Title: Tissue engineered organoid co-culture model of the cycling human endometrium in a fully-defined synthetic extracellular matrix.

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Abstract:

The human endometrium is a mucosal barrier that undergoes cycles of growth, differentiation, and breakdown in response to sex hormone fluctuations. Dynamic tissue responses to hormones are primarily driven by epithelial-stromal communication and its dysregulation is linked to myriad gynecological disorders. The lack of robust *in vitro* models for the long-term 3D co-culture of patient-derived endometrial epithelial and stromal cells hinders dissection of this crosstalk and thus impairs progress in disease treatment. Here, we describe a versatile synthetic extracellular matrix tailored to the endometrium that enables the *in vitro* modeling of human healthy and disease states across the menstrual cycle. We used a tissue-inspired approach to semi-empirically screen a parameter space that encompasses the biophysical and molecular features of the endometrial microenvironment. Leveraging cell-specific integrin expression profiles, we defined a modular polyethylene glycol (PEG)-based hydrogel that fosters hormone-driven expansion and differentiation of epithelial organoids co-cultured with stromal cells. Characteristic morphological and molecular responses of each cell type to hormone changes were observed when cells were co-encapsulated in hydrogels tuned to a stiffness regime similar to the native tissue and functionalized with a collagen-derived adhesion peptide (GFOGER) and a fibronectin-derived peptide (PHSRN-K-RGD). Using transcriptomic and functional assays, we demonstrate the system's ability to recapitulate menstrual-cycle specific reproductive events and identified that inflammation-induced dysregulation of epithelial proliferation is mediated via the stromal compartment. Altogether, we demonstrate the development of a fully synthetic matrix to sustain the dynamic changes of the endometrial microenvironment and support its applications to understand endometriotic diseases.

1. Introduction:

The endometrium is the mucosal lining of the uterus in which the establishment and maintenance of pregnancy occurs¹. It is a highly dynamic tissue that undergoes spatial and temporal changes in response to endocrine signaling by the ovarian sex hormones estradiol (E₂) and progesterone (P4). Cyclical changes in these hormones dictate the timing and functional capabilities of the endometrium to support nidation by driving cell-specific morphological and biochemical processes². Histologically, the endometrium is primarily composed of the hormone-responsive epithelium and specialized reticular fibroblasts (stromal cells) embedded in a dynamic extracellular matrix (ECM).³⁻⁵ Endocrine-mediated reproductive function is mediated through both direct and indirect mechanisms governed by the crosstalk between these cell populations.⁶⁻⁸ The idealized 28-day human menstrual cycle is characterized by three distinct phases in which the endometrium undergoes E₂-mediated tissue regeneration (the 'proliferative phase') followed by a 14-day P4-dominant phase (the 'secretory phase') that governs the differentiation processes necessary for the successful establishment of pregnancy (Fig 1A). In the absence of embryo implantation, a sharp withdrawal of hormones triggers a cascade of inflammatory processes that results in a rapid influx of leukocytes and cellular apoptosis that promote the shedding of the endometrial tissue (the 'menstrual phase') after which the cycle initiates again.^{3,4} Despite the fundamental role E₂ and P4 play in maintaining reproductive and systemic functions, a thorough understanding of the cellular mechanisms driving these processes remains elusive due in part to a lack of physiological models that recapitulate the complex multi-cellular human condition.^{3,9}

Experimental models capable of recapitulating epithelial-stromal crosstalk are necessary to understand human reproductive physiology and pathology. Endometrial stromal cells (ESCs) are historically the most utilized primary cell type for experimentation in reproductive biology due to their ability to be cultured and expanded *in vitro*. However, the isolation, culture, and expansion of primary endometrial epithelial cells has been a challenge. Recently, protocols to harvest, expand, and maintain primary endometrial epithelial cells as endometrial epithelial organoids (EEOs) have been developed. Organoids are self-organizing 3D cell structures that retain many physiologically relevant and functional features of their native tissues of origin.¹⁰ thus, EEOs provide opportunities to investigate human reproductive function, understand disease pathogenesis¹¹, and test therapeutic compounds for clinical applications.^{12,13} EEOs can be generated from isolated primary adult endometrial glands embedded in Matrigel, a complex, tumor-derived ECM primarily composed of heterogenous basement membrane (BM) proteins and growth factors (GFs), and a defined culture medium designed to enrich the expansion of the

stem/progenitor compartment.^{14,15} Clonally-generated EEOs retain columnar epithelial architecture of the native tissue and form polarized cyst-like structures that are genomic stable, hormone-responsive, and cell-heterogenous.^{12,13,16} However, the reliance on Matrigel to establish organoid systems presents a critical obstacle for numerous experimental, analytical, and therapeutic applications for dynamic and hormone-sensitive tissues such as the endometrium.^{17,18} Matrigel's inherent lot-to-lot variability, poorly-defined molecular composition, and limited tunability in biophysical and biomolecular properties have driven efforts to develop synthetic (or semi-synthetic) ECMs that are fully defined, modular, and tunable.^{11,19–27}

Although models that introduce the epithelial and stromal compartment in 3D using naturally-derived hydrogels have been developed, they are severely limited by a rapid ECM consumption and loss of ECM integrity, which ultimately leads to highly abbreviated and artificial experimental conditions.^{28–31} Moreover, these models often lack the biophysical and molecular composition of the native endometrium that can reliably support the morphogenic and simultaneous functional behavior of multiple cell types. Recently, the development of fully or semi-synthetic ECMs to culture enteric organoids were reported for both; murine^{24,32} and human^{18,22,27,32} tissues. We have previously demonstrated using cell lines that poly(ethylene glycol) (PEG)-based hydrogel systems can be applied to model the endometrial mucosal barrier.²⁶ We recently identified a completely synthetic hydrogel formulation designed for the culture, differentiation and passaging of human intestinal enteroids that also supported other epithelial-derived tissues, including endometrial epithelial cells²⁷. Furthermore, we have recently implemented this matrix to support the culture of pancreatic tumor organoids with a complex stroma.³³ Despite these advances, a fully-defined synthetic ECM that can support the long-term culture and function of multiple endometrial cell populations still predominates as an unmet need.

Inspired by the integrin expression and ECM composition found in the native tissue, we designed a hydrogel matrix that targets cell-specific integrins, supports cell-secreted ECM deposition, and mimics the biophysical properties of the endometrium. Here, we developed a bio-labile PEG-based synthetic ECM that supports the simultaneous culture of primary human EEOs and ESCs in a 3D co-culture model of the endometrium that recapitulates the sex hormone-induced phases of the human menstrual cycle. We validated the system using a 15-day *in vitro* co-culture that robustly modeled the phases associated with a human menstrual cycle. Moreover, we utilized this co-culture model to examine how inflammatory stimuli promoted disease-associated progesterone resistance and morphological aberrations suggestive of a dysfunctional endometrium. Altogether, this synthetic ECM supported the

112 generation of a multi-cellular tissue engineered endometrium that recapitulates key reproductive
113 physiological and pathological processes, representing a significant advance in our ability to
114 model and study human reproductive function and diseases *in vitro*.
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2. Methods:

2.1. Tissue Acquisition and Isolation of Human Endometrial Cells

All participants provided informed consent in accordance with a protocol approved by the Partners Human Research Committee and the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects (Protocol number IRB-P001994). Endometrial tissue was obtained from pipelle biopsies from reproductive age women (N=18, ages 18-45, Fig S15) undergoing laparoscopic surgery for non-malignant gynecologic indications. Study enrollment was limited to pre-menopausal women and excluded patients with an irregular or ambiguous cycle or a history of hormone use in the 3 months prior to surgery. Endometrial epithelial glands and stromal cells were isolated by enzymatic digestion and filter-separated as previously described³⁴ resulting in $\geq 95\%$ purity as assessed by positive staining for vimentin and morphological assessment. Briefly, single stromal cells were separated from intact epithelial glands fragments and cultured separately. Stromal cells were expanded in traditional 2D cultures (as described below) and epithelial glands were cultured as organoids using protocols adapted from Turco et al.³⁵ All cells were passaged a maximum of 5 times for all experimental *in vitro* assessments.

2.2. Cell Isolation and Endometrial Organoid Generation

Stromal cells were maintained in phenol red-free DMEM/F12 with 5% charcoal-stripped calf serum, 1 nM 17- β estradiol (E₂, Sigma Aldrich) and 1 $\times$ Pen-Strep solution (Sigma Aldrich). Some cell cultures were treated with 500 nM synthetic progesterone medroxyprogesterone acetate (MPA, Sigma Aldrich). Primary endometrial epithelial organoids (EEOs) were generated from the primary epithelial fragments (p0) according to established protocols and maintained in endometrial epithelial organoid expansion medium (EEO medium).^{12,13} EEO media is modified from elsewhere.³⁶ Briefly, EEO media was composed of advanced basal media (1X); rhEGF (50 ng/mL, Corning); rhNoggin (100 ng/mL, Peprotech); rhRspodin-1 (200 ng/mL, R&D systems); rhFGF-10 (50 ng/mL, Peprotech); Estradiol (E₂, 1nM, Sigma Aldrich); Nicotinamide (1mM); Insulin-Transferrin-Selenium (ITS, 1%, Invitrogen); N-Acetyl-L-Cysteine (1.25 mM); TGFB pathway inhibitor (A83-01, 500 nM, Peprotech); and Rock inhibitor Y-27632, (ROCKi, 10 μ M, Tocris) which was utilized for the initial culture set up and for 4 days post seeding. For seeding, epithelial cells, as fragments or single cell suspensions, were embedded in 70% Matrigel and cultured for 6 days, media was changed every two days (Fig S2). For passaging, 6-day old

organoids were incubated in Cell Recovery Solution (CRS, Gibco) for 30 mins at 4°C to dissolve Matrigel, then pelleted and digested with Tryp-LE supplemented with 1:100 DNase (40k units, Sigma) for 15 minutes in a 37°C water bath followed by mechanical dissolution to generate single cells. For expansion, single epithelial cells were seeded in Matrigel at a density of 1,000 cells/μL in 60μL droplets in non-tissue culture treated plates. To establish co-cultures of ESC and EEOs, ROCKi was omitted from EEO media. All media changes for the co-cultures occurred every 3 days for up to 15 days unless noted otherwise. Spent conditioned media from these cultures was collected and stored at -20°C for downstream analysis.

2.3. PEG hydrogel materials and peptides.

8-arm poly(ethylene glycol) (PEG) macromers (20 kDa) functionalized with vinyl sulfone (20 kDa PEG-VS) were purchased from JenKem Technology. All peptides were custom synthesized and purified (>95%) by Boston Open Labs (Cambridge, MA), GenScript (Piscataway, NJ), or CPC Scientific (Sunnyvale, CA). Peptides used in these studies include; a dithiol crosslinking peptide containing a matrix metalloproteinase (MMP)-sensitive substrate (Ac)GCRD-LPRTG-GPQGIWGQ-DRCG(Am) (**CL-LW**); a fibronectin (FN)-derived peptide containing both the canonical RGD motif from the 10th FN type III domain as well as the PHSRN synergy site from the 9th FN Type III repeat in a branched configuration akin to the biophysical presentation in FN, (Ac)PHSRNGGGK-GGGERCG(Ac) -GGRGDSPY(Am) (**PHSRN-K-RGD**);³⁷⁻³⁹ a Collagen I-derived peptide, (Ac)GGYGGGPG(GPP)₅GFOGER(GPP)₅GPC(Am) (**GFOGER**);^{22,40} a peptide with affinity for sequestering cell-produced FN, (Ac)KKGCRE-TLQPVYEYMGV(Am) (**FN-binder**);⁴¹ and a peptide with affinity for sequestering the basement membrane proteins type IV collagen and laminin, (Ac)GCRE-ISAFLGIPFAEPPMGPRRFLPPEPKKP(Am) (**BM-binder**).⁴² All peptides were reconstituted in acidic (pH 5.5) Milli-Q water (Millipore). The concentration of free thiols in all peptides was determined using Ellman's reagent (Sigma Aldrich).

2.4. Fabrication of synthetic PEG extracellular matrix.

All synthetic matrices were assembled to screen a range between 3-7 wt% PEG (w/v) using an 8-arm 20kDa PEG-VS backbone. Functionalization of the PEG-VS macromers (fPEG-VS) was performed stepwise via Michael-type addition reaction according to the concentrations determined prior to experimental set up. First, the PEG-VS macromer was diluted with 10X PBS-HEPES solution (1 M, pH 8.2) at 10% total solution volume and ultrapure H₂O. Second, the integrin binder peptides (**PHSRN-K-RGD**, **GFOGER**) and the matrix-binder peptides (**FN-binder**, **BM-binder**) were added to the reaction mixture, immediately vortexed and allowed to

react for 30 min at RT with at pH 7.8. Third, cells were counted, pelleted and gently resuspended with functionalized PEG-VS (fPEG-VS). Finally, upon cell resuspension, the crosslinker (CL-LW) was added to the mixture to initiate the gelation process, gently vortexed, and plated accordingly, typically in 3 μ L droplets in non-tissue culture treated 96-well plates unless otherwise specified. Cell-fPEG-VS mixture was incubated for at least 25 minutes in a humidified incubator at 37°C until hydrogels dollops were solidified. A test gel droplet was utilized as a marker for complete gelation before the addition of media.

2.5. Rheological characterization of hydrogels

To measure bulk bio-mechanical properties of crosslinked gels, 25 μ L of each gel matrix mixture was loaded into a 1 mL syringe that had the tip cut off at the 0.1 mL mark. The matrix was allowed to gel at 37 °C for 25 min and then extruded from the open syringe as a cylindrical disc and moved to a 24 well plate that contained 400 μ L of 1X PBS. The plate was incubated for 24 hours in a humidified incubator at 37 °C, 95% air, and 5% CO₂, to allow equilibrium swelling to occur prior to rheological characterization. This procedure generates hydrogel discs of 1.4 mm in thickness. The discs were sandwiched between an 8 mm sandblasted parallel plate geometry and sandblasted base. The shear modulus was determined by performing small-strain oscillatory shear measurements on an Anton Parr MCR 302 rheometer. The mechanical response was recorded by performing frequency sweep measurements (0.1–10 Hz) at a constant strain (0.05), at 37 °C. The elastic modulus (E) is reported as a measure of bulk matrix mechanical properties derived from the recorded storage modulus (G') in the linear elastic regime where: $E = 2G'(1 + \nu)$ with the assumption that $\nu = 0.5$ for ideal elastic hydrogel materials.

2.6. EEO generation in synthetic hydrogels

Endometrial organoids were established and expanded in Matrigel according to established protocols¹² with some minor modifications. Lumenized organoids with a diameter greater than 100 μ m were harvested with cold Cell Recovery Solution (CRS, ThermoFisher). Matrigel dollops were manually detached with a wide bore pipette tip, making sure to fully scrape the entire well to lift off the entire gel. The dollops were resuspended in cold CRS (5mL) for at least 30 mins on ice to dissolve remaining Matrigel, followed by centrifugation at xxxxx, which resulted in a clear pellet of organoids. Organoids were broken into single cell suspensions using 1mL of prewarmed TrypLE Express (ThermoFisher) supplemented with DNAase (10 μ L, 1:100) and continuous mechanical agitation until a single cell suspension of epithelial cells was observed.

Single cells were counted using a hemocytometer then resuspended in the functionalized PEG precursor (fPEG-VS) solutions prior to the addition of the crosslinker. In parallel, single cells were resuspended in Matrigel that served as an experimental control during matrix evaluation. In both cases, cells were encapsulated at a density of 500 cells/ μ L of matrix. Cell-matrix suspensions were seeded as 3 μ L droplets on a non-tissue treated 96 well plate and allowed to polymerize for 25 min in a humidified incubator at 37 °C, 95% air, and 5% CO₂. Complete gelation was examined visually on a test gel droplet prior to the addition of media. Gel droplets were maintained in 100 μ L of expansion EEO media supplemented with Y-27632 (10 μ M). Media was changed every three days.

2.7. Generation of co-culture model

Six-day old intact organoids (~100 μ m diameter) were grown and expanded in Matrigel and harvested as described above. To maintain EEO integrity, organoids were incubated in Cell Recovery Solution until Matrigel was fully dissolved. Careful resuspension of intact EEOs in DMEM/F12 was performed to prevent disrupting EEO integrity. In parallel, donor matched ESCs were expanded using traditional 2D polystyrene cell culture plates and grown to 80% confluency. ESCs were lifted from 2D culture plates using trypsin (1X) and processed to generate a single cell suspension. Intact EEOs and ESCs were prepared in parallel to encapsulate both cell types simultaneously and establish a co-culture in the PEG matrix solution. Cells were encapsulated at a density of 10,000 ESC and 10 intact EEOs per μ L of matrix. This epithelial-stromal ratio was determined experimentally based on the cell ratios utilized to generate established endometrial xenograft models (sub-renal capsule) described elsewhere^{43,44} and validated using whole-mount 3D image analysis of endometrial tissue we performed (Fig S5). Embedded cells in fPEG-vs were seeded in 3 μ L droplets and allowed to polymerize as described above in non-tissue culture treated 96-well plates. Co-culture was maintained in EEO media without Y-27632. Media was changed every three days and maintained for up to 15 days. It was noted that some basal components of the organoid medium contained P4 (N2 and B27) which may impact hormone responsive behavior, so a 'neutral' formulation of this organoid medium (nEEO medium) that completely lacked P4 was tested in some co-cultures. Matching monocultures of ESCs-only or EEOs-only were established in parallel for some experiments. Cultures were imaged daily.

2.8. EEO and co-culture efficiency and quantification

To quantify organoid diameter and total organoid formation efficiency, single epithelial cells were encapsulated in fPEG-VS hydrogels or Matrigel and cultured over 14 days. EEO formation efficiency was calculated as the percentage of organoids with a clear lumen with a diameter (>100 μ m) relative to Matrigel. 4X brightfield (BF) z-stack images of 10-day old endometrial organoids were captured using an EVOS M500 microscope (Invitrogen). The images were processed in Fiji using the time lapse Gaussian-based stacker focuser plugin to generate maximum intensity projections. Images were analyzed a deep learning based algorithm as described previously to produce a diameter distribution for each condition⁴⁵. Daily images of co-culture were manually curated to obtain relative growth rates of the organoids in single culture or co-culture by measuring EEO diameter and quantifying the daily fold change relative to day 1 of culture (video 4,5).

2.9. RNA isolation for quantitative real time PCR (qPCR) and bulk RNA-seq.

ESCs and intact EEOs cultured in Matrigel or synthetic hydrogels were used for molecular analysis. After 14 days of culture, intact EEOs were released from Matrigel and the synthetic ECM, using Cell Recovery Solution, or recombinant Sortase, respectively. Intact EEO-only cultures, ESC-only cultures, or Co-cultures were pelleted, resuspended in TRIzol reagent (ThermoFisher Scientific), and then stored at -80°C until processing. RNA was extracted using the Directzol RNA Mini-Prep kit (Zymo Research) per the manufacturer's protocols with the inclusion of an on-column DNase step using the PureLink DNase Set (ThermoFisher Scientific). At least eight replicates (3 μ l droplets) were pooled together to obtain sufficient RNA. cDNA was synthesized from ~1 μ g of total RNA using the High-Capacity RNA-to-cDNA Kit (ThermoFisher Scientific, 4387406) per manufacturer's protocols. TaqMan Fast Advanced Master Mix (ThermoFisher Scientific) was used in congruence with the cell-specific probes for qPCR. Gene expression was determined using the StepOnePlus real-time PCR system (Applied Biosystems) and calculated using the $\Delta\Delta$ Ct method using GraphPad Prism. Gene expression was first normalized using the housekeeping GAPDH gene in each sample, then the relative fold change relative to experimental control. High Throughput 3' Digital Gene Expression (3'DGE) RNAseq was performed on the isolated RNA using NovaSeq flowcells at the BioMicroCenter (MIT) and processed using the BMC/BCC 1.8 pipeline.

2.10. Secreted protein measurement by ELISA and Luminex

Spent media was stored at -80°C until analyzed by ELISA for prolactin (R&D systems). Protocols provided by the manufacturer were adapted to allow ELISAs to be performed in a

384-well plate (ThermoFisher) to minimize medium (sample) volume needed.²⁶ Multiplexed Luminex assays were performed to measure cytokines in 48- or 72-hour undiluted conditioned medium throughout the co-culture period using the magnetic Human Luminex XL Cytokine 45- PLEX pre-mixed kit (R&D systems). Protocols provided by the manufacturer were adapted to allow the assay to be performed in a 384 well plate to avoid introducing batch variability. Ten-point standard curves with culture medium and assay buffer blanks were included for quantification. Conversion from mean fluorescence intensity (MFI) values to absolute concentration was performed by interpolating 5-parameter logistical fits of standard curves for each analyte. Following the same downstream analysis protocol, secreted matrix metalloproteinases (MMP) concentrations were measured using the magnetic Human MMP Magnetic Luminex Performance Assay (R&D Systems). This assay required dilutions of cell culture supernatants in fresh culture medium at either 2x or 200x in order to detect the reported analytes within a quantifiable range.

2.11. Immunostaining and image analysis

Hydrogel samples were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) for 30 mins, permeabilized in 0.2% TritonX-100 in PBS (Sigma) for at least 30 mins at room temperature, and then blocked overnight with blocking buffer (1% BSA (Sigma), 5% normal donkey serum (Electron Microscopy Sciences) in PBS) at 4°C. Hydrogels were incubated with primary antibodies overnight while rocking at 4°C, rinsed with blocking buffer 3 times (5 min each while rocking at RT), and treated with secondary-conjugated antibodies overnight while rocking at 4°C. Specific antibodies can be found in the supplementary table of reagents. Images were captured using a ZEISS confocal Laser Scanning Microscope (LSM 880) or a Keyence BZ-X800 Fluorescence Microscope. Image analysis was performed using Fiji open software and 3D image processing was performed using Imaris (Bitmap) software (9.6.0).

2.12. Endometrial ECM proteomics

Endometrial ECM processing for proteomics, proteomics data collection, processing and analysis is described elsewhere (in preparation). Briefly, fresh endometrial biopsies were collected and processed using 50 µl of RapiGest (Waters Corporation) reconstituted in 50 mM NH₄HCO₃ at a concentration of 2mg/ml to digest the endometrial biopsy sample for 24 hours at 37°C. Next, urea (ThermoFisher) and dithiothreitol (ThermoFisher) were added to the sample to bring the mixture of a final concentration of 4 mM and 5 mM, respectively. Finally, iodoacetamide (MilliporeSigma) was added to the sample, for a final concentration of 15 mM

and kept in the dark, at 37°C, for 30 minutes. Samples were frozen at -80°C until all patient samples had been collected and processed. Samples were lyophilized overnight and then reconstituted in NH₄HCO₃. Samples were digested with Trypsin Gold, mass spectrometry grade (Promega), using a ratio of 1 µg trypsin to 50 µg sample protein as quantified using a Pierce BCA Protein Assay Kit (ThermoFisher). Samples were digested overnight at room temperature using a rotor for mixing and then placed in a speed vacuum to reduce sample volume. Dried down digested peptide samples were labeled with TMT10plex kit (Pierce). After labeling, the samples were mixed and purified with peptide desalting spin columns (Pierce). 1/5 of the sample was used for one LC/MS/MS analysis (Thermo Exploris480 mass spectrometer). The dried peptide mix was reconstituted in a solution of 2% formic acid (FA) for MS analysis. Peptides were loaded with the autosampler directly onto a 50cm EASY-Spray C18 column (Thermo Scientific). All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific; version IseNode in Proteome Discoverer 2.3.0.523). Sequest was set up to search uniprot_human_reviewed_032120.fasta (version March 21, 2020) assuming the digestion enzyme trypsin. Scaffold Q+ (version Scaffold_5.0.1, Proteome Software Inc.) was used to quantitate Label Based Quantitation peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 10.0% probability to achieve an FDR less than 1.0% by the Percolator posterior error probability calculation.⁴⁶ Normalization was performed iteratively (across samples and spectra) on intensities, as described in Statistical Analysis of Relative Labeled Mass Spectrometry Data from Complex Samples Using ANOVA.⁴⁷ Medians were used for averaging. The protein list was then compared to the curated human matrisome (<http://matrisome.org/>) list to look for ECM proteins. Samples were normalized using a mixed aliquot of all patient samples that was analyzed in each LC/MS-MS run. Overlapping ECM proteins found in all 3 LC/MS-MS runs were identified and the Limma package⁴⁸ in R was used to remove batch to batch effects.

2.13. Endometrial tissue single cell RNA sequencing (scRNAseq)

scRNAseq data collection, processing and analysis as described elsewhere (in preparation). Seq-Well was performed as described previously^{49,50}. Briefly, at least 15,000 cells were loaded onto each array preloaded with barcoded mRNA capture beads (ChemGenes). Arrays were washed with protein-free RPMI media, then sealed with plasma-treated polycarbonate membranes. Reverse transcription was performed with Maxima H Minus Reverse Transcriptase (ThermoFisher), excess primers were removed using an Exonuclease I digestion (New England Biolabs), and whole transcriptome amplification (WTA) by PCR was performed using KAPA Hifi

PCR Mastermix (Kapa Biosystems). WTA product was purified using Agencourt Ampure beads (Beckman Coulter) and 3' digital gene expression (DGE) sequencing libraries were prepared using Nextera XT (Illumina). Arrays were sequenced using custom primers on a Novaseq S4. All data were normalized, the top 2,000 variable genes identified. Initial clustering was performed, and cell types were assigned on the basis of marker genes identified using Seurat's Wilcoxon rank-sum test. All scripts to reproduce analyses are available for download (<https://github.com/bagoods>).

2.14. Bulk RNAseq analysis of In Vitro co-cultures

Aligned RNAseq transcript expected counts were analyzed in R (version 4.0.0) where data was modeled using the edgeR package (version 3.34.0).^{51,52} Counts were normalized by library size and filtered to exclude genes with less than 20 transcripts across all samples. Principal component analysis was performed using data batch-corrected by donor via the ComBat-seq method of the sva package (version 3.13) in R, and each gene was scaled to have unit variance before modeling.⁵³ Differential gene expression was calculated by modeling transcript data in a quasi-likelihood negative binomial generalized log-linear model using the edgeR function "glmQLFit". The use of a generalized log-linear model allowed for differences between hormone treatment groups to be compared while accounting for inter-donor variability. Genes were ranked by fold change in expression between hormone treatment groups to produce a ranked list of genes for gene set enrichment analysis (GSEA) (<http://www.broadinstitute.org/gsea/index.jsp>). Gene ontology (GO) term analysis was performed on ranked gene lists of genes with pvalues ≤ 0.05 . No adjustment was performed at this step due to the low sample number, high variability, and intent to use the RNA sequencing as a hypothesis generation step. The R package topGO (version 2.44.0) was used for GO term identification and the package clusterProfiler (version 4.0.2) was used for visualizations. A curated list of menstrual related genes was assembled from existing literature.^{54,55} A heatmap of $\log_2$ transformed data was created using the R package pheatmap (<https://CRAN.R-project.org/package=pheatmap>; version 1.0.12) with the "complete" clustering method.

2.15. Statistical analysis

Data are expressed as average $\pm$ standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 8. Unpaired t-tests assuming unequal variances and two-way ANOVAs with appropriate post-tests were performed. Holm-Sidak's multiple comparison of the mean or two-tailed Mann-Whitney tests when comparing two groups. qPCR data was analyzed

387 using multiple t-tests. Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p <$
388 0.0001 , and **** $P < 0.0001$.
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3. Results

3.1 Design of a fully synthetic ECM guided by multi-omic evaluation of human endometrium

The ECM is an integral and dynamic component of the endometrial microenvironment that mediates cellular and tissue-level function.⁵⁶ To engineer a fully synthetic ECM capable of supporting physiologically relevant culture of endometrial cells *in vitro*, we leveraged a multi-omic analysis of *ex vivo* human endometrial biopsies to characterize ECM composition and cell-ECM interactions. Specifically, we utilized single cell RNA sequencing (scRNAseq, N=6), and proteomic (LC-MS/MS, N=11) datasets from donors that represented the distinct phases across the menstrual cycle.

Previous immunohistology studies have identified that integrin expression as being highly dynamic across the human menstrual cycle⁵⁷. However, the transcriptomic expression of integrins of the endometrial microenvironment with single cell resolution has not been fully evaluated. Here, we leveraged scRNAseq to characterize the integrin expression of endometrial sub-populations within the cycling endometrium (Fig S1A-C) and identify the differentially expressed integrins between “stromal” and “epithelial” compartments (Fig 1C). These cellular compartments are known to modulate the functional changes in compositions of the ECM,^{58–60} therefore, we targeted these cell populations for the hydrogel design. Our analysis revealed that across the menstrual cycle epithelial compartment primarily expressed the $\alpha 1$, $\alpha 2$, and $\alpha 3$ integrin subunits that are associated collagen binding, as well as the $\alpha 6$ subunit, which mediates laminin (LMN) binding. ESCs primarily expressed the integrin $\alpha 5$ subunit, canonically involved in fibronectin binding, throughout both the proliferative and secretory phase (Fig 1C). The RGD-binding vitronectin receptor, integrin αV , was expressed by both cell populations and had a dynamic expression profile in the epithelial compartment throughout the menstrual cycle, corroborating previous findings that suggest it may play a specific role in embryo implantation.⁵⁷ We validated these cell-specific integrin expression profiles *in vitro* using cultured ESCs and epithelial cells (Fig S2D). Next, a proteomic analysis of the ECM composition was implemented to characterize the matrisome of proliferative and secretory phase human endometrium. Expression patterns largely varied according to cycle phase, suggestive of the ECM’s dynamic role in mediating key reproductive processes.^{61–63} Specifically, most collagens and laminin expression increased during the secretory phase while FN expression was higher in the proliferative phase (Fig 1B).

Altogether, the identification of collagens, fibronectin, and laminin rich matrix and its associated cellular integrin expression in endometrial cell populations of interest guided the design of fully defined synthetic matrix. To accommodate the culture of epithelial and the stromal compartment of the endometrium we targeted the engagement of the $\alpha 2\beta 1$ and $\alpha 5\beta 1$ integrins by utilizing synthetic peptide sequences that mimic the integrin binding sites of collagen (*GFOGER*, $\alpha 2\beta 1$)^{27,40} and fibronectin (*PHSRN-K-RGD*, $\alpha 5\beta 1$)^{24,26} respectively. While most other reports utilized a single peptide to maintain organoid²⁷ or stromal²⁶ cultures, we hypothesized that a dual-peptide approach with both peptides would enable the establishment of a co-culture platform. We functionalized an 8-arm poly(ethylene glycol) (PEG)-based hydrogel with a combination of GFOGER and PHSRN-K-RGD (MIX). Moreover, to support the dynamic hormone-induced nature of the endometrial laminin-based cell-ECM interactions, matrix-binding peptides targeting cell-secreted FN and basement membrane (B.M.) proteins (i.e., collagen IV/LMN) were also included in the matrix design (Fig 1D). We have previously shown that incorporation of these peptides enhanced the accumulation of fibrillar collagen and collagen IV/laminin-rich basement membrane deposition in cultures of endometrial cells.²⁶ Finally, a protease-sensitive crosslinking peptide (*CL-LW*) was incorporated to enable cell-mediated hydrogel remodeling during hormone-driven cellular morphogenesis and enable on-demand bio-orthogonal matrix dissolution for cell release^{26,38,64}. The tunable design of this hydrogel system enabled the semi-empirical evaluation of matrix formulations to identify an appropriate synthetic ECM that supports the culture and tissue-specific function of both endometrial stromal and epithelial cell populations throughout the menstrual cycle (Fig 1D).

3.2 Synthetic ECM supports simultaneous culture of primary human endometrial stromal and epithelial cells

3.2.1. Establishment and characterization of EEO cultures in a synthetic hydrogel

Protocols to establish and culture endometrial epithelial organoids (EEO) have transformed our ability to study human endometrial epithelial cells *in vitro*¹⁶. Despite this, the reliance on Matrigel as the gold-standard for EEO cultures limits experimental reproducibility and is not conducive to maintain physiologically representative co-cultures with endometrial stromal cells. To further characterize the biomolecular and biophysical properties on organoid establishment, survival, and growth, EEO generation was evaluated across a range of relevant synthetic matrix formulations (Fig 2A).

We previously showed a proof-of-principle that EEOs could emerge from single cells using a relatively stiff synthetic matrix²⁷. However, the characterization and hormone-induced phenotypes of these organoids was relatively unexplored. Moreover, the influences of matrix stiffness regimes that more closely mimic those of native endometrium were not investigated systematically. These first-generation hydrogels were substantially stiffer (~2000 Pa), than the native endometrial ECM (~250 Pa)⁶⁵ or Matrigel (~150-443 Pa)^{21,66}. First, establishment of EEO lines derived from single cells suspensions (scEEO) was demonstrated in synthetic matrices as previously described (Fig 2A,E). The elastic modulus of synthetic gels was tuned by varying the PEG polymer content from 3wt% (~300 Pa) to mimic native endometrial stiffness or to mimic a pathologic state by increasing it to 7wt% (~6 kPa) (Fig 2D). Although, increasing the matrix stiffness did not significantly impact the formation efficiency of EEOs in synthetic gels (Fig 2F-G), decreasing stiffness improved EEO morphology (Fig S4A) to match to those generated in Matrigel, suggesting that EEO clonal growth is robust across a range of physical properties (Fig 2E-G; Fig S3). Moreover, we evaluated formulations that probed the molecular (adhesion ligands) properties of the synthetic matrix using the dual-peptide approach. In agreement with our previous work, hydrogel functionalization with GFOGER, but not PHSRN-K-RGD, was necessary to robustly generate scEEOs in synthetic hydrogel (Fig 2C; Fig S3B). However, quantification of EEO diameter distribution and total EEO count across different dual-peptide 'MIX' formulations showed comparable scEEO formation efficiency compared to GFOGER alone or Matrigel (Fig 2F-G).

In the synthetic matrix, scEEOs retained proper polarity based on basolateral LMN deposition and apical F-actin accumulation, like EEOs cultured in Matrigel and epithelial glands *in vivo* (Fig 2H). Moreover, scEEOs in both synthetic gel and Matrigel maintained epithelial marker expression and mitotic activity similar to the native endometrial glands (Fig S4B-C) and sustained the capacity for hormone response based on expression of both estrogen receptor (ESR) and progesterone receptor (PGR) (Fig 2I-J). Further confirmation of the maturity of EEOs revealed distinct populations of motile ciliated and secretory epithelial cell that is comparable to *ex vivo* endometrial glands (Fig S4D, Video 1). These results were consistent across several tissue donors (N=10) and justified the use of soft hydrogels (3wt%) functionalized with GFOGER alone or dual presentation of GFOGER and PHSRN-K-RGD (MIX) in further experiments (Fig S3).

3.2.2. Establishment and characterization of ESC cultures in synthetic hydrogel

Following an analogous approach, the biomolecular and biophysical properties impacting ESC culture in 3D were evaluated (Fig 3A). ESCs were viable for at least 15 days of culture in a wide range of hydrogel conditions but demonstrated enhanced ECM remodeling capacity in softer hydrogel microenvironments (Fig 3B-D). Although all integrin ligands formulations supported ESC culture, hydrogels containing PHSRN-K-RGD demonstrated a modest increase in remodeling and overall stromal growth compared to matrices containing GFOGER alone (Fig 3E-F).

Stromal reproductive function was experimentally assessed by maintaining 3D cultures for a period 15 days, under sex hormone stimulation. In response to estrogen and a synthetic progestin treatment (E_2 and MPA), ESCs underwent decidualization, a differentiation process necessary for the establishment of pregnancy, characterized by the secretion of pro-gestational protein prolactin (PRL) and morphological changes toward a round, polyploid decidual cell⁶⁷. Although increased stiffness impacted ESC morphology and remodeling capacity, it did not alter ESC hormone response (Fig 3G). However, ESC decidualization response could be significantly suppressed by hormone treatment and inflammatory challenges (Fig 3H). Taken together, these results suggest that a softer and more physiologically relevant (3wt% PEG) synthetic matrix functionalized with GFOGER and PHSRN-K-RGD could support simultaneous culture of EEOs and ESCs in a multi-cellular model of the human endometrium.

3.2.3. Engineered synthetic matrices support stable co-culture of EEO and ESC with physiologic function

In the native endometrium both stromal and epithelial cells express hormone receptors, including PGR and ESR, and mediate tissue-level reproductive function via direct and indirect mechanisms involving multi-cellular communication.^{68,69,70,71} To generate a model capable of reproducing and studying these complex biological phenomena *in vitro*, the synthetic matrix formulation developed above was implemented to simultaneously culture matched EEOs and ESCs under a fully defined common media for up to 15 days (Fig 4A, Video 4). Co-cultures were exposed to hormone stimulation designed to mimic the proliferative (E_2 , 'E'), secretory (E_2 + MPA, 'PE'), or menstrual (72 hr PE withdrawal + RU-486, 'W') phases of the idealized human menstrual cycle (Fig 4C-D). All cultures were viable throughout the length of the experiments as demonstrated by live/dead staining (Fig S9) and a proliferative profile (Fig 6G). Immunostaining demonstrated the persistence of morphologically well-defined stromal and epithelial populations (Fig 4B, Video 2-3). Changes in EEO morphology characterized by thickening of the pseudo-stratified columnar epithelial layer and increased epithelial invaginations (Fig 4E, 5B) were

evident in the secretory phase cultures, and mimicked the structural development of endometrial glands observed *in vivo* (Fig 5A). Bulk RNA sequencing (RNAseq) analysis demonstrated that co-cultures were transcriptionally distinct from either ESC or EEOs cultured alone (Fig S6). Further analysis revealed that the treatment groups reproduced hallmark pathways associated with their respective menstrual phases (Fig 4F-H, S7). Under secretory phase conditions, a robust stromal decidualization response was observed. Specially, progesterone-induced gene expression, including PRL and ECM proteins, were significantly upregulated and cytokine-mediated inflammatory signaling were downregulated in PE treated groups (Fig 4F-H). Furthermore, canonical menstruation-associated pathways, including hypoxia⁷², inflammation⁶⁸ and coagulation⁷³ were also recapitulated in the withdrawal treatment group compared to E or PE. (Fig S7C-E).

Next, we utilized functional assays to validate the co-culture menstrual model. Co-cultures underwent decidualization, characterized by the secretion of PRL, in secretory groups, but was suppressed in the menses groups (Fig 5C-D). Further, progestin treatment resulted in both epithelial and stromal reduction in PGR staining not observed in the proliferative phase groups (Fig 5E). Epithelial-specific hormone response was demonstrated by secretion of progesterone associated endometrial protein (PAEP), a marker of the secretory epithelium (Fig 5F, S7). Co-cultures also showed a significant increase of apoptosis in the menses groups compared to the secretory treated groups supportive of the cell death observed during menstruation (Fig 5G-H, S8). Finally, protein secretion profiles of matrix metalloproteinases (MMPs) were measured from the spent media (Fig 5I, S13A) and recapitulated the remodeling dynamic changes observed *in vivo*.^{68,74} Altogether, this data demonstrates the development of a (I) fully defined and modular synthetic ECM that supports a long term, stable co-culture of EEOs and ESCs, and (II) an *in vitro* model of the cycling endometrium that is hormone responsive and recapitulates the phenotypic, morphologic, and biochemical changes associated with the idealized human menstrual cycle.

3.3 Inflammation disrupts endometrial function via a stromal dominant form of cellular communication

Chronic overexpression of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF α), and interleukin 1 beta (IL-1 β) have been associated with reproductive diseases⁷⁵⁻⁷⁷. Here, the endometrial co-culture models were used to examine inflammation-mediated disruption of endocrine function in the cycling endometrium. Proliferative, secretory, or menses co-cultures were exposed to a continuous dose of IL1 β (1 ng/mL) throughout the 15-day

experiment (Fig 4D). Progesterone action was significantly impaired in the co-cultures as demonstrated by the loss of PGR expression (Fig 6F), and the complete inhibition of stromal decidualization (Fig 5C). Although under inflammatory conditions ESC proliferation was suppressed (Fig 6G), ESCs remained active and developed an elongated morphology in 3D (Fig S10, Video 4) characterized by increased stromal remodeling and MMP activity (Fig 6D-E, Fig S13). Furthermore, columnar epithelial cell morphology was significantly disrupted, resulting in a thinner epithelial architecture (Fig 6B-C) reminiscent of the wound healing phenotype described in other epithelial cell populations.⁷⁹ Bulk transcriptomic data and multiplex protein analysis of secreted protein revealed a global up-regulation of pro-inflammatory cytokines and down regulation of anti-inflammatory cytokines in the inflamed cultures (Fig S11). Principal component analysis (PCA) and unsupervised hierarchical cluster analysis indicated clear separation between inflamed cultures and engagement of pathways associated with endometriotic diseases (Fig S13B).

To better understand the role of inflammation in disease progression we functionally examined viability, growth rates and proliferation in co-cultures and monocultures. In co-cultures, inflammation resulted in a significant increase EEO growth rate and subsequent decay compared to the EEO-monoculture controls (Fig 6I, S12) suggesting that epithelial response to inflammation is partially mediated by the stroma. To functionally characterize this behavior, we examined the proliferative profile of these cultures. We identified that IL-1 β significantly suppressed stromal proliferation, but had no impact on EEO proliferation when cultured separately. However, when co-cultured, IL-1 β significantly increased EEO proliferation (Fig 6G-H). A sharp growth and decay rates observed in the inflamed epithelium in the co-cultures was attributed to increased cell death and apoptosis in the IL-1 β treated groups (Fig S8, S9). Transcriptomic analysis of revealed more differential gene expression in co-cultures compared to monocultures, suggesting that the presence of both cell types enhance the response to cytokine challenge. Altogether, these results support that inflammatory and progesterone signal in the endometrium are primarily mediated via the stroma in both physiologic and pathologic conditions (Fig 6J). These findings demonstrate a platform that can recapitulate progesterone's mechanisms of action in the endometrium^{70,80} and dissects the mechanisms driving reproductive disease pathogenesis.

Discussion:

In the human endometrium, rapid spatial and temporal changes, primarily driven by E2 and P4, induce rapid alterations to ECM properties that are mechanically and molecularly linked to reproductive function.⁸¹ Tunable and bio-labile synthetic matrices that capture these dynamic ECM properties are required to dissect the mechanisms driving tissue function and disease pathogenesis.⁸² Hydrogels are well suited for 3D cell culture due to their ability to mimic the architecture and composition of the ECM. Matrigel, a heterogenous mixture of reconstituted basement membrane proteins, provides an efficient, yet restrictive, substrate to establish, expand, and study endometrial epithelial cells as organoids. The limitations of Matrigel have been thoroughly described elsewhere.^{21,83} Briefly, the variability of its composition, limited tunability and presence of xenogeneic contaminants severely impact its applications for multi-cellular models and translational research. Synthetic ECMs enable the tailoring the biophysical and molecular properties to mimic those of the native tissue^{25,81,84}. In this report, we used a multi-omic deconstructive approach to explore synthetic matrix regimes that closely simulated those of the native endometrial tissue and established a synthetic ECM that supports a multi-cellular *in vitro* 3D model of the human menstrual cycle.

Though efforts to generate Matrigel alternatives to culture endometrial cells have been presented^{85–89}, we report a fully synthetic hydrogel formulation that supports the long-term embedded culture of both primary EEOs and ESCs in 3D. Our previous work using endometrial cell lines identified that the fibronectin-mimetic (PHSRN-K-RGD) peptide supported the culture of endometrial fibroblasts in a synthetic hydrogel.²⁶ More recently, we reported the generation of epithelial organoids using the collagen-derived adhesion peptide GFOGER.²⁷ Here, we corroborated these findings and tailored the matrix to enable co-cultures of these cell populations that can mimic the idealized phases of the human menstrual cycle. By targeting the $\alpha 2 \beta 1$ and $\alpha 5 \beta 1$ integrins expressed by the epithelial and stromal cells, respectively, with both GFOGER and PHSRN-K-RGD (MIX) we observed improved scEEO formation efficiency ($70.6 \pm 13.2\%$) compared to the GFOGER-only matrices ($49.3 \pm 15.31\%$) (Fig 2F-G). These results corroborated our previous findings^{26,27,90} that focused on relatively stiff PEG hydrogels (> 2 kPa) that are drastically different than the native endometrium (~ 250 Pa⁶⁵) or of Matrigel of (~ 150 – 443 Pa).^{21,66} By modulating the total PEG concentration (wt%), while maintaining consistent crosslinking (45%) to more closely mimic the native ECM, we identified that decreasing hydrogel stiffness (3wt%, ~ 300 Pa) further improved the scEEO forming efficiency in both the MIX ($77.7 \pm 16.3\%$) or GFOGER-only formulations ($64.9 \pm 16.7\%$). Changing the elastic moduli of the tissue environment, either approaching or moving away from those of native tissues, can lead to

tremendous differences in cellular responses.^{81,84} We identified that stiffer ECM regimes (5wt%, ~2 kPa; 7wt%, ~6 kPa) strongly impacted EEO morphology and ESC remodeling compared to softer matrices. Robust stromal stellate spreading, and spherical organoid morphology was consistently maintained in the 3wt% hydrogels (Fig S2). Although hydrogel stiffness altered EEO morphology, organoid generation and diameter was not significantly impacted (Fig 2F-G). Moreover, we demonstrated a significant increase in stromal decidualization when cultured in 3D compared to 2D cultures, a finding whose mechanisms will require further investigation, yet may impact the future of culture and analysis of *in vitro* endometrial stromal cells (Fig 3G). To physiologically model the endometrium, we focused on the soft (3 wt%) hydrogels to generate the co-cultures, however, these stiffness-mediated phenotypes may provide insight into cell-ECM interactions in endometriotic diseases.⁹¹ We conclude that the dual-peptide 3wt%-MIX formulations is a novel approach to generate a synthetic matrix that is suitable for the culture of both the epithelial and stromal cells either as mono- or co-cultures. To test the efficacy of the synthetic matrix, we validated that expansion of scEEOs from original primary tissue is feasible in the synthetic ECM without the need to first expand in Matrigel (Fig S14). This confers the opportunity to investigate epithelial cell function otherwise masked by exogenous cytokines and growth factors found in Matrigel.

Numerous approaches to generate *in vitro* models of the endometrial microenvironment that attempt to dissect the intercellular communication that regulate reproductive function and disease have been reported. These models include de-cellularized scaffolds^{30,92}, explant tissue models^{29,31,88,93}, microfluidic organ-on-chip approaches⁹⁴⁻⁹⁶ and 3D culture models.^{26,29,30} Of these, renal graft models have been particularly useful in elucidating mechanisms by which hormones regulate epithelial and stromal function in the endometrium^{70,71,97,98}, yet their technical complexity and limited human translation make them challenging models to interpret and reproduce.^{44,70} Here, we report a modular, tunable, and scalable 3D *in vitro* human alternative that similarly captures tissue-level cellular communication, but enables a more robust analytical capability (Fig 6). To appropriately dissect stromal-epithelial communication, we defined stromal-epithelial cell ratios that were shown to approximate those observed *in vivo* (Fig S6).

Key reproductive events such as tissue regeneration, embryo implantation and menstruation are all mediated by inflammatory mediators such as cytokines, chemokines, and prostaglandins that regulate tissue function via cell-mediated communication^{1,31,99}. However, uncontrolled inflammation may promote the development of reproductive diseases such as endometriosis, infertility, and abnormal menstrual bleeding. Endometriosis is a reproductive disorder histologically characterized by the ectopic growth of endometrial tissue⁹ and is closely

associated with pro-inflammatory mediators such as TNF α and IL1 β as part of its pathogenesis.⁷⁵ Using the co-culture model of the cycling endometrium described, we demonstrated that exogenous stimulation by IL1 β recapitulated hallmarks of the endometriotic phenotype (Fig 6). As expected, reduced P4 sensitivity was observed in the inflamed co-cultures system, reflected by a complete loss of stromal decidualization (Fig 5C), increased epithelial growth (Fig 6G-H), and induction of additional pro-inflammatory cytokines (Fig S11). We observed that inflammation significantly reduced proliferation in the ESC, but increased matrix remodeling and induced an elongated morphology, suggestive of a fibrotic response¹⁰⁰. Likewise, epithelial cells underwent a morphologic change from a mature columnar epithelial structure to a thin squamous morphology in response to inflammation, suggestive of an wound healing phenotype⁷⁹. Together, these results demonstrate an altered cellular phenotype in the stromal and epithelial compartments due to inflammation may be closely linked to the invasive phenotype and fibrotic environment associated with endometriotic disease^{101–103}. Moreover, by examining the mitogenic effects of sex hormones and inflammation in our co-culture system, we demonstrate the novel mechanistic finding that inflammation does not impacts epithelial proliferation, differentiation, and function, but is mediated through the stromal fibroblasts (Fig 6J). Though intended as a proof of concept to demonstrate the capacity to dissect the mechanisms of cell-cell communication, the cell-specific mediators will warrant further analysis to identify the molecular factors driving this cellular response.

Altogether, this model provides a user-friendly and reproducible screening tool to explore the endocrine-mediated axis of cell-cell communication using organoid technologies that is amenable for therapeutic and contraceptive drug testing. Our approach to utilize pharmacologic PGR antagonists (RU-486) to induce menstruation supports the ability to examine clinical applications of selective hormone receptor modulators. Although this model robustly examines the role of the epithelial and stromal compartments, it is important to consider the role of other cell populations of the endometrial microenvironment, such as endothelial⁹⁰ and immune cells, to support reproductive function or pathogenesis. The modular and tunable nature of this 3D hydrogel platform makes the endometrial co-culture model is amenable for microfluidic and organ-on-chip applications to incorporate these other cell populations.

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Competing Interests statement: L.G.G. and V.H.G. have a patent application pending related to the hydrogel system. The rest of the authors have no competing interests.

Data availability. Source data are available upon request from the corresponding author.

4. References:

1. Aplin J. Cellular biochemistry of the endometrium. *Biol Uterus*. 1989.
2. Aplin JD, Ruane PT. Embryo-epithelium interactions during implantation at a glance. *J Cell Sci*. 2017;130(1):15-22. doi:10.1242/jcs.175943
3. Aplin JD. Cellular Biochemistry of the Endometrium. In: *Biology of the Uterus*. Vol 20. Karger Publishers; 1989:89-129. doi:10.1007/978-1-4684-5589-2_6
4. Noyes R, Hertig A, Rock J. Dating the endometrial biopsy. *Obstet Gynecol* 1950.
5. Loy SLY and TS. Normal cycling Endometrium:Molecular, Cellular, and Histologic Perspectives. In: *Endometriosis in Clinical Practice*. ; 2005:1-29.
6. Roy A, Matzuk MM. Reproductive tract function and dysfunction in women. *Nat Rev Endocrinol*. 2011;7(9):517-525. doi:10.1038/nrendo.2011.79
7. Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology*. 1999;140(10):4809-4820. doi:10.1210/endo.140.10.7070
8. Kelleher AM, Demayo FJ, Spencer TE. Uterine Glands: Developmental Biology and Functional Roles in Pregnancy. *Endocr Rev*. 2019;40(5):1424-1445. doi:10.1210/er.2018-00281
9. Cameron IT, Fraser IS, Smith SK (Stephen K. *Clinical Disorders of the Endometrium and Menstrual Cycle*. Oxford University Press; 1998.
10. Clevers H. Modeling Development and Disease with Organoids. *Cell*. 2016;165(7):1586-1597. doi:10.1016/j.cell.2016.05.082
11. Boretto M, Maenhoudt N, Luo X, et al. Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nat Cell Biol*. 2019;21(8):1041-1051. doi:10.1038/s41556-019-0360-z
12. Turco MY, Gardner L, Hughes J, et al. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol*. 2017;19(5):568-577. doi:10.1038/ncb3516
13. Boretto M, Cox B, Noben M, et al. Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability. *dev.biologists.org*. 2017. doi:10.1242/dev.148478
14. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology*. 2011;141(5):1762-1772. doi:10.1053/j.gastro.2011.07.050

15. Fujii M, Matano M, Toshimitsu K, et al. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell*. 2018;23(6):787-793.e6. doi:10.1016/j.stem.2018.11.016
16. Fitzgerald HC, Dhakal P, Behura SK, Schust DJ, Spencer TE. Self-renewing endometrial epithelial organoids of the human uterus. *Proc Natl Acad Sci U S A*. 2019;116(46):23132-23142. doi:10.1073/pnas.1915389116
17. Nakamura T, Sato T. Advancing Intestinal Organoid Technology Toward Regenerative Medicine. *CMGH*. 2018;5(1):51-60. doi:10.1016/j.jcmgh.2017.10.006
18. Cruz-Acuña R, Quirós M, Farkas AE, et al. Synthetic hydrogels for human intestinal organoid generation and colonic wound repair. *Nat Cell Biol*. 2017;19(11):1326-1335. doi:10.1038/ncb3632
19. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262-265. doi:10.1038/nature07935
20. Talbot NC, Caperna TJ. Proteome array identification of bioactive soluble proteins/peptides in Matrigel: relevance to stem cell responses. *Cytotechnology*. 2015;67(5):873-883. doi:10.1007/s10616-014-9727-y
21. Hughes CS, Postovit LM, Lajoie GA. Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics*. 2010;10(9):1886-1890. doi:10.1002/pmic.200900758
22. Cruz-Acuña R, García AJ, Cruz-Acuna R, Garcia AJ, Cruz-Acuña R, García AJ. Synthetic hydrogels mimicking basement membrane matrices to promote cell-matrix interactions. *Matrix Biol*. 2017;57-58:324-333. doi:10.1016/j.matbio.2016.06.002
23. Capeling MM, Czerwinski M, Huang S, et al. Nonadhesive Alginate Hydrogels Support Growth of Pluripotent Stem Cell-Derived Intestinal Organoids. *Stem Cell Reports*. 2019;12(2):381-394. doi:10.1016/j.stemcr.2018.12.001
24. Gjorevski N, Sachs N, Manfrin A, et al. Designer matrices for intestinal stem cell and organoid culture. *Nat Publ Gr*. 2016;539(7630):560-564. doi:10.1038/nature20168
25. Kratochvil MJ, Seymour AJ, Li TL, Paşca SP, Kuo CJ, Heilshorn SC. Engineered materials for organoid systems. *Nat Rev Mater*. 2019;4(9):606-622. doi:10.1038/s41578-019-0129-9
26. Cook CD, Hill AS, Guo M, et al. Local remodeling of synthetic extracellular matrix microenvironments by co-cultured endometrial epithelial and stromal cells enables long-term dynamic physiological function. *Integr Biol (United Kingdom)*. 2017;9(4):271-289.

doi:10.1039/c6ib00245e

27. Hernandez-Gordillo V, Kassis T, Lampejo A, et al. Fully synthetic matrices for in vitro culture of primary human intestinal enteroids and endometrial organoids. *Biomaterials*. 2020;254:120125. doi:10.1016/j.biomaterials.2020.120125
28. Pierro E, Minici F, Alesiani O, et al. Stromal-Epithelial Interactions Modulate Estrogen Responsiveness in Normal Human Endometrium1. *Biol Reprod*. 2001;64(3):831-838. doi:10.1095/biolreprod64.3.831
29. Arnold JT, Kaufman DG, Seppälä M, Lessey BA. Endometrial stromal cells regulate epithelial cell growth in vitro: A new co-culture model. *Hum Reprod*. 2001;16(5):836-845. doi:10.1093/humrep/16.5.836
30. Abbas Y, Brunel LG, Hollinshead MS, et al. Generation of a three-dimensional collagen scaffold-based model of the human endometrium. *Interface Focus*. 2020;10(2):20190079. doi:10.1098/rsfs.2019.0079
31. Osteen KG, Rodgers WH, Gaire M, Hargrove JT, Gorstein F, Matrisian LM. Stromal-epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium. *Proc Natl Acad Sci U S A*. 1994;91(21):10129-10133. doi:10.1073/pnas.91.21.10129
32. Rezakhani S, Gjorevski N, Lutolf MP. Low-Defect Thiol-Michael Addition Hydrogels as Matrigel Substitutes for Epithelial Organoid Derivation. *Adv Funct Mater*. 2020;30(48):2000761. doi:10.1002/adfm.202000761
33. Below CR, Kelly J, Brown A, et al. A microenvironment-inspired synthetic three-dimensional model for pancreatic ductal adenocarcinoma organoids. *Nat Mater*. 2021. doi:10.1038/s41563-021-01085-1
34. Osteen KG, Hill GA, Hargrove JT, Gorstein F. Development of a method to isolate and culture highly purified populations of stromal and epithelial cells from human endometrial biopsy specimens. *Fertil Steril*. 1989;52(6):965-972. doi:10.1016/S0015-0282(16)53160-4
35. Turco MY, Gardner L, Hughes J, et al. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol*. 2017;19(5):568-577. doi:10.1038/ncb3516
36. Turco MY, Gardner L, Kay RG, et al. Trophoblast organoids as a model for maternal–fetal interactions during human placentation. *Nature*. 2018;564(7735):263-281. doi:10.1038/s41586-018-0753-3
37. Lutolf MP, Lauer-Fields JL, Schmoekel HG, et al. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion

- characteristics. *Proc Natl Acad Sci U S A*. 2003;100(9):5413-5418. doi:10.1073/pnas.0737381100
38. Cambria E, Renggli K, Ahrens CC, et al. Covalent Modification of Synthetic Hydrogels with Bioactive Proteins via Sortase-Mediated Ligation. *Biomacromolecules*. 2015;16(8):2316-2326. doi:10.1021/acs.biomac.5b00549
39. Kuhlman W, Taniguchi I, Griffith LG, Mayes AM. Interplay between PEO tether length and ligand spacing governs cell spreading on RGD-modified PMMA-g-PEO comb copolymers. *Biomacromolecules*. 2007;8(10):3206-3213. doi:10.1021/bm070237o
40. Wojtowicz AM, Shekaran A, Oest ME, et al. Coating of biomaterial scaffolds with the collagen-mimetic peptide GFOGER for bone defect repair. *Biomaterials*. 2010;31(9):2574-2582. doi:10.1016/j.biomaterials.2009.12.008
41. Gao X, Groves MJ. Fibronectin-binding peptides. I. Isolation and characterization of two unique fibronectin-binding peptides from gelatin. *Eur J Pharm Biopharm*. 1998;45(3):275-284. doi:10.1016/S0939-6411(98)00010-1
42. Johnson G, Moore SW. Identification of a structural site on acetylcholinesterase that promotes neurite outgrowth and binds laminin-1 and collagen IV. *Biochem Biophys Res Commun*. 2004;319(2):448-455. doi:10.1016/j.bbrc.2004.05.018
43. Kurita T, Medina R, Schabel AB, et al. The activation function-1 domain of estrogen receptor α in uterine stromal cells is required for mouse but not human uterine epithelial response to estrogen. *Differentiation*. 2005;73(6):313-322. doi:10.1111/j.1432-0436.2005.00033.x
44. Cunha GR, Baskin L. Use of sub-renal capsule transplantation in developmental biology. *Differentiation*. 2016;91(4-5):4-9. doi:10.1016/j.diff.2015.10.007
45. Kassis T, Hernandez-Gordillo V, Langer R, Griffith LG. OrgaQuant: Human Intestinal Organoid Localization and Quantification Using Deep Convolutional Neural Networks. *Sci Rep*. 2019;9(1):12479. doi:10.1038/s41598-019-48874-y
46. Käll L, Storey JD, Noble WS. Non-parametric estimation of posterior error probabilities associated with peptides identified by tandem mass spectrometry. *Bioinformatics*. 2008;24(16):42-48. doi:10.1093/bioinformatics/btn294
47. Oberg AL, Mahoney DW, Eckel-Passow JE, et al. Statistical analysis of relative labeled mass spectrometry data from complex samples using ANOVA. *J Proteome Res*. 2008;7(1):225-233. doi:10.1021/pr700734f
48. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47-e47.

doi:10.1093/nar/gkv007

49. Hughes TK, Wadsworth MH, Gierahn TM, et al. Second-Strand Synthesis-Based Massively Parallel scRNA-Seq Reveals Cellular States and Molecular Features of Human Inflammatory Skin Pathologies. *Immunity*. 2020;53(4):878-894.e7. doi:10.1016/j.immuni.2020.09.015
50. Gierahn TM, Wadsworth MH, Hughes TK, et al. Seq-Well: A Portable, Low-Cost Platform for High-Throughput Single-Cell RNA-Seq of Low-Input Samples. *Nat Methods*. 2017;14(4):395-398. doi:10.1038/nmeth.4179.Seq-Well
51. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2009;26(1):139-140. doi:10.1093/bioinformatics/btp616
52. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res*. 2012;40(10):4288-4297. doi:10.1093/nar/gks042
53. Zhang Y, Parmigiani G, Johnson WE. ComBat-seq: batch effect adjustment for RNA-seq count data. *NAR Genomics Bioinforma*. 2020;2(3):1-10. doi:10.1093/nargab/lqaa078
54. Wang W, Vilella F, Alama P, et al. Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med*. 2020;26(10):1644-1653. doi:10.1038/s41591-020-1040-z
55. Lucas ES, Vrljicak P, Muter J, et al. Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. *Commun Biol*. 2020;3(1):37. doi:10.1038/s42003-020-0763-1
56. O'Connor BB, Pope BD, Peters MM, Ris-Stalpers C, Parker KK. The role of extracellular matrix in normal and pathological pregnancy: Future applications of microphysiological systems in reproductive medicine. *Exp Biol Med*. 2020;245(13):1163-1174. doi:10.1177/1535370220938741
57. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albeida SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *J Clin Invest*. 1992;90(1):188-195. doi:10.1172/JCI115835
58. Béliard A, Donnez J, Nisolle M, Foidart JM. Localization of laminin, fibronectin, E-cadherin, and integrins in endometrium and endometriosis. *Fertil Steril*. 1997;67(2):266-272. doi:10.1016/S0015-0282(97)81909-7
59. Bischof P, Redard M, Gindre P, Vassilakos P, Campana A. Localization of alpha 2, alpha 5 and alpha 6 integrin subunits in human endometrium, decidua and trophoblast. *Eur J*

- 878 *Obstet Gynecol Reprod Biol.* 1993;51(3):217-226. doi:10.1016/0028-2243(93)90038-E
- 879 60. Tabibzadeh S. Patterns of expression of integrin molecules in human endometrium
880 throughout the menstrual cycle. *Hum Reprod.* 1992;7(6):876-882.
881 doi:10.1093/oxfordjournals.humrep.a137753
- 882 61. Chen JI-C, Hannan NJ, Mak Y, et al. Proteomic Characterization of Midproliferative and
883 Midsecretory Human Endometrium. *J Proteome Res.* 2009;8(4):2032-2044.
884 doi:10.1021/pr801024g
- 885 62. Hood BL, Liu B, Alkhas A, et al. Proteomics of the Human Endometrial Glandular
886 Epithelium and Stroma from the Proliferative and Secretory Phases of the Menstrual
887 Cycle1. *Biol Reprod.* 2015;92(4). doi:10.1095/biolreprod.114.127217
- 888 63. Rai P, Kota V, Sundaram CS, Deendayal M, Shivaji S. Proteome of human endometrium:
889 Identification of differentially expressed proteins in proliferative and secretory phase
890 endometrium. *PROTEOMICS – Clin Appl.* 2010;4(1):48-59.
891 doi:https://doi.org/10.1002/prca.200900094
- 892 64. Valdez J, Cook CD, Ahrens CC, et al. On-demand dissolution of modular, synthetic
893 extracellular matrix reveals local epithelial-stromal communication networks. *Biomaterials.*
894 2017;130:90-103. doi:10.1016/j.biomaterials.2017.03.030
- 895 65. Abbas Y, Carnicer-Lombarte A, Gardner L, et al. Tissue stiffness at the human maternal-
896 fetal interface. *Hum Reprod.* 2019;34(10):1999-2008. doi:10.1093/humrep/dez139
- 897 66. Soofi SS, Last JA, Lliensiek SJ, Nealey PF, Murphy CJ. Elastic modulus of Matrigel as
898 determined by AFM. *J Struct Biol.* 2009;167(3):216-219.
899 doi:10.1016/j.jsb.2009.05.005.The
- 900 67. Wetendorf M, DeMayo FJ. The progesterone receptor regulates implantation,
901 decidualization, and glandular development via a complex paracrine signaling network. *Mol*
902 *Cell Endocrinol.* 2012;357(1-2):108-118. doi:10.1016/j.mce.2011.10.028
- 903 68. Critchley HO, Maybin JA, Armstrong GM, Williams ARW. Physiology of the endometrium
904 and regulation of menstruation. *Physiol Rev.* February 2020.
905 doi:10.1152/physrev.00031.2019
- 906 69. Mehaseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and
907 progesterone receptor isoform distribution through the menstrual cycle in uteri with and
908 without adenomyosis. *Fertil Steril.* 2011;95(7):2228-2235, 2235.e1.
909 doi:10.1016/j.fertnstert.2011.02.051
- 910 70. Cunha GR, Bigsby RM, Cooke PS, Sugimura Y. Stromal-epithelial interactions in adult
911 organs. *Cell Differ.* 1985;17(3):137-148. doi:10.1016/0045-6039(85)90481-6

71. Cooke PS, Buchanan DL, Young P, et al. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. *Proc Natl Acad Sci U S A*. 1997;94(12):6535-6540. doi:10.1073/pnas.94.12.6535
72. Reavey JJ, Walker C, Nicol M, et al. Markers of human endometrial hypoxia can be detected in vivo and ex vivo during physiological menstruation. *Hum Reprod*. 2021;36(4):941-950. doi:10.1093/humrep/deaa379
73. Davies J, Kadir RA. Endometrial haemostasis and menstruation. *Rev Endocr Metab Disord*. 2012;13(4):289-299. doi:10.1007/s11154-012-9226-4
74. Bruner KL, Rodgers WH, Gold LI, et al. Transforming growth factor beta mediates the progesterone suppression of an epithelial metalloproteinase by adjacent stroma in the human endometrium. *Proc Natl Acad Sci U S A*. 1995;92(16):7362-7366.
75. Wang Y, Nicholes K, Shih IM. The Origin and Pathogenesis of Endometriosis. *Annu Rev Pathol Mech Dis*. 2020;15:71-95. doi:10.1146/annurev-pathmechdis-012419-032654
76. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: Pathogenesis and treatment. *Nat Rev Endocrinol*. 2014;10(5):261-275. doi:10.1038/nrendo.2013.255
77. Horne AW, Saunders PTK. SnapShot: Endometriosis. *Cell*. 2019;179(7):1677-1677.e1. doi:10.1016/j.cell.2019.11.033
78. Chan RWS, Lee CL, Ng EHY, Yeung WSB. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. *Cell Prolif*. 2017;50(3). doi:10.1111/cpr.12330
79. Miyoshi H, VanDussen KL, Malvin NP, et al. Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. *EMBO J*. 2017;36(1):5-24. doi:10.15252/embj.201694660
80. Cooke PS, Buchanan DL, Young P, et al. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. *Proc Natl Acad Sci U S A*. 1997;94(12):6535-6540. doi:10.1073/pnas.94.12.6535
81. Griffith LG, Swartz MA. Capturing complex 3D tissue physiology in vitro. *Nat Rev Mol Cell Biol*. 2006;7(3):211-224. doi:10.1038/nrm1858
82. Guimarães CF, Gasperini L, Marques AP, Reis RL. The stiffness of living tissues and its implications for tissue engineering. *Nat Rev Mater*. February 2020:1-20. doi:10.1038/s41578-019-0169-1
83. Aisenbrey EA, Murphy WL. Synthetic alternatives to Matrigel. *Nat Rev Mater*. 2020;5(7):539-551. doi:10.1038/s41578-020-0199-8
84. Nicolas J, Magli S, Rabbachin L, Sampaolesi S, Nicotra F, Russo L. 3D Extracellular Matrix

- Mimics: Fundamental Concepts and Role of Materials Chemistry to Influence Stem Cell Fate. *Biomacromolecules*. 2020;21(6):1968-1994. doi:10.1021/acs.biomac.0c00045
85. Francés-Herrero E, Juárez-Barber E, Campo H, et al. Improved models of human endometrial organoids based on hydrogels from decellularized endometrium. *J Pers Med*. 2021;11(6). doi:10.3390/jpm11060504
86. López-Martínez S, Campo H, de Miguel-Gómez L, et al. A Natural Xenogeneic Endometrial Extracellular Matrix Hydrogel Toward Improving Current Human in vitro Models and Future in vivo Applications. *Front Bioeng Biotechnol*. 2021;9(March):1-20. doi:10.3389/fbioe.2021.639688
87. Zambuto SG, Clancy KBH, Harley BAC. A gelatin hydrogel to study endometrial angiogenesis and trophoblast invasion. *Interface Focus*. 2019;9(5). doi:10.1098/rsfs.2019.0016
88. Muruganandan S, Fan X, Dhal S, Nayak NR. Development of A 3D Tissue Slice Culture Model for the Study of Human Endometrial Repair and Regeneration. *Biomolecules*. 2020;10(1):136. doi:10.3390/biom10010136
89. Gargus ES, Rogers HB, McKinnon KE, Edmonds ME, Woodruff TK. Engineered reproductive tissues. *Nat Biomed Eng*. 2020;4(4):381-393. doi:10.1038/s41551-020-0525-x
90. Brown A, He H, Trumper E, Valdez J, Hammond P, Griffith LG. Engineering PEG-based hydrogels to foster efficient endothelial network formation in free-swelling and confined microenvironments. *Biomaterials*. 2020;243:119921. doi:10.1016/j.biomaterials.2020.119921
91. Gnecco JS, Brown AT, Kan EL, et al. Physiomimetic Models of Adenomyosis. *Semin Reprod Med*. 2020;38(2-3):179-196. doi:10.1055/s-0040-1719084
92. Olalekan SA, Burdette JE, Getsios S, Woodruff TK, Kim JJ. Development of a novel human recellularized endometrium that responds to a 28-day hormone treatment. *Biol Reprod*. 2017;96(5):971. doi:10.1093/BIOLRE/IOX039
93. Schäfer WR, Fischer L, Roth K, et al. Critical evaluation of human endometrial explants as an ex vivo model system: A molecular approach. *Mol Hum Reprod*. 2011;17(4):255-265. doi:10.1093/molehr/gaq095
94. 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(4):702-714. doi:10.1093/humrep/dez003

95. Gnecco JS, Pensabene V, Li DJ, et al. Compartmentalized Culture of Perivascular Stroma and Endothelial Cells in a Microfluidic Model of the Human Endometrium. *Ann Biomed Eng.* 2017;45(7):1758-1769. doi:10.1007/s10439-017-1797-5
96. Kimura H, Nakamura H, Akai T, et al. On-chip single embryo coculture with microporous-membrane-supported endometrial cells. *IEEE Trans Nanobioscience.* 2009;8(4):318-324. doi:10.1109/TNB.2009.2035275
97. Bigsby RM. Control of growth and differentiation of the endometrium: The role of tissue interactions. In: *Annals of the New York Academy of Sciences.* Vol 955. ; 2002:110-117. doi:10.1111/j.1749-6632.2002.tb02771.x
98. Buchanan DL, Setiawan T, Lubahn DB, et al. Tissue compartment-specific estrogen receptor- α participation in the mouse uterine epithelial secretory response. *Endocrinology.* 1999;140(1):484-491. doi:10.1210/endo.140.1.6448
99. Evans J, Salamonsen LA, Winship A, et al. Fertile ground: Human endometrial programming and lessons in health and disease. *Nat Rev Endocrinol.* 2016;12(11):654-667. doi:10.1038/nrendo.2016.116
100. Kendall RT, Feghali-Bostwick CA. Fibroblasts in fibrosis: novel roles and mediators. *Front Pharmacol.* 2014;5:123. doi:10.3389/fphar.2014.00123
101. Konrad L, Dietze R, Riaz MA, et al. Epithelial-Mesenchymal Transition in Endometriosis- When Does It Happen? *J Clin Med.* 2020;9(6). doi:10.3390/jcm9061915
102. Vigano P, Candiani M, Monno A, Giacomini E, Vercellini P, Somigliana E. Time to redefine endometriosis including its pro-fibrotic nature. *Hum Reprod.* 2017;33(3):347-352. doi:10.1093/humrep/dex354
103. Clancy KBH. Reproductive ecology and the endometrium: Physiology, variation, and new directions. *Am J Phys Anthropol.* 2009;140(SUPPL. 49):137-154. doi:10.1002/ajpa.21188

5. Figure Legends

Figure 1. A tissue-inspired multi-omic approach elucidates the design parameters to engineer a fully synthetic PEG hydrogel for the endometrium. (A) Representative histological hematoxylin and eosin (H&E) stained samples of the menstrual cycle phases. (B) Heatmap of proteomic analysis of the human endometrium showing ECM proteins (matrisome) across the menstrual cycle (n = 8 donors). Green, red and purple boxes highlight collagens, fibronectin, and laminins as dynamic proteins in the endometrium. (C) Dot plot analysis of specific integrins expression from single cell RNA-seq data (n = 6 donors, 6,659 cells). Examination of stromal and epithelial component integrin expression across the menstrual cycle (proliferative and secretory phases) Circle size and color indicate P-value and the means of the average expression value. (D) Schematic of the overall strategy to design and fabricate a synthetic ECM for the endometrium. 8-arm 20kDa PEG macromers are functionalized with cell matrix binders (FN, B.M.), integrin binder adhesion peptides, and polymerized with a protease-labile crosslinker. Conceptual schematic of the dual-peptide hydrogel design to support multiple cell types and descriptive outline of the key peptides utilized to interact with integrins.

Figure 2. Dual-peptide functionalization and softer synthetic matrix regimes enhance EEOs generation. (A) Schematic of the workflow for identifying a synthetic hydrogel formulation for EEO generation from single cells. (B) Representative images of scEEO and morphology in stiff (5wt%) in soft (3wt%) PEG hydrogels functionalized with GFOGER (3mM) compared to Matrigel. (C) Day-15 images of hydrogel formulations comparing adhesion peptides reveals that GFOGER is necessary to generate scEEOs. Dual-peptide functionalization with GFOGER (1.5mM) and PHSN-K-RGD (1.5 mM) did not negatively affect scEEO generation. (D) Quantification of the biomechanical properties (elastic moduli, Pa, n=3 gels) of the synthetic hydrogel formulations. Soft = 3wt% (~300 pa), 5wt% = (~2kPa) and 7wt% (~6kPa). (E) Time-lapse images of 10-day cultures of EEO derived from single cells in synthetic hydrogels (PEG 3wt%-MIX). (F) Quantification of the total lumenized EEO (>100 um diameter) count and EEO diameter distribution (F) after 14 days of culture in different hydrogel formulation and in Matrigel. (G) Quantification of the scEEO forming efficiency in synthetic hydrogels relative to Matrigel (N=8). (H) Characterization of EEO morphology and polarity of EEOs generated in synthetic ECM (3wt%-MIX) compared to Matrigel via immunofluorescent (IF) analysis of ECM deposition (LMN) and F-actin accumulation. (I-J) Characterization of protein and transcriptomic expression of hormone receptors in EEOs generated in synthetic hydrogels. (I) Representative images of PGR staining and (J) qPCR analysis of mRNA expression of PGR and ESR in EEOs treated with either E₂ (E) or E₂ + MPA (EP) for 14 days. Analysis compares ESR, PGR values relative to matching E treatment groups (control).

Figure 3. Development and characterization of a synthetic endometrial stromal cell culture. (A) Schematic of the workflow for culturing ESCs in 3D using a synthetic hydrogel. (B) Time-lapse images of embedded ESCs in synthetic matrix allow for long-term stable cultures. (C-D) Representative images of 14-day old 3D cultures of ESC in the synthetic hydrogel comparing different adhesion peptides and stiffnesses. (D) Maximum intensity projection images of F-actin

(green) reveal ESC remodeling (cell spreading) impacted primarily by hydrogel stiffness. (E-F) Representative 3D images and quantification (% fluorescence area) of ESC remodeling in synthetic ECMs. (G) Decidualization is not impacted by synthetic ECM (3wt% MIX). Biochemical measurement of prolactin (PRL) in the spent media from 14-day cultures treated with E₂ or E₂P4 in synthetic hydrogels of different stiffnesses (3wt%, 5wt%, 7wt%) is significantly higher in 3D compared to 2D. (H) Inflammation (IL1 β , 1 ng/mL) significantly suppressed decidualization in ESC cultures (E+ = E + IL1 β , PE+= PE+IL1 β , W = hormone withdrawal).

Figure 4. Establishment and Establishment of a co-culture model recapitulate the molecular signature of the human menstrual cycle in vitro. (A) Schematic and representative images of the co-cultures. EEOs (10 intact organoids/uL) and ESC (10k cells/uL) are expanded separately and embedded in the synthetic ECM and cultured as 3ul droplets for 15 days. A dual-peptide synthetic ECM (3wt% 20kDa-PEG) functionalized with GFOGER (1.5mM) and PHSRN-K-RGD (1.5mM) supports the establishment of the EEOs and ESC co-culture model. (B) Representative 3D images of day-15 endometrial EEOs (EpCAM = green) and ESC (Vimentin = red) in the co-cultures (scale =100 μ m). (C) Schematic and timeframe of the idealized 28-day menstrual cycle in vivo. (D) Experimental design for the validation of the co-culture endometrial model. Treatment groups are designed to mimic the hormonal changes in the phases of the menstrual cycle, including a pharmacologic induction of the menstruation using a PGR antagonist (RU-486, 10uM). Inflamed groups were co-treated with IL1 β (1ng/mL) throughout the length of the experiment. (E) IF imaging of the co-cultures and characterization of morphologic changes in response to hormone treatment reveals epithelial maturation in response to progestin. (F-H) Transcriptomic (bulk RNAseq) analysis of the co-cultures models. (F) A heatmap using a curated list of menstrual genes clusters samples based on hormone treatment group. (G-H) Hallmark pathways show gene expression changes consistent with corresponding phases of the menstrual cycle. Gene ontology (GO) analysis of the top significantly enriched GO terms that are downregulated (G) or upregulated (H) in response to progesterone. Data is shown with the -log of their P values. Cocultures treated with E₂ + MPA ("PE") have gene ontology (GO) expression profiles that align with the secretory phase of the menstrual cycle. All images and analysis were performed on day-15 co-cultures in the synthetic 3wt% 20kDa-PEG-Mix hydrogels (N=8).

Figure 5. Functional validation of the co-culture model recapitulates menstrual cycle-relevant reproductive processes. (A) Representative histological (IF) characterization of the proliferative and secretory phases of the menstrual cycle reveals morphologic and biochemical changes. (B) 3D renderings of the co-culture recapitulate the morphological changes are observed in the secretory treated organoids. (C-D) Stromal decidualization was assessed by measuring prolactin secretion in the medium. (C) Temporal profiles of decidualization normalized to fold change relative to day 0) were performed by measuring spent media (N=12) and can be suppressed by IL1 β treatment (1 ng/mL, E+, PE+). (D) PRL secretion is induced by PE treatment but suppressed in menses. (E) PGR staining in co-cultures at day 15 of treatment. (F) IF staining of PAEP (glycodelin) in co-cultures matches the epithelial secretory phenotype. (G-H) Cleaved caspase-3 (Casp-3) IF was performed in the cultures as a marker of apoptosis and quantified in (H). (I) MMPs were measured in the E vs PE co-cultures using a multiplex panel, day-15 MMP

profiles is shown. All images are of day-15 co-cultures in the synthetic ECM 3wt% 20kDa-PEG-Mix hydrogels.

Figure 6. Functional analysis of inflammation in co-cultures in synthetic hydrogel reveals an endometriotic phenotype mediated by cell-cell communication. (A) Live-dead 3D maximum intensity projections of co-cultures in the synthetic matrices in response to inflammation. (B-C) EEO morphology is impacted by inflammation. Representative images (B) and quantification (C) of epithelial thickness (N=3). (D-E) Representative IF images and quantification of the ESC morphology in the co-culture under inflamed and non-inflamed menstrual cycle conditions. (E) Increased ESC remodeling and decreased cell surface area was observed in in response to inflammation (N=3). (F) Day 15 PRL secretion is significantly inhibited in the inflamed groups. All images are of day-15 co-cultures in the synthetic ECM (3wt%-MIX). (G) Immunostaining analysis of the proliferative profiles of co-cultures and monocultures by EdU incorporation in response to hormones and inflammation (N=3). (H) Quantification of proliferative profiles calculated as a percentage of edu+, compared to DAPI stained nuclei for co-cultures and monocultures. (I) Real-time measurements of EEO diameter growth rates over 15 days (N=4) and quantified as fold change relative to the diameter from day 1 in control (E, PE, W) and inflamed (E+, PE+) conditions. (J) Conceptualization of the cellular mechanisms driving epithelial and stromal communication in the endometrium under physiologic (hormones) and pathogenic (inflamed) conditions. All images are of day-15 co-cultures in the synthetic ECM 3wt% 20kDa-PEG-Mix hydrogels.

6. Supplementary Figures Legends:

Supplementary Fig 1. Characterization of the human endometrium using single cell transcriptomic analysis of the endometrium. (A) Endometrial heterogeneity is shown as a t-distributed stochastic neighbor embedding (tSNE) projection colored by cell identities (left), and donor ID (top) and menstrual cycle stage (bottom). (B-C) Dot plot representation of the cycle phase dependent genes across the distinct cell populations (N=3). (C) Violin plots of the integrin expression across the endometrial cell populations (N=6). (D) Validation of cell specific integrin expression in cultured endometrial cells. ESC and EEOs were cultured in 2D in Matrigel respectively and analyzed by qPCR for integrin and hormone receptor expression relative to GAPDH (N=5).

Supplementary Fig 2. Workflow for EEO generation, hydrogel generation and screening. (A) Schematic of primary endometrial tissue processing workflow to generate organoids (B) (D) Schematic of detailed EEO generation and hydrogel screening protocols.

Supplementary Fig 3. Screening and quantification of EEO generation in synthetic matrices across donors. (A) Brightfield images of day-15 scEEO morphology cultured in hydrogel formulations. (B) Representative images of hydrogel screening of 5 different donors throughout the 14-day experiment derived from single cell. (C) Diameter of ten-day old endometrial organoids (n = 3 independent experiments) growing in the synthetic matrices described compared to EEOs cultured in Matrigel were quantified using a deep learning-algorithm (OrgaQuant) as described in the methods.

Supplementary Figure 4. Validation of EEOs cultured in PEG vs Matrigel. (A) IF images of scEEO morphology derived in soft synthetic matrices compared to Matrigel. (B) Characterization of scEEOs reveal epithelial-origin in synthetic matrices and in Matrigel. (C) Ki67 staining of EEOs cultured in synthetic ECM and in Matrigel in addition to a 3D projection of Ki67 stained ex vivo tissues. Inset is a gland cross-section. (D) Brightfield snaps from videos reflect epithelial heterogeneity and motile cilia. EEOs cultured in synthetic matrices and stained for acetylated α -tubulin reveal multi-ciliated and primary ciliated cells.

Supplementary Fig 5. Identifying cell ratios ex vivo for tissue modeling in vitro. 3D imaging of ex vivo tissues using tissue clearing methods and confocal imaging were analyzed using the Imaris (Bitmap 9.6.0) software to segment the epithelial and stromal compartments of the native endometrium (N=3 donors, 3 different volumes) using the 'Surface' function to masks individual cell regions in a volumetric image. (A) The 'Cells' function Single cell segmentation tools from the software were utilized to calculate the number of epithelial cells (Epi) per volume of epithelial glands (Epi/ μ L). (B) ESC single cell counting in 3D was performed using the 'Spots' function by labeling and counting the DAPI+ nuclei in the image. A similar approach was taken for co-culture in vitro models. (C) Cell concentration per unit area the native endometrium and the cell ration between epithelial cells and stromal cells. (D) 3D analysis of EEOs cultured in the synthetic matrices. (E) Cell density in vivo and in vitro comparing relative numbers and epithelial cell gland

volumes. (F) No significant differences in the ratio between single ESCs concentration (ESC/ μ l) and Epithelial volume (gland/volume ratio).

Supplementary Fig 6. Establishment and transcriptomic analysis of monocultures and cocultures. (A) Representative brightfield images of co-culture (N=6) and monocultures of EEOs (N=1) and ESCs (N=6). (B) Unsupervised PCA analysis of bulk RNAseq co-cultures and monocultures cluster by culture type. Ellipses bound the 95% confidence interval for each group. (C) Plotting $\log_2(\text{fold change})$ against $-\log_{10}(P \text{ value})$ in a volcano plot shows differentially expressed genes in cocultures as compared to cultures of ESC only.

Supplementary Fig 1. Functional validation of endometrial-coculture recapitulation of the menstrual cycle in vitro. (A) Immunofluorescent images of the organoid morphology under 'proliferative (E)' and 'secretory (PE)' hormone treatment groups for both EEOs and ESC. Morphological assessment of altered cellular response to hormones and to inflammation (IL1 β , 1ng/mL). (B) Immunofluorescent staining of PAEP of the day-15 co-cultures at a lower magnification reveals progesterone induced secretory phenotype in the EEOs. (C) Transcriptomic analysis of MMP reveals differential expression patterns in response to hormone treatments. (D-E) Gene Set Enrichment Analysis (GSEA) on a ranked list of genes from a generalized linear model of the coculture data. Hallmark pathways show gene expression changes consistent with corresponding phases of the menstrual cycle.

Supplementary Fig 2. Apoptosis is increased in the menstrual phase of the co-culture model and in the inflamed endometrium and corresponding viability is decreased in the inflamed group. (A-B) Comparison of apoptotic marker expression (cleaved caspase-3) immunofluorescent staining in co-cultures at day 15 in the menstrual cycle and inflamed groups in low (4X) and high (10X) magnification. (C) Quantification of ESC monoculture apoptosis relative to total nuclei count (N=3).

Supplementary Fig 9. Viability in the co-culture models at day 15. Low (4X) and high (10X) magnification of the Live (green) and Dead (red) assay in the hormone and inflammation and hormone treated groups comparing (A) cocultures and (B) ESC monocultures.

Supplementary Fig 10. Inflammation disrupts ESC remodeling and morphology. (A) Representative brightfield images of ESC monocultures in response to inflammation (IL1b, 1 ng/mL), reveals increased remodeling. (B) Immunofluorescent analysis of F-actin reveals morphologic changes associated with inflammatory response. Single cell segmentation (Red) elucidates the morphologic changes associated with hormone treatment and inflammation in 3D (N=3).

Supplementary Fig 11. Co-cultures treated with IL1 β have transcriptomic profiles consistent with inflamed endometrial states. (A) A volcano plot of $\log_2(\text{fold change})$ against $-\log_{10}(P \text{ value})$ for cocultures treated with inflammation as compared to non-inflamed controls. Transcript data is modeled using a generalized linear model to account for inter-donor and hormone treatment variability. (B) Differentially expressed genes were analyzed using gene ontology (GO) analysis to examine the most up- and down- regulated pathways. (C) A closer examination of the most significant GO terms reveals that canonical inflammatory genes are upregulated while PRL is

downregulated, as expected for an inflamed environment. (D) Gene Set Enrichment Analysis (GSEA) on a ranked list of all expressed genes similarly shows an enrichment of inflammatory pathways upon IL1 β treatment.

Supplementary Fig 12. Functional and molecular analysis of EEO growth rates. Inflamed co-cultures and monocultures were analyzed to examine the growth rates of EEOs under hormone treatments. Daily images were analyzed to quantify the diameter fold change of the organoids relative to day 1 of cultures. Co-cultures revealed increased growth and decay rates in response to inflammation compared to mono-culture (EEO only) controls (N=6).

Supplementary Fig 13. Temporal validation of inflammatory response on co-cultures via functional and protein analysis. Multiplex 45-panel of secreted cytokines measured in co-cultures throughout the 15-day experiment. Readings were measured every 3 days in control or inflamed conditions (N=3 replicates per day). (A) Daily changes in MMP panel protein secretion in co-cultures throughout the 15-day experiment. (B) Heatmap of temporal analysis of multiplex protein secretion. (C) Representative luminex panel of up regulated and downregulated cytokines in response to inflammation. Principal component analysis (PCA) and corresponding loading plots. All images are of day-15 co-cultures in the synthetic ECM (3wt%-MIX). (E = estradiol only, PE = E + MPA, M = PE withdrawal + RU486), E+ = E + IL1 β , and PE+ = PE+IL1 β , M+ = M + IL1 β).

Supplementary Fig 14. EEOs can be generated and expanded directly into synthetic ECM from primary tissues. (A) Schematic of epithelial gland collection and EEO generation and expansion using Matrigel and the synthetic matrix. (B) Brightfield images of EEOs generated in the synthetic matrix (3wt%-MIX) directly from freshly isolated gland fragments. (C) Characterization of Matrigel-free EEOs and immunofluorescent images of actin (green) staining. (D) Validation of expansion and passaging of EEOs (p1) using the synthetic matrix.

Supplementary Fig 15. Histological metadata and characterization of endometrial tissues collected. (A) Hematoxylin and eosin stain (H&E) of endometrial tissues from endometrial tissues. (B) Table outlining patient metadata and tissue processing.

Graphical abstract

Tissue engineering the endometrial microenvironment in 3D

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Figure 2. Dual-peptide functionalization and softer synthetic matrix regimes enhance EEOs generation.

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Supplementary Figures

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Supplementary Fig 4. Apoptosis is increased in the menstrual phase of the co-culture model and in the inflamed endometrium and corresponding viability is decreased in the inflamed group.

Supplementary Fig 9. Viability in the co-culture models at day 15.

Supplementary Fig 10. Inflammation disrupts ESC remodeling and morphology.

Supplementary Fig 12. Functional and molecular analysis of EEO growth rates.

Supplementary Fig 13. Temporal validation of inflammatory response on co-cultures via functional and protein analysis.

Supplementary Fig 14. EEOs can be generated and expanded directly into synthetic ECM from primary tissues.

Supplementary Fig 15.

B.

Donor ID (Control/Ovls)	DX Adenomyosis (Y)	DX Endometriosis (Y)	Other DX	Ex Vivo scRNAseq	Ex Vivo Proteomics	Cell Separation	Co-culture Model	Co-culture bulk RNAseq	H&E (Y)	AGE	Cycle Phase	Gravidy	Parity	Regular Cycles	Hormones in past 3 mo	NOTES
251	X		pelvic pain			X	X			42	Proliferative (CD13)			yes	no	
252			fibroid	X		X	X		X	39	Secretory			yes	no	
254		X				X			X	36	Secretory			yes	no	
255		X			X	X	X		X	40	Menues			yes	no	
259		X	Ov cyst	X		X	X	X	X	32	Secretory			yes	no	
260		X		X		X	X	X	X	27	Late secretory			yes	no	
261		X	Ovarian cyst	X		X			X	33	Proliferative			yes	no	
262			leiomyoma						X	38	Proliferative			yes	no	
263		X			X	X	X		X	40	Early Secretory	2	2	yes	no	weakly proliferated endometrium with focal stromal breakdown, squamous metaplasia of cervix, cyst, bloody, small biopsy
264			leiomyoma		X	X	X		X	43	Secretory	2	1	yes	no	leiomyomata, big biopsy, good
266					X	X	X		X	30	Proliferative	4	4	yes	no	
267	X	X			X				X	43	Proliferative	2	2	yes	no	leiomyoma
268	X		leiomyomas		X	X	X		X	44	Secretory	2	1	yes	no	PF is bloody, multiple leiomyomas, chronic inflammation of the cervix
270			cyst adenoma	X	X	X	X	X	X	42	Early Secretory	1	0	yes	no	scRNA Fresh and frozen, mucinous cystadenoma with focal dystrophic calcifications, corpus luteal cyst
271			leiomyoma	X	X	X	X	X	X	36	Secretory	3	2	yes	no	
272			AUB	X	X	X	X	X	X	42	Proliferative	2	2	no	no	PTC bloody
273	X	X			X	X			X	39	Late Secretory	2	2	yes	no	late secretory
274	X	X			X				X	41	Proliferative	0	0	yes	no	

Videos

Motile cilia in vivo and in EEOs

Motile cilia in synthetic PEG ECM (3wt% MIX)

Video 1

Ex vivo gland

EEOs in Matrigel

EEO monolayers

Proliferative phase co-culture

Video 2

Secretory phase co-culture

Video 3

Video 4

Video 5

- 1
- 2