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# Human *in vitro* endometriosis microphysiological system

<https://doi.org/10.1515/cdbme-2025-0197>

**Abstract:** Endometriosis is a debilitating condition that causes severe pain and infertility, among other morbidities. It affects 10% of reproductive-aged women worldwide and poses a significant public health challenge due to its prevalence and the lack of effective treatments and diagnostic tools. The study of endometriosis is restricted by the difficulty in modelling its multifactorial nature. To address this challenge, we developed a microphysiological system capable of reproducing key features of the menstrual cycle and endometriosis. In this publication, we describe such system and proof-of-concept data using simplified *in vitro* models that validate its use.

**Keywords:** Microphysiological system, endometriosis, *in vitro*, woman health.

## 1 Introduction

Endometriosis is a chronic and debilitating condition that affects approximately 10% of reproductive-aged women worldwide [1]. It occurs when the endometrium, which lines the inside of the uterus, grows in areas outside of the uterus, such as the peritoneum, ovaries, fallopian tubes and colon. The misplaced tissue can cause severe pain, infertility and other morbidities [1], [2].

The development of endometriosis is inevitably linked to menstruation, but its pathogenic mechanisms remain poorly understood [2]. Historically, the most biologically relevant models of endometriosis have been menstruating non-human primates and rodents. These carry significant costs and ethical concerns as well as limitations of translating findings to the clinic due to species differences and experimental

methodologies such as the administration of exogenous hormones and menstrual tissue transplantation [3]. The use of human-based *in vitro* models has at once, met ethical standards and improved translational outcomes. Indeed, human-based research approaches have revealed some mechanistic insights into endometriosis pathophysiology and identified potential therapy targets and risk factors [3].

The biggest challenge in modelling endometriosis *in vitro* concerns replicating the multifactorial nature of the disease, i.e. different tissues and cell types involved, as well as varying hormonal concentrations [3], [4]. To answer this challenge, we have developed a sophisticated human-based *in vitro* microphysiological system (MPS) dedicated to studying endometriosis. In this paper, we describe the three modules that make up this MPS and report preliminary data showing the replication of specific features of endometriosis, including endometrial cell response to hormones and tissue integration.

## 2 Endometriosis microphysiological system

The MPS consists of three interconnected modules, each dedicated to simulating different aspects of the female reproductive system and endometriosis (see Figure 1). The first module concerns a programmable fluidic unit that delivers reproductive hormones at concentrations that aim to mimic each stage of the menstrual cycle to the second module, where a human-based 3D model of the endometrium is cultured. Models of typically affected tissue are cultured in the third module, where invasion by the endometrial tissue occurs. Altogether, the MPS is designed for microscopy imaging in a non-invasive manner, but it also allows tissue recovery for the study of protein and gene expression.

### 2.1 Endocrine fluidic unit (module 1)

The endocrine fluidic unit is depicted in Figure 1A. It consists of programmable peristaltic pumps and electronic valves that allow the management of up to 4 different types of medium.

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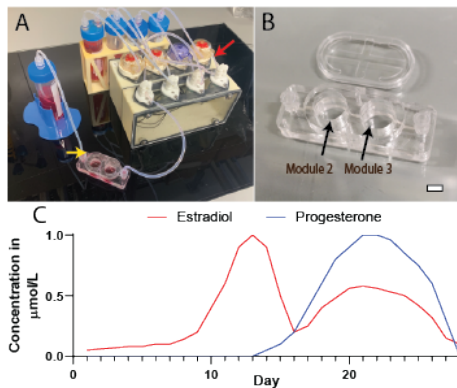
In this configuration, we can deliver specific concentrations of estradiol and progesterone to the cells cultured in the biochip (Module 2 & 3), depicted in Figure 1B. Figure 1C, shows the hormone concentrations that can be reproduced with this setup, which match reference hormonal concentrations over a 28-day menstrual cycle described in Stricker *et al.*, 2006 [5].

## 2.2 Tissue-on-chip (module 2 and 3)

We have called the biochip where tissue constructs are cultured, the Tissue-on-chip. It includes both endometrium tissue and invaded tissue constructs (modules 2 and 3).

The endometrium tissue model is cultured in a first chamber that is connected to a second chamber via a fluidic channel, where the invaded tissue is cultured, see Figure 1B. The fluidic channel not only allows endometrial cells to invade the tissue in the second chamber with the help of the flow produced by the endocrine fluidic unit but also exposes both tissues to the same hormonal conditions.

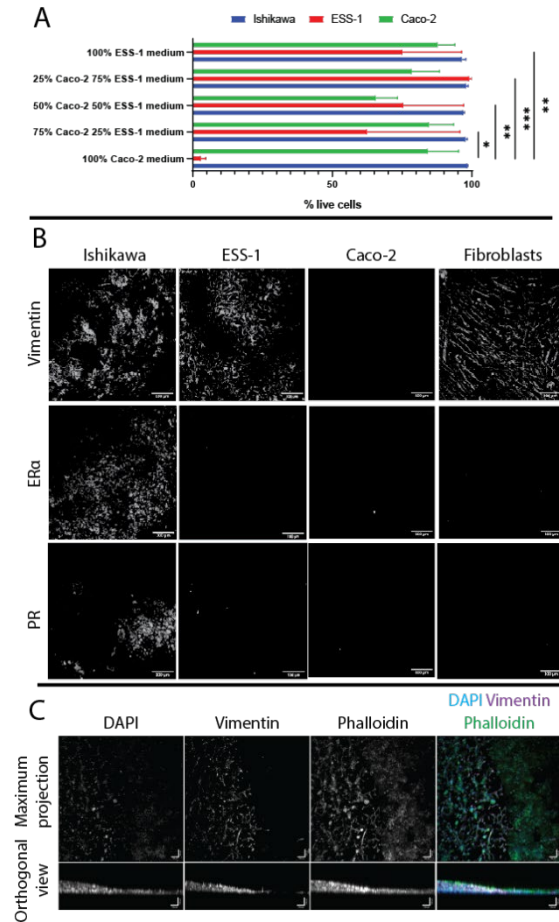
The Tissue-on-chip was designed to be transparent and to fit most microscope stages, making it ideal for use with light, fluorescence and confocal microscopy. Furthermore, the Tissue-on-chip has a removable lid, allowing recovery of the cultured tissue for the study of protein and gene expression.



**Figure 1:** A) MPS with 3 modules. Red arrow points out the fluidic unit. Yellow arrow points out Tissue-on-chip. B) Tissue-on-chip. C) Concentrations of estradiol and progesterone that can be reproduced with module 1 in a 28-day cycle.

## 3 3D endometrium model

To generate a 3D model of the endometrium, we have selected human ESS-1 endometrial stromal sarcoma cells (RRID:CVCL\_1205) and human Ishikawa endometrial adenocarcinoma epithelial cells (RRID:CVCL\_2529) to test the feasibility of building an endometrial construct on which



**Figure 2:** A) Percentage of live cells cultured in different mixes of medium. N=3 per condition. P values were determined by ordinary 2-way ANOVA and comparisons done using the Bonferroni method (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). B) Immunofluorescence images of Vimentin, ER $\alpha$  and PR expression by Ishikawa, ESS-1, Caco-2 and Fibroblasts. C) Immunofluorescence images of 3D model of endometrium. Field of view shows around 50% of the construct. Scale bars: 100  $\mu\text{m}$ .

to base our MPS on. These cell lines were chosen as they are immortalized cell lines and easier to manipulate compared to iPSC-derived cells. We have used human fibroblasts (ATCC: CRL-2429 CCD-112Sk) and human Caco-2 colorectal adenocarcinoma cells (ATCC: HTB-37) as control cell lines.

We performed medium compatibility tests to assess if whether different cell lines could be viably cultured within the same biochip. We stained Ishikawa, ESS-1 and Caco-2 cells with Calcein AM (ThermoFisher, C1430) to mark live cells and Propidium Iodide (Fluka, 81845) to mark dead cells and calculated the percentage of live cells in each mix of ESS-1 medium (RPMI 1640 (Gibco, 11875-093) with 10% inactivated Fetal Bovine Serum (FBS) (Gibco, A5256701)), and Caco-2 medium (MEM, GlutaMax Supplement (ThermoFisher, 41090028), 1% MEM NEAA (ThermoFisher, 111400359) and 10% inactivated FBS (Gibco, A5256701)).

We found that culture of all cell types is viable when using 100% ESS-1 medium, see Figure 2A.

To confirm that Ishikawa and ESS-1 cells could be used to generate 3D endometrium models that are responsive to varying concentrations of estradiol and progesterone, we investigated the expression hormone receptors by these cells by immunolabeling cells with anti-estrogen receptor  $\alpha$  (ER $\alpha$ ) antibody (ThermoFisher, MA1-310) or anti-progesterone receptor (PR) antibody (ThermoFisher, MA1-411) and with anti-mouse Alexa Fluor 488 (Invitrogen, A-11001). We found that ESS-1 and Ishikawa cell lines express ER $\alpha$ , but PR is only expressed by Ishikawa cells, see Figure 2B.

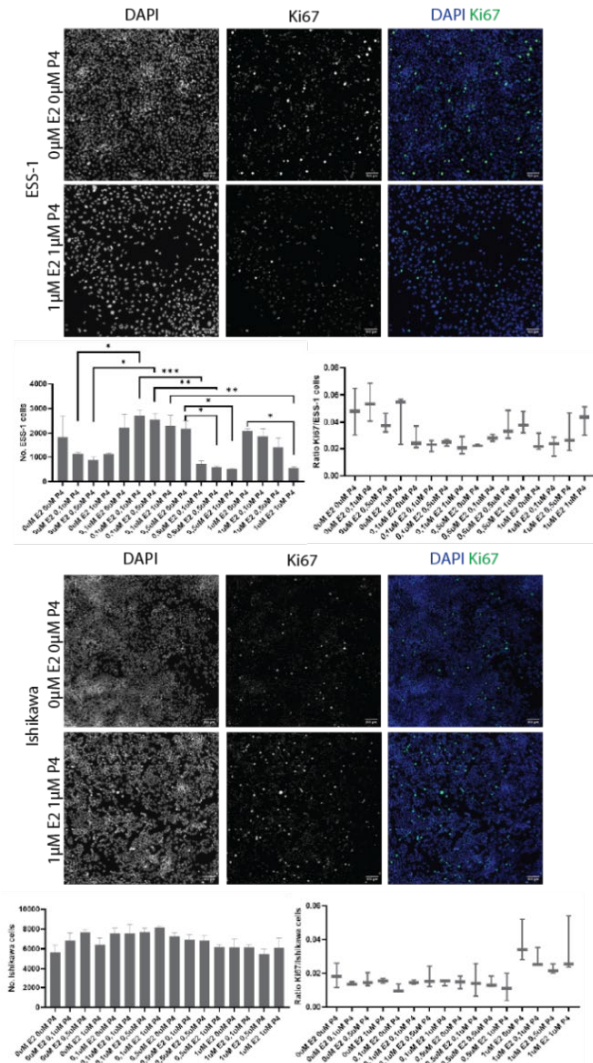
Altogether, the data described above indicates that it is feasible to use these cell lines to construct an endometrial model responsive to varying hormonal concentrations. Thereby, we have built a 3D endometrium construct that consists of ESS-1 cells embedded in a collagen I (Gibco, A1048301) and laminin (Gibco, 23017015) 3D matrix, which is surrounded by Ishikawa cells.

### 3.1 Cell-specific response to hormonal exposure

To assess if ESS-1 and Ishikawa cell growth is affected by exposure to sex hormones, we incubated cells with varying concentrations of  $\beta$ -estradiol (E2) (BioReagent, P8783) and progesterone (P4) (BioReagent, E2758) for 4 days and assessed cell number and percentage of dividing cells. Cell numbers were calculated by counting number of cell nuclei stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma, D9542), while cell division was evaluated by calculating the percentage of cell nuclei labelled with anti-Ki67 (BD Biosciences, 556003) and anti-mouse Alexa Fluor 488 (Life Tech, A-1101), see Figure 3.

Results show that Ishikawa cell growth is not affected by varying hormonal concentrations. Differently, the number of ESS-1 cells is significantly reduced with increasing concentrations of progesterone when  $\beta$ -estradiol concentration is constant. However, it is worth noting that ESS-1 cell number is significantly increased when they are incubated with low concentrations of both  $\beta$ -estradiol (0.1  $\mu$ M) and progesterone (0.1  $\mu$ M, 0.5  $\mu$ M) when compared to being incubated with just one hormone.

The percentage of dividing cells is not significantly altered in each condition, indicating that hormonal incubation affects cell growth by impacting cell viability rather than cell division. Nevertheless, these results suggest that endometrium constructs built with these cell lines will display different phenotypes under varying hormonal concentrations.



**Figure 3:** ESS-1 and Ishikawa cells labelled with DAPI and Ki67. Plots show number of cell nuclei and percentage of Ki67 positive cells in each 16 conditions. N=3 per condition. P values were determined by ordinary 2-way ANOVA and comparisons done using the Bonferroni method (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Scale bars: 100  $\mu$ m.

## 4 Invaded tissue

Endometriosis is characterized by the invasion of different tissues, including the colon, by the endometrial cells [1], [2], [6]. Thus, we have designed Module 3 to be able to support the culture of different tissue constructs. Here, we have used Caco-2 cells as a simplified *in vitro* model of invaded colorectal epithelium, thereby mimicking bowel endometriosis, to assess the integration capacity of endometrial cells.

## 4.1 Endometrial cells can integrate a colorectal epithelium model

To assess the integration capacity of Ishikawa cells, we have fluorescently labelled cells with Hoechst 33342 (Invitrogen, H3570) and seeded them on confluent Caco-2 layer at different amounts (0, 10, 100 and 1000 cells). Integration was calculated by counting the number of labelled cells in a field of view (images not shown) and comparing to the theoretical value corresponding to maximum integration. The theoretical value of maximum integration was calculated (see eq 1).

$$\text{Max integration} = A_{\text{field of view}} \times \frac{N_{\text{seeded cells}}}{A_{\text{well}}} \quad (1)$$

Where Max integration corresponds to the number of labelled cells equivalent to maximum integration,  $A_{\text{field of view}}$  is the area of the field of view (0.006 cm<sup>2</sup>),  $N_{\text{seeded cells}}$  is the number of seeded Ishikawa cells, and  $A_{\text{well}}$  is the area of the cultureware where Caco-2 cells are cultured (0.33 cm<sup>2</sup>).

Using this method, preliminary data indicates that Ishikawa cells can integrate Caco-2 monolayer with an efficacy between 40 and 100%, see table 1.

**Table 1:** Number of Ishikawa cells counted in each condition, theoretical value equivalent to maximum integration, and calculated integration efficacy in percentage. N=3 per condition.

| No. seeded cells | Counted (N ± SD) | Max integration | Integration efficacy |
|------------------|------------------|-----------------|----------------------|
| 0                | 0 ± 0            | 0               | -                    |
| 10               | 0 ± 0.6          | 0               | -                    |
| 100              | 2 ± 1            | 2               | 100 ± 50 %           |
| 1000             | 13 ± 5.6         | 18              | 72.2 ± 31.1 %        |

## 5 Conclusion

Endometriosis poses a significant public health challenge due to the lack of effective treatments and diagnostic tools. Its multifactorial nature makes it difficult to model it in research settings. Historically, the most biologically relevant models have been based on menstruating non-human primates and rodents, which carry significant ethical and economic costs and are not translatable to the human condition.

Here we report a novel MPS dedicated to modelling cyclical hormonal changes and tissue invasiveness typical of endometriosis. By using human cells, we show proof-of-concept data, including endometrial cell response to reproductive hormones and endometrial cell integration in a colorectal epithelium model, that validates the use of MPS.

Although this data is promising, we understand that further work must be done to model endometriosis accurately.

Indeed, ESS-1 and Ishikawa cell response to reproductive hormones differs from the physiological endometrium changes during the menstrual cycle, likely due to their immortalized characteristics and the fact they were only exposed to two of the hormones involved in this cycle. Additionally, although Ishikawa cells, representative of the endometrium epithelium, can integrate a colorectal epithelium model, we have yet to investigate if ESS-1 cells, representative of the endometrium stroma, have similar behaviours. The endometrial stromal cells represent the largest fraction primarily involved in tissue invasion [2]. Further experimentation is also required to assess the migratory capabilities of the used endometrial cells.

Altogether, we envision that our MPS can be applicable for use with more relevant *in vitro* models, such as ones derived from endometrial progenitor cells, which can give rise to all cell lineages of the endometrium [4], or iPSCs, that could be used to derive endometrium and invaded tissue models.

### Author Statement

We thank the HES-SO for funding this research (Grant 129917) and we thank Dr. Govindan and Dr. Loussert-Fonta for initial inputs. The authors state no conflict of interest.

## References

- [1] P. Parasar, P. Ozcan, and K. L. Terry, 'Endometriosis: Epidemiology, Diagnosis and Clinical Management', *Curr Obstet Gynecol Rep*, vol. 6, no. 1, pp. 34–41, Mar. 2017, 10.1007/s13669-017-0187-1.
- [2] R. O. Burney and L. C. Giudice, 'Pathogenesis and pathophysiology of endometriosis', *Fertility and Sterility*, vol. 98, no. 3, pp. 511–519, Sep. 2012, 10.1016/j.fertnstert.2012.06.029.
- [3] H. Malvezzi, E. B. Marengo, S. Podgaec, and C. D. A. Piccinato, 'Endometriosis: current challenges in modeling a multifactorial disease of unknown etiology', *J Transl Med*, vol. 18, no. 1, p. 311, Dec. 2020, 10.1186/s12967-020-02471-0.
- [4] A. Gołabek-Grenda and A. Olejnik, 'In vitro modeling of endometriosis and endometriotic microenvironment – Challenges and recent advances', *Cellular Signalling*, vol. 97, p. 110375, Sep. 2022, 10.1016/j.cellsig.2022.110375.
- [5] R. Stricker, R. Eberhart, M.-C. Chevailler, F. A. Quinn, P. Bischof, and R. Stricker, 'Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT® analyzer', *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 44, no. 7, Jan. 2006, 10.1515/CCLM.2006.160.
- [6] G. C. Miller, A. Sokolova, M. L. Bettington, C. Rosty, and I. S. Brown, 'Colorectal endometriosis – a challenging, often overlooked cause of colorectal pathology: a clinicopathological review of 114 cases', *Pathology*, vol. 56, no. 6, pp. 795–803, Oct. 2024, 10.1016/j.pathol.2024.04.006.