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Endometrium - Collagenase

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Single-cell reconstruction of the early maternal-fetal interface in humans. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M et al. Nature 2018;563;7731;347-353.

Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describe the tissue dissociation procedures from human endometrium and pregnancy endometrium samples. This protocol is adapted from *Vento-Tormo et al. 2018 Nature* with some modification from Prof. Ashley Moffett (Department of Pathology, University of Cambridge).

Guidelines

Human samples including tissue, blood and bodily fluids have the potential to harbour HG2 and Hazard Group 3 (HG3) organisms, specifically Blood Borne Viruses (BBVs,); and for brain tissue, CNS tissue and CSF, prions. In the UK we can work with such samples at CL2 on the condition that we do not intend to culture any of the organisms that might be contained in the samples and that the samples haven't already been identified by tests or diagnosis as containing HG3 organisms.

Materials

MATERIALS

- RPMI 1640 Medium **Thermo Fisher Scientific** **Catalog #**11875093
- Parafilm, 4X125' **Bio Basic Inc.** **Catalog #**PF002.SIZE.1
- Falcon® Conical Tubes, 50 mL 500 Tubes **STEMCELL Technologies Inc.** **Catalog #**38010
- DNAse I **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**4716728001
- Fetal bovine serum
- PBS **Invitrogen - Thermo Fisher**
- HypoThermosol® FRS Preservation solution **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H4416
- Collagenase V **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C9263
- Hams F12 **Thermo Scientific** **Catalog #**11765054
- 100 µm Cell Strainer **Falcon** **Catalog #**352360
- 10X RBC Lysis Buffer (Multi-species) **eBioscience** **Catalog #**00-4300-54

Safety warnings

Samples are unscreened human tissues, please adhere to Biological Safety at Containment Level 2 work procedures.

Prepare collagenase mix

1 Collagenase mix recipe:

	Product	Stock	Final volume (20ml : 3ml/sample)	Concentration
	RPMI or Hams F12 + 10% FBS	9 ml RPMI or Ham's F12 + 1 ml FBS	8.9 ml	
	Collagenase V	10 mg/ml	1 ml	1 mg/ml
	DNase I	10 mg/ml	100 ul	0.1 mg/ml

Tissue dissociation and digestion

2 Note: Flash-frozen tissue with isopentane for Spatial Transcriptomics work. {optional}
Note: If tissue is going to be transported, do it with preservation solution (HypoThermosol® FRS) at 4 °C . Store sample for fixing in formalin (RNA Scope) & nuclei sequencing (flash-frozen) {optional}.

Scrape off the blood vessels on remaining tissues. Note: We can skip this step if the donor is perfused.

3 Wash tissue with PBS {optional}.

4 Place wet tissue under a petri dish. Take 2 scalpels and roughly mince up the tissue. This step is crucial to increase the efficiency of the digestion.

5 Transfer contents to 50ml falcon containing the collagenase mix (~ 3 mL /tissue but it will depend on the size of the tissue)

6 Tighten lid and then seal with parafilm.

7 Incubate at 37 °C for 00:45:00 . Shacking during the incubation is recommended.

8 Resuspend with 20 mL RPMI 10%.

9 Filter sample through small strainer (100um) – do not discard retained tissue. The retained tissue will be used for "Endometrium-Trypsin" protocol.

10 Filtered material: Centrifuge at 450 g, 00:05:00 (0.5 rcf, 00:05:00).

11 Wash with 10 mL of PBS twice.

11.1 {optional- for biopsies we do this}
Resuspend sample with 2 mL to 4 mL of 1X RBC lysis buffer mix and incubate for 00:10:00 .

*RLB preparation: Dilute 10X RLB stock with water.

After RBC lysis, add 10 mL of RPMI 10% and centrifuge 450g for 00:05:00

Wash twice with 10ml of PBS.

12 Resuspend with 1 mL RPMI 10%, count cells and sort for cell population (refer to **"Cell staining for flow cytometry and sorting"** protocol)

13 {Optional} Resuspend cells in freezing medium to a concentration of 1×10^{7} cells and aliquot into cryogenic storage vials.