



Establishing three dimensional endometrial assembloids for the investigation of reproductive disorders

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Introduction

- ❖ In an era of precision medicine, there is a requirement for in vitro models that reflect the genetic, cellular and pathophysiological hallmarks of individual patients.
- ❖ Organoids, three-dimensional cultures that have been generated from primary tissue and remain genetically stable, provide a novel platform to investigate disease, although still lack important contributions from the microenvironment^{1,2}.
- ❖ Assembloids are recently developed co-cultures of patient-derived endometrial organoid that also include patient derived stromal cells arranged into three dimensional representations of the endometrium^{3,4}.
- ❖ In this study, we established endometrial assembloids to provide pre-clinical testing platforms for the investigation of endometrial biology, its relationship to endometrial pathophysiology and association with reproduction.

References

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Methods

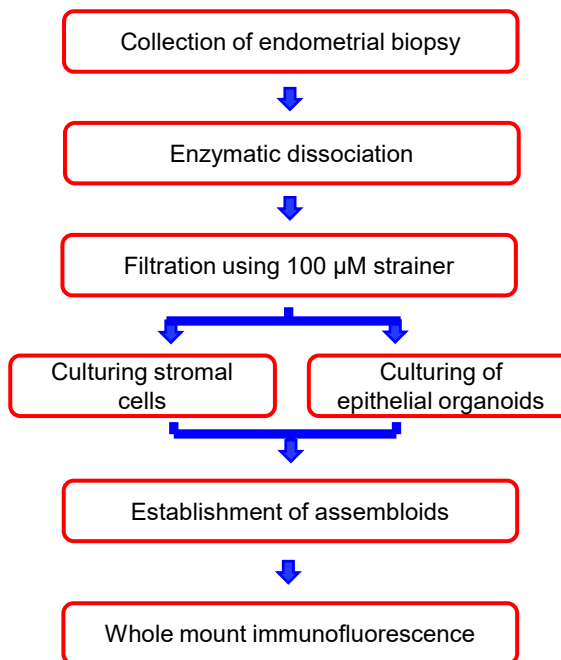
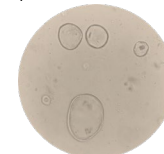


Figure 1: Establishment of an assembloid co-culture system. A schematic outline of the endometrial epithelial and stromal cell isolation from endometrial biopsy and establishment of co-culture system.

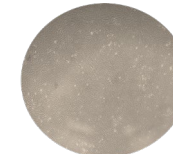
Results

Isolation and culture of stromal cells and organoids from human endometrial biopsy specimen

- ❖ Epithelial and stromal cells isolated using standard size exclusion techniques



Organoid, Bright field 10x



Stromal cells, Bright field 10x

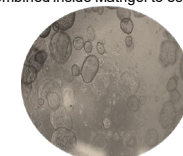
Figure 2: Bright field microscopy images of the isolated endometrial organoids and stromal cells using standard size exclusion techniques.

Establishment of assembloids

- ❖ Endometrial stromal cells and gland organoid pellets were mixed at a ratio of 1:1 and seeded in matrigel

Organoids		Stromal Cells	
7101269	ESC 1299	ESC1306	ESC973
5820096	ESC 1299	ESC1306	ESC973
1012641	ESC 1299	ESC1306	ESC973

Table 1: Establishment of endometrial assembloids system. Both epithelial and stromal cultures in various combinations were processed to individual cells and recombined inside Matrigel to establish three dimensional assembloids.



Day 14
Organoid 5820096



Day 14
Organoid 5820096 +ESC973

Figure 3: Representative bright field images of organoid culture and co-culture at day 14.

Characterization of organoids and assembloids

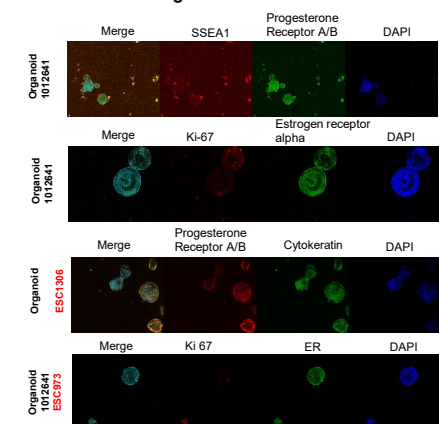


Figure 4: Identification of the phenotype of cultured organoids and assembloids using whole mount immunofluorescence staining. Established human endometrial gland organoids recapitulated characteristics of parental tissue.

Conclusions

- ❖ We demonstrated that the production of endometrial assembloids that closely recapitulates the characteristics of parental tissue.
- ❖ Using assembloids, we found success in long-term culture and the recapitulation of endometrium in vitro.
- ❖ Assembloids remained hormonally responsive.
- ❖ Assembloids can be used for in vitro models of endometrial and reproductive disorders.