Published in final edited form as:

Lab Chip. 2016 August 2; 16(16): 3065–3073. doi:10.1039/c6lc00259e.

A microphysiological model of the human placental barrier

Cassidy Blundell¹, Emily R. Tess¹, Ariana S. R. Schanzer¹, Christos Coutifaris², Emily J. Su³, Samuel Parry⁴, and Dongeun Huh^{1,*}

¹Department of Bioengineering, University of Pennsylvania, Philadelphia, PA

²Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

³Department of Maternal-Fetal Medicine, University of Colorado Denver, Denver, CO

⁴Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Abstract

During human pregnancy, the fetal circulation is separated from maternal blood in the placenta by two cell layers – the fetal capillary endothelium and placental trophoblast. This placental barrier plays an essential role in fetal development and health by tightly regulating the exchange of endogenous and exogenous materials between the mother and the fetus. Here we present a microengineered device that provides a novel platform to mimic the structural and functional complexity of this specialized tissue *in vitro*. Our model is created in a multilayered microfluidic system that enables co-culture of human trophoblast cells and human fetal endothelial cells in a physiologically relevant spatial arrangement to replicate the characteristic architecture of the human placental barrier. We have engineered this co-culture model to induce progressive fusion of trophoblast cells and to form a syncytialized epithelium that resembles the syncytiotrophoblast *in vivo*. Our system also allows the cultured trophoblasts to form dense microvilli under dynamic flow conditions and to reconstitute expression and physiological localization of membrane transport proteins, such as glucose transporters (GLUTs), critical to the barrier function of the placenta. To provide a proof-of-principle for using this microdevice to recapitulate native function of the placental barrier, we demonstrated physiological transport of glucose across the microengineered maternal-fetal interface. Importantly, the rate of maternal-to-fetal glucose transfer in this system closely approximated that measured in *ex vivo* perfused human placentas. Our “placenta-on-a-chip” platform represents an important advance in the development of new technologies to model and study the physiological complexity of the human placenta for a wide variety of applications.

Graphical Abstract

We present a microphysiological model that reconstitutes the functional unit of the human placenta.

*To whom correspondence should be addressed: huhd@seas.upenn.edu, Tel: 1-215-898-5208.

Introduction

The placenta is a highly specialized organ in the human body that plays an integral role in the development and maintenance of pregnancy^{1,2}. As evidenced by the recent launching of the Human Placenta Project by the US National Institutes of Health, the current dearth of knowledge about the human placenta is providing an impetus to improve our ability to probe and understand the inner workings of this vital organ. Specifically, collective research efforts emerging in this area focus on developing new technologies to examine and monitor human-specific placental structure and function during health and disease³. These studies are further justified by the poor physiological relevance and predictive power of existing animal models that have proven problematic for the study of the human placenta^{4,5}.

Motivated by these unmet challenges and emerging opportunities in reproductive biology and medicine, we have developed a microengineered cell culture system that enables a new approach to modeling the salient features of the human placenta (Figs. 1A,B). The primary role of the placenta is to mediate the exchange of various endogenous and exogenous substances between the mother and fetus during pregnancy^{6,7}. Central to this critical organ function is a multilayered membranous structure that consists of the syncytiotrophoblast and the fetal capillary endothelium, separated by a thin interstitium (Fig. 1C). This specialized barrier, which separates the maternal intervillous space and fetal circulation, is responsible for regulating the rate and selectivity of placental transport. Aberrant changes in its structure and function are implicated in various complications of pregnancy, such as preeclampsia and intrauterine growth restriction^{8,9}. Our microsystem provides a novel platform to emulate this essential unit of the placenta by allowing human placental cells to grow and organize into a multilayered living tissue that replicates the native architecture of the maternal-fetal interface. Specifically, this model is a compartmentalized microfluidic system consisting of two closely apposed microchannels to enable culture of human trophoblasts and human placental villous endothelial cells on opposite sides of a thin semipermeable membrane under dynamic flow conditions (Fig. 1D). The design of this device also makes it possible to engineer the soluble microenvironment of the maternal compartment to induce syncytialization of the trophoblast cells and to reproduce their differentiated morphological and biochemical phenotypes. We show progressive fusion of the cultured trophoblast cells and their increased production of human chorionic gonadotropin during the course of syncytialization induced by the activation of the protein kinase A pathway.

To further demonstrate the potential of this biomimetic microengineering approach, we present our “placenta-on-a-chip” system as a promising alternative to existing models for the study of physiological transport function of the human placenta. Current research on placental transport relies heavily on *in vitro* techniques based on Transwell cell culture inserts that provide a polarized environment conducive to barrier formation¹⁰. This traditional approach, however, has been used predominantly for establishing monoculture of trophoblast cells to study their transport function. Consequently, existing Transwell models do not mimic the multi-layered structure of the placental barrier, failing to recapitulate endothelial contribution to physiological barrier function¹¹. Furthermore, static culture conditions in these models do not reconstitute the dynamic flow environment of the placenta *in vivo* that has been shown to influence cellular phenotypes in the placental barrier¹². As an alternative approach, researchers utilize *ex vivo* models involving controlled perfusion of the whole human placenta¹³. Although this technique has gained widespread acceptance in placental transport studies, significant challenges remain due to the requirement for human specimens, the limited amount of time that tissue remains viable, and the inconsistent results produced by these models¹⁴. The whole organ strategy also makes high-resolution analysis at the cellular and tissue levels difficult, which is often required for a mechanistic understanding of placental transport. As a new approach to address the shortcomings of these existing techniques, we demonstrate the feasibility of leveraging our microengineered platform to model physiological biomolecular transport across the maternal-fetal interface. Using glucose as a model substance, we show that our microengineered placental barrier is capable of mediating net directional molecular transport from the maternal to fetal compartments. Our data also reveal the advantage of this biomimetic microsystem over Transwell inserts for accurately predicting the physiological rate of glucose transport in the human placenta.

Methods

Cell culture

The BeWo b30 human trophoblast cell line¹⁵ was obtained from Dr. Nicholas Illsley of Hackensack University Medical Center and was cultured in DMEM/F-12K medium (GE Healthcare) containing 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin (Gibco). Human primary placental villous endothelial cells (HPVECs) were isolated from term placentas as described previously¹⁶ and maintained in EGM-2 medium containing 2% FBS (Lonza).

For static Transwell cell culture studies, Transwell inserts (24-well plate; pore size = 0.4 μm ; surface area = 0.33 cm^2) were coated with human fibronectin solution (0.1 mg/ml in PBS). For cell seeding, the insert was first inverted, and a drop of HPVEC suspension (50,000 cells/insert) was placed on the basal surface of the insert membrane. The seeded insert was then placed in a cell culture incubator for 1 hour for cell attachment. Following HPVEC adhesion, the insert was washed and placed back in the well plate, and the basal compartment was filled with endothelial media. Subsequently, the apical chamber of the insert was filled with a BeWo cell suspension (50,000 cells/insert), incubated for 1 hour, and washed to seed the upper side of the membrane with trophoblast cells.

Microdevice fabrication

The upper and lower layers of the microdevice were fabricated using standard soft lithography techniques. Briefly, poly(dimethylsiloxane) (PDMS) (Sylgard, Dow Corning) base was mixed with curing agent at a weight ratio of 10:1 and degassed to remove air bubbles. The mixture was then cast on a silicon master containing photolithographically prepared microchannel features made of SU-8 (MicroChem). The microchannel dimensions were 1 mm (width) $\times$ 1.5 cm (length) $\times$ 135 μ m (height). A biopsy punch was used to create 1 mm-diameter holes through the upper PDMS slab to gain fluidic access to the upper and lower microchannels.

To assemble the device, the two PDMS layers were bonded to a semipermeable polycarbonate membrane containing 1 μ m pores (GE Healthcare) using adhesive PDMS mortar¹⁷. To create this layer, PDMS precursor was mixed with curing agent at a weight ratio of 10:3 and spin-coated on a 100 mm Petri dish at 2500 rpm for 5 minutes. Subsequently, both the upper and lower microdevice layers were gently placed on the dish to transfer the spin-coated mortar film onto the surfaces of the PDMS slabs containing the microchannel features. This step was followed by bonding of the polycarbonate membrane to the upper PDMS slab. These two layers were then aligned and attached to the lower PDMS slab, and cured at room temperature overnight to ensure complete bonding.

Microfluidic cell culture

The assembled microdevice was first sterilized using UV irradiation. Following sterilization, the surface of the intervening porous membrane was coated with extracellular matrix (ECM) by filling and incubating the microchannels with a human fibronectin solution (0.1 mg/ml in PBS) at 37C for at least 4 hours. The channels were then rinsed with PBS to remove the ECM solution prior to cell seeding. To form the fetal endothelium, we introduced a suspension of trypsinized HPVECs (4×10^6 cells/ml) into the lower microchannel and immediately inverted the device to allow the cells to settle to the original lower side of the porous membrane. Subsequently, the seeded microdevice was incubated at 37C for 1 hour to enable cell attachment and spreading. During this period, the inlet and outlet access ports were blocked to prevent unwanted convective motion of culture medium in the microchannels. Once attachment of HPVECs was confirmed, the device was flipped back, and the upper microchannel was seeded with BeWo cells suspended in DMEM/F-12K at a concentration of 4×10^6 cells/ml. After incubation at 37C for 1 hour, the microdevice was connected to syringe pumps that generated continuous flow of culture media in the upper and lower microchannels at a volumetric flow rate of 100 μ L/hr.

Analysis of intercellular junctions

In order to assess the formation of intercellular junctions, the trophoblast cells and HPVECs were fixed in 4% paraformaldehyde (PFA) for 15 minutes, permeabilized in 0.25% Triton X-100 for 10 minutes, and then incubated in 2% bovine serum albumin (BSA) for 1 hour. All steps were performed at room temperature. The trophoblast cells and HPVECs were incubated with anti-E-cadherin (Life Technologies) and anti-VE-cadherin antibodies (Cell Signaling Technologies), respectively. These primary antibodies were diluted in 2% BSA and incubated in the microdevice for 1 hour at room temperature. Next, the samples were

thoroughly washed with PBS. Secondary antibodies (Life Technologies) were diluted in 2% BSA, incubated for 45 minutes at room temperature, and then washed with PBS. Nuclei were labeled using DAPI subsequent to the secondary antibody incubation. Following staining, the membrane was carefully removed from the microdevice and mounted onto a coverslip. Images were acquired using an inverted microscope (Zeiss Axio Observer) and a confocal laser-scanning microscope (Leica TCS SP8). Image processing and three-dimensional rendering were carried out using Volocity (PerkinElmer).

Analysis of microvilli formation

Following 72 hours of culture, trophoblast cells grown in Transwell and microfluidic devices were fixed, permeabilized, and incubated with 2% BSA as described above. Next, the samples were stained with Alexa 488-conjugated phalloidin (Life Technologies) for 30 minutes at room temperature and washed to visualize F-actin in the microvilli. The cell nuclei were counterstained with DAPI. A confocal laser-scanning microscope (Leica TCS SP8) was used to acquire Z-stack images. For quantification of relative fluorescence, Z-stack data were converted to a maximum intensity projection image and manual thresholding was performed in FIJI to isolate fluorescent pixels¹⁸. Histogram counting was used to determine the relative amount of actin fluorescence in static and dynamic conditions.

Trophoblast syncytialization

Following formation of a confluent epithelial monolayer on the membrane surface in the upper microchannel, we treated the apical side of the epithelium with forskolin to activate the protein kinase A pathway in the cultured trophoblasts. A stock solution of forskolin (Sigma; 5 mg/mL in DMSO) was diluted with F-12K medium to a final concentration of 50 μ M and perfused through the upper microchannel. After 72 hours of forskolin treatment, the trophoblast cells were fixed in 4% PFA, permeabilized in Triton-X 100, and then incubated with 2% BSA in PBS for immunofluorescence staining. To analyze changes in junctional protein expression, the samples were incubated with anti-E-cadherin antibody (Life Technologies) in 2% BSA, followed by secondary antibody and DAPI. Additionally, media perfusate was collected at 48, 72, and 96 hours from both untreated and forskolin-treated devices. The collected samples were analyzed using a human chorionic gonadotropin beta (β -hCG) ELISA kit (Abcam) to quantify the levels of β -hCG produced by the trophoblast population at each time point.

Measurement of barrier permeability after syncytialization

Barrier function of the syncytialized epithelium was assessed by measuring the transport of 3 kDa fluorescein isothiocyanate-dextran (FITC-dextran; Life Technologies) between the maternal and fetal compartments. FITC-dextran (0.1 mg/mL in DMEM/F-12K media) was introduced to the upper maternal microchannel and perfused for 3 hours. The media perfusate was collected from both microchannels during this period and the fluorescence intensity of the collected samples was quantified using a microplate reader (Tecan). The amount of dextran transport was assessed based on the mean fluorescence intensity in the outflow from the lower fetal microchannel.

Visualization of cell membrane transporters

After 3 days of microfluidic culture, cells were processed for immunofluorescence imaging as described above in order to evaluate the presence and spatial distribution of glucose transporters in the microengineered placental barrier. Briefly, cells were fixed on-chip in 4% PFA, permeabilized in Triton-X 100, and incubated in 2% BSA in PBS. The samples were then incubated with mouse anti-glucose transporter 1 antibody (Abcam), followed by secondary antibody (Life Technologies). Images were acquired using a confocal laser-scanning microscope (Leica), and image processing was carried out using Volocity software (PerkinElmer). Assessment of transporter membrane localization was performed using FIJI¹⁸. The apical and basal membranes were manually segmented in 10 representative images, and the mean fluorescence intensity was measured in each image. These values were adjusted for background fluorescence.

Analysis of glucose transport

To analyze glucose transport across the maternal-fetal interface, the maternal compartment was perfused with culture medium containing 10 mM glucose. This increased glucose concentration was generated by adding D-glucose (Gibco) to F-12K medium. Media on the fetal side contained 5.5 mM of glucose during perfusion. Outflow from the maternal and fetal microchannels was collected over a period of 2 hours and analyzed by a glucose meter (Accu-Chek Aviva) to measure glucose concentration. These studies were carried out to measure the rate of transport across three types of barriers: (1) a bare membrane in a cell-free device, (2) a monolayer of BeWo cells without the endothelium, and (3) an epithelial-endothelial barrier formed by co-culture of BeWo cells and HPVECs. For each group, barrier function was quantified by the percent increase in fetal glucose concentration over the period of perfusion. Additionally, the percent rate of transfer was calculated for the co-culture model using the following equation previously described in placental transport studies¹⁹,

$$\% \text{ rate of transfer} = \frac{\Delta G_F}{\Delta C_M} \times 100$$

where C_F and C_M denote changes in glucose concentration in the fetal and maternal compartments during perfusion, respectively. This value was compared to the percent rate of transfer previously measured in the human placenta to investigate the physiological relevance of our model.

To measure glucose transport in Transwell, the apical and basal chambers were filled with 200 μL of epithelial media containing 10 mM glucose and 500 μL of endothelial media with 5.5 mM glucose, respectively. A 50 μL sample was taken from each chamber every 30 minutes over the course of transport experiments. The concentration of glucose in the samples was measured using a handheld glucose meter (Accu-Chek Aviva). The percent rate of transfer was calculated as described above.

Statistical Analysis

Results are reported as mean $\pm$ S.E.M. Statistical significance was assessed using a two-tailed Student's t-test or analysis of variance (ANOVA). Each experiment was repeated at least three times.

Results and Discussion

Reconstituting the microarchitecture of the human placental barrier

The function of the maternal-fetal interface as a mediator of placental transport is imparted primarily by its multilayered physical structure, characterized by the trophoblast epithelium and the fetal endothelium held in close apposition. Our microengineered system made it possible to replicate this critical microarchitecture of the placental barrier by permitting compartmentalization of its two key cell types in the maternal and fetal circulations. The trophoblast and endothelial cell populations introduced into the microchannels established firm adhesion to the ECM-coated membrane and began to spread within a few hours of cell seeding. During perfusion culture, these cells proliferated in a continuous manner to form fully confluent monolayers in both the upper and lower chambers, which covered the entire surface of the membrane within 24 hours of initial cell seeding. The resulting bi-layer tissue closely resembled the trophoblast-endothelial interface of the chorionic villus *in vivo* (Figs. 2A,B). Despite porosity of the interstitial membrane, we did not observe cell transmigration between the microchannels, presumably due to the small size of the membrane pores (1 μ m). Under perfusion culture conditions, the microengineered placental barrier was maintained without a significant loss of cell viability for prolonged periods (> 1 week).

Next, we evaluated the formation of cell-cell junctions to assess structural integrity of the barrier. A specific focus of this analysis was on the visualization of VE-cadherin expression in the fetal endothelium and E-cadherin in the trophoblast cells. Immunofluorescence imaging of the bilayer tissue cultured for 3 days clearly showed a network of continuous and well-defined junctional complexes throughout both the trophoblast and endothelial layers (Figs. 2C,D). Importantly, expression of the junctional proteins was found to be uniform across the entire cell culture membrane, showing no indication of localized regions with immature junctions. Considering that intercellular adhesion plays an essential role in placental transport *in vivo*, these results together demonstrate the capability of our model to recapitulate not only the relative spatial arrangement of the maternal and fetal tissue in the placental barrier but also the structural integrity necessary for its function.

Microfluorimetric analysis of the placental barrier in our device also revealed evidence of extracellular matrix deposition by trophoblast cells. In the human placenta, laminin is a critical component of the trophoblast basement membrane in the chorionic villus that contributes to barrier integrity²⁰. Confocal microscopy of the trophoblast cells cultured in our device for 6 days showed extensive extracellular deposition of laminin (Fig. 2E). Moreover, this deposition was localized to the basal side of the cells, forming a thin layer of laminin between the epithelium and underlying semipermeable membrane (Fig. 2F), which was reminiscent of the basal lamina *in vivo*.

Microvilli formation in the microengineered placental barrier

Another vital structural feature of the placental barrier is the membrane protrusions on the apical surface of trophoblasts facing the maternal intervillous space. These microvilli increase the overall surface area available for placental transport, enabling highly efficient exchange of nutrients and waste products between the mother and the fetus^{6,21}. The microvillous surface also contains key molecular transporters, as well as hormone receptors that influence cellular function. Our microfluidic device allowed the cultured trophoblast cells to recapitulate this critical phenotype.

As illustrated in Figs. 3A and 3B, the apical surface of the trophoblasts cultured in the maternal chamber for 3 days was covered with a dense layer of microvilli that appeared as fine hair-like protrusions in the fluorescence micrographs. Although cell-to-cell variability was observed in the extent of microvilli formation, this morphological differentiation was evident in the vast majority of the cells. In contrast, the trophoblast cells grown in Transwell for the same period exhibited reduced expression of microvilli (Figs. 3C,D). Image analysis of these cells showed a smaller number of microvilli per cell, as well as marked morphological alterations characterized by decreased villus length and thickness. This significant difference may be attributed to the effect of fluid mechanical forces that have recently been shown to trigger microvilli formation in trophoblast cells¹². Continuous flow of culture medium in the maternal chamber of our microdevice generates fluid shear stress that approximates the level of hemodynamic shear stress in the intervillous space^{12, 22}. Presumably, this physiological biomechanical cue stimulates the trophoblasts to form microvilli, whereas static culture conditions in Transwell inserts fail to provide the dynamic microenvironment required for this process. Considering that the microvilli serve as a key regulator of placental transport, these observations illustrate that our system is advantageous over traditional *in vitro* models not only for reproducing the structural phenotype of the placental barrier but also for mimicking its physiological barrier function.

Syncytialization of the microengineered placental barrier

With the progression of pregnancy, cytotrophoblast cells covering the chorionic villi of the human placenta differentiate and fuse to form a multinucleated syncytiotrophoblast (Fig. 4A). This terminally differentiated syncytium forms the continuous outer lining of the chorionic villi and comes in direct contact with maternal blood in the intervillous space. This process of syncytialization is a hallmark of placentation and plays a central role in physiological function of the placental barrier as a key regulator of material exchange between the maternal and fetal circulations²³. While the underlying molecular pathways of syncytialization are not fully understood, studies have shown that activation of adenylate cyclase, which is the regulatory subunit of protein kinase A, by 3',5'-cyclic monophosphate (cAMP) or forskolin induces BeWo cells and primary villous cytotrophoblast cells to fuse and acquire differentiated phenotypes of the syncytiotrophoblast^{24,25}.

Based on these previous findings, we engineered the soluble microenvironment of trophoblast cells using forskolin-supplemented media to induce syncytialization. When the BeWo cells in the maternal compartment were exposed to forskolin, they began to undergo cell-cell fusion as illustrated by nuclear aggregation that was evident at 72 hours of forskolin

treatment (Fig. 4B). This response occurred in approximately 50% of the BeWo population and did not exert deleterious effects on cell viability. Concurrent to the fusion of trophoblast cells was a loss of epithelial cell-cell junctions. As shown in Fig. 4B, microscopic inspection of the trophoblasts showed significant downregulation of E-cadherin throughout the epithelial layer. Interestingly, this reduced expression of junctional proteins did not compromise the structural integrity of the barrier. On the contrary, syncytialization in our model led to improved barrier function. When we measured paracellular permeability using FITC-dextran, the amount of dextran transport across the barrier decreased over the course of forskolin exposure (Fig. 4C). These observations closely match characteristic alterations in the morphology and barrier function of BeWo cells during their acquisition of syncytiotrophoblast-like phenotypes²⁶, indicating successful syncytialization of the trophoblast epithelium in our model.

One of the most important functional consequences of trophoblast syncytialization in the human placenta is the production of hormones that play a crucial role in the progression of both placental and fetal development²⁷. As a representative example of a placental hormone, human chorionic gonadotropin (hCG) is secreted by the syncytiotrophoblast and serves as a biochemical marker of *in vitro* trophoblast differentiation. Therefore, we sought to measure the production of the β subunit of hCG in the maternal compartment of our model as a way to quantitatively examine syncytialization. In the absence of forskolin, analysis of maternal outflow yielded no detectable β -hCG (Fig. 4D). In contrast, administration of forskolin triggered production of hCG by trophoblast cells within 48 hours, and the hormone levels continued to increase over the course of 96 hours of forskolin treatment (Fig. 4D). Interestingly, the extent of increase was substantially greater in the first 48 hours, implying positive feedback control of cell differentiation during the initial period of stimulation. This may be explained by the previous discovery that hCG produced by differentiated trophoblast cells activates adenylyl cyclase to increase intracellular cAMP and thus to further promote trophoblast differentiation²⁸. Taken together, these results demonstrate that our placenta-on-a-chip platform enables both morphological and functional differentiation of trophoblast cells to reconstitute the syncytium of the placental barrier.

Glucose transport across the microengineered placental barrier

Glucose from the maternal circulation is a primary source of energy for fetal growth and development during pregnancy. The maternal-to-fetal transport of glucose across the placental barrier is mediated by facilitated diffusion via a family of membrane-bound glucose transporters (GLUTs)^{29,30}. GLUT1 is the most abundant type of glucose transporter in the human placenta and is found in the syncytium of the placental barrier. Its expression has been shown to increase over the second half of pregnancy to meet the increased rate of fetal growth³¹. While GLUT1 transporters are expressed in both the apical and basolateral surfaces of the syncytiotrophoblast layer, studies have revealed their asymmetric localization, with a greater proportion located on the apical microvillous membrane facing the maternal intervillous space³². Our model recapitulates this pattern of GLUT1 expression. Immunofluorescence analysis demonstrated robust expression of GLUT1 transporters in the population of differentiated trophoblast cells comprising the microengineered syncytium

(Fig. 5A). More importantly, there was increased GLUT1 expression on the apical side of the epithelium, resembling the native spatial distribution of the transporter (Figs. 5B,C).

Based on this evidence of transporter expression and localization, we then carried out quantitative analysis of glucose transport in the placenta-on-a-chip system. In this study, facilitated diffusion of glucose from the maternal to fetal compartment was induced by creating a concentration gradient across the microengineered tissue interface (Fig. 5D). As a control condition, we first evaluated glucose transport in a model consisting of the upper and lower microchannels separated by a bare membrane. Measurements taken from this acellular system were used to establish the baseline permeability of the porous membrane to maternal glucose. To assess the contribution of the epithelium to barrier function, another control group was generated by similar devices used for monoculture of trophoblasts in the maternal compartment. As shown in Fig. 5E, the presence of the epithelial barrier in this group led to approximately 50% reduction in the percent increase in fetal glucose concentration as compared to the baseline data obtained from the acellular model. When fetal endothelial cells were included to establish a co-culture model, permeability decreased further due to the additional cell layer, and the increase in fetal glucose was evaluated to be roughly 30% of that measured in the trophoblast monoculture group. These results demonstrate the ability of the differentiated trophoblasts in our model to mediate glucose transport. It also indicates that the fetal endothelium may have a previously underappreciated effect on the rate of glucose transport by providing an additional barrier to facilitated diffusion.

Our analysis revealed that the percent rate of glucose transfer from the maternal to the fetal compartments was 34.8%. Importantly, this value lies within the range of glucose transfer rates measured in the perfused *ex vivo* human placenta (26.5–38.3%) (Fig. 5F)³³, which is regarded as the gold standard for the study of human placental transport¹¹. Co-culture of trophoblasts and villous endothelial cells in Transwell inserts yielded decreased transport at a reduced rate of 22.5% (Fig. 5F), failing to fall within the physiological range. These data serve to validate the physiological relevance of the placenta-on-a-chip system and to demonstrate its advantage over conventional *in vitro* techniques. The ability of our model to accurately approximate transport rates in the human placenta is particularly compelling, as animal data poorly predict glucose transfer across the human placental barrier due to interspecies differences in the molecular underpinnings of transport function. For example, glucose transport in the murine and rodent placenta is mediated predominantly by GLUT3, whereas GLUT1 is the primary glucose transporter in the human placental barrier³⁴. Hence, our data illustrate the feasibility of using the placenta-on-a-chip system as an alternative to existing animal models to simulate physiological glucose transport across the intact human placental barrier.

Conclusion

In this paper, we have presented a microphysiological system that reconstitutes the most essential unit of the human placenta. The design of this placenta-on-a-chip microdevice was inspired by the anatomy of the critical tissue-tissue interface that separates the maternal and fetal circulations in pregnancy. This system has the potential to serve as a powerful platform for *in vitro* investigation of the human placenta in that it allows for precise spatiotemporal

control of placental cells and their extracellular microenvironment to simulate, directly visualize, and quantitatively analyze human-specific placental structure and function.

These capabilities provide new opportunities to create cell-based screening assays for evaluating the placental transfer of pathogens, drugs, chemicals, and environmental toxins. Our approach may also enable the development of specialized *in vitro* models to mimic aberrant changes in the placental barrier under pathological conditions of pregnancy. These types of microengineered systems will greatly improve our ability to mechanistically examine and understand placental barrier dysfunction that has been identified as an important feature of various pregnancy-related diseases.

Our current model, however, still leaves room for improvement to realize its full potential. Although BeWo cells used in this study have proven effective for recapitulating key aspects of the maternal-fetal interface, questions remain regarding the validity of using these cancer-derived cells to represent the normal epithelium of the placental barrier. To address this limitation, subsequent iterations of this system should include culture of primary villous trophoblast cells for improved physiological relevance. Future studies should also investigate transport of other essential nutrients, such as amino acids and fatty acids, across the microengineered placental barrier to further validate the predictive capability of our model. The effects of fluid flow on microvilli formation and placental transport documented in this and previous studies warrant more rigorous investigation of how placental structure and function are regulated by hemodynamic forces and other microenvironmental cues. In addition, opportunities remain in reconstituting the dynamic structural remodeling and functional adaptation of the placental barrier during the progression of pregnancy.

Despite the limited scope of our study, however, the results described here clearly demonstrate the potential of microengineering technologies for the study of the human placenta. We believe that our work represents a critical step to pioneer innovative research approaches that may improve our fundamental understanding of human reproductive health and disease.

Acknowledgments

This research was supported by the March of Dimes Prematurity Research Center at the University of Pennsylvania and the National Institutes of Health (NIH) Director's New Innovator Award to D.H. (1DP2HL127720-01). We thank N.P. Iilsley and A. Schwartz for providing the BeWo b30 clone, R. Simmons for helpful discussions, V. Good and R. Leite for technical assistance in cell sourcing, and M. Helfrick for administrative support. We also thank M. Farrell, J. Seo, J. Cho, and M. Mondrinos for their valuable input.

References

1. Gude NM, Roberts CT, Kalionis B, King RG. Thrombosis Research. 2004; 114:397–407. [PubMed: 15507270]
2. Jones HN, Powell TL, Jansson T. Placenta. 2007; 28:763–774. [PubMed: 17582493]
3. Dilworth MR, Sibley CP. Placenta. 2013; 34:S34–S39. [PubMed: 23153501]
4. Carter AM. Placenta. 2007; 28(Suppl A):S41–7. [PubMed: 17196252]
5. Moffett A, Loke C. Nat Rev Immunol. 2006; 6:584–594. [PubMed: 16868549]
6. Lager S, Powell TL. Journal of Pregnancy. 2012; 2012:179827–14. [PubMed: 23304511]
7. Syme MR, Paxton JW, Keelan JA. Clin Pharmacokinet. 2004; 43:487–514. [PubMed: 15170365]

8. Sibley CP. The Journal of Physiology. 2009; 587:3431–3440. [PubMed: 19417095]
9. Spencer RN, Carr DJ, David AL. Prenat Diagn. 2014; 34:677–684. [PubMed: 24799349]
10. Sastry B. Adv Drug Deliv Rev. 1999; 38:17–39. [PubMed: 10837744]
11. Elad D, Levkovitz R, Jaffa AJ, Desoye G, Hod M. Traffic. 2013; 15:122–126. [PubMed: 24127903]
12. Miura S, Sato K, Kato-Negishi M, Teshima T, Takeuchi S. Nat Commun. 2015; 6:1–11.
13. Omarini D, Pistotti V, Bonati M. J Pharmacol Toxicol Methods. 1992; 28:61–66. [PubMed: 1482787]
14. Mathiesen L, Mose T, Mørck TJ, Nielsen JKS, Nielsen LK, Maroun LL, Dziegiel MH, Larsen LG, Knudsen LE. Reproductive Toxicology. 2010; 30:138–146. [PubMed: 20096346]
15. Wice B, Menton D, Geuze H, Schwartz AL. Exp Cell Res. 1990; 186:306–316. [PubMed: 2153559]
16. Su EJ, Cheng YH, Chatterton RT, Lin ZH, Yin P, Reierstad S, Innes J, Bulun SE. Biology of Reproduction. 2007; 77:517–525. [PubMed: 17538076]
17. Chueh BH, Huh D, Kyrtos CR, Houssin T, Futai N, Takayama S. Analytical chemistry. 2007; 79:3504–3508. [PubMed: 17388566]
18. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. Nat Meth. 2012; 9:676–682.
19. Lee JS, Romero R, Han YM, Kim HC, Kim CJ, Hong JS, Huh D. The Journal of Maternal-Fetal & Neonatal Medicine. 2015; 29:1046–1054. [PubMed: 26075842]
20. Amenta PS, Gay S, Vaheri A, Martinez-Hernandez A. Collagen and Related Research Clinical and Experimental. 1986; 6:125–152. [PubMed: 3731745]
21. Wang, Y. ... Series on Integrated Systems Physiology: from 2010.
22. Burton GJ, Woods AW, Jauniaux E, Kingdom JCP. Placenta. 2009; 30:473–482. [PubMed: 19375795]
23. Huppertz B, Borges M. Methods Mol Biol. 2008; 475:135–147. [PubMed: 18979242]
24. Gerbaud P, Pidoux G. Placenta. 2015; 36:S35–S42. [PubMed: 25564303]
25. Gerbaud P, Taskén K, Pidoux G. Front Pharmacol. 2015; 6:202. [PubMed: 26441659]
26. Liu F, Soares MJ, Audus KL. Am J Physiol. 1997; 273:C1596–604. [PubMed: 9374645]
27. Costa MA. Reprod Biomed Online. 2016; 32:14–43. [PubMed: 26615903]
28. Costa MA. Molecular and Cellular Endocrinology. 2016; 420:180–193. [PubMed: 26586208]
29. Tadokoro C, Yoshimoto Y, Sakata M, Fujimiya M, Kurachi H, Adachi E, Maeda T, Miyake A. Histol Histopathol. 1996; 11:673–681. [PubMed: 8839758]
30. Baumann MU, Deborde S, Illsley NP. Endocrine. 2002; 19:13–22. [PubMed: 12583599]
31. Jansson T, Wennergren M, Illsley NP. J Clin Endocrinol Metab. 1993; 77:1554–1562. [PubMed: 8263141]
32. Illsley NP. Placenta. 2000; 21:14–22. [PubMed: 10692246]
33. Brandes JM, Tavoloni N, Potter BJ, Sarkozi L, Shepard MD, Berk PD. The American Journal of Obstetrics & Gynecology. 1983; 146:800–806. [PubMed: 6869452]
34. Hahn T, Barth S, Weiss U, Mosgoeller W, Desoye G. FASEB J. 1998; 12:1221–1231. [PubMed: 9737725]

Figure 1.

A. Schematic of a human fetus and placenta within the uterine cavity. The placenta is anchored to the uterine wall and connected to the developing fetus via the umbilical cord. **B.** Cross-sectional view of the placenta illustrates the placental cotyledons. Each cotyledon consists of a stem chorionic villus and its branches. These villi are in direct contact with maternal blood in the intervillous space. **C.** The maternal intervillous space is separated from the lumen of the fetal capillary by the maternal-fetal interface composed of the syncytiotrophoblast, basal lamina, and villous endothelial cells. **D.** The three-dimensional microarchitecture of the placental barrier is reconstituted within our microengineered system. The placenta-on-a-chip consists of upper and lower microchannels separated by a thin, semipermeable membrane. Trophoblast cells are cultured in the upper microchannel on the apical side of the membrane, and villous endothelial cells are grown in the lower microchannel on the basal side of the membrane.

Figure 2.

A. Three-dimensional rendering of the microengineered placental barrier. The trophoblast and endothelial cell populations are stained for E-cadherin (red) and VE-cadherin (green), respectively. Nuclei are shown in blue. Scale bar: 30 μm . **B.** Cross-sectional view of the same microengineered barrier. Scale bar: 30 μm . **C.** The trophoblast cells form a continuous network of epithelial adherens junctions (E-cadherin, red). Nuclei are stained with DAPI (blue). Scale bar: 20 μm . **D.** The placental villous endothelium also displays intact cell-cell junctions (VE-cadherin, red). Green and blue show actin and nuclear staining, respectively. Scale bar: 20 μm . **E.** The trophoblast cells produce laminin (green) during culture in our microdevice. The image shows cells on Day 6. Scale bar: 30 μm . **F.** Cross-sectional view of laminin deposition. Laminin is stained green, and trophoblast nuclei are labeled in blue. The location of the cell culture membrane is indicated by the dotted line. Scale bar: 10 μm .

Figure 3.

A. Trophoblast cells cultured under dynamic flow conditions in the placenta-on-a-chip show widespread microvilli formation on the apical cell surface. Scale bar: 15 μm . **B.** Three-dimensional rendering of microvilli on the surface of BeWo cells in the placenta-on-a-chip. Scale bar: 20 μm . **C.** Microvilli in BeWo cells cultured in Transwell appear finer and significantly less abundant. Scale bar: 15 μm . **D.** Quantification of relative fluorescence of microvilli F-actin (green) in static and dynamic culture conditions. Cell nuclei were counterstained with DAPI (blue). The intensity of fluorescence generated by microvilli was increased substantially when the trophoblast cells were grown under flow conditions in the placenta-on-a-chip, as compared to static Transwell culture (****, $p < 0.0001$).

Figure 4.

Trophoblast syncytialization. A. In the human placenta, cytotrophoblast cells in early gestation go through a process of cell fusion to form a multinucleated syncytiotrophoblast. The resulting syncytium makes up the outer lining of the chorionic villi that comes into contact with maternal blood in the intervillous space. **B.** The BeWo cells cultured in our model exhibit high levels of E-cadherin (red) expression prior to forskolin treatment (left). Continuous epithelial exposure to forskolin over 72 hours induces trophoblasts to undergo cell-cell fusion, leading to nuclear aggregation and decreased expression of epithelial junctions. **C.** During forskolin-induced syncytialization, the permeability of the microengineered barrier to dextran decreases over time. * represents a statistically significant difference between 24 h and 72h ($p < 0.05$; a.u. represents arbitrary unit), as well as between 48h and 72h (**, $p < 0.01$). **D.** The BeWo cells produce increasing levels of β -human chorionic gonadotropin (β -HCG) over 120 hours of forskolin treatment (red bars). Hormone secretion by untreated cells is negligible.

Figure 5.
A. The syncytial epithelium in our model expresses high levels of GLUT1 transporters (red). Blue shows DAPI staining. Scale bar: 10 μ m. **B.** This cross-sectional view of the trophoblasts shows the asymmetric localization of GLUT1 transporters to the apical membrane. Scale bar: 5 μ m. **C.** Immunofluorescence staining of GLUT1 on the apical side is substantially stronger than that on the basal side (****, $p<0.0001$). a.u. represents arbitrary unit. **D.** A concentration gradient of glucose (green dots) is generated across the microengineered placental barrier to drive glucose transport from the maternal to fetal compartments. **E.** Percent increase in fetal glucose concentration for the bare membrane, trophoblast monoculture, and co-culture conditions (*, $p<0.05$). **F.** The percent rate of glucose transfer in the placenta-on-a-chip device is similar to that measured in the perfused *ex vivo* placenta. The *in vitro* placental barrier formed in Transwell fails to reconstitute the physiological glucose transport rates.