HubLE | **Methods**

Culture of MLO-A5 and MLO-Y4 osteocyte cell lines

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MLO-A5 and MLO-Y4 are murine cell lines routinely used to study osteocytes[1,2]. This HubLE Method describes the protocol for the routine culture of these cell lines.

Materials

- Rat tail type-I collagen solution
- 0.02M Acetic acid solution (sterile)
- Phosphate buffered saline (PBS) Ca^{2+} and Mg^{2+} free, pH7.4
- Penicillin/Streptomycin
- Foetal bovine serum (FBS)
- Calf serum (CS)
- Complete media (CM): Minimum Essential Medium Eagle - Alpha modification (αMEM) supplemented with penicillin (100U/mL) and streptomycin (100µg/mL) + 2.5%FBS + 2.5%CS
- Trypsin-EDTA
- Dimethyl Sulfoxide (DMSO)

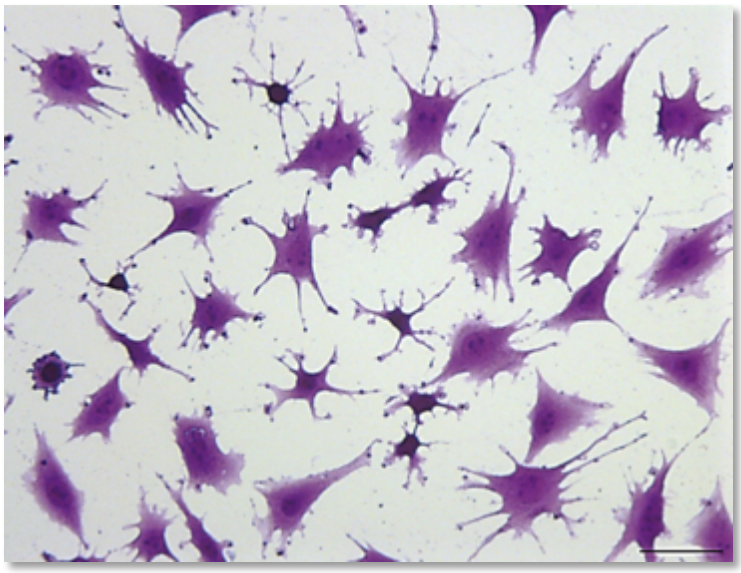

Fig. 1 Typical morphology of MLO-Y4 cells grown in collagen-coated flasks. The cells have been fixed with 10% buffered formalin and stained with 0.1% crystal violet. Scale: 100µM

Method

Update

1. **Coating the flask/plate with collagen**
   - 1.2 Dilute sterile type-1 collagen solution to 0.15mg/ml in 0.02M acetic acid. ***[Tip 1]***
   - 1.3 To coat tissue culture treated flasks/plates, use 0.2ml/cm^2 collagen solution and leave for 1 hour at room temperature, then remove.
   - 1.4 Rinse with sterile PBS twice, plates can now be used immediately or air-dried and stored at 4°C for use later. ***[Tip 2]***. If using non-sterile solutions, expose the coated flasks to UV light for 45 mins in a laminar flow-hood, prior to use.
2. **Maintenance of MLO-A5 and MLO-Y4**
   - 2.1 Defrost 5x10^5 to 1x10^6 cells just long enough to thaw. Mix the cells with 10ml of CM and centrifuge at 250g for 5 minutes.
   - 2.2 Resuspend pellet in CM and culture cells at 37°C in a humidified atmosphere with 95% air and 5% CO_2. **[*Tip 3*]**
   - 2.3 To passage cells, usually 3-4 days post seeding, discard the media and rinse the cells twice with PBS. Add 3ml trypsin-EDTA to each T75 flask and incubate at 37°C for 5 minutes.
   - 2.4 Check for detachment of cells under the microscope and then collect them in 10ml CM and centrifuge at 250g for 5 minutes.
   - 2.5 Discard the supernatant and resuspend the cell pellet in 2ml of CM. Perform a viable cell count using Trypan Blue. **[*Tip 4*]**
   - 2.6 To freeze cells, resuspend the cell pellet in freezing media containing 60% α-MEM, 30% FBS and 10% DMSO. Store 1x10^6 cells/ml in each cryovial and freeze at -80°C using a cell freezing container. For long term storage, transfer the cryovials into liquid nitrogen.

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Tips

Update

1. Rat tail type 1 collagen: Use ice-cold pipette tips when working with rat tail type 1 collagen.
2. Excess collagen solution from the flasks/wells can be reused up to 5 times and should be stored at 4°C. With each use the efficiency of coating may decrease, and increasing coating incubation time is suggested. The coated flasks and plates can also be stored at 4°C for up to a month.
3. Cells in collagen-coated T75 flasks can be grown till 70% confluence before passaging. MLO-Y4 cells should not be allowed to grow above 70% confluency to maintain their phenotype. Care should be taken to avoid high flow rates during media changes, as osteocyte-like cells are extremely sensitive for fluid flow-derived shear stress.
4. Typically, 2.0-2.5x10^6 cells are harvested from a T75 flask with routine seeding of 0.5–1x10^6 cells per T75 flask.

References

Update

1. Kato, Y., Boskey, A., Spevak, L., Dallas, M., Hori, M. & Bonewald, L.F. (2001) Establishment of an osteoid preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. J Bone Miner Res, 16, 1622-1633.
2. Kato, Y., Windle, J.J., Koop, B.A., Mundy, G.R. & Bonewald, L.F. (1997) Establishment of an osteocyte-like cell line, MLO-Y4. J Bone Miner Res, 12, 2014-2023.

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