A Gelatin Hydrogel Model of the Endometrium and Trophoblast Invasion

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Statement of Purpose: The endometrium is the lining of the uterus and site of embryo implantation. This dynamic tissue undergoes rapid cycles of growth, breakdown, and remodeling each menstrual cycle with the goal of preparing the tissue for a potential pregnancy. If conception occurs, the blastocyst will breach the endometrial epithelium and embed into the underlying stroma. A complex molecular dialogue between endometrial cells and trophoblast cells from the blastocyst will coordinate the extent of invasion. Defective implantation can result in a variety of pregnancy disorders, such as preeclampsia or intrauterine growth restriction. Despite the importance of early implantation in the establishment and maintenance of pregnancy, much remains unknown about this process due to the ethical constraints of studying it in humans as well as the challenge of observing this process that occurs approximately 10 days post-conception. Three-dimensional biomaterial models provide the unique opportunity to perform mechanistic studies of implantation in tunable and scalable platforms with the complexity necessary to appropriately model the endometrium and trophoblast invasion. Using methacrylamide-functionalized gelatin (GelMA) hydrogels, we developed an endometrial model consisting of an endometrial epithelial culture overlaying a hormone-responsive endometrial perivascular niche [1]. We report high-throughput microarray experiments to identify extracellular matrix (ECM) proteins that facilitate improved endometrial epithelial cell adhesion, then adapt a microbial transglutaminase (mTg) approach to immobilize these proteins to our endometrial model. We use trophoblast invasion patterns within GelMA hydrogels to reveal effects of soluble factors from the maternal-fetal interface (epidermal growth factor, nodal, pregnancy-specific glycoproteins) on trophoblast motility.

Materials and Methods: Polyacrylamide microarrays were fabricated consisting of single and pairwise combinations of 10 ECM proteins. Endometrial epithelial cells were seeded onto microarrays for 24 hours, fixed, stained with Hoechst, and imaged using a Zeiss Axioscan Z1. Analysis of cellular adhesion was performed using an image analysis pipeline on R. GelMA hydrogels were coated with ECM proteins by combining 0.5 mg/mL mTg and ECM proteins and were imaged using a Leica DMi8 Yokogawa W1 spinning disc confocal microscope outfitted with a Hamamatsu EM-CCD digital camera. Endometrial perivascular niche cultures were fabricated by combining human endometrial microvascular endothelial cells (HEMECs) and human endometrial stromal cells (HESCs) in a 1:1 ratio with 500,000 cells/mL. Hydrogels were cultured for 7 days in the presence of VEGF or decidualization hormones and then imaged using a Zeiss LSM 710 Confocal Microscope. Swan71 trophoblast cells were seeded into round bottom plates for 48 hours to create spheroids that were then encapsulated within GelMA hydrogels. Trophoblast invasion was quantified as outgrowth area using FIJI by manual tracing with data were analyzed across groups on each day of culture.

Results: We report three critical stages for developing an dynamic endometrial model. Epithelial patterning. Endometrial epithelial adhesion in microarray culture was improved in microarrays coated with basement membrane associated factor pairs (Fig. 1A). Microbial transglutaminase provides a route to immobilize identified proteins on the hydrogel surface (e.g., Laminin, Fig. 1B). Ongoing efforts are quantifying the effect of Col1+III and ColIV+TenascinC on endometrial epithelial cell immobilization, expansion, and functional activity (cytokeratin-18), comparing results to a conventional basement membrane cocktail (ColIV+laminin). Stromal cell decidualization. GelMA hydrogels support decidualization of encapsulated endometrial stromal cells (HESCs) in co-culture with endometrial endothelial cells (HEMECs) (Fig. 1C). HESCs are elongated and express CD10 (red) but become decidualized when cultured with hormones and undergo a characteristic morphological change to a round morphology and demonstrate decreased expression of CD10. Decidualization also affected the complexity of the endothelial cell networks, causing HEMECs to spread less when HESCs became decidualized. Trophoblast invasion. GelMA hydrogels support quantitative analysis of trophoblast motility. Trophoblast motility is strongly enhanced in the context of EGF (Fig. 1D) but reduced in response to stressors such as cortisol (not shown); interestingly, pregnancy specific glycoproteins (PSGs) have differential effects, with PSG9 reducing but PSG1 increasing motility.

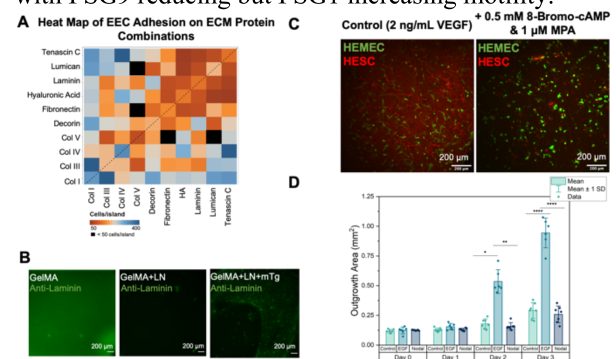

Figure 1. (A) Epithelial cell adhesion heatmap. (B) Laminin coated GelMA hydrogels. (C) Maximum intensity projection of HEMEC/HESC co-cultures in GelMA hydrogels. (D) Trophoblast outgrowth area.

Conclusions: We report a biomaterial endometrial model consisting of an endometrial epithelial culture and endometrial perivascular niche. We use this platform to investigate patterns of endometrial epithelial adhesion, stromal perivascular remodeling, and trophoblast motility.

References:

1. Zambuto, S., *Interface Focus,* 2019;**9**: 5.
2. Zambuto, S., *Tissue Eng*, accepted 2020.
3. Zambuto, S., *biorxiv*, 2020.