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Instrumenting a Fetal Membrane on a Chip as Emerging Technology for Preterm Birth Research

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Abstract

Preterm birth (PTB) is clinically defined as process of giving birth before 37 weeks of gestation and is a leading cause of death among neonates and children under the age of five. Prematurity remains a critical issue in developed countries, yet our understanding of the pathophysiology of PTB remains largely unknown. Among pregnancy complications, subclinical infections such as chorioamnionitis (CAM) are implicated in up to 70% of PTB cases. Specifically, CAM is characterized by the infection of the fetal membranes that surround the developing fetus and extend from the placenta, and is often associated with preterm, premature rupture of the fetal membranes (PPROM). The fetal membrane plays a key structural role in maintaining the fetal and maternal compartments of the gravid uterus. However, our understanding of the mechanisms of PPROM and the spatio-temporal progress of CAM remains vastly unknown. A lack of human-derived models have hindered our understanding of the mechanism that govern spontaneous PTB. Thus, in this short review, we discuss the emerging microfabrication technologies, specifically, organ-on-chip (OoCs) models, that seek to recapitulate the cellular and molecular context of the gestational membranes in vitro. These models show promise to facilitate the investigation of pathologic mechanisms that drive these disease conditions by mimicking the interactive contribution of the major cell types that make up the microenvironment of the fetal membrane and enable high throughput screening. Herein, we histologically characterize the microenvironment of the fetal membrane as a metric for scaling to recapitulate the functional components of the human fetal membrane. We review the current OoC models of the gravid uterus and conceptualize an “Instrumented Fetal Membrane on a Chip” (IFMOC) design as a prototype for PPROM and CAM research. Lastly, we discuss further applications of these OoC models for toxicological or pharmacological screening and personalized medicine. Fetal membrane OoCs offer an innovative and valuable platform to explore complex interactions between multiple drug types, toxic substances, and/or pathogenic microbes and their potential impacts on pregnancy outcomes. Further work will be required by integrating technological and analytical capabilities in order to characterize the fetal membrane microenvironment for preterm birth research.

Introduction

Annually, nearly 15 million preterm births (PTB) occur worldwide (1), making prematurity the leading cause of death in neonates and the second-leading cause in children under 5 years old (2, 3). While there are many causes of PTB, it is most often caused by subclinical infection of the membranes that surround the developing fetus and extend from the placenta, a condition referred to as infectious chorioamnionitis (CAM). This usually the result of bacteria ascending from the vagina to invade the fetal membranes (**Figure 1**) (4). The fetal membranes are a critical protective barrier during normal pregnancy and are composed of three structural layers, including the fetal-derived amnion and chorion and the maternal-derived decidua (5). Despite this relatively simple organ structure, little is known about how fetal membranes participate in immune defense or how microbes evade these defenses.

Defining the host-microbial interactions within the fetal membrane at a cellular and molecular level will reveal actionable targets for early diagnosis, prevention and treatment of CAM. As many as 70% or more of preterm births are associated with CAM, particularly when the delivery occurs before 30 weeks of gestation (6). Babies exposed to CAM in utero are at increased risk for neonatal sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, cerebral palsy and retinopathy of prematurity (7). Unfortunately, CAM is often asymptomatic and difficult to diagnose in time to prevent maternal and fetal adverse outcomes. Furthermore, subsets of pregnant women with microbial contamination of amniotic fluid carry their pregnancy to term, suggesting host factors likely influence the risk for CAM-associated PTB (8). Additionally, antibiotic therapies have shown discrepancy between populations of women and have failed, for the most part, to prevent preterm birth (9, 10). A common pregnancy complication that stems from CAM and is a major contributor to the burden of PTB is preterm premature rupture of the fetal membranes (PPROM). Although fetal membrane rupture is an essential part of the delivery process, PPROM at less than 34 weeks of gestation is responsible for approximately 25% of premature births (11). Our limited understanding of the early steps involved in disease pathogenesis impedes solutions to this immense problem.

There is a significant deficit in tractable model systems of human fetal membranes. Most studies of human fetal membrane immunology employ traditional cell and ex vivo tissue culture models (12, 13), and are limited by either loss of the biological context or an inability to maintain ex vivo tissues for prolonged periods. Tissue culture also lacks the capability to dissect the roles of individual cell types within the context of a tissue microenvironment. Typically,

independent cell and tissue culture experiments are conducted and terminated at discrete time points and do not convey the molecular pathways involved throughout the course of infection. This disjointed approach creates challenges for understanding the dynamic host-microbial relationship. In addition, these culture systems often rely on relatively large culture media volumes employed which may dilute paracrine signals involved in cellular crosstalk. Animal models are beneficial for physiologic studies, but the placenta and fetal membranes have tremendous differences in anatomy and physiology amongst mammalian species. These biological differences from humans limit progress towards translational solutions (14, 15). Thus, there is an essential need to develop robust models of human CAM that can eliminate species-specific differences, incorporate all relevant cell subtypes to accelerate research in immunology and microbiology (16, 17).

Microfluidic organotypic model systems, commonly referred to as Organ-on-Chip (OoC) technologies, are expected to have a major impact on drug discovery, screening, and assessment of efficacy and safety (18, 19). Such 3D platforms may recapitulate inter- and intra-cell signaling and the physiological context of tissue dynamics by compartmentalizing the major cellular components for quantitative and qualitative analysis (18). Apart from their potential role in clinical pharmacology, such OoC models can be used to study the effects of environmental insults (e.g., toxins, radiation, or malnutrition) or infections on human health (20). Reproductive tract organotypic culture models are emerging to meet this need (21). In this review, we describe existing OoC of the gravid uterus and provide conceptual insight into an emerging tool, the instrumented fetal membrane-on-chip (IFMOC), a new model our team is developing. An IFMOC could provide a living, continuously perfused model of the fetal membrane that can be leveraged to shed new light on many physiological and pathophysiological processes, including host-microbial interactions that occur during CAM and PTB. Here, we use PPROM as an example of a gestational membrane disease process that can be examined using this OoC model.

Existing organ-on-chip (OoC) models of the gravid uterus

Advances in biomedical engineering are increasingly deployed in microphysiological models geared toward mimicking multiple organs in vitro in many disciplines including reproductive biology (21). By utilizing microfluidic technologies, OoC devices can provide controlled perfusion inside microfluidic structures, which can then refine cell and medium volume ratios and emulate bloodstream-like flow (to continuously supply nutrients and remove wastes while mimicking hemodynamic forces) (22). One major goal is to hasten the speed and improve the accuracy of toxicity testing in preclinical drug development (21), however, such models might also be used to gain new insight into tissue level physiological processes and disease pathogenesis (20). Applications of these technologies as innovative tools for reproductive research have recently emerged as discussed below.

References cited in this review relevant to OoC models of the gravid female reproductive tract were obtained by searching the MEDLINE database for English language articles using PubMed (United States National Library of Medicine (Bethesda, MD)) for all years available. The following search terms or combination of terms were used: “endometrium”, “embryo”, “microfluidic”, “organ on chip”, “placenta”, “pregnancy”, and “reproductive tract”. Additional references were obtained through bibliographies cited in manuscripts. Literature was reviewed through December 2016.

OoC models of embryo implantation

In this review, we focused on the post-implantation embryo; however, microfluidic models of the pre-implantation embryo have been developed and reviewed elsewhere (23-25). Although early attempts at modeling embryo implantation using a microfluidic 2-chamber device were presented in abstract form in 2007 (24, 26, 27), it was not until 2009 that Kimura and colleagues developed a static, 2-chamber OoC capable of culturing a single mouse embryo on a bed of endometrial stromal cells (22). Their device consisted of an upper polydimethylsiloxane (PDMS) chip with multiple cell trap wells and a lower PDMS chamber with a microchannel for chemical supply along with a polyester microporous membrane from a commercially available culture insert plate (22). This approach was believed at the time to be the first single embryo co-culture device for the management of mammalian embryos (22). In 2014, Chen and collaborators advanced the field for co-culturing a single embryo with a lawn of endometrial stromal cells, using a fabricated PDMS dual-chamber device coated with type IV collagen and subjecting the device to a constant

media flow (28). This model enhanced the quality of embryo culture by utilizing microfluidic technologies. A more recent, but similar approach was taken by Chang et al. to develop a comprehensive stand-alone microfluidic platform, "Womb-on-a Chip", that mimics the microenvironment and incorporates key parameters of embryo implantation, including mild biomechanical forces (29). This model used PDMS based microfluidic chambers to directly co-culture primary endometrial stromal fibroblasts with murine embryos with the ability to visualize the implantation process in real time.

To our knowledge, these devices have not been applied to the clinical problem of PTB, but were developed to address the root mechanisms of infertility and improve assisted reproductive technology (ART). However, there is potential to use such devices to better understand early events during pre-conception and the establishment of pregnancy that may lead preterm birth.

OoC models of the placenta

The human placenta is an understudied organ that has long been appreciated to play a major role in important complications of pregnancy, including prematurity, infection, intrauterine growth restriction, (pre)eclampsia and gestational diabetes (30-33). More recently, attention has focused on the placenta for its potential role in the developmental origins of health and disease (DOHaD), a paradigm that relates early life exposures (including gestational health) to lifespan and disease risk in offspring (34, 35). The possibility that placental pathology could be a critical root mechanism for major causes of morbidity and mortality in adults (36, 37), such as cardiovascular disease, obesity, diabetes, and neurocognitive problems, creates a new importance for defining normal placental structure and function. Knowledge of placental molecular biology lags behind that of other organs; however, this is not surprising, since it is not feasible to sample an individual human placenta at multiple time points throughout pregnancy. Thus, there is a need for new models of placental biology that can be applied to understanding both normal and disease states.

Recently, two groups have published placenta OoC models (38, 39). The placental chip system reported by Lee, et al. was developed using a technique known as soft lithography, resulting in a microfluidic system made of two PDMS chambers separated by a thin extracellular matrix membrane. An immortalized trophoblast cell line (JEG-3) combined

with primary human umbilical vein endothelial cells (HUVEC) were used to represent two of the major non-immune cell types of the placenta in the device. These cells were seeded onto the opposite sides of the extracellular matrix membrane and cultured under dynamic flow conditions. Confluent layers of trophoblasts and endothelial cells were generated in close apposition in order to mimic the human placental maternal-fetal interface (39). Functional validation of this system was performed by measuring glucose transport across the trophoblast-endothelial interface over time. The permeability of the barrier was analyzed and compared to that obtained from acellular devices and additional control groups comprised of either epithelial or endothelial layers alone (39).

The OoC model developed by Blundell, et al., was very similar to the Lee report, utilizing a two-chamber PDMS device generated via soft lithography and populated with a primary vascular cell type (human primary placental villous endothelial cells (HPVECs)) and a placental choriocarcinoma epithelial cells (BeWo, ATCC), separated by a porous membrane coated with extracellular matrix components (38). As in the Lee study (39)(38)(39), the investigators demonstrated functionality of the device in terms of permeability, hormone production and nutrient transport (38). Both models of the trophoblast-vascular interface represent important advances in microscale modeling of the human placenta. However, a limitation of these OoC models was the simplified cellular community structure, lacking immune cells and other relevant non-immune cells such as decidual stromal cells. It is anticipated that these types of placental OoCs will further advance our understanding of maternal-fetal nutrient and waste transport, drug toxicity, immunology/tolerance, and the pathogenesis of infectious diseases as well as other complications of reproduction.

In a recent report, Sticker, et al., developed a multi-chambered microfluidic device that further enhance the development of complex in vitro cell cultures for placental research. These models will be essential for developing a robust culture system that provides the individual assessment of each cell type (40, 41). Professor Peter Erlt's group fabricated a 4-chamber device using a photosensitive thermoset (OSTEMER 322-40) as a porous membrane and used it to establish a compartmentalized tri-culture of human umbilical vein endothelial cells (HUVEC), BeWo cells, and adipose tissue-derived human mesenchymal stem cells (adMSCs) (40). These organs-on-chips offer an opportunity to enhance the multicellular in vitro models of complex tissues, including the placenta and fetal membranes, and demonstrates the increasing complexity of in vitro tissue modeling. Altogether, these models represent the driving interest in the development of OoCs of the gravid uterus to better understand maternal-fetal interactions.

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186 **Potential OoC Models of the Fetal Membrane**

187 Existing models of the human fetal membrane

188 Current studies of human fetal membrane immunology employ traditional cell and tissue culture models. Cell culture
189 is limited by the loss of the biological context provided by neighboring cells and matrix, while tissue culture is crippled
190 by an inability to maintain viability ex vivo for prolonged periods of time (without contamination). To advance fetal
191 membrane research, transwell platforms have been implemented to dissect the maternal and fetal polarization of these
192 membranes (42-44). In these models, the ex vivo human membrane tissue is used to recreate the membrane of a
193 transwell assay and thereby offers the ability to determine differences between the apical (amniotic) and basal
194 (choriodeciual) compartments of the fetal membrane tissue. Although this method allows for whole fetal membrane
195 cultures in similar anatomical context as would be seen in vivo including unidirectional pathway of an infection, its
196 reliance on inclusive human tissue acquisition, short term cultures, as well as intra-sample and donor-to-donor
197 variability, impact the reproducibility of results and limit its applications for mechanistic studies.

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199 Tissue culture also lacks the capability to dissect the roles of individual cell types within the context of whole tissue.
200 Typically, independent cell and tissue culture experiments are conducted and terminated at discrete time points
201 throughout the course of infection. This disjointed approach creates challenges for understanding the dynamic host-
202 microbial relationship. In addition, these culture systems suffer from dilutional effects imposed by the relatively large
203 culture media volumes employed (16). Animal models have been invaluable tools; however, they are also limited,
204 presenting endocrine, anatomical and immunological differences from humans (14, 15). Thus, there is an urgent need
205 to develop better human translational CAM models to eliminate species-specific differences and accelerate research
206 in immunology and microbiology (16, 17). Development of innovative OoCs of the fetal membrane would provide a
207 foundation for robust in vitro human studies that could supplement in vivo animal and ex vivo human studies (**Table**
208 **1**).

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Table 1. Potential advantages of an instrumented fetal membrane on a chip (IFMOC) device
Creates a highly defined, living model of human fetal membrane that can be maintained for days-to-weeks
The ability to define the contribution(s) of individual cell types to the immunology of intact membranes, facilitating high-resolution mapping of autocrine and paracrine signaling networks within this compartment
The potential to incorporate transgenic and gene-deficient cell types within the membranes and to define the contribution of particular genes and gene-networks to human reproductive immunology (and physiology)
The capacity to better model covariates such as fetal sex or race/ethnicity at the tissue level
The ability to incorporate the IFMOC into novel imaging tools and downstream analytics while preserving the capacity to perform longitudinal studies throughout the course of infection: from colonization to invasion

The Microenvironment of Fetal Membranes

Fetal membranes are a deceptively simple tissue structure (5, 45), composed primarily of decidual stromal cells, chorionic trophoblasts, fibroblasts (mesenchymal cells), a monolayer of amniotic epithelial cells, resident immune cells and a collagen-rich extracellular matrix. While the exact fetal membrane thickness and cell densities are variable between individuals, the overall cell ratios and histologic analysis between the somatic cells demonstrate a consistent composition (**Figure 2**). In our models, we have purposely omitted the structural mesenchymal cells for the sake of simplicity and focused on the functional reproductive cells. We also summarize the calculated histological composition of the fetal membrane and its cellular subtypes (**Figure 3a.**). These descriptive parameters provide an idealized scaling design for OoCs that completely or partially recapitulate the cellular components of the fetal membrane. It is important to keep in mind that these approximations do not consider subpopulations within each cell type and may change temporally and spatially between each sample. Immune cells exist within this structure, where approximately 35% of immune cells are T lymphocytes, 14% are NK cells, 9% are monocyte/macrophage cells and 2% are B cells (46, 47). $CD68^+$ Macrophages are therefore the major innate immune phagocyte in uninfected fetal membranes (48-51) and make up approximately 9-13% of total cells residing in the fetal membrane (**Figure 3a**). However, how macrophages govern host defense and inflammatory responses is uncertain. An interesting feature of fetal membranes is the coexistence of maternal-derived decidual macrophages and fetal-derived macrophages (a.k.a.

placental macrophages or Hofbauer cells) (52-55). Although studies have identified epigenetic differences between maternal and fetal macrophages isolated from the same gestational tissues (53), we lack knowledge regarding functional differences between macrophage subtypes in the context of infection. Animal data suggest that macrophages drive PTB in the setting of inflammation or infection but have not determined the contributions of maternal and fetal macrophages to this process (56-59). By integrating most of these cells in a tissue-level context that recapitulates the dynamic crosstalk of these somatic and immune cells, it may be possible to understand the roles of each cell type in the regulation of inflammation, maintaining homeostasis and avoiding CAM.

As noted above, CAM is a major cause of PTB (60), preterm premature rupture of membranes (PPROM) (61), stillbirth (62), and neonatal sepsis (63, 64). Bacterial CAM provokes inflammatory responses that trigger early labor (65) and contribute to a fetal inflammatory response syndrome (FIRS) (60). Unfortunately, CAM is often clinically silent until an adverse event occurs (66). Developing accurate, early diagnostic tests and preventive and/or therapeutic interventions requires a better understanding of mechanisms of disease pathogenesis, particularly events occurring early during host-microbial interaction. While the above section described published OoC models of the gravid uterus, there is not, to our knowledge, a functional OoC of the human fetal membrane. Using OoC technologies it may be possible to develop a living fetal membrane model to characterize cellular interactions and response to infections **(19)**. Conceptually, a highly defined in vitro model of human fetal membrane that can be maintained for days-to-weeks with an ability to define the contribution(s) of individual cell types to the immunology of intact membranes would facilitate the high-resolution mapping of autocrine and paracrine signaling networks that occur within this tissue. This compartmentalized model would enable both phenotypic and biochemical analytics to characterize fetal membrane barrier integrity as a method to study CAM mediated disease conditions (e.g. PPROM).

In order to reduce the burden of intra-sample variability, commercially available cell lines are commonly used as displayed by the OoCs described above. Primary cells obtained from donors or patients also can be implemented within these models. Depending on the source of cells utilized, fetal membrane OoCs could developed to model either healthy or abnormal pregnancies. Primary cells could be employed, for example, from fetal membranes obtained from preterm labors or PPROM and used to study disease pathogenesis. Furthermore, induced pluripotent stem cells (iPSCs) could be obtained from mother-child pairs, even years postpartum, and differentiated into the unique fetal membrane

cell types for use in a membrane OoC. Genetic engineering tools, such as CRISPR/Cas9, can be harnessed to manipulate the contribution of specific genes to membrane biology. Thus, an effective fetal membrane OoC model might be modified to use diverse cell types gain a deeper insight into disease pathogenesis or to understand normal reproductive processes. This ability to incorporate primary cells into OoCs supports both precision and personalized medicine initiatives.

Herein, we provide an example of an idealized OoC model of the fetal membrane (**Figure 3b.)**; however, the design and complexity of models will certainly be subject to alternative configurations to satisfy the driving experimental question. Our team is currently developing an instrumented fetal membrane-on-chip (IFMOC) device to recapitulate anatomical, biochemical and reproductive processes of the fetal membrane that physically separates maternal and fetal components (**Figure 4**). We envision that the IFMOC will model important biological variables such as fetal sex and/or race/ethnicity at the tissue level and incorporate novel imaging tools and downstream analytics into longitudinal studies throughout the course of infection: from colonization to loss of membrane integrity**.**

Development of a first generation IFMOC

Deconstructing fetal membranes into component cell types in the highly-controlled environment of the IFMOC will provide the capacity to define the contribution of cell-type-specific signaling in response to infection. A major advantage of the IFMOC over native ex vivo fetal membranes is the ability to tightly control cell populations to reduce intra-sample variability (**Figure 4a**). Using microfluidics technologies, we can load, perfuse and stimulate each cell type separately, and inject, recirculate or sample media from each compartment to maintain short or long-term culture experimental designs. We have recently developed a universal dual-chamber microfluidic device using a high-resolution porous membrane to establish a model of the non-gravid endometrial perivascular stroma, which has provided the technology to establish a prototype of the first generation IFMOC. The microfabrication protocol describes a PDMS two-chamber device divided by high-resolution semipermeable resin-based (1002F) transparent membranes (67, 68). This membrane allows for both diffusion of molecules and passage of bacteria. It simultaneously serves as basal lamina to compartmentalize each cell type.

As a proof of principle of the applications of this device, our group used primary human umbilical vein endothelial cells (HUVECs) and endometrial stromal fibroblasts to model the perivascular stroma of the endometrium (68). Using this microfluidic platform, we functionally measured barrier integrity and hormone sensitivity of the stromal fibroblasts to undergo the process of decidualization in a long-term culture that mimicked the length and hormonal changes associated with an idealized menstrual cycle. These results provide the potential of this microfluidic model to maintain long-term cultures, mimics physiological responses to hormones, offers the potential to model endothelial (or epithelial barrier function) and ensures sufficient sensitivity of the device to biochemically measure paracrine molecules from the conditioned media (68). By applying these microfabrication and engineering technologies to the cells that make up the fetal membrane, we can begin to integrate the first generation of the IFMOC to model the compartmentalization of chorion/decidua and amniotic components of the fetal membrane in vitro while significantly reducing the total volumes required to establish a co-culture. As mentioned, this reduction may provide a stronger signaling network between compartmentalized cells and thus a more robust crosstalk. In the first series of experiments, we have established co-cultures of amniotic epithelial cells and decidual cells using the two-chamber device (**Figure 4b**). Similarly, we have established amniotic epithelial cell co-cultures with trophoblasts (not shown). The goal remains to compartmentalize each cell type to assess their individual contribution to fetal membrane homeostasis and function, perhaps using existing multi-chambered devices as in the work by Sticker and colleagues (40). We have initially focused the IFMOC to identify possible roles of the choriodecidua in regulating amniotic epithelial barrier integrity in response to bacterial infections during CAM-induced PPROM. After optimization of co-culture of the adherent primary cells inside the IFMOC, (i.e., decidual cells, chorionic trophoblasts, mesenchymal fibroblasts, and amniotic epithelial cells) we will introduce macrophages, as a representative leukocyte, to assess the immunological interaction in CAM. However, the capability to introduce other key immunological cells (e.g., neutrophils) is feasible within this microfluidic platform.

Assessment of response to infection using IFMOC

To utilize the IFMOC as a qualitative and quantitative tool, we propose introducing electrical sensors or biological reporters to assess for markers of alterations in metabolic activity (glucose consumption, extracellular acidification and lactate production) and oxidative stress (superoxide generation). As a demonstration of feasibility of this approach, we have used a microfluidic multianalyte microphysiometer (MAMP) to assess macrophage metabolic responses to

infection (69). On-chip metabolism can be measured by utilizing screen-printed electrodes for microphysiometry, allowing real-time assessment of glucose utilization and lactate production, as above (70, 71). Inflammatory mediators and metalloproteinases produced during CAM may lead to PPROM and can be quantified through secretion sampling of spent media through direct enzyme linked immunoassay (ELISA) or high throughput proteomics such as protein multiplex arrays, as well as fluorescence based biosensors and functional enzymatic activity assays (72, 73). As described above, barrier integrity of the amniotic epithelium is important for membrane physiology and quantitative models of this component of the fetal membrane will provide insight into the putative pathophysiological mechanisms of PPROM. This approach will facilitate our ability to test our sub-hypothesis regarding the contribution of macrophages to the pro- and anti-inflammatory balance and effect of inflammation on the integrity of the fetal membrane.

Discussion

Maternal-fetal health is a field of research that is difficult to study due to both complex ethical and physiological dilemmas, which have undermined our understanding of pregnancy related disease processes including preterm birth. Specifically, the lack of translatable and physiological models that recapitulate human conditions hinder both the ability to examine the mechanisms behind reproductive disorders, such CAM that result in PTB, and identify therapeutic targets. As mentioned above, the fetal membrane plays an integral physiologic function to create a barrier and encapsulate the embryo to form a maternal-fetal interface. Under the correct temporal signals, the orchestrated rupture of this membrane is a necessary process during term deliveries. However, PPROM is a major contributor to prematurity and accounts for approximately 25% (74) of all PTBs. To facilitate our understanding of the pathophysiology of these diseases, we must identify the interactive contributions of the major cell types that comprise the microenvironment of the fetal membrane. This objective requires more robust, quantitative models that recapitulate the human condition. Current models to understand this issue include animal models, human ex vivo tissue approaches, and in vitro cultures of human reproductive cells. Emerging approaches, including OoC and microfluidic technologies offer innovative technologies to enhance the in vitro modelling of human organs and tissues. In recent years, significant interest has been put forth by several agencies including the Environmental Protection Agency (EPA), the Human Placenta Project (National Institutes of Health)(75) and the National Center for Advancing Translational

Sciences (NCATS) to develop these organotypic models of the reproductive tract, including the gravid uterus. Thus, we introduce the IFMOC as an approach to compartmentalize the cells that form the fetal membrane in order understand the immune-endocrine mechanisms behind CAM and PPROM induced preterm birth.

The integration between engineering and reproductive biology promises to provide novel robust models as instrumented tools for discovery research and predictive toxicology for environmental chemicals and developing drugs. The ability to dissect the intercellular communication at the tissue-level in vitro offers the ability to examine how, for example, endocrine disrupting chemicals, may alter the gravid uterus and drive it towards a pathogenic state (19, 21). Akin to the ex vivo fetal membrane experimental models described above, an IFMOC will provide a "living" model of the fetal membrane, including tissue polarity, analysis of cell specific paracrine networks, and membrane barrier function (**Figure 2**). These functional quantitative and qualitative outcomes will facilitate our understanding of bacterial colonization and transmittance from the maternal side to the amnion. Although infections are a common causes of PTB, only a subset of women develop CAM-induced PTB, suggesting that other stressors are at play.

We and others have observed that environmental toxicants may cause disruptions of immune-endocrine pathways during infection-related processes which may contribute to disease pathogenesis, although, human epidemiological data has been less conclusive (76-78). Animals models have shown that environmental toxicant exposures, such as endocrine disputing chemicals such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD or dioxin) enhance the inflammatory response of pregnant dams resulting in a high incidence of spontaneous preterm birth in response to low level lipopolysaccharide (LPS) stimuli compared to vehicle treated controls (79, 80). The mechanisms behind this phenomenon remain elusive. An IFMOC may provide insight into the disparity between infectious stimuli and PTB by identify adverse outcome pathways from interacting toxicant and CAM pathogenic mechanisms.

As mentioned, a key advantage of OoCs over current in vitro models include its microfluidic nature to maintain individualized chamber perfusion, aid in maintaining long-term cultures, reduce total culture volumes and introduce hemodynamic forces (e.g. shear stress) if desired. However, these microfluidic technologies also offer the possibility to interconnect different OoCs in tandem to mimic the systemic communication between organs. As an example, by interconnecting the IFMOC downstream of a liver organotypic model (e.g., liver-on-a-chip) it may provide a more

robust physiologic response by modelling how xenobiotics pass through the liver, where they may be metabolized, prior to reaching the fetal membrane. These serial interactions between organs may continue to enhance the complexity of in vitro systems and provide innovative modelling avenues for reproductive research.

Lastly, it is important to note that like any in vitro model, the OoCs do have intrinsic limitations in that they cannot fully reproduce all biological and physiological processes. In our first generation IFMOC, we focused on the macrophages as a representative immune cell that plays a critical role in both physiological and pathological processes of the fetal membrane. However, additional or alternative cell types, such as neutrophils or natural killer cells, can be incorporated within the platform to characterize their contribution to inflammatory processes in response to infections. Some technical limitations involve the properties of polydimethylsiloxane (PDMS), the prototypical material from which many of these devices are fabricated, including our first generation IFMOC. While PDMS serves as the gold standard for microfabrication as a biocompatible and air permeable substrate, its hydrophobic nature is prone to absorb lipophilic molecules (81, 82). This effect may hinder the immediate ability to perform detailed pharmacokinetic/pharmacodynamic (PK/PD) analysis of certain drugs; nonetheless these OoCs can provide an initial phenotypic screening tool until novel alternative materials become available. Lastly, the idealized IFMOC described herein focuses primarily on paracrine communication as a means of crosstalk between cells; however, we must consider that cell-cell contact and cell-ECM play a critical role to mediate tissue homeostasis. While these models may not be able to replicate all physiologic conditions that are found in vivo, OoCs are an innovative emerging technology with the promise of enhancing current human in vitro studies to generate more comprehensive understanding into tissue homeostasis and disease pathogenesis.

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Figure Legends

FIGURE 1. Chorioamnionitis and pregnancy complications. (A.) A simplified model of the pregnant uterus, with the fetal membranes (red) extending from the placenta and surrounding the developing fetus, which is suspended in amniotic fluid. (B.) Bacteria are drawn colonizing the vagina, ascending through the cervical canal, and invading the fetal membranes to provoke an inflammatory response (chorioamnionitis). Infection can spread to the fetus causing fetal inflammatory response syndrome (FIRS) or the placenta (placentitis). Complications of chorioamnionitis include preterm premature rupture of the fetal membranes (PPROM), preterm birth, stillbirth or neonatal sepsis.

FIGURE 2. Histologic characterization of the fetal membrane structure. The fetal membrane is composed of representative layers that include the chorion (A.) primarily consisting of trophoblasts, the decidua (B.), and an amniotic epithelial monolayer (C.). Resident immune cells, including, macrophages (D.), structural mesenchymal cells and extracellular matrix make up the remainder of the microenvironment. The histologic dimensions of each component was approximated by analyzing at least four representative images (original magnification 20X) from six different 2mm punch biopsies of fetal membranes from human term non-laboring pregnancies using a protocol approved by the Vanderbilt University Institutional Review Board. Analysis includes standard deviation of the sample. Macrophages density assessed by measuring positive staining for CD68 by immunohistochemistry. For a representative of the total leukocyte distributions, please refer to (44).

FIGURE 3. Conceptualization for an instrumented fetal membrane on a chip (IFMOC). Allosteric and functional scaling are critical facets of tissue modeling in order to incorporate the appropriate cell types at physiologic ratios. The idealized cellular microenvironment and tissue composition are summarized in (A.) as an aid to scale and develop innovative models of the fetal membrane. (B.) A conceptualized schematic of an IFMOC may recapitulate the microfluidic scaling and compartmentalize the cellular composition of the fetal membrane in a multi-culture system. These models may provide insight into intercellular crosstalk and pathophysiology of CAM and PPROM.

FIGURE 4. A prototype of the first generation IFMOC. (A.) Fetal membranes are primarily composed of amnion epithelial cells, chorion trophoblasts, residing leukocytes and decidual stromal cell. Our interest in macrophages stems

633 from a sub-hypothesis to examine their role in inflammatory processes of the fetal membrane, but it is important to
634 note, that any immune cell of interest can be incorporated within this system. (B.) A schematic of the development of
635 the first generation IFMOC using a two-chamber microfluidic device for analysis of inflammatory networks and
636 membrane barrier integrity. (C.) Immunofluorescent images of a compartmentalized co-culture of amnion epithelial
637 cells and primary decidualized stromal cells. Scale bar represents 400 µm, unless otherwise noted.
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