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Chondrocyte isolation from human cartilage

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ABSTRACT

A standard methodology used within our group for extracting chondrocytes from human cartilage obtained as waste tissue at the time of joint replacement. Chondrocytes obtained through this method are used at P1 (at most P2) for in vitro assays. This is adapted from other published methods based on the requirements of our group for a consistent and straightforward method for obtaining viable cells.

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
DMEM/F-12, HEPES	31330038	Thermo Fisher
Collagenase	C9722	Sigma Aldrich

MATERIALS TEXT

All reagents and surfaces should be sterile

Reagents

PBS (Ca²⁺ and Mg²⁺ free)

Collagenase (Sigma, C9722)

10% DF12 - (Standard Medium)

DMEM/F12 with glutamine and HEPES (31330038, ThermoFisher) containing 10% v/v Heat Inactivated FCS and 1000IU/ml penicillin/100µg/ml streptomycin. If using other media, add HEPES to 0.4% w/v.

Collagenase Solution

1mg/ml (w/v) collagenase in Standard Medium

Equipment

Scalpels

Forceps

Cutting board

10cm Petri Dishes (Cell culture treated)

37C incubator w/shaker (not CO₂)

0.2µm syringe filters

0.45µm syringe filters

50ml syringes

50ml tubes

5ml tubes

Centrifuge

70µm cell sieves

Collagenase preparation

- 1 Make up Collagenase solution (1mg/ml) in standard medium, allow to dissolve and sterile filter through a 0.45um followed by 0.2um syringe filter. The 0.45um filter step is essential to enable efficient filtration. 15m

Collagenase mixture can be used fresh or immediately frozen and used up to 6 months after preparation. Store in aliquots as there is loss of activity with freeze-thaw cycles.

Cartilage preparation

- 2 Wash cartilage-bone cut thoroughly in PBS by shaking for 1 minute. 1m
- 3 Repeat wash step 1m
- 4 Place cartilage-bone cut on a sterile cutting board and use a clean scalpel to scrape off any remaining blood or non-cartilage tissue from the cartilage surface
- 5 Cutting flat along the bone, remove full thickness pieces of cartilage from the bone surface and place in standard medium in a petri dish
- 6 Cut cartilage pieces (in media) in to approx 3mmx3mmxfull thickness pieces using a scalpel.

Digestion

- 7 Place cartilage pieces in to collagenase digestion mix (10% v/v cartilage:digestion mix) in a 50ml tube
- 8 Incubate cartilage-collagenase mix for 12-15 hours at 37C with shaking (45-60rpm).

Cell Isolation for culture

- 9 After digestion, pass mixture through a 70um cell sieve
- 10 Centrifuge filtered solution for 5 minutes at 300xg
- 11 Wash pellet, resuspend and repeat centrifugation step
- 12 Resuspend cells in 1ml standard medium until pellet is completely resuspended. Take volume up to 10ml and mix thoroughly by pipetting.
- 13 Count cells and adjust volume to 4×10^4 cells/ml in standard medium

- 14 Plate 10ml cell:media mixture into relevant number of 10cm cell-culture treated petri dishes and leave for 3-7 days to adhere at 37C in a CO₂ incubator

Post-isolation

- 15 Once cells have adhered (this can take 3-7 days) refresh media and continue to refresh media every 2-3 days until cells reach 80% confluence.

- 16 Once cells are 80% confluent EITHER:

- 16.1 Freeze: By washing cells in standard medium, scraping in approx 5ml per plate, and centrifuging in 15ml tubes before resuspending in ice cold 10%DMSO in FBS prior to immediate freezing.

- 16.2 OR:

Passage cells by washing cells in standard medium, scraping in approx 5ml per plate, and centrifuging in 15ml tubes before resuspending in appropriate volume of medium for a 1:2 split (ie each 10cm dish becomes 2x10cm dishes). Cells should ideally be used at this passage.



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