Modelling Fallopian Tube Tissue with Endometriosis using 3D-Bioprinting

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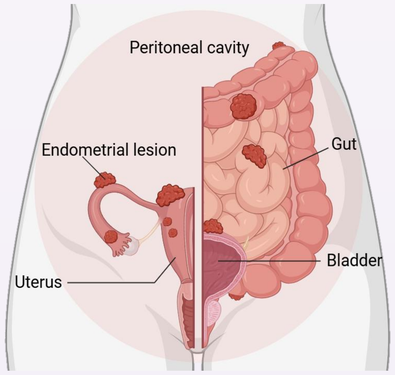
1. BACKGROUND

Endometriosis is a disease of the female reproductive system where endometrial cells grow outside the uterus and form inflammatory and painful lesions.

Symptoms include painful periods, heavy bleeding, infertility, stomach problems (diarrhea, constipation or bloating), abdominal and back pain, and pain during sex.

There is no cure for endometriosis. Symptom management involves surgery and hormonal therapy, providing short term relief due to a high recurrence rate.

- ✓ **Emerging Bioengineering Models:** Organoids, microfluids, scaffolds, and hydrogels.
- × **Existing limitations:** No current model exist to replicate tubal endometriosis.



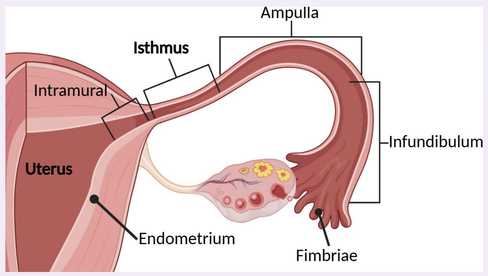
Retrograde menstruation occurs when menstrual blood flows backward through the fallopian tubes into the pelvic cavity, in addition to exiting through the vagina. This can lead to lesion formation on the peritoneum and surrounding organs as endometrial cells implant and grow outside the uterus.

It takes 10+ years to diagnose endometriosis!

2. OBJECTIVE

The objective is to create a **diseased fallopian tube tissue model** with endometriosis using, **extrusion 3D bioprinting,** as a tool to study **disease progression** and potential **treatments**.

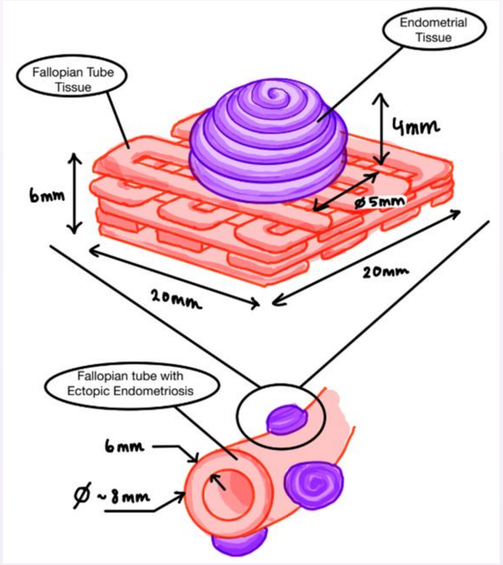
Focus: Replicating **isthmus** segment, since it connects directly to the uterus and plays a crucial role in fertility.



3. PROPOSED DESIGN

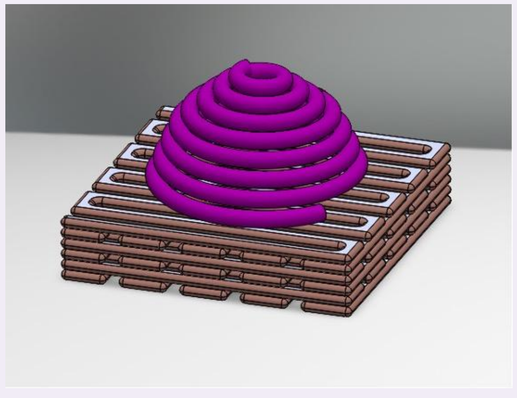
The proposed design is based on the following measurements:

- Each FT is approximately 0.2-0.6 inches in diameter with a 6mm thickness
  - ➢ ***Model is designed with a thickness of 6mm***
- Typical endometriosis cases consist of superficial lesions that are less than 5mm wide and 2-4mm in depth
  - ➢ ***Model is designed 5mm wide and 4mm deep***
- Each well in a 6 well plate is approximately 35mm in diameter
  - ➢ ***Model is designed to be 20mm in length and 20mm width***



4. CAD MODEL

The CAD 3D model was created using SolidWorks and is representative of the extrusion path that is taken by the bioprinter.



5. MATERIALS

80% of endometriosis lesions happen in the mucosa layer, and 53.85% of lesions in the serosa

Layer	Material/Cell type	Function
Mucosa	ECM: Matrigel (GFR)	Support epithelial cell attachment, growth, and differentiation
	Primary fallopian tube epithelial cells (FTECs)	Form innermost mucosal epithelium, differentiate into ciliated and secretory epithelial cells, support hormone response and barrier function
	Immortalized FTECs (FT2821J, FT194)	
Muscularis	ECM: Collagen I	Scaffold for stromal cells, support cell organization & epithelial folding
	hS1 Fibroblast-like stromal cells	Support ECM remodeling, provide growth factors for epithelial cells
Serosa	Fibroblasts and Immune Cells	Replicate inflammatory response and fibrosis
	Hydrogel with mesothelial cells derived from iPSCs	Scaffold to mimic serous layer around the FT
Superficial lesions	Epithelial and stromal cells	Mimic superficial peritoneal lesions

6. BIOFABRICATION METHOD

Extrusion and Digital Light Processing were the considered 3D bioprinting techniques. Extrusion was determined to be the best technique and four printers were compared.

Bioprinter Model	Cost ($CAD)	Ability to use multiple cell types	Requires technical expertise for set-up	Resolution
Cellink Bio X	55,000	Yes	No	1µm
EnvisionTEC 3DBioplotter	150,000	Yes	Yes	1µm
Allevi 3DBioplotter	40,000	No	No	50µm
Cellink Inkredible	5,000	Yes	No	10µm

Due to its relatively low cost, easy set-up, high precision and resolution, and ability to use multiple cell types, the Cellink Inkredible was selected as the optimal biofabrication method!

7. TESTING METHODS

To accurately replicate the microenvironment, biological function, and immune response, the following key factors must be considered:

- **Hormonal effects:** Cyclic progesterone and estrogen levels drive cell proliferation, differentiation, shedding and regeneration
- **Inflammation:** Model replicates blood accumulation, hemorrhage, and tissue injury triggering a tumor-like immune response
- **Cytokines:** Immune proteins injected to simulate inflammatory response; assess proinflammatory markers and immune cell infiltration
- **Angiogenesis:** Supports new blood vessel growth to sustain lesions; VEGF used to promote vascular development
- **Cell viability:** Visual evaluation of cell viability via live/dead staining using dyes and fluorescence microscopy

In vitro testing will compare model to patient tissue; gene expression, cell viability, and inflammatory markers will be analyzed.

8. CONCLUSIONS & FUTURE WORK

Benefits	Limitations
Focuses on superficial lesions in the isthmus	Limited to one FT segment
More realistic than 2D cultures	Lacks full organ complexity and vascularization
Includes hormonal cycles, cytokines, inflammation, and angiogenesis	Difficult to replicate dynamic in vivo hormonal and immune interactions
The Inkredible bioprinter is affordable, scalable, and adaptable Allows for multi-material layering	Lacks temperature control and interchangeable nozzles
More accurate than animal models	Best suited for short-term studies due to challenges in maintaining cell viability over time

Future Adoption:

- Move from theory to application by printing and testing the 3D model
- Adapt the model for different regions, lesion types, stages, and severities of endometriosis

9. ACKNOWLEDGEMENTS

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