Article:

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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 cause a fetal inflammatory response syndrome (FIRS) and can spread to the fetus and/or the placenta (placentitis). Complications of chorioamnionitis include premature preterm 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 maternal decidua (B.), and an amniotic epithelial monolayer (C.). Resident immune cells (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 obtained from human non-laboring term placentas after informed consent 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 and at physiological 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 decidua stromal cell. Our interest in macrophages stems from a sub-hypothesis to examine their role in inflammatory processes of the fetal membrane, but it is important to note, that any immune cell of interest can be incorporated within this system. (B.) A schematic of the development of the first generation IFMOC using a two-chamber microfluidic device for analysis of inflammatory networks and membrane barrier integrity. (C.) Immunofluorescent images of a compartmentalized co-culture of amniotic epithelial cells and primary decidualized stromal cells. Scale bar represents 400 µm, unless otherwise noted.

Figures

Figure 1:

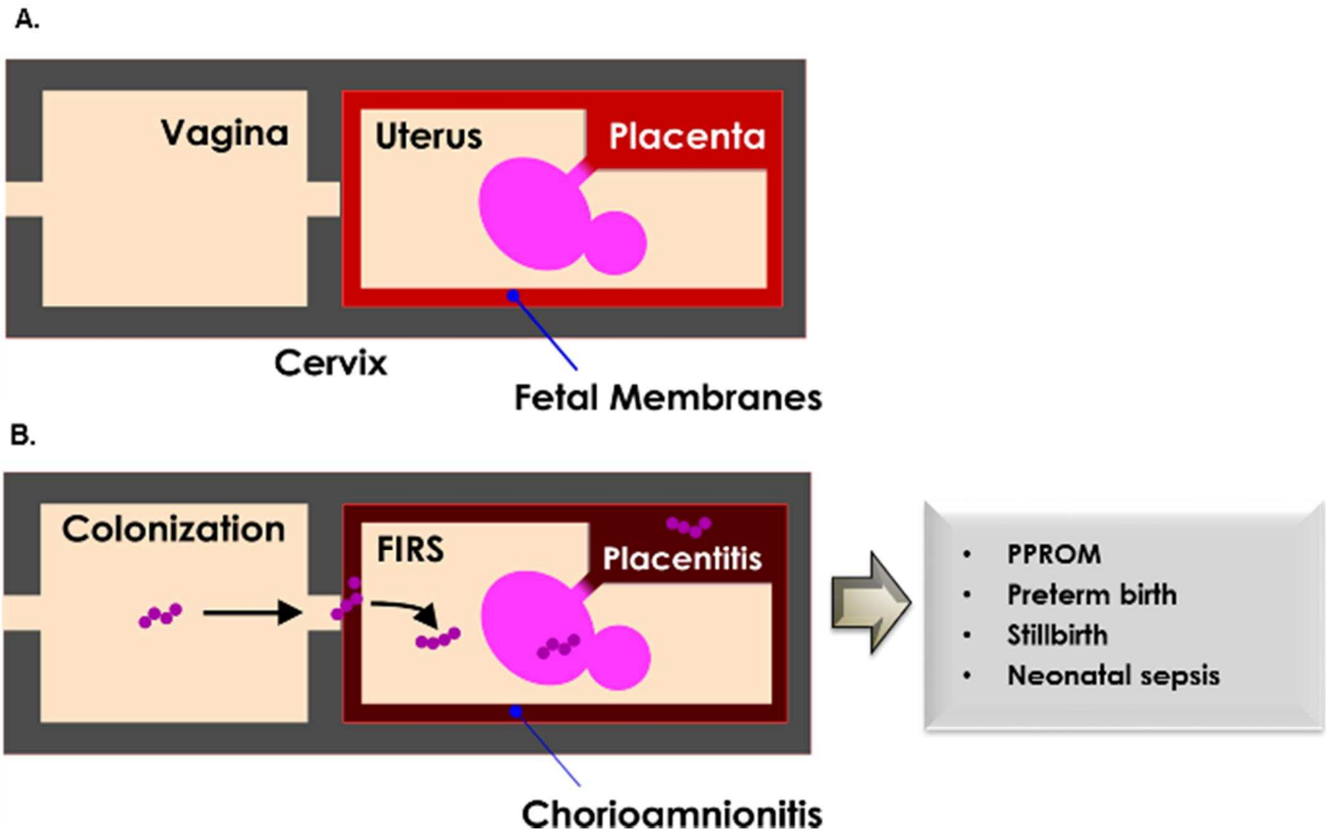


Figure 2:

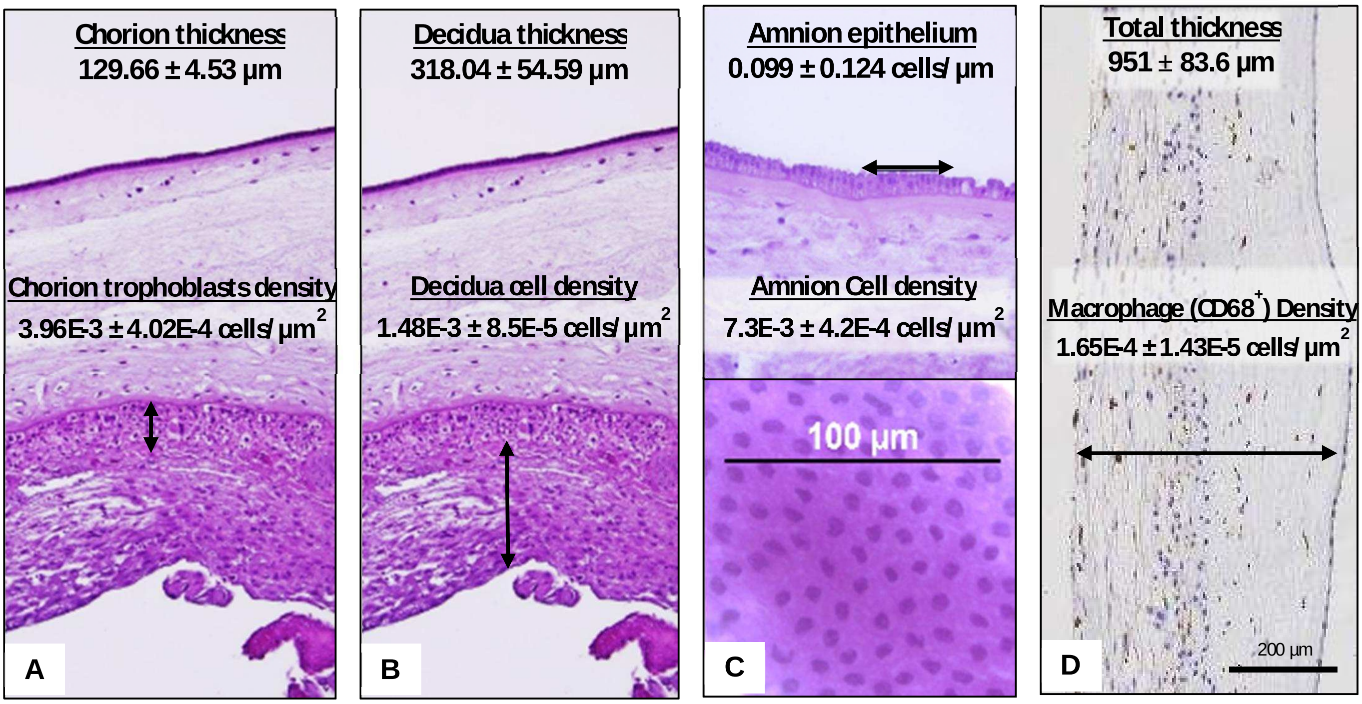
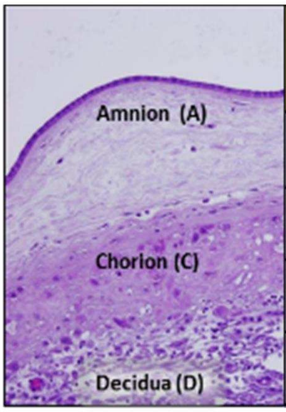

Chorion thickness
129.66 ± 4.53 µm
Chorion trophoblasts density
3.96E-3 ± 4.02E-4 cells/ µm2
A
Decidua thickness
318.04 ± 54.59 µm
Decidua cell density
1.48E-3 ± 8.5E-5 cells/ µm2
B
Amnion epithelium
0.099 ± 0.124 cells/ µm
Amnion Cell density
7.3E-3 ± 4.2E-4 cells/ µm2
100 µm
C
Total thickness
951 ± 83.6 µm
Macrophage (CD68+) Density
1.65E-4 ± 1.43E-5 cells/ µm2
200 µm
D

Figure 3:

A.



Fetal membrane cell types		Cell density (cells/µm²)	Tissue thickness (µm)	Tissue composition[a] (%)
Amniotic epithelium		7.3E-03 ± 4.2E-04	0.099 ± 0.124*	0.6
Chorion trophoblasts		3.96E-03 ± 4.02E-04	129.66 ± 4.53	45
Immune cells (e.g. Mϕ)		1.65E-04 ± 1.43E-05	951 ± 83.6¥	9-13[44]
Decidual cells		1.48E-03 ± 8.5E-05	318.04 ± 54.59	41

*Cells/µm, ªApproximation, ¥Total FM thickness
Note: mesenchymal fibroblasts not included

B.

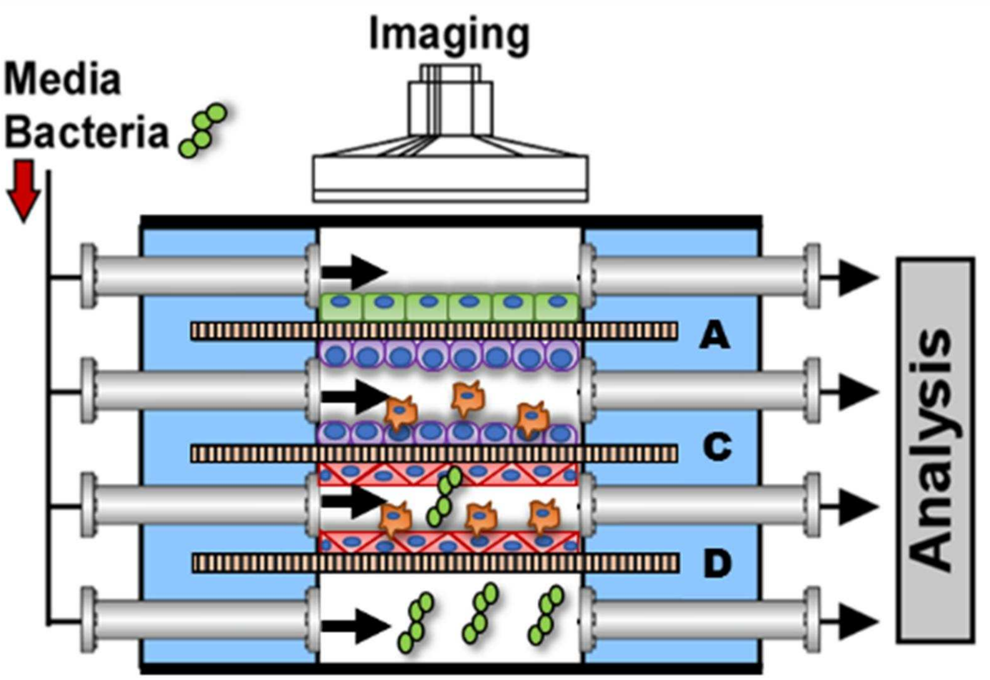
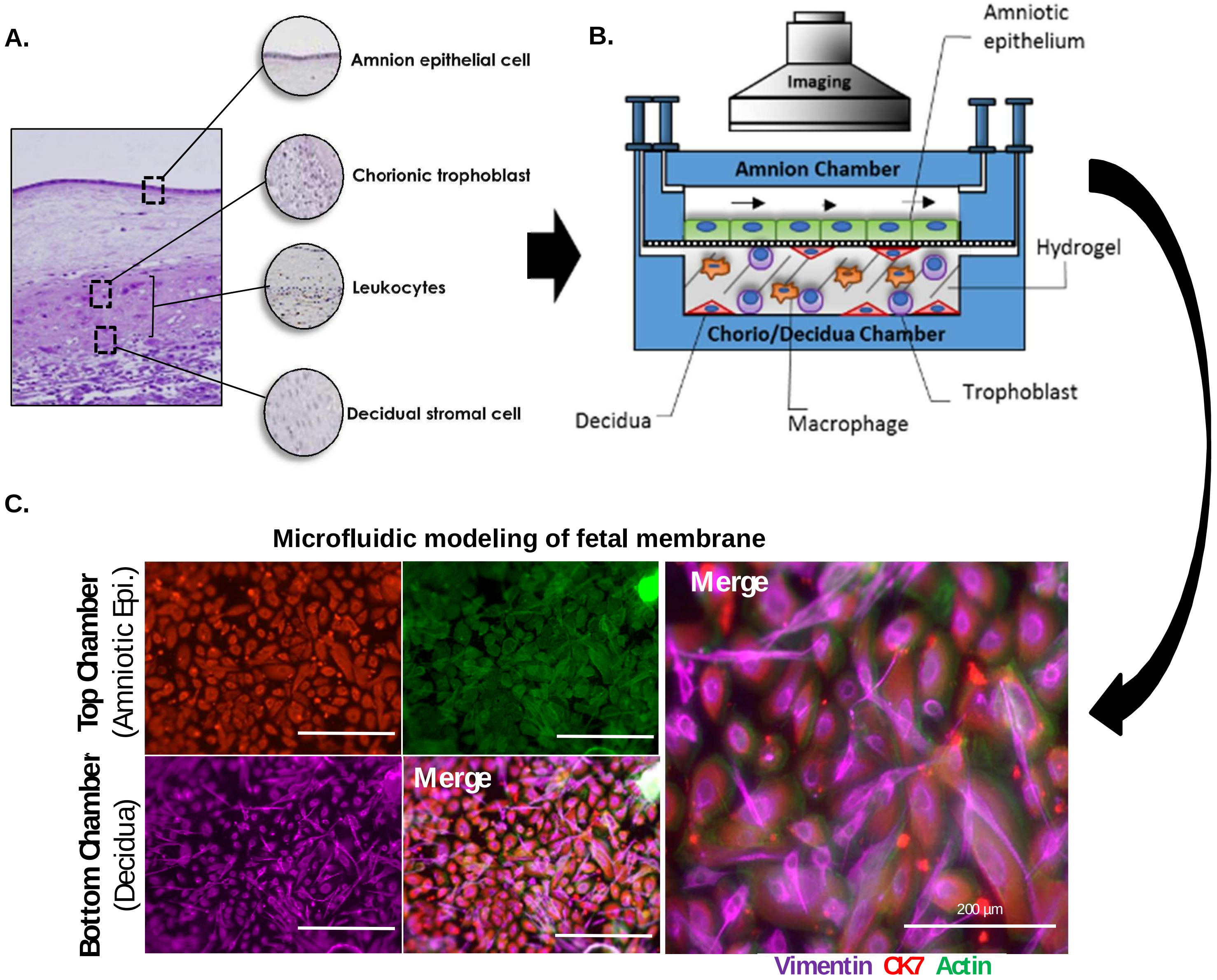


Table 1:

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

Figure 4:


A.
Amnion epithelial cell
Chorionic trophoblast
Leukocytes
Decidual stromal cell
B.
Imaging
Amniotic epithelium
Amnion Chamber
Hydrogel
Chorio/Decidua Chamber
Trophoblast
Decidua
Macrophage
C.
Microfluidic modeling of fetal membrane
Top Chamber (Amniotic Epi.)
Bottom Chamber (Decidua)
Merge
Merge
200 µm
Vimentin CK7 Actin