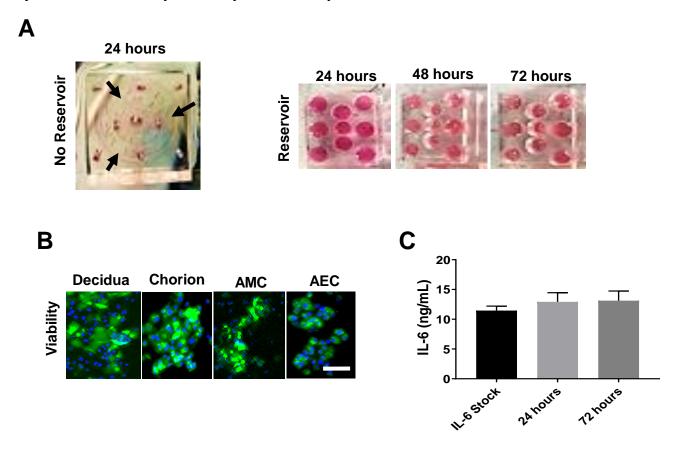
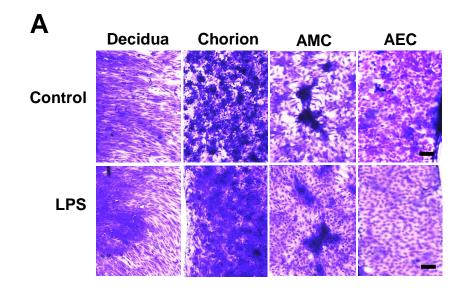
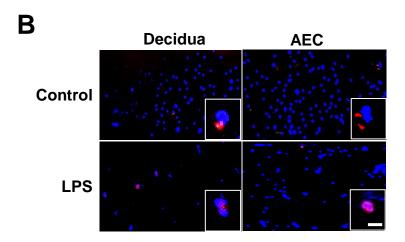
Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2020



## Supplemental Figure 1: Initial FMi-OOC device characterization for testing cell culture and media collection capabilities.

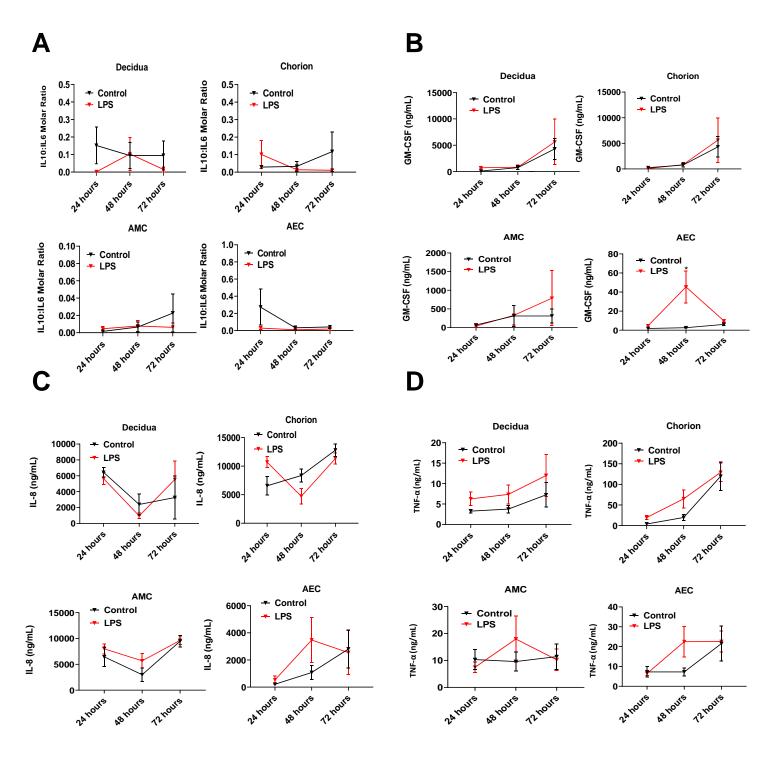
- A) The FMi-OOC device without the top media reservoir layer, showing fast evaporation of media within 24 h, where bubbles in the cell culture chambers can be seen (black arrows). The FMi-OOC device with the on-chip reservoir layer attached on top, which enabled maintaining media levels within the cell culture chambers and reservoirs for up to 72 h. Scale bar =  $50 \mu m$ .
- **B)** Example images of cells grown within the FMi-OOC for 72 h, showing good viability as indicated by strong calcein AM stain (green) but no ethidium homodimer-1 stain (red).
- **C)** ELISA measured pro-inflammatory cytokine IL-6 concentrations within the FMi-OOC, showing no difference over a 72 h period, indicating no or limited absorption of cytokines to the PDMS device or reservoir system (n = 3). Values are expressed as mean  $\pm$  SEM.





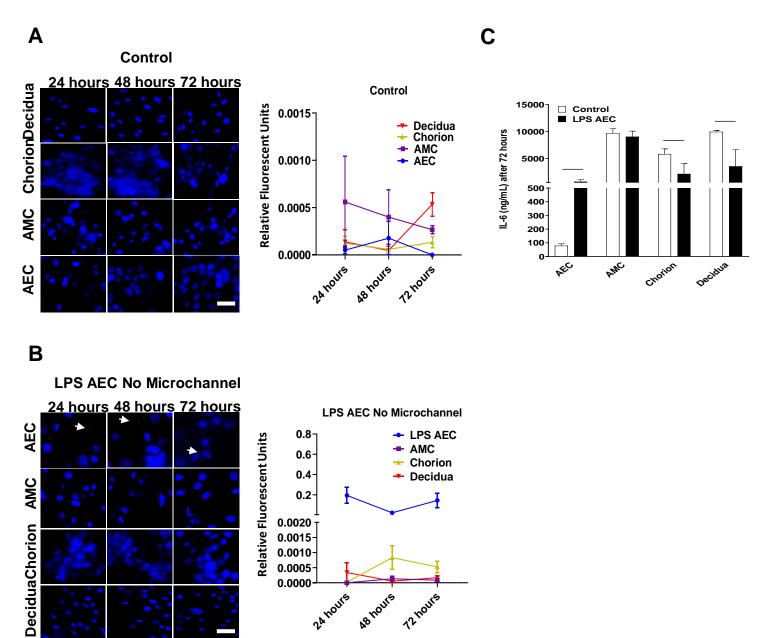
Supplemental Figure 2: LPS-induced NF-kB translocation but showing no cell death within the FMi-OOC system.

- A) Maternal and fetal cells remained viable, as noted by the retention of crystal violet stain, even after LPS treatment on the maternal side for 72 h. Scale bar =  $50 \mu m$ .
- **B)** LPS-treated decidua and AECs within the FMi-OOC induced NF-kB (red) translocation from the cytoplasm to the nucleus, documenting its activation (n = 3). Scale bar = 10  $\mu$ m. Blue DAPI, Red NF-kB.



Supplemental Figure 3: Production and propagation of pro- and anti-inflammatory mediators in the FMi-OOC.

- **A)** Multiplex measured pro- and anti-inflammatory cytokines, IL-6 and IL-10, converted to their molar ratios and graphed for each cell type and time point (n = 4).
- **B)** Multiplex measured pro-inflammatory cytokine GM-CSF (AEC: P=0.032), **C)** IL-8, and **D)** TNF- $\alpha$  in each cell culture chamber after 24 h, 48 h, and 72 h (n=4). Values are expressed as mean  $\pm$  SEM.



Supplemental Figure 4: Experimental controls for the LPS propagation studies within the FMi-OOC system

A) To ensure that we did not have any non-specific binding of LPS antibodies, control FMi-OOCs were stained following the immunocytochemistry protocol but without the primary antibody. LPS was not detected in any cell or time point as seen by no red LPS dots (n = 3). Scale bar = 10 µm. Blue – DAPI, Red – LPS. Values are expressed as mean intensities  $\pm$  SEM. **B-C)** To confirm that our model relied on LPS trafficking through the interconnecting microchannels to mimic ascending infection *in utero*, an FMi-OOC was designed without microchannel connections between the cell culture chambers. AECs treated with LPS (100 ng/mL) (red; white arrows) for 72 h neither propagated LPS nor inflammation (**C**) to other cell chambers due to the lack of microchannels. However, AECs were found to have LPS at all time points (**B**) and showed higher inflammation after 72 h compared to other cell chambers (**C**). The line above the bar graph means at least 2-fold change. Scale bar = 10 µm. Blue – DAPI, Red – LPS. Values are expressed as mean intensities  $\pm$  SEM.