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Single-Cell Isolation of Human Knee Meniscus

Forked from Single-Cell Isolation of Human Articular Cartilage

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

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Abstract

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

Protocol materials

- Dulbeccos Phosphate Buffered Solution [DPBS] **Gibco - Thermo Fisher Scientific Catalog #**14190-144
- Bovine Calf Serum [CS] **VWR International (Avantor) Catalog #**10158-358
- Antibiotic-Antimycotic 9100×0 [Anti-Anti] **Thermo Fisher Scientific Catalog #**15240062
- Penicillin-Streptomycin-Glutamine [PSG] **Corning Catalog #**30-009-CI
- DMEM with L-Glutamine 4.5g/L Glucose and Sodium Pyruvate **Fisher Scientific Catalog #**MT-10-013-CV
- Collagenase Type II **Worthington Biochemical Corporation Catalog #**L5004177
- Ethylenediamine tetraacetic acid [EDTA] **Fisher Scientific Catalog #**BP120-500
- Bovine Serum Albumin [BSA] **Fisher Scientific Catalog #**9048-46-8

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 dx.doi.org/10.17504/protocols.io.6qpvr614zvmk/v1

2 Meniscal tissue is washed with Room temperature
Dulbeccos Phosphate Buffered Solution [DPBS] **Gibco - Thermo Fisher Scientific Catalog #**14190-144
supplemented with 10%
Bovine Calf Serum [CS] **VWR International (Avantor) Catalog #**10158-358, 1%
Antibiotic-Antimycotic 9100×0 [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 03:30:00 of digestion. 6h 30m

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

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 1×10^6cells/mL for single cell RNA-seq library preparation.