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Single-Cell Isolation of Human Articular Cartilage

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1

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This is a protocol that describes the process of isolating single cells from healthy knee cartilage for single cell RNAseq.

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Dulbecco's Phosphate Buffered Solution [DPBS] (Gibco, 14190-144)

Dulbecco's Modified Eagle Medium [DMEM] (Corning; 10-013-CV)

Bovine Calf Serum [CS] (VWR; 10158-358)

Antibiotic : Antimycotic [Anti-Anti] (GeminiBio; 400-101)

Penicillin-Streptomycin-Glutamine [PSG] (Corning; 30-009-CI)

Collagenase Type II (Worthington; L5004177)

Feather Disposable Scalpel #21 (Electron Microscopy Sciences; 72042-21)

100 um strainer (Fisher Scientific; 22363549)




40 um strainer (Fisher Scientific; 22363547)

50 mL centrifuge tubes (BioPioneer; CNT-50)

Ethylenediamine tetraacetic acid [EDTA] (Fisher Scientific; BP120-500)

DNase I (RNase-free) (Takara; 2270B)

Bovine Serum Albumin [BSA] (Fisher Scientific; 9048-46-8)

- 1 ~1.5 g of articular cartilage from healthy donor knees (grades 0-1) is resected from the medial condyle of the proximal femur. For details regarding the tissue harvesting procedure please see dx.doi.org/10.17504/protocols.io.14egn7996v5d/v1.
- 2 Cartilage shavings are washed with  **Room temperature** Dulbecco's Phosphate Buffered Solution (DPBS) supplemented with 10% calf serum (CS), 1% Antibiotic : Antimycotic (Anti-Anti) and 1% Penicillin-Streptomycin-Glutamine (PSG).
- 3 Cartilage shavings are minced with a #21 Feather disposable scalpel, and digested in 20mL^{2h} Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% Anti-Anti and 2% collagenase type II with 100 rpm shaking at  **37 °C** for  **02:00:00** .
- 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 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).
- 7 Resuspended single cells are treated with DNase I at a concentration of 100 ug/mL for 🕒00:15:00 at 🌡 Room temperature . 15m
- 8 Single cells are spun down at 1200 rpm for 🕒00:05:00 at 🌡 Room temperature 5m
- 9 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).
- 10 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 1×10^6 cells/mL for single cell RNA-seq library preparation.