**Protocol for Isolation and Culture of Articular Chondrocytes**

Modified from Brittberg and Petersen

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Transport harvested cartilage in 0.9 normal saline or 1x PBS (**with Pen/Strept**).

1) Prepare DMEM/collagenase solution (**Bioreactor mixture**):
    a. Add 10ml DMEM containing **20%** FBS (+P/S)
    b. Add collagenase (1 mg/ml) (clostridial collagenase, Gibco #17100-017)
    c. Add deoxyribonuclease I (0.1mg/ml) (Sigma, DN25)
    d. Filter into Falcon tube
2) Mince cartilage (300-500mg) with sterilized straight razor blade on lid of a cell culture dish in 1ml DMEM (decreases cell adherence).
3) Wash in DMEM, spin at 1000rpm x5 min (Eppendorf 5810 in the cold room).
4) Resuspend cell pellet in DMEM/collagenase (Bioreactor mixture)
5) Place in 50ml bioreactor flask.
6) Digest with low speed spinner for 5-6 hours.
7) Filter through cell strainer (100 um) (should be mostly digested).
8) Wash with DMEM x1 (spin x2).
9) Plate with DMEM containing **20%** FBS (+P/S).

**Note**: Brittberg uses 1mg/ml of collagenase with overnight digestion
Can also use 2.5mg/ml for 5 hours
Or use 5mg/ml for 2.5 hours
Shorter incubation with collagenase tends to increase cell yield and cell happiness

**REFERENCES**:

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