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Single-Cell Isolation of Human Knee Meniscus

Forked from Single-Cell Isolation of Human Articular Cartilage

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ABSTRACT

This is a protocol that describes the process of isolating single cells from human knee meniscus for scRNA-seq.

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FORK NOTE

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1 ~1g of tissue from healthy donor knees (Grades 0-1) is collected from the meniscus. For details regarding

the tissue harvesting procedure please see
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2 Meniscal tissue is washed with **Room temperature**
Dulbeccos Phosphate Buffered Solution [DPBS] **Gibco - Thermo Fisher Catalog #14190-144**
Bovine Calf Serum [CS] **VWR international Ltd Catalog #10158-358** supplemented with 10% , 1%
Antibiotic-Antimycotic 9100x0 [Anti-Anti] **Thermo Fisher Scientific Catalog #15240062** and 1%
Penicillin-Streptomycin-Glutamine [PSG] **Corning Catalog #30-009-CI** .

3 The tissue is then finely minced with a #21 Feather disposable scalpel, and digested in 20mL 30m
DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate **Fisher Scientific Catalog #MT-10-013-CV**
supplemented with 1% Anti-Anti and 2%
Collagenase Type II **Worthington Biochemical Corporation Catalog #L5004177** using 100 rpm
shaking at **37 °C** for **00:30:00** .

4 Cells are gently passed through a 100 µm filter into a 50 mL centrifuge tube followed by gentle passage through a 40 µm filter into a fresh 50 mL centrifuge tube.

5 Filtered cells are spun down at 1200 rpm for **00:05:00** at **Room temperature** 5m

6 Carefully remove and collect the collagenase supernatant; the collagenase is reused in subsequent steps.

7 Cells are then resuspended in DMEM supplemented with 10% CS, 1% Anti-Anti and 1% PSG and stored at **37 °C** .

8 Repeat the digestion, spin down and filtration steps for **01:00:00** and then **02:00:00** , for a total of 6h 30m
03:30:00 of digestion.

9 Combine all collected cells and spin down at 1200 rpm for **00:05:00** at **Room temperature** . 5m

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10 The supernatant is carefully removed, and the remaining cell pellet is delicately resuspended in 10mL of **Room temperature** DPBS supplemented with 5% CS and 5 mM Ethylenediamine tetraacetic acid [EDTA] **Fisher Scientific Catalog #BP120-500** .

11 Single cells are spun down at 1200 rpm for **00:05:00** at **Room temperature** . 5m

12 The supernatant is carefully removed, and the remaining cell pellet is delicately resuspended in 10 mL of DPBS supplemented with 0.04% Bovine Serum Albumin [BSA] **Fisher Scientific Catalog #9048-46-8** .

13 The Invitrogen Countess II FL automated cell counter is used to quantify single cells and determine cell viability. Live cells are determined by trypan blue staining. If >70% cell viability is confirmed, the single cell suspension is diluted to a concentration of $1x10^6$cells/mL for single cell RNA-seq library preparation.

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