

Minireview

The role of extracellular matrix in normal and pathological pregnancy: Future applications of microphysiological systems in reproductive medicine

Blakely B O'Connor¹, Benjamin D Pope¹, Michael M Peters¹, Carrie Ris-Stalpers² and Kevin K Parker¹ 

¹Disease Biophysics Group, Wyss Institute for Biologically Inspired Engineering; Harvard John A. Paulson School of Engineering and Applied Sciences; Harvard University, Cambridge, MA 02138, USA; ²Department of Gynecology and Obstetrics, Academic Reproduction and Development, Amsterdam UMC, University of Amsterdam, Amsterdam 1105, The Netherlands

Corresponding author: Kevin K Parker. Email: kkparker@seas.harvard.edu

Impact statement

Extracellular matrix in the womb regulates the initiation, progression, and completion of a healthy pregnancy. The composition and physical properties of extracellular matrix in the uterus and at the maternal-fetal interface are remodeled at each gestational stage, while maladaptive matrix remodeling results in obstetric disease. As *in vitro* models of uterine and placental tissues, including micro- and milli-scale versions of these organs on chips, are developed to overcome the inherent limitations of studying human development *in vivo*, we can isolate the influence of cellular and extracellular components in healthy and pathological pregnancies. By understanding and recreating key aspects of the extracellular microenvironment at the maternal-fetal interface, we can engineer microphysiological systems to improve assisted reproduction, obstetric disease treatment, and prenatal drug safety.

Abstract

Remodeling of extracellular matrix in the womb facilitates the dramatic morphogenesis of maternal and placental tissues necessary to support fetal development. In addition to providing a scaffold to support tissue structure, extracellular matrix influences pregnancy outcomes by facilitating communication between cells and their microenvironment to regulate cellular adhesion, migration, and invasion. By reviewing the functions of extracellular matrix during key developmental milestones, including fertilization, embryo implantation, placental invasion, uterine growth, and labor, we illustrate the importance of extracellular matrix during healthy pregnancy and development. We also discuss how maladaptive matrix expression contributes to infertility and obstetric diseases such as implantation failure, preeclampsia, placenta accreta, and preterm birth. Recently, advances in engineering the biotic-abiotic interface have potentiated the development of microphysiological systems, known as organs-on-chips, to represent human physiological and pathophysiological conditions *in vitro*. These technologies may offer new opportunities to study human fertility and provide a more granular understanding of the role of adaptive and maladaptive remodeling of the extracellular matrix during pregnancy.

Keywords: Extracellular matrix, pregnancy, placenta, maternal-fetal interface, obstetric diseases

Experimental Biology and Medicine 2020; 245: 1163–1174. DOI: 10.1177/1535370220938741

Introduction

Before conception and throughout gestation, the womb integrates a multitude of signals to become a receptive environment capable of supporting embryonic growth and development. While much attention has been given to the role of hormones and growth factors during this transformation,^{1,2} cells in the womb also receive important biophysical and biochemical cues from their surrounding extracellular matrix. Extracellular matrix is composed of

fibrous proteins and viscous proteoglycans that provide a three-dimensional scaffold within which cells adhere.^{3,4} Once viewed as a passive substrate,⁵ the extracellular matrix is now recognized as a key regulator of embryonic development, organ growth, and disease progression.^{6,7} While it is well accepted that extracellular matrix regulates cell differentiation and morphogenesis in the embryo proper,⁸ the role of extracellular matrix in regulating the structural and functional changes that occur in the womb

and at the maternal–fetal interface is not yet fully appreciated.

During pregnancy, extracellular matrix maintains the structural integrity of the uterus, facilitates embryo adhesion, and regulates placental invasion into the endometrium to form the maternal–fetal interface. To mediate these processes, the extracellular matrix attaches to the cells via membrane bound adhesion molecules including integrins and selectins.^{9,10} Through these connections, cells sense the physical characteristics of their microenvironment, including substrate stiffness, pressure, shear, and stretch, and convert these mechanical cues into chemical and electrical signals that regulate cell structure and behavior.^{11–13} Throughout gestation, the extracellular matrix is dynamically remodeled, constantly deposited and degraded to support evolving tissue functions.¹⁴ The spatiotemporal regulation of this remodeling is critical; if matrix molecules in the womb are abnormally expressed, pathological conditions of reproduction and pregnancy often occur.^{15–19} For example, placenta accreta spectrum disorder—an obstetric disease characterized by unrestricted placental invasion—is thought to arise from maladaptive matrix signaling from fibrotic scar tissue in the womb. As a result, placental overgrowth poses a great risk of maternal hemorrhage, often necessitating surgical removal of the placenta and uterus upon delivery.²⁰ While this condition was once extremely rare, placenta accreta is becoming more prevalent as the rate of cesarean deliveries increases,²¹ and there are few options for more conservative treatments.²² Thus, there is a pressing need to better understand the interactions between cells and extracellular matrix at the maternal–fetal interface in the progression of healthy pregnancies to inform efforts to treat obstetric diseases.

In this review, we argue that extracellular matrix in the uterus and at the maternal–fetal interface is a primary determinant of pregnancy outcome. To support this claim, we review the functions of the extracellular matrix and matrix-mediated cellular processes in the context of key milestones that occur throughout gestation. We discuss how extracellular matrix in the womb facilitates cellular adhesion and migration and supports tissue growth mediated by mechanical signaling. We also review extracellular matrix composition and remodeling typical of a healthy pregnancy and discuss cases where aberrant expression or maladaptation of the remodeling process results in reproductive and obstetric complications such as implantation failure, preeclampsia, placenta accreta, and preterm labor. Finally, we discuss the recreation of native matrix in *in vitro* models of the female reproductive tract to study developmental toxicities and to facilitate the discovery of novel treatments for infertility and obstetric diseases.

Extracellular matrix facilitates fertilization and implantation

Even before conception, extracellular matrix in the reproductive tract lays the foundation for a successful pregnancy. In mammalian ovaries, oocytes mature in follicles surrounded by cumulus cells that produce a matrix cushion that encapsulates and protects the egg.²³ In response to an

ovulatory surge in luteinizing hormone, this cushion thickens to form the zona pellucida.²⁴ The zona pellucida is a transparent membrane which is composed of a hyaluronic acid and specialized glycoproteins that protects the egg during release from the ovary and facilitates fertilization.²⁵ If matrix molecules or their crosslinkers are disrupted or missing from the zona pellucida, oocyte release and fertilization are impaired, resulting in infertility or sterility.^{26–28} Matrix composition is affected by a number of factors, including disrupted endocrine function during the menstrual cycle and genetic abnormalities that affect matrix protein production.^{29,30}

A few days after an egg is fertilized, the resulting conceptus must implant into the endometrium, the inner lining of the uterus, to continue normal growth and development. Because both embryonic and endometrial signaling pathways must be synchronized, the timing of implantation is critical.^{31,32} If implantation occurs two or three days early or late, the risk of spontaneous abortion or pregnancy complications increases dramatically.^{33,34} Steroid hormones³⁵ and other signaling molecules are coordinated during the menstrual cycle to create a “window of receptivity”, a period when conditions are optimal for embryo implantation. The composition of extracellular matrix in the endometrium helps to define this window of receptivity (Table 1, Figure 1(a)), which is marked by a reduction of matrix molecules that prevent adhesion and an increase in integrin expression to facilitate attachment between the embryo and uterine wall.³⁶

Throughout the menstrual cycle, endometrial matrix composition shifts in response to cycling levels of estrogen and progesterone to balance factors that prevent or potentiate embryonic adhesion.⁶² Before ovulation, the surface of the endometrium is coated with anti-adhesion glycoproteins, such as mucin and Tenascin-C, that inhibit cell attachment until secretion is suppressed or cleared by cell surface proteases.^{40,63} After ovulation, the endometrium undergoes a process known as decidualization, where endometrial fibroblasts differentiate into secretory decidual cells that produce extracellular matrix and secrete factors that nourish the embryo.⁶⁴ As steroid hormone levels increase, decidual cells deposit matrix proteins such as laminin, entactin, fibronectin, collagen IV, and heparan sulfate in the glandular areas of the endometrium to promote embryonic attachment and invasion.^{42,65} Heparan sulfate, in particular, has been shown to facilitate implantation as it is recognized by selectins found on the outer layer of the blastocyst.⁶⁶ Decidual cells also synthesize more collagen V, which has high affinity for heparan sulfates and is thought to stabilize growth factors found in the extracellular microenvironment.^{67,68} Together, the reduction of anti-adhesive glycoproteins and the synthesis of proteoglycans which promote cell–matrix and matrix–matrix interactions help to create a receptive and adhesive environment for implantation.

Coinciding with the secretion of specific matrix molecules during decidualization, transiently expressed integrins in the endometrium also signify the window of receptivity for implantation. In decidual cells, integrin activation potentiates cytoskeletal remodeling and focal

Table 1. Distribution of select extracellular matrix proteins in the womb.

	Matrix	Location/structure	Adaptive remodeling	Refs	
Endometrium	Collagen I	Interstitial fibers	–	14,37	
	Collagen III	Interstitial fibers	↓ in decidualization	14	
	Collagen V	Interstitial fibers	↑ in decidualization	14	
	Fibrillin	Decidual cells	↑ in 1st & 2nd trimester	38	
	Fibulin	Decidual cells	↓ during 1st trimester	39	
	Elastin	–	–	38	
	Tenascin C	Stroma	↓ in secretory phase	40	
	Hyaluronan	Stroma and vessels	↓ in secretory phase	41	
	Fibronectin	Basement membrane & PCM	↑ in decidualization	42	
	Laminin	Basement membrane & PCM	↑ in decidualization	42	
	Collagen IV	Basement membrane & PCM	↑ in decidualization	42	
	Heparan Sulfate	Basement membrane & PCM	↑ in decidualization	42	
	Placenta	Collagen I	Stromal fibers & vessels	↓ in 3rd trimester	43–45
		Collagen III	Stromal thin beaded fibers	–	44
		Collagen V	Stromal thin filaments	–	44
Fibrillin		Villous stroma	↑ in 3rd trimester	43,46	
Fibulin		Villous/extravillous trophoblast	↑ 1st, 2nd,3rd trimester	47	
Elastin		Stroma and vessels	–	43,48	
Tenascin C		Stroma and vessels	–	43	
Hyaluronan		Stroma and vessels	–	49	
Fibronectin		Stroma & basement membrane	↑ in 3rd trimester	44	
Laminin		Stroma & basement membrane	↑ in 3rd trimester	43–45	
Collagen IV		Stroma & basement membrane	↑ 1st, 2nd,3rd trimester	43,44	
Heparan sulfate		Stroma & basement membrane	–	50	
Myometrium		Collagen I	Smooth muscle cells	↓ in 3rd trimester	51
		Collagen III	Smooth muscle cells & fibers	↑ in pregnancy	51
		Collagen V	Smooth muscle cells	–	51,52
	Fibrillin	Colocalized with elastin fibers	↑ in pregnancy	53	
	Fibulin	–	–	–	
	Elastin	Outer myometrium	↑ in pregnancy	54,55	
	Tenascin C	Basement membrane and smooth muscle	↑ in pregnancy	56,57	
	Hyaluronan	Cervical tissue	↑ at term & labor	58	
	Fibronectin	Connective tissue & PCM	↑ in late pregnancy	51,59	
	Laminin	Basement membrane	↑ in pregnancy	60	
	Collagen IV	Basement membrane	↑ in late pregnancy	53,59	
	Heparan sulfate	Cervix & PCM	↑ in labor	61	

PCM: pericellular matrix.

adhesion assembly that stabilizes embryo apposition and attachment to the uterine wall, which are important initial steps in implantation.⁶⁹ Evidence from animal models has shown that implantation efficiency is impaired when certain integrins are inhibited or lacking. For example, mice embryos lacking integrin β_1 , which is required for implantation, do not develop beyond the point when normal embryos implant.⁷⁰ Further, mice and rabbits that receive an intrauterine injection of integrin $\alpha_V\beta_3$ blocking antibody have fewer successful implantations than control animals injected with bovine serum albumin or antibodies for non-RGD peptides.^{71,72} Integrins α_V and β_3 are also thought to be involved in regulating human implantation, as both integrins are temporarily upregulated during decidualization and ovulation.¹⁰

Aberrant expression of these integrins has been observed in women with a history of infertility and endometriosis, an inflammatory disease of the inner lining of the uterus. In some cases, integrin β_3 expression is delayed, resulting in a window of endometrial receptivity that is not synchronized with the developmental stage of the embryo trying to implant.¹⁰ In other cases, integrin β_3

expression is reduced in endometrial lesions, which may contribute to the subfertility often seen in women with endometriosis.⁴⁰ Reduced sensitivity to progesterone in endometriosis further disrupts the balance of matrix deposition and degradation necessary for decidualization and implantation.^{73–75} Because of their role in implantation, integrins and adhesive extracellular matrix molecules are potential biomarkers to improve assisted reproductive technologies and fertility treatments. Taken together, these observations demonstrate that extracellular matrix composition and binding site availability contribute to endometrial receptivity and thereby regulate embryo implantation.

Extracellular matrix guides placental invasion

After a blastocyst apposes and attaches to inner lining of the uterus, extraembryonic cells that will form the placenta begin to migrate into and remodel the endometrium. These invasive cells, called extravillous trophoblasts, travel along fibrillar matrices in uterine tissue, degrading and depositing new matrix proteins as they anchor the embryo to the uterine wall (Figures 1(b) and 2(a) and (b)).⁷⁶ In concert

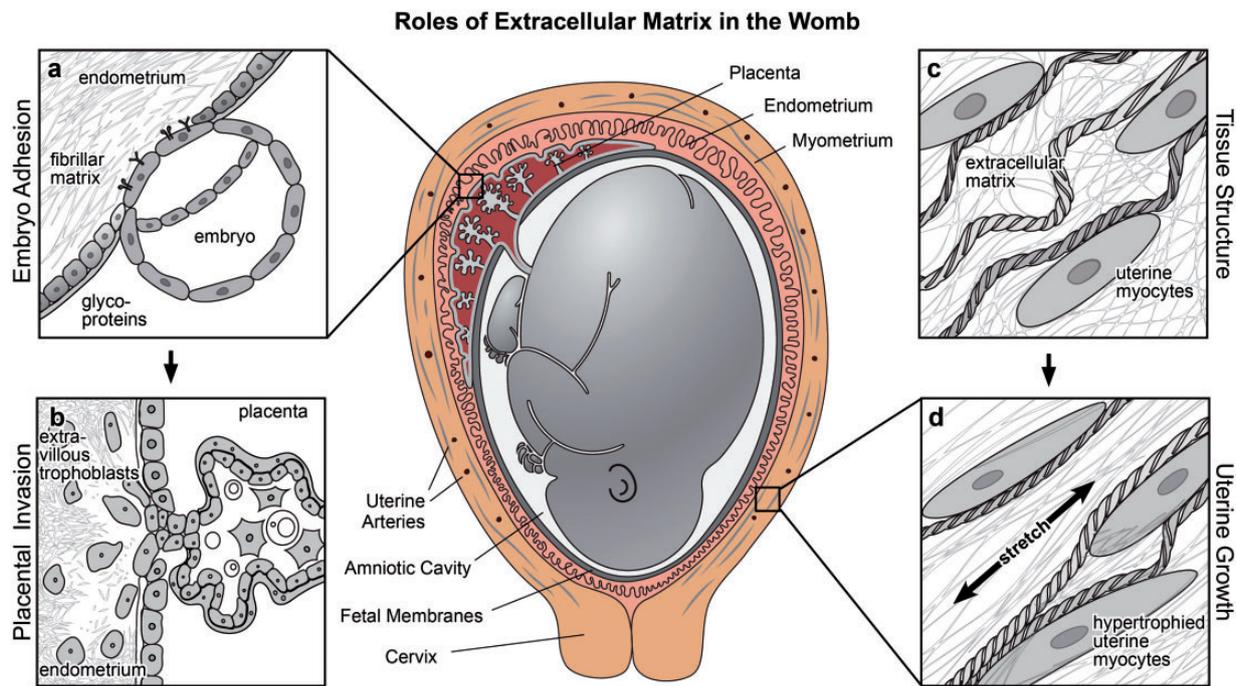


Figure 1. Roles of extracellular matrix in the womb. Extracellular matrix provides structural support in the womb and mediates important cellular processes including adhesion, migration, invasion, and mechanical signaling. (a) Extracellular matrix regulates embryonic adhesion by defining a window of receptivity for implantation. (b) Extracellular matrix guides placental invasion and is dynamically remodeled when extravillous trophoblasts invade the endometrium. (c–d) Extracellular matrix forms a fibrillar scaffold to reinforce tissue strength and extensibility, propagating mechanical signals that induce stretch-mediated hypertrophy and uterine contractile activation at the onset of labor.

with decidual cells and maternal immune cells, extravillous trophoblasts use enzymes such as matrix metalloproteinases (MMPs) to degrade the native matrix and remodel uterine spiral arteries, ensuring sufficient blood flow from the maternal to the fetal compartment.^{43,79,80} As the placenta develops, it secretes hormones that further stimulate matrix deposition and protease secretion in both placental trophoblasts and maternal endometrial cells.⁸¹ Ultimately, the degree of tissue remodeling and vascularization is regulated by a delicate balance of MMP expression, enzymatic activation, and abundance of tissue inhibitors of metalloproteinases (TIMPs),⁸² which are controlled by a combination of steroid hormones as well as genetic and epigenetic programming.^{83,84}

The spatiotemporal regulation of integrins and MMP expression in the placenta and endometrium is critical for proper trophoblast invasion. In early pregnancy when the placenta is most invasive, these extravillous trophoblasts secrete mostly MMP-2, a gelatinase that targets collagens and basement membrane proteins near the surface of the endometrium.⁸⁵ By the end of the first trimester, these cells also secrete MMP-9 to degrade interstitial collagens deeper in the uterus.⁸⁶ Expression of integrins and MMPs also varies among trophoblasts derived from different regions of the placenta.^{87,88} To illustrate this differential expression, we parsed integrin and MMP expression data from a genome-wide transcription study of sorted trophoblast cells isolated from first trimester placentas (Figure 2(c) to (e)).⁷⁷ Here, we see that extravillous trophoblasts express more fibronectin-binding integrins and basement membrane degrading proteases than other cell types in the

placenta, which is necessary to penetrate endometrial basal lamina. If MMPs or their target matrix proteins are missing or are overabundant, the placenta cannot invade properly, resulting in obstetric syndromes that increase risks of both maternal and fetal morbidity.^{18,89–91}

Insufficient placental invasion is thought to contribute to preeclampsia, an obstetric disease characterized by the onset of hypertension, proteinuria, and maternal inflammation after the 20th week of pregnancy.⁹² During preeclampsia, shallow trophoblast invasion and incomplete remodeling of the spiral arteries result in reduced placental volume and increased blood pressure that often lead to fetal growth restriction.^{93,94} Aberrant integrin and MMP expression at the maternal fetal interface are one explanation for this restricted invasion. For example, placental cells isolated from patients with preeclampsia express lower levels of integrins and MMPs that bind to and degrade laminin, collagen, and fibronectin compared to cells from normal pregnancies.^{19,90} Genetic deficiency in MMP-9—a proteinase that cleaves collagens that are abundant in the womb—impairs trophoblast invasion and decidualization in mice, producing a preeclamptic phenotype with intrauterine growth restriction, hypertension, and proteinuria.⁸⁹ Interestingly, not all matrix degrading enzymes are reduced in preeclampsia. Patients with preeclampsia have elevated levels of extracellular matrix metalloproteinase inducers circulating in their bloodstream, while their placentae express a broader range of MMPs, suggesting a possible compensatory mechanism to overcome insufficient invasion and vascular remodeling.^{95,96} However, matrix fragments generated by excessive proteases can cause

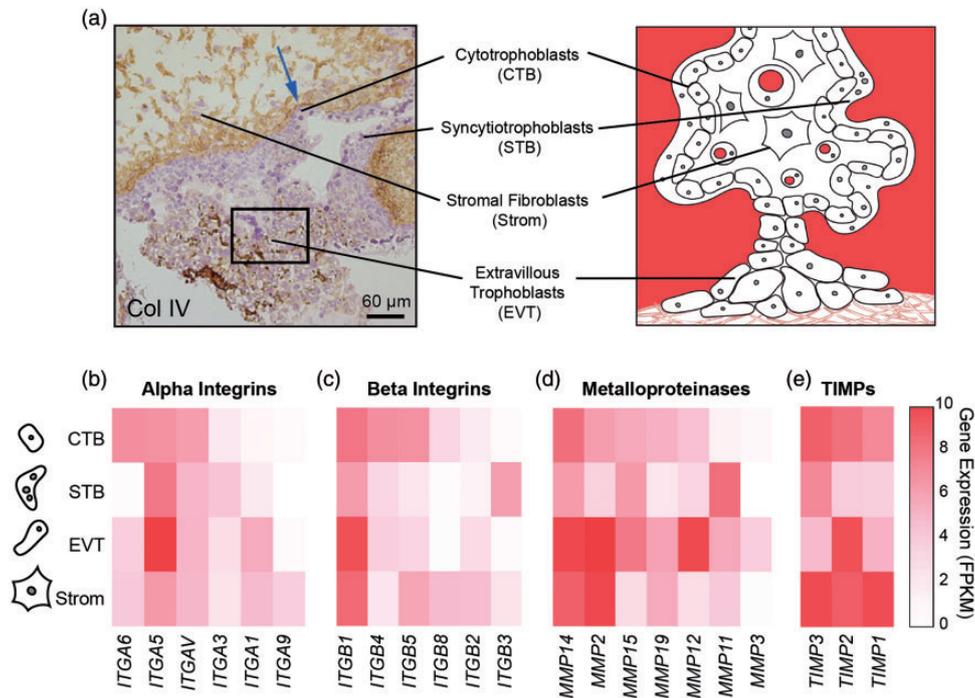


Figure 2. Integrin and matrix metalloproteinase gene expression in placental cell types. (a) First trimester placental villi stained for Collagen IV and counterstained with hematoxylin and simplified illustration (right). Arrow indicates basement membrane staining and box represents globular Col IV in extravillous column (reprinted with permission from Oefner *et al.*⁷⁸). (b–e) Heatmaps of alpha (panel b) and beta (panel c) integrin and matrix metalloproteinase (panel d) and tissue inhibitors of matrix metalloproteinases (panel e) transcription levels in placental cells (RNA sequencing data were extracted from a previously published dataset⁷⁷) For clarity, only genes with more than 3.6 fragments per kilobase per million mapped reads (FPKM) in at least one cell type are included.

inflammation by triggering additional immune cell activation and recruitment.^{97,98} Therefore, maladaptive proteinase expression and aberrant remodeling of the extracellular matrix at the maternal–fetal interface contribute to both insufficient invasion at the onset of preeclampsia and overactive maternal immune response seen in the later stages of the disease.⁹⁹

Maladaptive matrix proteinase expression in the womb is also associated with excessive trophoblast invasion as seen in placenta accreta spectrum disorder. Instead of adhering to and remodeling maternal tissues to connect to the maternal circulation, these placentae penetrate the deep muscular layers of the uterus and sometimes even enter the abdominal cavity, resulting in a cancer-like growth. Overly invasive placentas show increased expression of MMPs and lower levels of enzyme degradation inhibitors.¹⁰⁰ Specifically, extravillous trophoblasts from patients with placenta accreta spectrum disorder retain the highly invasive phenotype of first trimester trophoblasts well into the third trimester of gestation.¹⁰¹ Although the disease is characterized by improper trophoblast invasion, placenta accreta spectrum disorder is not considered to be a disease of placental origin. On the contrary, risk factors for placenta accreta spectrum include past uterine surgeries such as cesarean section and uterine fibroid removal, suggesting disruption in maternal endometrial tissue architecture is a contributing factor in the disease.¹⁰² Disorganized and fibrotic extracellular matrix in uterine scar tissues is weaker than that of healthy tissue and presents points of entry for excessive invasion.¹⁰³

To improve uterine tissue integrity and reduce scar formation after cesarean deliveries, matrix-based scaffolds have emerged as an approach to facilitate wound healing in the womb.^{104–106} By recapitulating the healthy matrix that supports cellular migration and integration, tissue-engineered wound healing approaches may reduce scar formation in the uterus after cesarean delivery, thereby ameliorating the risk of developing placenta accreta spectrum disorder in subsequent pregnancies. Altogether, these results emphasize the importance of uterine matrix integrity in regulating trophoblast invasion and underscore the potential for novel approaches to improve scarless wound healing after cesarean delivery.

Extracellular matrix remodeling during uterine growth and labor

In the uterus, layers of smooth muscle and extracellular matrix wrap circumferentially and longitudinally around the uterine cavity.¹⁰⁷ Within these layers, the concentration and structure of extracellular matrix change throughout pregnancy to enable dramatic growth and expansion without increasing intrauterine pressure.⁵⁴ Strikingly, the matrix surrounding the outer layer of the uterus possesses not only the strength to withstand forces generated during labor but also the flexibility to expand to a volume almost 500 times its original size.¹⁰⁷ Matrix strength derives from elaboration by uterine cells that remodel the extracellular fibrils that reinforce tissues (Figure 1(c)). For example, small collagen fibrils are degraded and reassembled

within the uterus to form larger collagen bundles that increase tissue strength.^{37,51,60} Thicker elastic fibers are similarly formed by increased expression of elastin and fibrillin to accommodate tissue strain.⁵⁵ Further, basement membrane proteins are upregulated near the end of term, with increased deposition near hypertrophied muscle cells to improve cellular integration within the tissue.⁵⁹ Interestingly, this increase in extracellular matrix deposition is only temporary. Towards the end of pregnancy, the body begins to degrade the additional matrix and soften uterine tissue,¹⁰⁸ thereby increasing connectivity between uterine muscle cells to achieve synchronized contractions for labor.^{109,110}

The dynamic deposition and remodeling of extracellular matrix proteins are important for maintaining a healthy pregnancy and enabling a safe labor and delivery. If extracellular matrix composition is disrupted or lacking, the structural integrity of the uterus is impaired.¹¹¹ For example, women with Ehlers-Danlos syndrome, a family of diseases that disrupts connective tissues in the body, are more likely to experience obstetric complications including premature rupture of membranes and preterm birth.¹¹² Pregnant patients with severe forms Ehlers-Danlos syndrome also face an increased risk of uterine rupture and maternal mortality.¹¹³ The hypothesized mechanism of premature membrane rupture is a defect in collagen synthesis, which weakens the chorionic membrane of the affected fetus.¹¹⁴ Aside from genetic defects that impair collagen synthesis, uterine integrity is also compromised in women with who have had a previous cesarean delivery.^{115,116} After a cesarean section, fibrotic scar tissue with increased but disrupted collagen at the site of the incision leads to uterine tissue weakness that is more prone to rupture in subsequent pregnancies.¹¹⁷⁻¹¹⁹ While cesarean scar niche formation directly effects myometrial tissue mechanical properties, including elasticity, extensibility, and strength, the close proximity of uterine tissues with the chorionic and amniotic membrane can influence their mechanical properties as well.^{111,119} Together, these clinical observations suggest that extracellular matrix plays an important role in maintaining uterine integrity throughout pregnancy and delivery.

Throughout gestation, uterine growth is mediated by a combination of steroid hormone dynamics and mechanical stretch. In early pregnancy, uterine myocyte hyperplasia and matrix deposition are triggered by hormones released by the ovaries and the nascent placenta. Specifically, both estrogen and progesterone stimulate increased extracellular matrix production and remodeling in the womb that increase tissue strength and extensibility.¹¹¹ As pregnancy progresses, mechanical stretch plays a more significant role by modulating uterine response to hormonal cues and transmitting physical signals that modulate gene expression and cellular function. Importantly, uterine stretch caused by the growing fetus is necessary for the normal induction of matrix protein expression.⁹⁸ Even in the absence of steroid hormones, stretch alone can induce uterine growth and protein production; for example, when a balloon was used to inflate the uterus of ovariectomized rabbits, uterine cell mass and protein expression increased

as if the animals were pregnant.³⁷ Mechanical stretch also regulates uterine muscle hypertrophy that enables uterine contractility in the later stages of pregnancy and labor (Figure 1(d)).^{120,121} After first proliferating in early pregnancy, uterine myocytes then grow and expand by hypertrophy, simultaneously becoming more excitable and contractile in preparation of labor. Importantly, these muscle cells do not contract synchronously until active labor begins.¹²² For labor to begin, the extracellular matrix that once reinforced tissue strength must be degraded to enable electromechanical coupling between uterine myocytes. Like extracellular matrix production, matrix degradation is also regulated by a combination of hormonal and mechanical signals. Injections of progesterone in the final days of pregnancy can postpone matrix degradation and the onset of labor in rats.⁵⁹ The activity of matrix degradation enzymes is also sensitive to stretch,¹²³ further contributing to the mechanosensitive matrix remodeling and tissue softening that is necessary for labor to begin.

Due to its regulation of labor onset in normal pregnancies, mechanical signaling has been investigated as a potential cause of preterm labor and preterm birth. Preterm birth—or delivery that occurs before 37 weeks gestation—affects between 5% and 18% of pregnancies and is the leading cause of infant mortality and morbidity.^{85,124} Of these preterm deliveries, approximately two-thirds are due to preterm labor and premature rupture of fetal membranes, and one-third represent medical interventions to treat preeclampsia or fetal growth restriction.¹²⁵ Mechanical signaling and maladaptive remodeling of extracellular matrix in the myometrium or fetal membranes contribute to certain cases of spontaneous preterm labor. For example, twin or multiple gestation pregnancies are more likely to result in preterm birth, in part because the uterus stretches and remodels more rapidly than in singleton pregnancies.¹²⁶⁻¹²⁸ Pregnant patients with a history of endometriosis also face increased risk of premature rupture of membranes or preterm birth.¹²⁹ Just as reduced progesterone sensitivity in early pregnancy impairs decidualization and implantation, aberrant expression of MMPs and TIMPs near term can cause precocious softening of the cervix and amnion.¹¹¹ Further, uterine collagenase and elastase expression in both term and preterm labor are also upregulated in response to stretch and can induce contraction of uterine smooth muscle cells necessary for labor.¹¹⁰ In sum, matrix degrading enzymes are critical for the completion of normal labor and represent possible pharmacological targets for preventing or postponing preterm labor.

Recapitulating the maternal–fetal interface *in vitro*

While extracellular matrix performs key roles in pregnancy, it remains difficult to fully elucidate how matrix aberrations lead to obstetric disease and how to correct them to improve pregnancy outcomes. Difficulties stem from the limitations of clinical trials in pregnant women and species variation in reproductive anatomy and gestation in animal models.¹³⁰ Further, the multivariate interactions that influence extracellular matrix remodeling, including

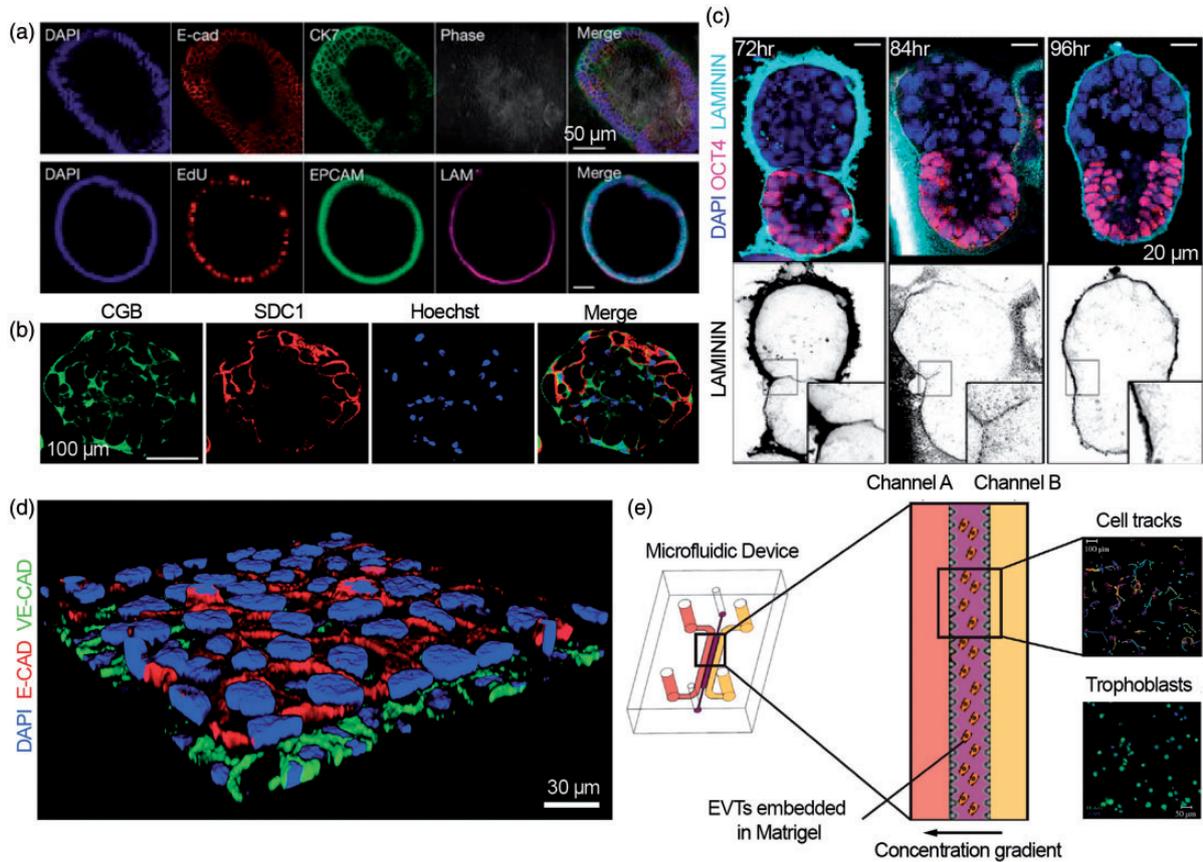


Figure 3. *In vitro* models of the maternal–fetal interface and embryogenesis. (a) Primary endometrial cells suspended in matrigel self-organize into organoids in chemically defined medium (adapted from Turco *et al.*¹³¹). (b) Human trophoblast stem cells form three-dimensional syncytiotrophoblast aggregates that produced human chorionic gonadotropin (CGB) and syndecan-1 (SDC) a trophoblast marker (adapted from Okae *et al.*⁷⁷). (c) Mouse embryonic stem cells and extra-embryonic trophoblast stem cells create embryo-like structures in three-dimensional matrigel scaffolds (adapted from Harrison *et al.*¹³⁵). (d) Microphysiological model of the placental barrier composed of trophoblasts and endothelial cells on a fibronectin-coated membrane, stained with E-cadherin and VE-cadherin, respectively (adapted from Blundell *et al.*¹⁵¹). (e) Microfluidic model of trophoblast invasion, with primary extravillous trophoblasts (EVTs) embedded in Matrigel between two channels that create a chemokine gradient (adapted from Abbas *et al.*¹⁵³ under Creative Commons CC BY 4.0).

hormonal and immune signaling, are difficult to control and manipulate *in vivo*. Therefore, there is a need for alternative approaches to study the contributions of extracellular matrix remodeling in healthy and pathological pregnancies.

Addressing the limitations of *in vivo* experiments, complex reproductive tissue structures have already been generated from stem cells and primary cell cultures *in vitro*. For example, human endometrial and decidual cells have been used to model maternal tissues (Figure 3(a)),¹³¹ while cytotrophoblast aggregates have been used to recapitulate placental development (Figure 3(b)).^{77,132} While *in vitro* fertilization has enabled the study of early embryogenesis for many years,^{133,134} later stages of embryonic development have recently been modeled *in vitro* by combining mouse embryonic and trophoblast stem cells (Figure 3(c)).¹³⁵ Thus far, these organoid models have relied on tissue self-assembly within undefined mixtures of animal-derived matrices.

In conjunction with advances in stem cell biology, microphysiological systems, or organs on chips,^{136–140} have emerged as human tissue models with the potential to control and study cell–cell and cell–matrix interactions.^{141–146}

When applied to the female reproductive tract,¹⁴⁷ endometrial,¹⁴⁸ placental,¹⁵¹ and fetal membrane¹⁴⁹ models have provided insight into reproductive diseases,¹⁵⁰ prenatal drug safety,¹⁵² and basic developmental biology.¹³² Featuring multiple cell types and continuous perfusion, these systems can quantify cellular migration, invasion, and barrier function in response to dynamic stimuli (Figure 3(d) and (e)).^{151,153–156}

By recreating aspects of native extracellular matrix, microphysiological systems are ideal for probing the contributions of matrix cues on cellular behavior at the maternal–fetal interface.^{157–159} Using native tissue composition as a guide,¹⁶⁰ experiments in microphysiological systems have demonstrated that matrix proteins including fibronectin, laminin, and collagen regulate placental and amniotic cell adhesion, morphology, differentiation, and function.^{159,161,162} In addition to structural proteins, matrix-binding and matrix-degrading proteins can be spatially and temporally controlled *in vitro* to study their effects on cell migration, invasion, and hormone secretion.^{163–165} Beyond protein composition, matrix stiffness can also be tuned,¹⁶⁶ allowing co-culture of endometrial epithelial and stromal cells that require different local

microenvironments,¹⁶⁷ and generation of three dimensional models to study endometrial vascularization and trophoblast invasion.¹⁶⁸ These studies demonstrate that microphysiological systems can be used prior to *in vivo* testing to elucidate the functions of extracellular matrix at the maternal–fetal interface.

Even with advances in stem cells and tissue engineering, microphysiological systems of the maternal–fetal interface have yet to realize their full potential to advance reproductive medicine. To realize this potential, microphysiological systems should incorporate and continue to study the effects of extracellular cues at the maternal–fetal interface. To inform and improve the design and accuracy of these systems, structural and mechanical properties of the womb microenvironment should also be further characterized throughout development and disease.¹⁶⁹ As microphysiological systems move towards exclusively human tissues and matrix, minimum essential elements of the extracellular microenvironment should be identified to balance precision with scalability for clinical applications. By engineering the biotic–abiotic interface of stem cell-derived tissues to match their native counterparts, microphysiological systems of the maternal–fetal interface will provide a platform for personalized reproductive medicine.

Conclusions

The extracellular matrix coordinates cellular differentiation and morphogenesis in the womb. Though often overlooked, maladaptive extracellular matrix composition and remodeling in womb are contributing factors for obstetric complications that threaten both maternal and fetal health. To close the gap in our understanding and appreciation of the role of extracellular matrix in pregnancy, we propose to use microphysiological systems to further probe the relationship between maternal and fetal cells and their physical microenvironment. By incorporating physiologically relevant cues from the extracellular matrix, these *in vitro* models can be used to improve reproductive technologies and identify therapeutic interventions for obstetric diseases. We envision the knowledge gained from these systems can be used to engineer more receptive environments for assisted reproduction, to develop wound dressings to scarlessly heal surgical incisions, and to better predict prenatal drug safety, thereby improving women's reproductive health.

Authors' contributions: All authors contributed to the design, writing, review, and editing of the manuscript.

ACKNOWLEDGMENTS

The authors thank Michael Rosnach for schematic design and illustrations, as well as Dan Drennan, Luke MacQueen, Dan Needleman, Suzanne Smith, and John Zimmerman for constructive feedback on the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the John A. Paulson School of Engineering and Applied Sciences at Harvard University; the Wyss Institute for Biologically Inspired Engineering at Harvard University; the Harvard Materials Research Science and Engineering Center [grant number DMR-1420570]; and the Eunice Kennedy Shriver National Institute of Child Health and Human Development [award number F31HD095594]. B. D. Pope is a Good Ventures Fellow of the Life Sciences Research Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ORCID iD

Kevin K Parker  <https://orcid.org/0000-0002-5968-7535>

REFERENCES

1. Napso T, Yong HEJ, Lopez-Tello J, Sferruzzi-Perri AN. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. *Front Physiol* 2018;**9**:1091
2. Knöfler M. Critical growth factors and signalling pathways controlling human trophoblast invasion. *Int J Dev Biol* 2010;**54**:269–80
3. Muiznieks LD, Keeley FW. Molecular assembly and mechanical properties of the extracellular matrix: a fibrous protein perspective. *Biochim Biophys Acta* 2013;**1832**:866–75
4. Yanagishita M. Function of proteoglycans in the extracellular matrix. *Acta Pathol Jpn* 1993;**43**:283–93
5. Piez KA. History of extracellular matrix: a personal view. *Matrix Biol* 1997;**16**:85–92
6. Vining KH, Mooney DJ. Mechanical forces direct stem cell behaviour in development and regeneration. *Nat Rev Mol Cell Biol* 2017;**18**:728–42
7. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014;**15**:786–801
8. Mammoto T, Ingber DE. Mechanical control of tissue and organ development. *Development* 2010;**137**:1407–20
9. Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 1993;**260**:1124–7
10. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *J Clin Invest* 1992;**90**:188–95
11. Pelham RJ, Wang Y-L. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci U S A* 1997;**94**:13661–5
12. Mouw JK, Yui Y, Damiano L, Bainer R, Lakins J, Acerbi I, Ou G, Wijekoon A, Levental K, Gilbert P, Hwang E, Chen Y, Weaver V. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. *Nat Med* 2014;**20**:360–7
13. Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat Rev Mol Cell Biol* 2009;**10**:75–82
14. Aplin JD, Jones C. Extracellular matrix in endometrium and decidua. In: Genbaev O, Kloppper A, Beaconsfield R. (eds) *Placenta as a model and a source*. Boston, MA: Springer US, 1989, pp. 115–28

15. Sahoo SS, Quah MY, Nielsen S, Atkins J, Au GG, Cairns MJ, Nahar P, Lombard JM, Tanwar PS. Inhibition of extracellular matrix mediated TGF- β ; signalling suppresses endometrial cancer metastasis. *Oncotarget* 2017;**8**:71400-17
16. Zbucka M, Miltyk W, Bielawski T, Surazynski A, Palka J, Wolczynski S. Mechanism of collagen biosynthesis up-regulation in cultured leiomyoma cells. *Folia Histochem Cytobiol* 2007;**45**:S181-5
17. Malik M, Britten J, Segars J, Catherino WH. Leiomyoma cells in 3-dimensional cultures demonstrate an attenuated response to fasudil, a rho-kinase inhibitor, when compared to 2-dimensional cultures. *Reprod Sci* 2014;**21**:1126-38
18. Brosens I, Pijnenborg R, Vercruyse L, Romero R. The 'great obstetrical syndromes' are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193-201
19. Kim M-S, Yu JH, Lee M-Y, Kim AL, Jo MH, Kim MG, Cho S-R, Kim Y-H. Differential expression of extracellular matrix and adhesion molecules in fetal-origin amniotic epithelial cells of preeclamptic pregnancy. *PLoS One* 2016;**11**:e0156038
20. Timor-Tritsch IE, Monteagudo A, Cali G, Palacios-Jaraquemada JM, Maymon R, Arslan AA, Patil N, Popiolek D, Mittal KR. Cesarean scar pregnancy and early placenta accreta share common histology. *Ultrasound Obstet Gynecol* 2014;**43**:383-95
21. Jauniaux E, Silver RM, Matsubara S. The new world of placenta accreta spectrum disorders. *Int J Gynaecol Obstet* 2018;**140**:259-60
22. Fox KA, Shamshirsaz AA, Carusi D, Secord AA, Lee P, Turan OM, Huls C, Abuhamad A, Simhan H, Barton J, Wright J, Silver R, Belfort MA. Conservative management of morbidly adherent placenta: expert review. *Am J Obstet Gynecol* 2015;**213**:755-60
23. Russell DL, Salustri A. Extracellular matrix of the cumulus-oocyte complex. *Semin Reprod Med* 2006;**24**:217-27
24. Familiari G, Heyn R, Relucenti M, Sathananthan H. Structural changes of the zona pellucida during fertilization and embryo development. *Front Biosci* 2008;**13**:6730-51
25. Salustri A, Yanagishita M, Hascall VC. Synthesis and accumulation of hyaluronic acid and proteoglycans in the mouse cumulus cell-oocyte complex during follicle-stimulating hormone-induced mucification. *J Biol Chem* 1989;**264**:13840-7
26. Irving-Rodgers HF, Rodgers RJ. Extracellular matrix in ovarian follicular development and disease. *Cell Tissue Res* 2005;**322**:89-98
27. Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, Kawamura K, Suzuki T, Kimata K. Defect in SHAP-hyaluronan complex causes severe female infertility. A study by inactivation of the bikunin gene in mice. *J Biol Chem* 2001;**276**:7693-6
28. Ishizuka Y, Takeo T, Nakao S, Yoshimoto H, Hirose Y, Sakai Y, Horikoshi Y, Takeuji S, Tsuchiyama S, Nakagata N. Prolonged exposure to hyaluronidase decreases the fertilization and development rates of fresh and cryopreserved mouse oocytes. *J Reprod Dev* 2014;**60**:454-9
29. Hassani F, Oryan S, Eftekhari-Yazdi P, Bazrgar M, Moini A, Nasiri N, Sharifi-Zarchi A. Downregulation of extracellular matrix and cell adhesion molecules in cumulus cells of infertile polycystic ovary syndrome women with and without insulin resistance. *Cell J* 2019;**21**:35-42
30. Huang H-L, Lv C, Zhao Y-C, Li W, He X-M, Li P, Sha A-G, Tian X, Papasian CJ, Deng H-W, Lu G-X, Xiao H-M. Mutant ZP1 in familial infertility. *N Engl J Med* 2014;**370**:1220-6
31. Sharkey AM, Smith SK. The endometrium as a cause of implantation failure. *Best Pract Res Clin Obstet Gynaecol* 2003;**17**:289-307
32. Koot YEM, Teklenburg G, Salker MS, Brosens JJ, Macklon NS. Molecular aspects of implantation failure. *Biochim Biophys Acta* 2012;**1822**:1943-50
33. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* 1999;**340**:1796-9
34. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. *N Engl J Med* 1988;**319**:189-94
35. Mancini V, Pensabene V. Organs-on-chip models of the female reproductive system. *Bioengineering* 2019;**6**:pii: E103
36. Aplin JD. Adhesion molecules in implantation. *Rev Reprod* 1997;**2**:84-93
37. Zorn TT, Bevilacqua EAF, Abrahamsohn PA. Collagen remodeling during decidualization in the mouse. *Cell Tissue Res* 1986;**244**:443-8
38. Fleming S, Bell SC. Localization of fibrillin-1 in human endometrium and decidua during the menstrual cycle and pregnancy. *Hum Reprod* 1997;**12**:2051-2056
39. Winship A, Cuman C, Rainczuk K, Dimitriadis E. Fibulin-5 is upregulated in decidualized human endometrial stromal cells and promotes primary human extravillous trophoblast outgrowth. *Placenta* 2015;**36**:1405-11
40. Klemmt PAB, Carver JG, Koninckx P, McVeigh EJ, Mardon HJ. Endometrial cells from women with endometriosis have increased adhesion and proliferative capacity in response to extracellular matrix components: towards a mechanistic model for endometriosis progression. *Hum Reprod* 2007;**22**:3139-47
41. Salamonsen L, Shuster S, Stern R. Distribution of hyaluronan in human endometrium across the menstrual cycle. *Cell Tissue Res* 2001;**306**:335-340
42. Wewer UM, Faber M, Liotta LA, Albrechtsen R. Immunochemical and ultrastructural assessment of the nature of the pericellular basement membrane of human decidual cells. *Lab Invest* 1985;**53**:624-33
43. Chen C, Aplin J. Placental extracellular matrix: gene expression, deposition by placental fibroblasts and the effect of oxygen. *Placenta* 2003;**24**:316-25
44. Amenta PS, Gay S, Vaheri A, Martinez-Hernandez A. The extracellular matrix is an integrated unit: ultrastructural localization of collagen types I, III, IV, V, VI. *Coll Relat Res* 1986;**6**:125-152
45. Tenney B. A study of the collagen of the placenta. *Am J Obstet Gynecol* 1935;**29**:819-825
46. Jacobson SL, Kimberly D, Thornburg K, Maslen C. Localization of fibrillin-1 in the human term placenta. *J Soc Gynecol Investig* 2:686-90
47. Gauster M, Berghold VM, Moser G, Orendi K, Siwetz M, Huppertz B. Fibulin-5 expression in the human placenta. *Histochem Cell Biol* 2011;**135**:203-213
48. Baran Ö, Tuncer M, Nergiz Y, Akkuş M, Erdemoğlu M, Büyükbayram H. An increase of elastic tissue fibers in blood vessel walls of placental stem villi and differences in the thickness of blood vessel walls in third trimester pre-eclampsia pregnancies. *Open Med* 2010;**5**:227-234
49. Matejevic D, Neudeck H, Graf R, Müller T, Dietl J. Localization of hyaluronan with a hyaluronan-specific hyaluronic acid binding protein in the placenta in pre-eclampsia. *Gynecol Obstet Invest* 2001;**52**:257-9
50. Chen C-P, Liu S-H, Lee M-Y, Chen Y-Y. Heparan sulfate proteoglycans in the basement membranes of the human placenta and decidua. *Placenta* 2008;**29**:309-316
51. Pulkkinen MO, Lehto M, Jalkanen M, Nääntö-Salonen K. Collagen types and fibronectin in the uterine muscle of normal and hypertensive pregnant patients. *Am J Obstet Gynecol* 1984;**149**:711-7
52. Abedin MZ, Ayad S, Weiss JB. Type V collagen: the presence of appreciable amounts of $\alpha 3(V)$ chain in uterus. *Biochem Biophys Res Commun* 1981;**102**:1237-1245
53. Rehman KS, Yin S, Mayhew BA, Word RA, Rainey WE. Human myometrial adaptation to pregnancy: cDNA microarray gene expression profiling of myometrium from non-pregnant and pregnant women. *Mol Hum Reprod* 2003;**9**:681-700
54. Metaxa-Mariatou V, McGavigan CJ, Robertson K, Stewart C, Cameron IT, Campbell S. Elastin distribution in the myometrial and vascular smooth muscle of the human uterus. *Mol Hum Reprod* 2002;**8**:559-65
55. Leppert PC, Yu SY. Three-dimensional structures of uterine elastic fibers: scanning electron microscopic studies. *Connect Tissue Res* 1991;**27**:15-31
56. Jamaluddin MFB, Nagendra PB, Nahar P, Oldmeadow C, Tanwar PS. Proteomic analysis identifies tenascin-C expression is upregulated in uterine fibroids. *Reprod Sci* 2019;**26**:476-486
57. Kida H, Taga M, Minaguchi H, Hanazono M, Ohashi T, Sakakura T, Kusakabe M. The change in tenascin expression in mouse uterus during early pregnancy. *J Assist Reprod Genet* 1997;**14**:44-50

58. El Maradny E, Kanayama N. The role of hyaluronic acid as a mediator and regulator of cervical ripening. *Hum Reprod* 1997;**12**:1080-1088
59. Shynlova O, Mitchell JA, Tsampalieros A, Langille BL, Lye SJ. Progesterone and gravidity differentially regulate expression of extracellular matrix components in the pregnant rat myometrium. *Biol Reprod* 2004;**70**:986-92
60. Nishinaka K, Fukuda Y. Changes in extracellular matrix materials in the uterine myometrium of rats during pregnancy and postparturition. *Acta Pathol Jpn* 1991;**41**:122-32
61. Hjelm AM, Barchan K, Malmström A, Ekman-Ordeberg GE. Changes of the uterine proteoglycan distribution at term pregnancy and during labour. *Eur J Obstet Gynecol Reprod Biol* 2002;**100**:146-151
62. Aplin JD, Ruane PT. Embryo-epithelium interactions during implantation at a glance. *J Cell Sci* 2017;**130**:15-22
63. Constantinou PE, Morgado M, Carson DD. Transmembrane mucin expression and function in embryo implantation and placentation. In: Geisert R, Bazer F. (eds) *Regulation of implantation and establishment of pregnancy in mammals: tribute to 45 year anniversary of Roger V. Short's "maternal recognition of pregnancy"*, advances in anatomy, embryology and cell biology. Switzerland: Springer, Cham, 2015, pp. 51-68
64. Gellersen B, Brosens I, Brosens J. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med* 2007;**25**:445-53
65. Wewer UM, Damjanov A, Weiss J, Liotta LA, Damjanov I. Mouse endometrial stromal cells produce basement-membrane components. *Differentiation* 1986;**32**:49-58
66. Yucha RW, Jost M, Rothstein D, Robertson N, Marcolongo MS. Quantifying the biomechanics of conception: L-selectin-mediated blastocyst implantation mechanics with engineered 'trophospheres'. *Tissue Eng Part A* 2014;**20**:189-96
67. Aplin JD, Charlton AK, Ayad S. An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and first trimester of pregnancy. *Cell Tissue Res* 1988;**253**:231-40
68. Iwahashi M, Muragaki Y, Ooshima A, Yamoto M, Nakano R. Alterations in distribution and composition of the extracellular matrix during decidualization of the human endometrium. *J Reprod Fertil* 1996;**108**:147-55
69. Burghardt RC, Burghardt JR, Taylor JD, Reeder AT, Nguen BT, Spencer TE, Bayless KJ, Johnson GA. Enhanced focal adhesion assembly reflects increased mechanosensation and mechanotransduction at maternal-conceptus interface and uterine wall during ovine pregnancy. *Reproduction* 2009;**137**:567-82
70. Fässler R, Meyer M. Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev* 1995;**9**:1896-908
71. Illera MJ, Cullinan E, Gui Y, Yuan L, Beyler SA, Lessey BA. Blockade of the alpha(v)beta(3) integrin adversely affects implantation in the mouse. *Biol Reprod* 2000;**62**:1285-90
72. Illera MJ, Lorenzo PL, Gui Y-T, Beyler SA, Apparao KBC, Lessey BA. A role for alphavbeta3 integrin during implantation in the rabbit model. *Biol Reprod* 2003;**68**:766-71
73. Burney RO, Talbi S, Hamilton AE, Vo KC Nyegaard M, Nexhat CR, Lessey BA, Guidice LC. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* 2007;**148**:3814-26
74. Kim TH, Yoo JY, Choi KC, Shin J-H, Leach RE, Fazleabas AT, Young SL, Lessey BA, Young SL, Lessey BA, Yoon H-G, Jeong J-W. Loss of HDAC3 results in nonreceptive endometrium and female infertility. *Sci Transl Med* 2019;**11**:pii:eaaf7533
75. Bruner-Tran KL, Eisenberg E, Yeaman GR, Anderson TA, McBean J, Osteen KG. Steroid and cytokine regulation of matrix metalloproteinase expression in endometriosis and the establishment of experimental endometriosis in nude mice. *J Clin Endocrinol Metab* 2002;**87**:4782-91
76. Singh H, Aplin JD. Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation. *J Anat* 2009;**215**:3-13
77. Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, Kabayama Y, Suyama M, Sasaki H, Arima T. Derivation of human trophoblast stem cells. *Cell Stem Cell* 2018;**22**:50-63.e6
78. Oefner CM, Sharkey A, Gardner L, Critchley H, Oyen M, Moffett A. Collagen type IV at the fetal-maternal interface. *Placenta* 2015;**36**:59-68
79. Zhou Y, Genbacev O, Fisher SJ. The human placenta remodels the uterus by using a combination of molecules that govern vasculogenesis or leukocyte extravasation. *Ann N Y Acad Sci* 2003;**995**:73-83
80. Ji L, Brkić J, Liu M, Fu G, Peng C, Wang Y-L. Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. *Mol Aspects Med* 2013;**34**:981-1023
81. Tapia-Pizarro A, Argandona F, Palomino WA, Devoto L. Human chorionic gonadotropin (hCG) modulation of TIMP1 secretion by human endometrial stromal cells facilitates extravillous trophoblast invasion in vitro. *Hum Reprod* 2013;**28**:2215-27
82. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010;**141**:52-67
83. Wang X, Miller DC, Harman R, Antczak DE, Clark AG. Paternally expressed genes predominate in the placenta. *Proc Natl Acad Sci U S A* 2013;**110**:10705-10
84. Fowden AL, Coan PM, Angiolini E, Burton GJ, Constancia M. Imprinted genes and the epigenetic regulation of placental phenotype. *Prog Biophys Mol Biol* 2011;**106**:281-8
85. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science* 2014;**345**:760-5
86. Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, Grobely D, Damsky CH, Fisher SJ. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *J Cell Biol* 1991;**113**:437-49
87. Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. *Science* 1997;**277**:1669-72
88. Damsky CH, Fitzgerald ML, Fisher SJ. Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vivo. *J Clin Invest* 1992;**89**:210-22
89. Plaks V, Rinkenberger J, Dai J, Flannery M, Sund M, Kanasaki K, Ni W, Kalluri R, Werb Z. Matrix metalloproteinase-9 deficiency phenocopies features of preeclampsia and intrauterine growth restriction. *Proc Natl Acad Sci U S A* 2013;**110**:11109-14
90. Lian IA, Toft JH, Olsen GD, Langaas M, Bjørge L, Eide IP, Børdaahl PE, Austgulen R. Matrix metalloproteinase 1 in pre-eclampsia and fetal growth restriction: reduced gene expression in decidual tissue and protein expression in extravillous trophoblasts. *Placenta* 2010;**31**:615-20
91. Perez-Garcia V, Fineberg E, Wilson R, Murray A, Mazzeo CI, Tudor C, Sienerth A, White JK, Tuck E, Ryder EJ, Gleeson D, Siragher E, Wardle-Jones H, Staudt N, Wali N, Collins J, Geyer S, Busch-Nentwich EM, Galli A, Smith JC, Robertson E, Adams DJ, Weninger WJ, Mohun T, Hemberger M. Placentation defects are highly prevalent in embryonic lethal mouse mutants. *Nature* 2018;**555**:463-8
92. Martinez-Fierro ML, Hernández-Delgado GP, Flores-Morales V, Cardenas-Vargas E, Mercado-Reyes M, Rodriguez-Sanchez IP, Delgado-Enciso I, Galván-Tejada CE, Galván-Tejada JI, Celaya-Padilla JM, Garza-Veloz I. Current model systems for the study of preeclampsia. *Exp Biol Med* 2018;**243**:576-85
93. Boyd PA, Scott A. Quantitative structural studies on human placentas associated with pre-eclampsia, essential hypertension and intrauterine growth retardation. *Br J Obstet Gynaecol* 1985;**92**:714-21
94. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertens* 2013;**62**:1046-54
95. Romão M, Weel IC, Lifshitz SJ, Peraçoli MTS. Elevated hyaluronan and extracellular matrix metalloproteinase inducer levels in women with preeclampsia. *Arch Gynecol Obstet* 2014;**289**:575-9
96. Vetraino IM, Roby J, Tolley T, Parks WC. Collagenase-I, stromelysin-I, and matrilysin are expressed within the placenta during multiple stages of human pregnancy. *Placenta* 1996;**17**:557-63
97. Hunninghake G, Davidson J, Renard S, Szapiel S, Gadek JE, Crystal RG. Elastin fragments attract macrophage precursors to diseased sites in pulmonary emphysema. *Science* 1981;**212**:925-7
98. Steadman R, Irwin MH, St J, John PL, Blackburn WD, Heck LW, Abrahamson DR. Laminin cleavage by activated human neutrophils

- yields proteolytic fragments with selective migratory properties. *J Leukoc Biol* 1993;**53**:354–65
99. Ilekis JV, Tsilou E, Fisher S, Abrahams VM, Soares MJ, Cross JC, Zamudio S, Illsley NP, Myatt L, Colvis C, Constantine MM, Haas DM, Sadovsky Y, Weiner C, Rytting E, Bidwell G. Placental origins of adverse pregnancy outcomes: potential molecular targets: an executive workshop summary of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. *Am J Obstet Gynecol* 2016;**215**:S1–S46
 100. Kocarslan S, Incebiyik A, Guldur ME, Ekinci T, Ozardali HI. What is the role of matrix metalloproteinase-2 in placenta percreta? *J Obstet Gynaecol Res* 2015;**41**:1018–22
 101. DaSilva-Arnold SC, Zamudio S, Al-Khan A, Alvarez-Perez J, Mannion C, Koenig C, Luke D, Perez AM, Petroff M, Alvarez M, Illsley NP. Human trophoblast epithelial-mesenchymal transition in abnormally invasive placenta. *Biol Reprod* 2018;**99**:409–21
 102. Silver RM, Landon MB, Rouse DJ, Leveno KJ, Spong CY, Thom EA, Moawad AH, Caritis SN, Harper M, Wapner RJ, Sorokin Y, Miodovnik M, Carpenter M, Peaceman AM, O'Sullivan MJ, Sibai B, Langer O, Thorp JM, Ramin SM, Mercer BM, National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Maternal morbidity associated with multiple repeat cesarean deliveries. *Obstet Gynecol* 2006;**107**:1226–32
 103. Roeder HA, Cramer SF, Leppert PC. A look at uterine wound healing through a histopathological study of uterine scars. *Reprod Sci* 2012;**19**:463–73
 104. Kuo CY, Baker H, Fries MH, Yoo JJ, Kim PCW, Fisher JP. Bioengineering strategies to treat female infertility. *Tissue Eng Part B Rev* 2017;**23**:294–306
 105. Song T, Zhao X, Sun H, Li X, Lin N, Ding L, Dai J, Hu Y. Regeneration of uterine horns in rats using collagen scaffolds loaded with human embryonic stem cell-derived endometrium-like cells. *Tissue Eng Part A* 2015;**21**:353–61
 106. Ding L, Li X, Sun H, Su J, Lin N, Péault B, Song T, Yang J, Dai J, Hu Y. Transplantation of bone marrow mesenchymal stem cells on collagen scaffolds for the functional regeneration of injured rat uterus. *Biomaterials* 2014;**35**:4888–900
 107. Ramsey EM. Anatomy of the human uterus. In: T Chard, Grudzinskas JG (eds). *Uterus*. New York, NY: Cambridge University Press, 1994; pp. 18–40
 108. Myers KM, Elad D. Biomechanics of the human uterus. *Wires Syst Biol Med* 2017;**9**:e1388
 109. Lombardi A, Makieva S, Rinaldi SF, Arcuri F, Petraglia F, Norman JE. Expression of matrix metalloproteinases in the mouse uterus and human myometrium during pregnancy, labor, and preterm labor. *Reprod Sci* 2018;**25**:938–49
 110. Walsh SW, Nugent WH, Solotskaya AV, Anderson CD, Grider JR, Strauss JF III. Matrix metalloproteinase-1 and elastase are novel uterine agents acting through protease-activated receptor 1. *Reprod Sci* 2018;**25**:1058–66
 111. Nallasamy S, Yoshida K, Akins M, Myers K, Iozzo R, Mahendroo M. Steroid hormones are key modulators of tissue mechanical function via regulation of collagen and elastic fibers. *Endocrinology* 2017;**158**:950–62
 112. Pezaro S, Pearce G, Reinhold E. Hypermobile Ehlers-Danlos syndrome during pregnancy, birth and beyond. *Br J Midwifery* 2018;**26**:217–23
 113. Murray ML, Pepin M, Peterson S, Byers PH. Pregnancy-related deaths and complications in women with vascular Ehlers-Danlos syndrome. *Genet Med* 2014;**16**:874–80
 114. Hermanns-Lê T, Piérard GE. Skin ultrastructural clues on the impact of Ehlers-Danlos syndrome in women. *J Dermatological Res* 2016;**1**:34–40
 115. Alamo L, Vial Y, Denys A, Andreisek G, Meuwly J-Y, Schmidt S. MRI findings of complications related to previous uterine scars. *Eur J Radiol Open* 2018;**5**:6–15
 116. Antila-Långsjö RM, Mäenpää JU, Huhtala HS, Tomás EI, Staff SM. Cesarean scar defect: a prospective study on risk factors. *Am J Obstet Gynecol* 2018;**219**:458.e1–458.e8
 117. Landon MB, Hauth JC, Leveno KJ, Spong CY, Leindecker S, Varner MW, Moawad AH, Caritis SN, Harper M, Wapner RJ, Sorokin T, Miodovnik M, Carpenter M, Peaceman AM, O'Sullivan MJ, Sibai B, Langer O, Thorp JM, Ramin SM, Mercer BM, Gabbe SG, National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Maternal and perinatal outcomes associated with a trial of labor after prior cesarean delivery. *N Engl J Med* 2004;**351**:2581–9
 118. Motomura K, Ganchimeg T, Nagata C, Ota E, Vogel JP, Betran AP, Torloni RM, Jayaratne K, Jwa SC, Mittal S, Recidoro ZD, Matsumoto K, Fujieda M, Nafiou I, Yunis K, Qureshi Z, Souza JP, Mori R. Incidence and outcomes of uterine rupture among women with prior cesarean section: WHO multicountry survey on maternal and newborn health. *Sci Rep* 2017;**7**:44093
 119. Buhimschi CS, Zhao G, Sora N, Madri JA, Buhimschi IA. Myometrial wound healing post-Cesarean delivery in the MRL/MpJ mouse model of uterine scarring. *Am J Pathol* 2010;**177**:197–207
 120. Shynlova O, Kwong R, Lye SJ. Mechanical stretch regulates hypertrophic phenotype of the myometrium during pregnancy. *Reproduction* 2010;**139**:247–53
 121. Shynlova O, Williams SJ, Draper H, White BG, MacPhee DJ, Lye SJ. Uterine stretch regulates temporal and spatial expression of fibronectin protein and its alpha 5 integrin receptor in myometrium of unilaterally pregnant rats. *Biol Reprod* 2007;**77**:880–8
 122. Garfield RE, Maner WL. Physiology and electrical activity of uterine contractions. *Semin Cell Dev Biol* 2007;**18**:289–95
 123. Zhou D, Lee HS, Villarreal F, Teng A, Lu E, Reynolds S, Qin C, Smith J, Sung KLP. Differential MMP-2 activity of ligament cells under mechanical stretch injury: an in vitro study on human ACL and MCL fibroblasts. *J Orthop Res* 2005;**23**:949–57
 124. Strauss JF III. Extracellular matrix dynamics and fetal membrane rupture. *Reprod Sci* 2013;**20**:140–53
 125. de Laat MWM, Pieper PG, Oudijk MA, Mulder BJM, Christoffels VM, Afink GB, Postma AV, Ris-Stalpers C. The clinical and molecular relations between idiopathic preterm labor and maternal congenital heart defects. *Reprod Sci* 2013;**20**:190–201
 126. Turton P, Arrowsmith S, Prescott J, Ballard C, Bricker L, Neilson J, Wray S. A comparison of the contractile properties of myometrium from singleton and twin pregnancies. *PLoS One* 2013;**8**:e63800
 127. Biggio JR, Anderson S. Spontaneous preterm birth in multiples. *Clin Obstet Gynecol* 2015;**58**:654–67
 128. Challis JRG, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, Whittle WL, Newnham JP. Prostaglandins and mechanisms of preterm birth. *Reproduction* 2002;**124**:1–17
 129. Kobayashi H, Kawahara N, Ogawa K, Yoshimoto C. A relationship between endometriosis and obstetric complications. *Reprod Sci* 2020;**1**–8
 130. Gundling WE, Wildman DE. A review of inter- and intraspecific variation in the eutherian placenta. *Philos Trans R Soc Lond B Biol Sci* 2015;**370**:20140072
 131. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO, Simons BD, Hemberger M, Koo B-K, Moffett A, Burton GJ. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 2017;**19**:568–577
 132. Haider S, Meinhardt G, Saleh L, Kunihs V, Gampel M, Kaindl U, Ellinger A, Burkard TR, Fiala C, Pollheimer J, Mendjan S, Latos PA, Knöfler M. Self-renewing trophoblast organoids recapitulate the developmental program of the early human placenta. *Stem Cell Rep* 2018;**11**:537–551
 133. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization in vitro of human oocytes matured in vitro. *Nature* 1969;**221**:632–635
 134. Edwards RG, Steptoe PC, Purdy JM. Fertilization and cleavage in vitro of preovulatory human oocytes. *Nature* 1970;**227**:1307–1309
 135. Harrison SE, Sozen B, Christodoulou N, Kyprianou C, Zernicka-Goetz M. Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis in vitro. *Science* 2017;**356**:pii:eaal1810
 136. Lind JU, Busbee JA, Valentine AD, Campalini FS, Yuan H, Yadid M, Park S-J, Kotikian A, Nesmith AP, Campbell PH, Vlassak JJ, Lewis JA,

- Parker KK. Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. *Nat Mater* 2017;**16**:303–308
137. Herland A, van der Meer AD, FitzGerald EA, Park T-E, Sleeboom JFF, Ingber DE. Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip. *PLoS One* 2016;**11**:e0150360
138. Gliberman AL, Pope BD, Zimmerman JF, Liu Q, Ferrier JP Jr, Kenty JHR, Schrell AM, Mukhitov N, Shores KL, Buganza Tepole A, Melton DA, Roper MG, Parker KK. Synchronized stimulation and continuous insulin sensing in a microfluidic human islet on a chip designed for scalable manufacturing. *Lab Chip* 2019;**19**:2993–3010
139. Nesmith AP, Wagner MA, Pasqualini FS, O'Connor BB, Pincus MJ, August PR, Parker KK. A human in vitro model of duchenne muscular dystrophy muscle formation and contractility. *J Cell Biol* 2016;**215**:47–56
140. Alford PW, Nesmith AP, Seywerd JN, Grosberg A, Parker KK. Vascular smooth muscle contractility depends on cell shape. *Integr Biol* 2011;**3**:1063
141. Angelini TE, Hannezo E, Trepas X, Marquez M, Fredberg JJ, Weitz D. Glass-like dynamics of collective cell migration. *Proc Natl Acad Sci U S A* 2011;**108**:4714–9
142. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat Biotechnol* 2014;**32**:760–772
143. Benam KH, Dauth S, Hassell B, Herland A, Jain A, Jang K-J, Karalis K, Kim HJ, MacQueen L, Mahmoodian R, Musah S, Torisawa Y, van der Meer AD, Villenave R, Yadid M, Parker KK, Ingber DE. Engineered in vitro disease models. *Annu Rev Pathol Mech Pathol* 2015;**10**:195–262
144. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp Biol Med* 2014;**239**:1061–72
145. Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park T-E, Dauth S, Mannix R, Budnik N, Shores K, Cho A, Nawroth JC, Segre D, Budnik B, Ingber DE, Parker KK. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat Biotechnol* 2018;**36**:865–874
146. Novak R, Ingram M, Marquez S, Das D, Delahanty A, Herland A, Maoz BM, Jeanty SSF, Somayaji MR, Burt M, Calamari E, Chalkiadaki A, Cho A, Choe Y, Chou DB, Crouce M, Dauth S, Divic T, Fernandez-Alcon J, Ferrante T, Ferrier J, FitzGerald EA, Fleming R, Jalili-Firoozinezhad S, Grevesse T, Goss JA, Hamkins-Indik T, Henry O, Hinojosa C, Huffstater T, Jang K-J, Kujala V, Leng L, Mannix R, Milton Y, Nawroth J, Nestor BA, Ng CF, O'Connor B, Park T-E, Sanchez H, Sliz J, Sontheimer-Phelps A, Swenor B, Tompson G II, Touloumes GJ, Tranchemontagne Z, Wen N, Yadid M, Bahinski A, Hamilton GA, Levner D, Levy O, Przekwas A, Prantil-Baun P, Parker KK, Ingber DE. Robotic fluidic coupling and interrogation of multiple vascularized organ chips. *Nat Biomed Eng* 2020;**4**:407–20
147. Young AN, Moyle-Heyman G, Kim JJ, Burdette JE. Microphysiologic systems in female reproductive biology. *Exp Biol Med* 2017;**242**:1690–1700
148. Weimar CHE, Post Uiterweer ED, Teklenburg G, Heijnen CJ, Macklon NS. In-vitro model systems for the study of human embryo-endometrium interactions. *Reprod Biomed Online* 2013;**27**:461–76
149. Richardson L, Gnecco J, Ding T, Osteen K, Rogers LM, Aronoff DM, Menon R. Fetal membrane organ-on-chip: an innovative approach to study cellular interactions. *Reprod Sci* 2019. doi: 10.1177/1933719119828084
150. Zhu Y, Yin F, Wang H, Wang L, Yuan J, Qin J. Placental barrier-on-a-chip: modeling placental inflammatory responses to bacterial infection. *ACS Biomater Sci Eng* 2018;**4**:3356–3363
151. Blundell C, Tess ER, Schanzer ASR, Coutifaris C, Su EJ, Parry S, Huh D. A microphysiological model of the human placental barrier. 2016;**16**:3065–73
152. Blundell C, Yi Y-S, Ma L, Tess ER, Farrell MJ, Georgescu A, Aleksunes LM, Huh D. Placental drug transport-on-a-chip: a microengineered in vitro model of transporter-mediated drug efflux in the human placental barrier. *Adv Healthcare Mater* 2018;**7**:1700786
153. Abbas Y, Oefner CM, Polacheck WJ, Gardner L, Farrell L, Sharkey A, Kamm R, Moffett A, Oyen ML. A microfluidics assay to study invasion of human placental trophoblast cells. *J R Soc Interface* 2017;**14**:pii:20170131
154. Lee JS, Romero R, Han YM, Kim HC, Kim CJ, Hong J-S, Huh D. Placenta-on-a-chip: a novel platform to study the biology of the human placenta. *J Matern Neonatal Med* 2016;**29**:1046–1054
155. Gnecco JS, Ding T, Smith C, Lu J, Bruner-Tran KL, Osteen KG. Hemodynamic forces enhance decidualization via endothelial-derived prostaglandin E2 and prostacyclin in a microfluidic model of the human endometrium. *Hum Reprod* 2019;**34**:702–714
156. Gnecco JS, Pensabene V, Li DJ, Ding T, Hui EE, Bruner-Tran KL, Osteen KG. Compartmentalized culture of perivascular stroma and endothelial cells in a microfluidic model of the human endometrium. *Ann Biomed Eng* 2017;**45**:1758–1769
157. Shao Y, Taniguchi K, Gurdziel K, Townshend RF, Xue X, Yong KMA, Sang J, Spence JR, Gumucio DL, Fu J. Self-organized amniogenesis by human pluripotent stem cells in a biomimetic implantation-like niche. *Nat Mater* 2017;**16**:419–425
158. Ma Z, Sagrillo-Fagundes L, Tran R, Parameshwar PK, Kalashnikov N, Vaillancourt C, Moraes C. Biomimetic micropatterned adhesive surfaces to mechanobiologically regulate placental trophoblast fusion. *ACS Appl Mater Interfaces* 2019;**11**:47810–47821
159. Wong MK, Shawky SA, Aryasomayajula A, Green MA, Ewart T, Selvaganapathy PR, Raha S. Extracellular matrix surface regulates self-assembly of three-dimensional placental trophoblast spheroids. *PLoS One* 2018;**13**:e0199632
160. Gnecco JS, Anders AP, Cliffel D, Pensabene V, Rogers LM, Osteen K, Aronoff DM. Instrumenting a fetal membrane on a chip as emerging technology for preterm birth research. *Curr Pharm Des* 2017;**23**:6115–6124
161. Kilburn BA, Wang J, Duniec-Dmuchowski ZM, Leach RE, Romero R, Armant DR. Extracellular matrix composition and hypoxia regulate the expression of HLA-G and integrins in a human trophoblast cell line. *Biol Reprod* 2000;**62**:739–747
162. Richardson L, Jeong S, Kim S, Han A, Menon R. Amnion membrane organ-on-chip: an innovative approach to study cellular interactions. *FASEB J* 2019;**33**:8945–8960
163. Klaffky EJ, Gonzales IM, Sutherland AE. Trophoblast cells exhibit differential responses to laminin isoforms. *Dev Biol* 2006;**292**:277–289
164. Castellucci M, Kaufmann P, Bischof P. Extracellular matrix influences hormone and protein production by human chorionic villi. *Cell Tissue Res* 1990;**262**:135–142
165. Desforges M, Harris LK, Aplin JD. Elastin-derived peptides stimulate trophoblast migration and invasion: a positive feedback loop to enhance spiral artery remodelling. *Mol Hum Reprod* 2015;**21**:95–104
166. Grevesse T, Versaevael M, Circelli G, Desprez S, Gabriele S. A simple route to functionalize polyacrylamide hydrogels for the independent tuning of mechanotransduction cues. *Lab Chip* 2013;**13**:777
167. Cook CD, Hill AS, Guo M, Stockdale L, Papps JP, Isaacson KB, Lauffenburger DA, Griffith LG. Local remodeling of synthetic extracellular matrix microenvironments by co-cultured endometrial epithelial and stromal cells enables long-term dynamic physiological function. *Integr Biol* 2017;**9**:271–289
168. Zambuto SG, Clancy KBH, Harley B. A gelatin hydrogel to study endometrial angiogenesis and trophoblast invasion. *Interf Focus* 2019;**9**:20190016
169. Abbas Y, Carnicer-Lombarte A, Gardner L, Thomas J, Brosens JJ, Moffett A, Sharkey AM, Franze K, Burton GJ, Oyen ML. Tissue stiffness at the human maternal–fetal interface. *Hum Reprod* 2019;**34**:1999–2008