Single Cell Isolation from Human Ovarian Tissue

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ABSTRACT

This protocol outlines steps to obtain a single cell suspension from fresh human ovarian tissue. Here, we do not outline steps for removing the ovarian cortex from the organ and slicing 1 mm thick sections of cortex and medulla, but begin with 1 mm thick pieces of ovarian cortex. Tissue is digested enzymatically using Collagenase IA and DNase I then rinsed in preparation for staining and/or sorting. All steps should be performed using wide-bore pipette tips to avoid shear force damage to cells in suspension.

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MATERIALS TEXT

Supplies for tissue preparation: glass petri dish, disposable #10 surgical blade, DPBS -/- (optional: tissue chopping device for mincing tissue).
Supplies for digestion: Small spatulas, 70 and 30 µm strainers, 30 and 60 mm petri dishes, conical tubes (1.5, 5, and 15 mL), sterile DPBS +/+ and -/-, BSA (Fisher BP9706), FBS (Fisher 10-082-147), Collagenase IA (Sigma C2674), DNase I (Sigma D4138), wide-bore pipette tips, incubating shaker, centrifuge.

Preparations

1 Prepare enzymes: Dilute DNase I stock (2% w/v) to 2 mg/mL by combining 10 µL stock with 90 µL DPBS +/+. Mix well and store on ice for the duration of cell isolation. Collagenase stock at 100 mg/mL should be stored on ice for the duration of cell isolation.

2 Pre-weigh an empty 30 mm dish. This will be used to measure the mass of tissue prior to digestion.

3 Prepare solutions for digest and rinsing.

3.1 Prepare Inactivation Solution (15 mL DPBS -/- with 10% FBS). Keep solution on ice for duration of experiment.

3.2 Aliquot 15 mL DPBS +/+ for digest solution. It is crucial that DPBS +/+ be used, as the magnesium and calcium aid in enzymatic digestion. Keep in a bead bath for duration of experiment to keep solution warm.

3.3 Prepare red blood cell (RBC) lysis buffer. Dilute 1 mL RBC lysis buffer stock into 9 mL sterile DI water. Keep solution warm in bead bath for duration of experiment.

3.4 Prepare rinse solutions. Make 15 mL 1% (w/v) BSA in DPBS -/- and 15 mL 0.04% (w/v) BSA in DPBS -/-. Sterile filter each solution. Store on ice for duration of experiment.

Tissue Cutting and Digest

4 Starting with 1 mm thick pieces of ovarian cortex or medulla, cut all tissue into ~1mm^3 pieces either a) mechanically using a #10 blade in a glass petri dish or b) using a tissue chopper.

5 After cutting, move tissue to a cell strainer (any pore size) and rinse tissue with DPBS -/- twice to wash off any media or previous solutions.

6 Using a spatula, transfer the cut tissue to the pre-weighed 30 mm petri dish. Weigh tissue in pre-weighed dish to obtain tissue mass. Add 2 mL DPBS +/+ to the dish, then add 10 µL Collagenase IA (stock = 100 mg/mL) and 10 µL diluted DNase I for a solution of 0.5 mg/mL Collagenase IA and 0.01 mg/mL DNase I in DPBS +/+.

7 Place 30 mm dish with tissue in a secondary container and tape down if necessary. Tape dish to shaker and digest for 30 minutes at 150 rpm and 37ºC. Tissue pieces should be freely moving in the digest solution to obtain proper enzymatic digestion and leverage mechanical disruption for cell isolation.

8 After 30 minutes, strain tissue and supernatant through a 70 µm strainer over a 60 mm dish. Put the tissue pieces back into the 30 mm digestion dish using a spatula. Quickly add 2 mL DPBS +/+ to the dish to avoid leaving tissue out of solution.

9 Add 2 mL ice-cold Inactivation Solution to the 60 mm dish of supernatant to quench digestion, then strain through a 30 µm cell strainer into another 60 mm dish. Collect strained supernatant (single cells) and store in a 5 mL tube on ice. Once cells are dissociated and in solution like this, it is crucial to keep cell suspension as cold as possible.

10 Add 10 µL Collagenase IA (stock = 100 mg/mL) and 10 µL diluted DNase I to the digest dish. Return digest dish to shaker and digest for 30 minutes at 150 rpm and 37ºC.

11 After 30 minutes, strain tissue and supernatant through a 70 µm strainer over a 60 mm dish. Set aside strainer with tissue pieces. Add 2 mL ice-cold Inactivation Solution to the 60 mm dish of supernatant to quench digestion, then strain through a 30 µm cell strainer into another 60 mm dish. Collect strained supernatant (single cells) and store in a 5 mL tube on ice.

12 If needed, aliquot each 5 mL tube from first and second 30 minute digests into smaller tubes to centrifuge. Centrifuge at 100g for 5 minutes at room temperature.

Rinsing and Preparation for Staining/Sorting

13 Resuspend cell pellet(s) in 1X RBC Lysis Buffer. 3 mL working volume per tube is suggested for cell pellets from a 5 mL tube; 1 mL working volume per tube is suggested for cell pellets from a 1.5 mL tube. Incubate at room temperature for 2 minutes, then centrifuge at 100g for 5 minutes at room temperature.

14 Resuspend pellet(s) in 1% BSA solution to remove cell debris, then centrifuge at 100g for 5 minutes. Repeat, for a total of two rinses in 1% BSA solution. Working volume recommendations: 1 mL for 1.5 mL tubes and 3 mL for 5 mL tubes.

15 Resuspend pellet(s) in 0.04% BSA solution before proceeding to staining and/or sorting. Working volume recommendations: 1 mL for 1.5 mL tubes and 3 mL for 5 mL tubes.