Isolation of muscle precursor cells from whole muscle

Reagents

- Media:
    - Collection and digest: DMEM + p/s (100 U/mL penicillin, 100 μg/mL streptomycin)
    - Post-digest trituration: DMEM + 10% FBS + 1x p/s
    - Growth media: Ham's F10 + 20% FBS + 1x p/s
- Pronase (Calbiochem cat# 53702)
    - Prepare 1% solution (w/v) in sterile DMEM. Filter (0.45 μm), then store 2 mL aliquots at –20°C
- Pasteur pipets
- Steriflip 100μm filters (50 mL) (Fisher SE1M002M8)
- Stir plate in 37°C incubator or Envirogenie at 37°C
    - Use stirplate in incubator with 5% CO_2 if possible. For Envirogenie, add HEPES (25 mM final , 1:40 dillution) to digest media (i.e., add 500uL HEPES to 20mL digest).
- Sterile, wide-bore serological pipets: Can use 25 mL pipet, break off end of 5 mL pipet at the seam, or break 5mL pipet closer to end using a hemostat.
- Collagen-coated 100mm tissue culture dishes.

Procedure:

NOTE: Protocol is based on muscle from one mouse. For multiple mice, muscle from each mouse should be digested in separate dish and can be pooled at the end if desired.

Before you start: Prepare 3 100mm dishes - 2 containing 5 mL DMEM + p/s and 1 containing sterile PBS. Equilibrate DMEM dishes in incubator for 10-15 min. Sterilize all tools to be used for muscle excision.

1) Dissect hindlimb muscle from both legs of one mouse, rinse briefly in sterile PBS, and place into p100 containing DMEM + p/s.

*** from this point on, all steps to be performed in hood under sterile conditions***

2) Clean muscles of excess connective tissue and tendons and transfer to 2nd p100 containing 5 mL DMEM + p/s.

3) Mince the muscle into fine pieces using razor blades held with hemostats for 1-1.5 min.

4) Transfer the minced muscle to a 15ml centrifuge tube (may need a wide-bore pipet) and centrifuge using tabletop centrifuge for 2 minutes at speed 800-1000 x g.

5) Aspirate medium and bring volume up to 10mL with DMEM + p/s. replace cap, invert several times to loosen pellet and mix, decant into p100 dish. Repeat with 8 mL

DMEM + p/s. Add 2ml of 1% pronase to bring final volume of 20ml (0.1% pronase final). Add stir bar (1") cleaned with EtOH.

6) Place plate on stir plate and digest at 37°C for 1 hour while stirring
    a. Stir at medium speed to give gentle agitation without splashing. If using Envirogenie, set stirring speed to 150 RPM.

7) Transfer digest to a 50ml centrifuge tube (may need wide-bore pipet) and spin for 3 min at 800-1000 x g. Aspirate medium.

8) Resuspend muscle in 7-10 mL DMEM + 10% FBS + p/s (use more media for more muscle). Using a 25mL pipet, triturate the muscle many times (15-20) to loosen cells.

9) Let pieces settle, then remove as much media as possible using a 6" Pasteur pipet (avoid muscle pieces) and transfer to a clean 50mL tube. Repeat 1-2 times if necessary.

10) Pass supernatant through a Steriflip 100μm vacuum filter and wash filter with 5ml of DMEM + 10% FBS + p/s
    a. let as much as possible flow through just by gravity first, then apply gentle vacuum.

11) Spin for 5 minutes at speed 1000 x g.

12) Resuspend pellet in 10 mL of growth media and transfer to 100mm dish. Add 5ng/mL basic FGF.

Notes on maintaining and expanding MPCs

1) Do not refeed cells until day 3 after plating. Thereafter, refeed every other day with growth media supplemented with 5ng/mL basic FGF.
2) Do not expect to see many MPCs cells until 4 days after isolation.
3) When cells on 100mm dish approach ~80% confluence, trypsinize and replate ALL cells on a 150mm dish.
4) For early passages, keep ALL cells and split into 2 plates if necessary. Once cells become more pure MPCs, extra cells may be discarded.
5) Early on, cells will be predominantly MPCs but will also contain some fibroblasts and other cell types. Most other cell types will begin to die off over time. 2 ways to get rid of fibroblasts include:
    a. Preplating: after trypsinizing cells, plate on UNCOATED tissue culture dish and allow fibroblasts to "sit down" for 30-45 min. Transfer floating cells to a COLLAGEN-COATED plate. Don't preplate for too long because MPCs will eventually start to sit down too. *This method is recommended during early phases of MPC expansion* (NOTE: MPCs don't like repeated pre-plating)
    b. Limited trypsinization: During trypsinization, leave trypsin on plate for only a fraction of normal time (10-30 seconds). This will allow MPCs to be removed while leaving most fibroblasts behind. *This method is only recommended during later stages of MPC expansion* (i.e. when you have lots of MPCs), since a number of MPCs will also be left behind.