Examining the Effect of Phthalates on a Novel 3D *In Vitro* Ovarian Endometriosis Spheroid Model

Hannah S. Theriault[1,6], Sarah Ibrahim[2], Alison C. Nunes[6], Sia Mittal[3,6], Jenny Martinez[1,6], Kathryn B.H. Clancy[4,6], Romana A. Nowak[2], Brendan A.C. Harley[3,5,6]

[1] Department of Bioengineering; [2] Department of Animal Sciences; [3] Department of Chemical and Biomolecular Engineering; [4] Department of Anthropology; [5] Cancer Center at Illinois; [6] Carl R. Woese Institute for Genomic Biology at the University of Illinois Urbana-Champaign (UIUC), Urbana, USA

Endometriosis is a chronic inflammatory gynecological disease affecting 1 in 10 menstruators. It is identified laparoscopically by the presence of endometrial-like tissue around the pelvic cavity. These lesions cause dysmenorrhea, menorrhagia, fertility issues, and an increased risk of ovarian cancer (with ovarian endometriosis). Often, it takes a decade from symptom onset to diagnosis, thus, not much is known about the early stages of these lesions and their potential causes. Researchers believe that there is a positive correlation between phthalate exposure and endometriosis risk. Phthalates are known endocrine disruptors used in many everyday products to create flexible plastics. Di(2-ethylhexyl) phthalate (DEHP) is a particularly interesting research target for endometriosis as it is one of the most common phthalates and has been observed in animal models to alter estrogen synthesis. Previous *in vitro* work has examined the impact of DEHP on healthy endometrial cells and cancer-derived endometrial cells. Here we propose a novel method for studying the effects of DEHP on a spheroid co-culture model of ovarian endometriosis incorporating both an epithelial and stromal component derived from endometriotic lesions in in methacrylamide-functionalized gelatin (GelMA). Endometriotic cells used for these experiments include azurite-blue tagged human endometrial-endometriotic stromal cells (azb-iEC-ESCs; Fazleabas Lab, Michigan State University) and GFP-transduced human endometriotic epithelial cells (12z; ABM). Endometriotic spheroids were formed using cohorts of GFP-12z cells and azb-iEC-ESCs (1:3 respectively) in 5k cell spheroids. These spheroids were encapsulated in GelMA precursor and UV polymerized. GelMA was synthesized utilizing a previously established method by the Harley Lab. GelMA was characterized with NMR (Agilent 600MHz NMR), and compressive testing (Instron 5943). 3D hydrogels were prepared with 5wt% or 7wt% GelMA, PBS, and 0.1% w/v lithium phenyl phosphinate photoinitiator. GelMA was photopolymerized under a UV light (λ = 365 nm, 7.14 mW cm–2, AccuCure Spot System ULM-3-365) for 30s. Spheroids were cultured in a 50/50 mixture of 12z complete culture medium (CCM) and iEC-ESC CCM. For this pilot experiment, media (0 μM DEHP, 1 μM DEHP, 0.1 μM DEHP, and 0.01 μM DEHP treatments) was changed every two days and spheroids were imaged on D0, D2, and D8. GelMA synthesized at 5wt% and UV polymerized for 30s fell within the stiffness of natural, healthy ovarian tissue (3.3 ± 2.5 kPa). 7wt% fell above our desired stiffness. We identified a ratio of epithelial and stromal endometriotic cells that mimic histological stains of lesions and supports outgrowth of spheroids within our system. We identified physiological doses of DEHP and observed initial differences in outgrowth where control and 0.1 μM DEHP experienced the most outgrowth by D8. Here we have engineered a model to encapsulate ovarian endometriosis spheroids in physiologically relevant extracellular matrix to investigate the effects of DEHP. We observed visual differences in spheroid outgrowth of control vs. DEHP treatments. In this pilot study we observed minimized outgrowth in 1 μM DEHP and 0.01 μM DEHP treatments, and future replicates will shape conclusions about the relationship between DEHP exposure and endometriosis outgrowth. We propose future repetitions of this experiment that include a vehicle control (DMSO), an expansion of treatments (inclusion of MEHP, a primary metabolite of DEHP), and an expansion of replicates (n >6).