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Single-Cell Isolation of Human Articular Cartilage

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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 healthy knee cartilage for single cell RNAseq.

Materials

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)

Troubleshooting

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 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 . 2h

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