleic acids can form a bio-corona around MNPs that are transported *via* the maternal blood to the placenta (Cao et al., 2022). A bio-corona can play an important role in the transport of molecules and particles across the placental barrier and their toxicity (Gruber et al., 2020; Monopoli et al., 2011). The effect of weathering and formation of bio- and eco-coronas on MNP uptake and placental toxicity should be thoroughly investigated.

The identification of the drivers of toxicity of environmentally relevant MNPs is extremely challenging. This is mainly due to the scarcity of well-characterized relevant reference materials, challenges in isolating and characterizing environmental MNPs in complex environmental samples, and the lack of analytical methods for the detection and quantification of non-fluorescent particles (specifically in the nanosized range) in human samples as well as in laboratory setting. Such tools and relevant reference materials are urgently needed to study realistic exposure scenarios and to reduce the current uncertainty in the human risk assessment of MNPs.

This review shows that there are a number of suitable, human-relevant placenta models to characterize the toxicity of MNPs under experimental conditions that could be utilized now to address the most pressing knowledge gaps. These studies should: 1) include fit-for-purpose placental models; 2) focus on the placental transfer and placental function; 3) investigate environmentally relevant particles, *i.e.* polymers other than PS, weathered particles and MNPs with eco- and bio-corona; and 4) use harmonized reference materials and detection methods. The next step forward to better understand actual risks in humans would be to perform more comprehensive risk assessment studies. Here, longitudinal human biomonitoring and epidemiological studies with birth cohorts are required in which maternal and fetal exposures and health outcomes should be further characterized.

## CRediT authorship contribution statement

**Hanna M. Dusza:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Jeske van Boxel:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Majorie B.M. van Duursen:** Conceptualization, Resources, Writing – review & editing, Supervision. **Markus M. Forsberg:** Conceptualization, Writing – review & editing. **Juliette Legler:** Conceptualization, Resources, Writing – review & editing, Supervision. **Kirsi H. Vähäkangas:** Conceptualization, Writing – review & editing.

## Data availability

No data was used for the research described in the article.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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