Minireview

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

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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

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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 hormones[35] 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 $ß_1$, 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 $\alpha_V ß_3$ 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 $ß_3$ 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 $ß_3$ 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 $ß_3$ 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

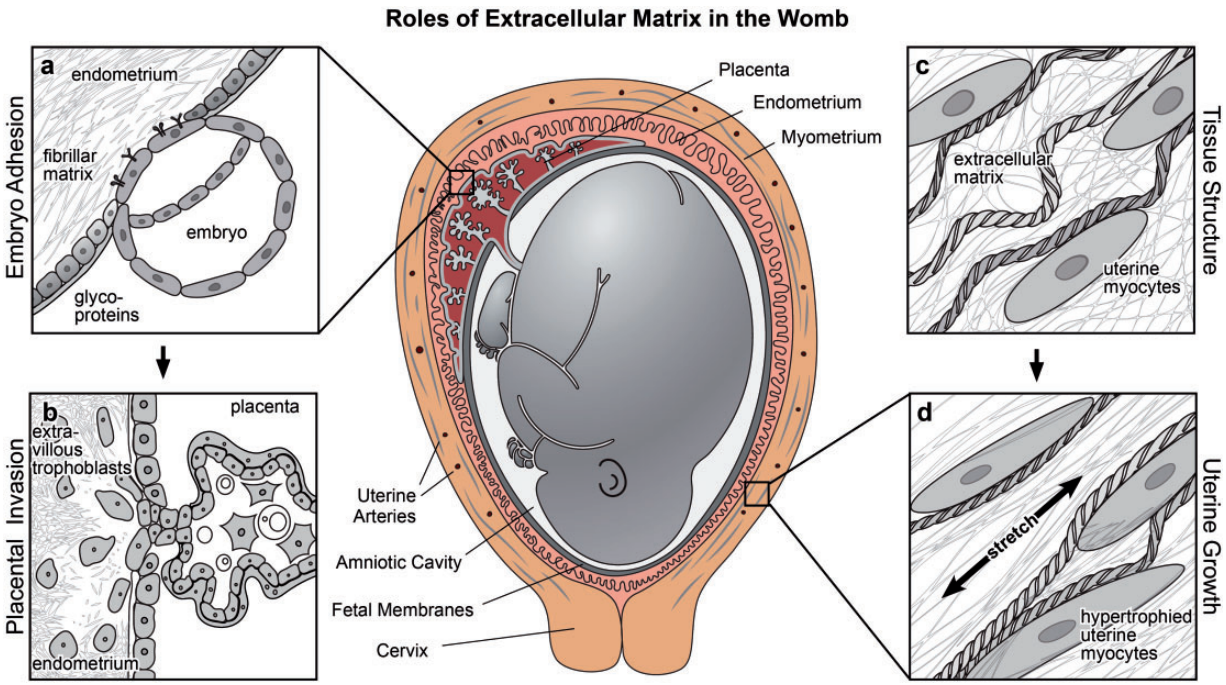

Figure 1. Roles of extracellular matrix in the womb. Extracellular provides structural support in the womb and mediates important cellular processes including adhesion, migration, invasion, and mechanical signaling. (a) Extracellular matrix regulates embryonic adhesion by defining a window of receptivity 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

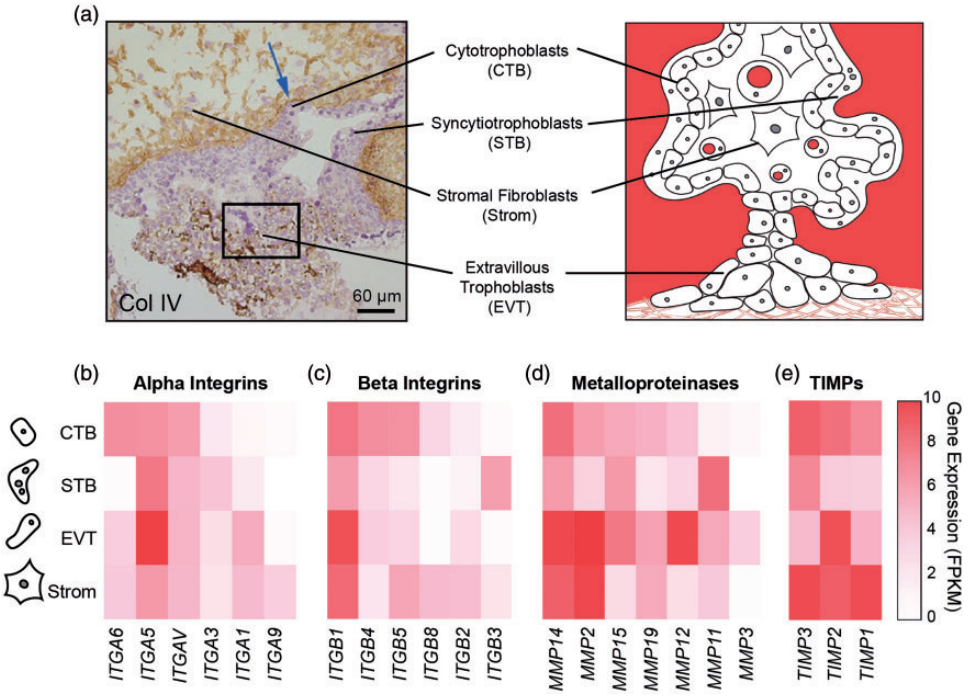

Figure 2. Integrin and matrix metalloproteinase gene expression in placental cell types. (a) First trimester placental villi stained for Collagen IV and counterstained with hematoxylin and simplified illustration (right). Arrow indicates basement membrane staining and box represents globular Col IV in 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 dataset[77]) For clarity, only genes with more than 3.6 fragments per kilobase of transcript 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 cavitity.[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

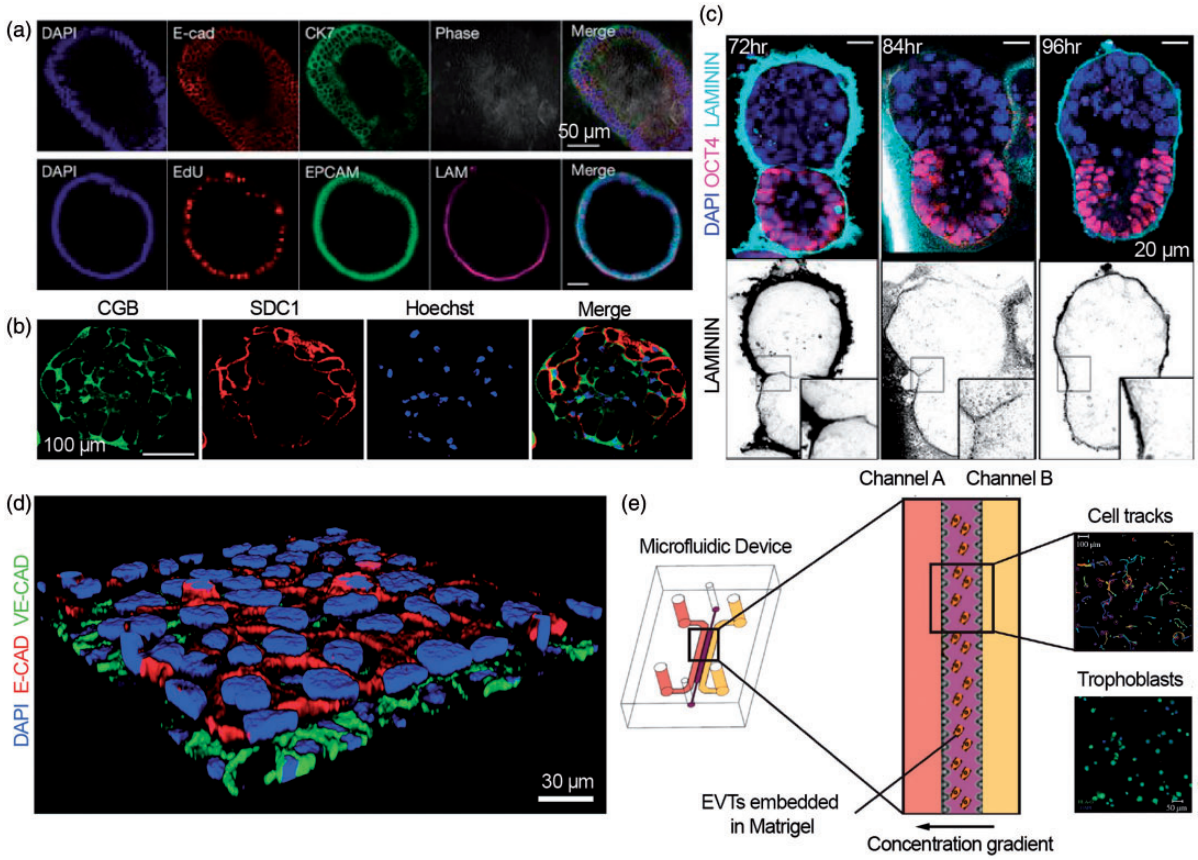

Figure 3. *In vitro* models of the maternal–fetal interface and embryogenesis. (a) Primary endometrial cells suspended in matrigel self-organize into organoids in chemically defined medium (adapted from Turco *et al*.[131]). (b) Human trophoblast stem cells form three-dimensional syncytiotrophoblasts 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 stems 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 membrane[149] 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.

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