Modelling the Human Placental Interface In Vitro

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Acting as the primary link between mother and fetus, the placenta is involved in regulating nutrient, oxygen, and waste exchange; thus, healthy placental development is crucial for a successful pregnancy. In line with the increasing demands of the fetus, the placenta evolves throughout pregnancy, making it a particularly difficult organ to study. Research into placental development and dysfunction poses a unique scientific challenge due to ethical constraints and the differences in morphology and function that exist between species. An alternative is to create an in vitro model of the human placenta. Advancements in the differentiation of human induced pluripotent stem cells (hiPSCs), microfluidics, and bioprinting have each contributed to the development of new models, which can be designed to closely match physiological in vivo conditions. By including relevant placental cell types and control over the microenvironment, these in vitro models can reveal clues to the pathogenesis of placental dysfunction and facilitate drug testing across the maternal-fetal interface.

Keywords: placenta ; maternal-fetal interface ; trophoblast invasion ; bioprinting ; microfluidics ; placenta-on-a-chip ; in vitro models

1. Introduction

The human placenta is a crucial organ that supports fetal development throughout gestation. Placental growth and function are precisely regulated to ensure effective circulation of oxygen and nutrients, removal of waste, generation and release of metabolites, and protection against diseases, infections, and xenobiotic transfer to the fetus[1]. Considering its vital role, it is essential to understand placental development and the causes of its dysfunction. However, due to ethical concerns, our understanding of the placenta is largely derived from explants at term or from unsuccessful pregnancies. Explants have provided many clues into pathological pregnancies, such as fetal growth restriction, pre-eclampsia, and stillbirth at varied stages of disease[2][3][4]. However, explants begin to degenerate within hours after collection, making experimentation with human tissue challenging. Efforts have been made to develop accurate animal models[5]; however, considerable differences between species make it difficult to develop a non-primate animal model that fully mimics human placentation[6][7]. Rodent models are useful for understanding specific aspects of placentation, but many processes are difficult to assess in vivo. Ultimately, bioengineered in vitro models promise to bridge the gap between species and offer precise control over the microenvironment to recapitulate specific aspects of human placentation in health and disease[8].

2. Development and Functions of the Human Placenta

The placenta, a fetal organ, forms shortly after fertilization and continues to change throughout pregnancy in response to the metabolic demands of the fetus. Placentation begins post-fertilization when the blastocyst attaches to the inner layer (endometrium) of the uterus. The blastocyst then begins to invade the endometrium with the help of its outer layer of cells, termed trophoblasts. This fetal trophoblastic layer is divided into two cell types, an external multinucleated syncytiotrophoblast layer (the invasive trophoblasts) and an inner cytotrophoblast layer. About two weeks after fertilization, the external syncytiotrophoblast forms preliminary fluid-filled villi structures directed outward, towards the decidual layer of the mother's uterus (**Figure 1a**). Then, the cytotrophoblasts proliferate and migrate through the syncytiotrophoblastic layer to form the primary villi[9]. Soon, these villi expand and become vascularized with fetal placental vessels. Meanwhile, trophoblasts remodel the maternal spiral arteries of the decidua, which become dilated, allowing for maternal blood to fill the intervillous space. As a result, there is a large surface area for the exchange of nutrients traveling from the mother's circulation into the intervillous spaces, through the trophoblast layers, and into the closed placental circulation of the villi, which nourishes the fetus via the umbilical cord[10][11]. By the second trimester, the main features of the mature placenta are formed (**Figure 1b**).

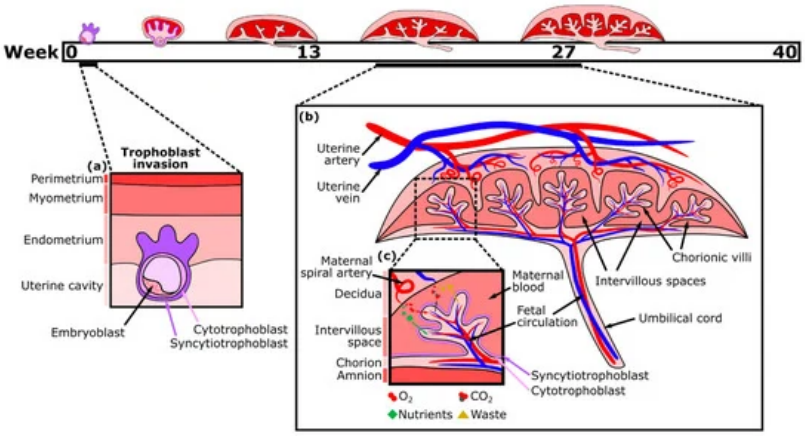

Figure 1. *Placental development timeline, trophoblast invasion, and mature placental structure. (**a**) Diagram of trophoblast invasion around day 9, wherein the syncytiotrophoblast layer surrounding the embryoblast begins to invade the endometrium. (**b**) Mature placental structure showing maternal and fetal vasculature. (**c**) Focus on exchange of nutrients between open maternal blood and closed fetal circulation across the two trophoblastic layers.*

Nutrient exchange between maternal and fetal blood is facilitated largely by the syncytiotrophoblast, which is one continuous multinucleated layer of cells (**Figure 1c**). The ability of nutrients to cross this layer depends on transporter proteins and its thickness, which is reduced near the vascularized parts of the villi[12]. Small hydrophobic molecules, such as oxygen and carbon dioxide, can easily diffuse across plasma membranes in response to differences in the concentration gradient between maternal and fetal blood, which varies with the maternal blood supply, environment, and rate of blood flow. Partial pressure of oxygen in the maternal blood is considerably higher than in the fetal blood, while carbon dioxide is more abundant in the fetal blood. Oxygen exchange is also facilitated by fetal hemoglobin having a higher affinity for oxygen than that of an adult[13]. Consequently, oxygen diffuses through the placenta from the maternal to the fetal blood, while carbon dioxide diffuses in the opposite direction. Transport of large (molecular weight > 1 kDa) and hydrophilic molecules, however, is size restricted and diffusion limited, therefore various transporter proteins are necessary to increase flux[14][15].

The placenta also functions as an immunological barrier, countering the maternal immune response that would normally cause rejection of the fetus, finally leading to spontaneous abortion[16]. Still, maternal antibodies (immunoglobulin G (IgG)) are actively transported across the placenta by neonatal Fc receptors (FcRns), conferring protection against infections to the fetus and the neonate during the first months of life [17].

In addition to its barrier function, the placenta acts as an endocrine organ. For example, to prevent the progression of the menstrual cycle and the loss of the endometrial lining, the syncytiotrophoblast releases human chorionic gonadotropin (HCG). HCG prolongs the life of the corpus luteum, which is thus able to continue releasing progesterone and promote the healthy function of endometrial vasculature, preventing its deterioration and loss[18]. The placenta also produces placental growth hormone (PGH), which is structurally very similar to pituitary growth hormone and eventually completely replaces it[19]. Importantly, the placenta supports pregnancy and fetal growth by selectively secreting the steroids estrogen and progesterone[19].

3. In Vitro Models of the Placental Barrier

A variety of in vitro models have been developed to study different aspects of placental biology; however, one aspect of particular focus is the maternal-fetal interface as a barrier (**Figure 2a**). Cells derived from gestational choriocarcinoma[20][21] or immortalized trophoblasts[22] have been widely used to represent both villous and extravillous trophoblasts as cultured monolayers. The monolayers have been grown on plates or semipermeable membranes (transwell inserts) and have been employed to investigate hormone secretion, transcellular transport of glucose, environmental toxicants, and susceptibility to parasite infection[23][24][25]. Despite their simplicity, cell monolayer models can be an effective first step to studying the mechanisms and properties of the human placental barrier. However, by focusing on just one aspect of the placenta (trophoblasts), these models lack physiological complexity and a comparable cellular microenvironment. Kreuder et al. addressed some of these drawbacks by including essential components of the placental villi, such as fibroblasts and endothelial cells[26], as represented in **Figure 2b**. Their model involved bioprinting a methacrylated gelatin membrane (GelMA), which mimics extracellular matrix (ECM) features, containing primary placental fibroblasts, to simulate villous stroma. BeWo trophoblasts and primary human placental endothelial cells were cultured on either side of this printed membrane, thus representing a more complex model of human placental villi[26]. Barrier properties were assessed by two permeability assays: one using a fluorescently labeled molecule to measure solute flux, and the other by impedance-

based measurements using a transepithelial electrical resistance (TEER) system. Their results showed that the bioprinted membrane presents physiological ECM-like features, such as a lower elasticity, which resembles that of placental tissue, in comparison with filter membrane-based systems. Moreover, TEER values were higher when BeWo trophoblasts were cultured on the membrane containing fibroblasts rather than in monotypic cultures, demonstrating a reduction in permeability (reduced leakiness) due to the incorporation of the stromal compartment.

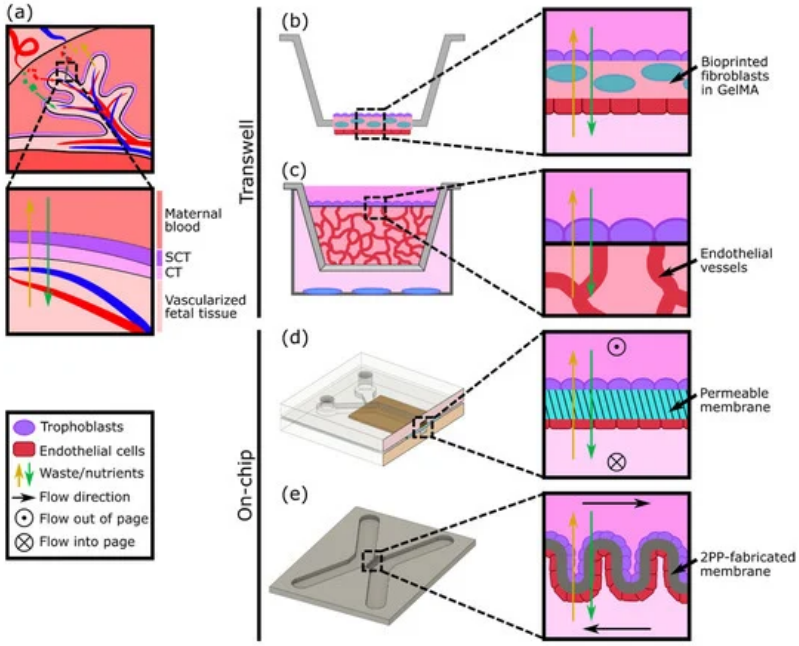

Figure 2. *Modeling the placental barrier in vitro. (**a**) Diagram of transport between maternal and fetal blood supplies across the syncytiotrophoblast (SCT) and cytotrophoblast (CT). (**b**) A modified transwell model using a bioprinted layer of fibroblasts with trophoblasts and endothelial cells cultured on either side (based on design in [26]). (**c**) A transwell model with a layer of trophoblasts cultured on top of vasculature in a 3D gel matrix. Other cell types (blue) can be cultured below the transwell to test the effect of cell secretions (based on design in [27]). (**d**) Endothelial cells and trophoblasts can be cultured on either side of a permeable membrane in a PDMS microfluidic device with flow (based on designs in [17][28]). (**e**) Endothelial cells and trophoblasts may also be cultured on either side of a 2PP-fabricated membrane to achieve different geometries (based on design in [29]).*

In vitro models of the placental barrier have also recently incorporated 3D vascularized networks. For example, Nishiguchi et al. used a modified transwell system to seed a layer of laminin and collagen-coated trophoblasts (either BeWo or primary cytotrophoblasts) onto a thick layer of self-assembled capillary networks, formed from primary fibroblasts (normal human dermal fibroblasts (NHDFs)) and human umbilical vein endothelial cells (HUVECs) in a fibrin hydrogel[27] (**Figure 2c**). This model was employed to examine cell damage signaling across the barrier by exposing rat embryonic cortical neurons to conditioned medium collected from the in vitro placental barriers assembled with direct or indirect contact with the vascular bed. Although the vessels were not perfused, the presence of the vasculature resulted in a reduction in neuron dendrite length, providing evidence of crosstalk between the trophoblasts and the endothelium.

Considering the multilayered structure and physiologic microenvironment critical to placental barrier function, other groups have also attempted to generate more complex models, including both an endothelial (representative of the fetal vessels) and trophoblast component. For instance, cocultures of endothelial and trophoblast cells have been combined in sandwiched monolayers on chip, which allow for exposure to fluid flow mimicking the hemodynamic shear stress present in maternal and fetal compartments (**Figure 2d**). Blundell et al. generated a two-layer polydimethylsiloxane (PDMS) device with two channels separated by a thin porous membrane, which allows for constant perfusion with culture media[28]. BeWo trophoblasts were cultured on the upper side of the membrane and human placental vascular endothelial cells (HPVECs) on the lower side. This coculture model recapitulated structural features of the maternal-fetal interface and showed the expression and physiological localization of placental transporter proteins. The authors observed more complete formation of dense microvilli projections on the apical surface of the trophoblast cells when cultured under fluid shear stress conditions, when compared with static culture. Moreover, they found that inclusion of the fetal endothelium was crucial to replicate physiological maternal-fetal glucose transport, as confirmed by comparing with the glucose transfer rates measured across two other types of barriers: a cell-free barrier and a trophoblast monolayer without endothelium[28]. In a similar approach, Lee et al. cultured JEG-3 trophoblasts and HUVECs on either side of a solidified collagen membrane and subjected each side to dynamic flow conditions. The system facilitated cell proliferation and the formation of confluent monolayers into a placental barrier model, which demonstrated different glucose transport rates

depending on the presence of the epithelium and in accordance with findings from Blundell's model[17]. More recently, a similar microfluidic two-channel design with a polyethylene (PETE) membrane separating monolayers of BeWo trophoblasts and HUVECs was tested to examine caffeine transport across the placenta, a molecule that cannot be fully metabolized by a developing fetus[29]. This study provided new insights into the extent of caffeine transfer from mother to fetus and demonstrated the utility of the system for future xenobiotic compound testing.

Another way to achieve the complex geometry of the placental villous membrane, while bypassing the use of flat cell monolayers, was proposed by Mandt et al., who developed a barrier model using a high-resolution three-dimensional (3D) printing method called two-photon polymerization (2PP)[30]. A villi-like convoluted surface within a microfluidic device with two separate channels was shaped by 2PP from a modified gelatin-based hydrogel material (GelMA), mimicking the basal membrane of the placenta (**Figure 2e**). To mimic the fetal and maternal compartments, HUVEC and BeWo trophoblasts were then seeded on either side of the membrane and cultured under constant flow. The authors studied transcellular transport across this barrier and demonstrated in vivo-like properties by showing the permeability of sugar-sized molecules (riboflavin, 350 Da) and the impermeability of larger ones (dextran, 200 kDa).

4. In Vitro Models of Trophoblast Invasion

Improper trophoblast invasion into the endometrial spiral arteries is often associated with pregnancy complications, including pre-eclampsia and fetal growth restriction[31]. Thus, understanding how spiral artery remodeling impacts the early steps of placental development is crucial. A variety of in vitro models specifically designed to replicate the process of trophoblast invasion (**Figure 3a**) have been developed. Transwell assays have been extensively used to assess trophoblast invasiveness and generally involve the observation of cell migration through a Matrigel layer, often towards a chemoattractant (**Figure 3b**)[32][33][34][35]. However, these 2D adherent cell systems do not fully replicate the invasion process in an anatomically relevant manner. Recently, the inclusion of self-assembled spheroids of extravillous trophoblasts has allowed for complex 3D cell-cell interactions, bringing important insight into the mechanisms underlying trophoblast migration and invasion (**Figure 3c**). For instance, You et al. demonstrated that endometrial signaling is essential to promote and guide trophoblast invasion by observing that trophoblasts were able to migrate from the spheroids and invade the Matrigel only when there was an underlying layer of human endometrial stromal cells[36].

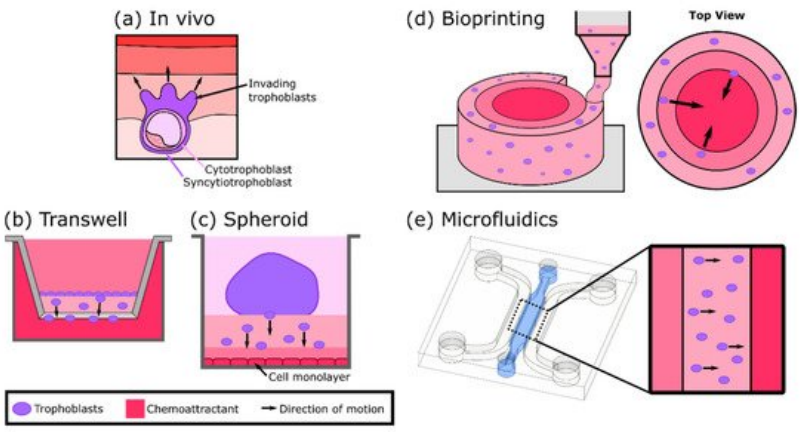

Figure 3. *Summary of trophoblast invasion models. (**a**) Diagram of trophoblast invasion of the endometrium in vivo. (**b**) A transwell model with a monolayer of trophoblasts invading a gel towards the chemoattractant in the well (based on designs in [32][33][34][35]). (**c**) A spheroid model in a well plate with trophoblasts invading the underlying gel towards a monolayer of cells (based on design in [36]). (**d**) A bioprinted model consisting of concentric rings of a gel, with trophoblasts in the outermost layer and a chemoattractant in the innermost layer (based on designs in [37][38][39]). (**e**) A microfluidic device with trophoblasts suspended in a gel in the center channel with medium flowing through the channels on either side (based on design in [40][41]). The chemoattractant is included in only one media channel in this design.*

Bioprinting allows for controlled 3D spatial patterning of cells, biomaterials, ECM components, and growth factors in order to generate tissue analogues and has led to the development of a number of physiologically relevant trophoblast invasion assays (**Figure 3d**). One such study utilized bioprinting to generate a cylindrical hydrogel model containing placental basement membrane (BM) proteins, including collagen, laminin, and fibronectin, and a central source of chemoattractant (epidermal growth factor (EGF)) at the center and an outer layer of cytotrophoblasts[37]. The results showed that trophoblast cell migration was significantly higher in the presence of BM proteins when compared with the empty hydrogel, demonstrating the importance of the ECM microenvironment in trophoblast invasion. Similarly, Ding et al. bioprinted multi-ring and multi-strip hydrogel systems incorporating EGF and adjacent layers with and without

encapsulated cells (invasive trophoblasts, HTR-8/SVneo). Their strategy enabled the recapitulation and modulation of in vivo 3D cellular microenvironments and the study of trophoblast migration in different geometries[38]. Considering that EGF is downregulated in pre-eclampsia (PE)[42], Kuo et al. generated a cylindrical 3D-printed GelMA hydrogel loaded with different concentrations of the growth factor to study the migratory response of trophoblasts in the development of PE. Their results showed that trophoblast migration increases in response to higher EGF concentrations[39]. Since insufficient trophoblast invasion is a primary feature of PE, this model represents a useful tool in the identification of novel therapeutic targets for its treatment.

Besides bioprinting, microfluidic models have also been useful in tracking and quantifying the dynamics of trophoblast cell migration since they can be designed to generate stable gradients on chip (**Figure 3e**). As an example, Abbas et al. embedded primary trophoblasts in Matrigel and demonstrated the impact on their migratory behavior by including a gradient of granulocyte-macrophage colony-stimulating factor (GM-CSF)[40]. In fact, with the addition of the gradient, trophoblast cells exhibited increased directionality and motility, suggesting that GM-CSF is a key cytokine in the regulation of trophoblast invasion. The system was later improved by including endothelial cells to elucidate their effect on trophoblast invasiveness[41]. Moreover, invasion-stimulation was induced with folic acid, and trophoblast tracking was facilitated by the incorporation of fluorescent cell tagging. The results showed that trophoblast invasiveness was enhanced in the presence of endothelial cells, suggesting that the release of cytokines and growth factors from the endothelium has a role in trophoblast migration.

5. Three-Dimensional Models to Study Placental Dysfunction, Infections, and Maternal-Fetal Toxicology

Besides the defective remodeling of the spiral arteries, many other aspects of placental dysfunction are associated with altered placental development and the onset of pregnancy complications. These events, which include the impairment of villous tree maturation[43], the detrimental effects of pathogen infection [44], and the response to drugs and environmental cues[45][46], still need further investigation. As discussed previously, the advent of technologies such as bioprinting and microfluidic-based organs-on-a-chip have facilitated the recapitulation of critical placental functions and stages of development, raising the possibility to apply these models to study the mechanisms underlying these aberrant events. For example, a work from Haase et al. brought new insight into placental vasculopathy, showing that pericytes (mural cells of the microvasculature) contribute to growth restriction of fetal microvessels grown in microfluidic devices[47]. Moreover, the results showed PE-like effects, including upregulation of inflammatory cytokines, hyperproliferation of stromal cells, dysfunctional barrier properties, and immune cell infiltration.

Microfluidic technologies have also been implemented to explore the impact of pathogenic infections during pregnancy. Zhu et al. generated a multilayered microfluidic placental barrier-on-a-chip model to investigate the placental inflammatory responses to bacterial infection[48]. When *Escherichia coli* was applied to the maternal side of the chip, trophoblast cells triggered an acute inflammatory response by secreting interleukin-1α, IL-1β, and IL-8 cytokines, followed by the adhesion of maternal macrophages. More recently, a microfluidic organ-on-a-chip model comprising the decidua and the fetal chorionic and amnionic membranes was generated to track the propagation of infection and inflammation across the maternal-fetal interface[49]. This four-chamber system containing primary cells from the maternal-fetal interface and a collagen matrix mimics cellular features seen in the native tissue, such as morphology, cellular transitions, migration, and production of nascent collagen. The ascending infection and consequent inflammation were tracked by examining the propagation of lipopolysaccharide (LPS) from the decidua to the amnion. The results demonstrated the disruption of the maternal-fetal interface integrity during ascending infection due to an imbalanced immune response, an event that is associated with preterm birth[50].

It is particularly challenging to study the effects of drugs on the structure and function of the placental barrier since they cannot be tested on pregnant women. The thalidomide disaster of the 1960's[51] unveiled that the placenta is not an impenetrable barrier and allows xenobiotics to cross from the maternal to the fetal circulation, leading to congenital abnormalities. Therefore, it has become crucial to ensure that potential therapeutic agents and common medications do not impact human fetal development. To overcome this, placenta-on-a-chip technologies have been adopted to study the transport of drugs across the placental barrier, demonstrating their potential use as preclinical drug efficacy and drug safety testing tools. For example, the microfluidic model by Blundell et al., described earlier, was used to investigate the diffusion of heparin and the gestational diabetes drug glyburide across the maternal-fetal interface[52], demonstrating the capability to recapitulate the native function of efflux transporters and the limited drug intake of an in vivo placenta. Finally, increasing evidence also indicates that nanoparticles can cross the placenta barrier, eliciting a toxic effect[53]. For instance, the impact of exposure to titanium dioxide, a common nanomaterial used in plastics, medicines, food products, cosmetics, and toothpastes, has recently been investigated using a micro-engineered 3D placental model[54]. The results

showed disruption of placental barrier integrity and adhesion of maternal immune cells in the presence of this nanomaterial.

Overall, the employment of 3D micro-engineered models creates a suitable and controlled approach towards the understanding of the effects of drug treatments and disease conditions on placental function.

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