# Towards understanding endometriosis: Generation of an endometrial organoid and stromal fibroblast biobank

Anna Wallis[1], Kamila Marciniak[1], Hongorzul Davaapil[1], Maryna Panamarova[1], Hazel Rogers[1], Joanne Doran[1], Amy Yeung[1] , Carmen Sancho Serra[2], Magda Mareckova[2], Iva Kelava[2], Roser Vento-Tormo[2]

[1] Cellular Modelling, Cellular Operations, Scientific Operations, Wellcome Sanger Institute, Cambridge, UK
[2] Cellular Genetics Department, Wellcome Sanger Institute, Cambridge, UK

## Abstract & Background

The endometrium is the inner epithelial lining of the uterus, which plays a key role in fertility where it thickens and provides an optimal environment for the developing embryo. In the absence of pregnancy, part of the endometrial lining is shed and the cycle restarts. Endometriosis is a condition which is characterised by the presence of ectopic endometrial tissue outside of the uterine cavity, including the intestines and bladder. During the menstrual cycle, these lesions thicken and bleed in response to changing levels of oestrogen and progesterone, leading to inflammation and a wide variety of symptoms; notably, pelvic pain, fatigue and infertility. Despite affecting ~10% of females of reproductive age, endometriosis remains poorly understood, with limited diagnostic and treatment options. This is further complicated by the lack of animal models, as the function of the human endometrium cannot be recapitulated in typical *in vivo* models.

In order to better understand the disease, *in vitro* models that recapitulate the human endometrium need to be developed. This project aims to establish a biobank of healthy and diseased endometrium and their matching stromal fibroblasts. We have based our protocol for derivation from existing published work, and have adapted it for higher-throughput processing and expansion. Endometrial organoids and their associated stromal fibroblasts can then be used in future experiments for hormone treatment, drug testing and co-culture experiments; these will shed light on a poorly-understood aspect of women's reproductive health, and hopefully lead to better treatment and management options.

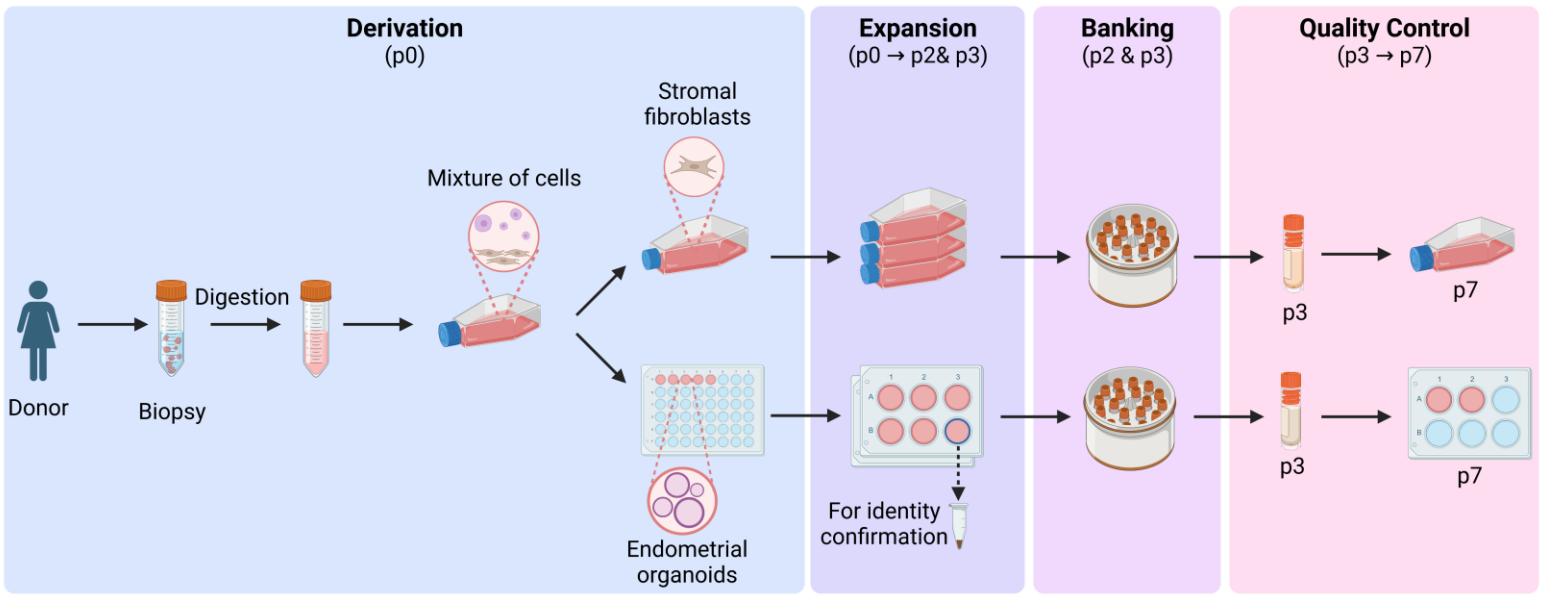


**Figure 1.** Project pipeline: from Derivation to Quality Control (QC). Figure created using *Biorender.com*

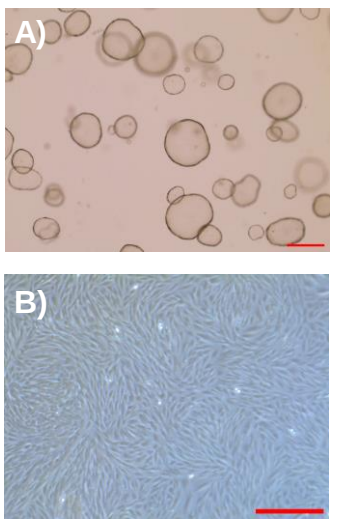


**Figure 2**. **A)** Endometrial organoids and **B)** stromal fibroblasts. 500µm scale bars.

## Method

Matched endometrial organoid and stromal fibroblast lines were derived from biopsies (**Fig 1**) using an adaptation of an established protocol[1]. During derivation, the endometrial epithelial cells were separated from the stromal fibroblasts and seeded in matrigel. Matrigel is a 3D matrix that enables the cells to form round, hollow endometrial organoids **(Fig 2A)**, whereas fibroblasts were grown in flasks and have a spindle-like morphology **(Fig 2B)**.

The organoids and fibroblast lines were expanded and banked at passage 2 and 3. In order to confirm endometrial epithelial identity, marker expression analysis was performed on all organoid lines to ensure that organoids were positive for endometrial epithelial markers, and negative for markers of other cell types (e.g. bladder, intestine). These lines then progressed to Quality Control (QC), where we determined whether they were capable of extended propagation. Once QC was successful, these lines could then be used for downstream applications. Current work has focused on eutopic biopsies (i.e. tissue from the uterus – not from ectopic lesions).

## Optimising the passage method

**Mechanical or enzymatic?** Passaging was previously done using mechanical disruption which presented difficulties regarding scalability and ergonomics. We therefore compared whether organoids could be broken down appropriately using an enzymatic reagent - TrypLE. Organoids were broken down more quickly and consistently via enzymatic dissociation with TrypLE compared to mechanical disruption (**Fig 3**).

**Digestion time?** TrypLE digestion time was also optimised. Slow and fast-growing organoid lines were digested using TrypLE for 4-14 minutes at 37°C and analysed in an Incucyte SX5 for 7 days (**Fig 4**). The digestion times that resulted in optimal growth rate for slow- and fast-growing lines were 8 and 12 minutes, respectively. Organoids are currently dissociated using TrypLE incubation for 8 or 10 minutes, depending on the growth of the line. As a precaution, organoid dissociation is monitored after 4 minutes during the first passage, as patient-to-patient variability would also need to be considered.

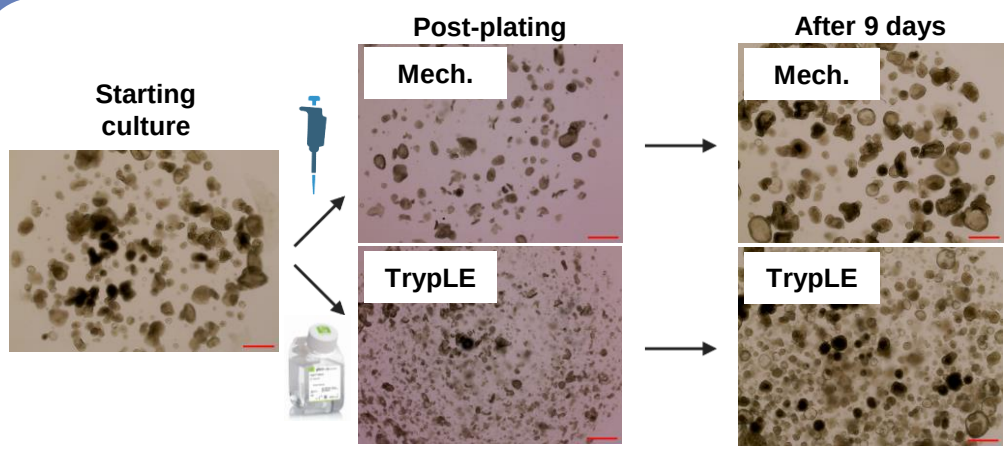


**Figure 3.** Comparison of passaging method – mechanical disruption (top) and enzymatic digestion using TrypLE (bottom). 500µm scale bars.

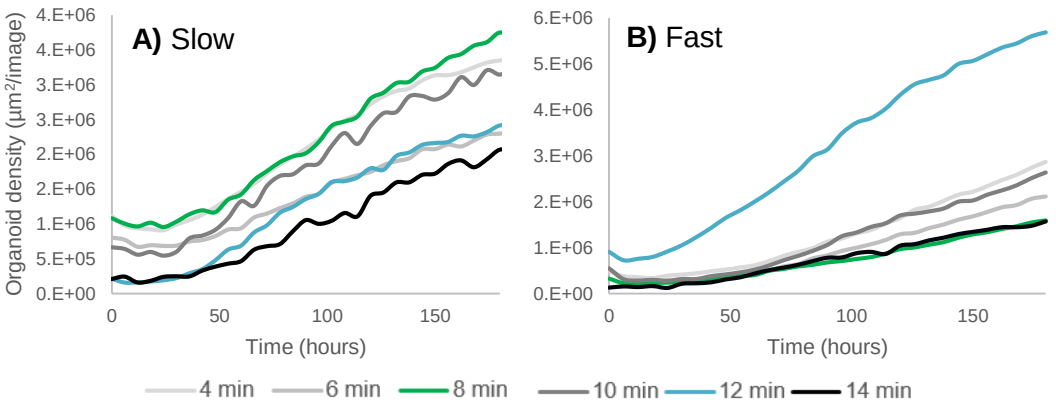


**Figure 4.** Incucyte analysis of organoid density after slow- **(A)** and fast- **(B)** growing lines were disrupted in TrypLE for various durations. Error bars removed for clarity.

## Future work

**Deriving models from ectopic lesions:** Our current work has focused on healthy & diseased eutopic endometrium. In the next phase of the project we will be deriving organoid models from the ectopic lesions of patients with endometriosis.

**Co-culture:** Matched endometrial organoids and fibroblasts will be grown together in co-culture to provide *in vitro* models that recapitulate the physiological niche and heterogeneity of the endometrium.

**References:**
[1] Turco, M., et al. Nat Cell Biol 19, 568–577 (2017)

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