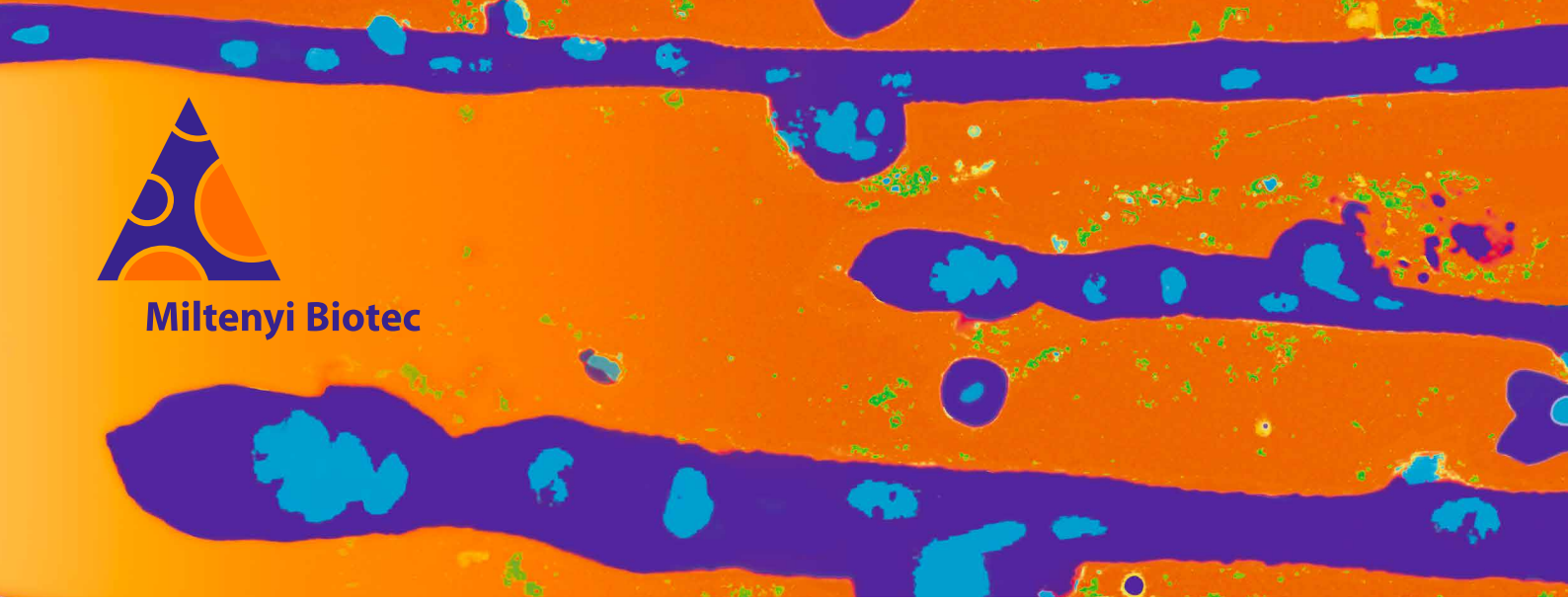

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Adult muscle stem cell isolation

Dissociation of mouse skeletal muscle to isolate adult muscle stem cells

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Background

Muscle stem cells (MuSCs), also known as satellite cells, are responsible for maintenance and regeneration of skeletal muscle mass[1–8]. Satellite cells were first identified in 1961 by their anatomical location, as mononuclear cells residing at the periphery of muscle fibers, between the myofiber membrane and the basal lamina[9,10]. They are mostly quiescent in healthy adults, but become activated upon muscle injury[8]. Following activation, they proliferate and some of their progeny differentiate to generate fusion-competent muscle cells, while others self-renew to replenish the stem cell pool[11–14]. Several years ago, the Blau laboratory characterized adult murine MuSCs by flow cytometry, in conjunction with quantitative *in vivo* functional assays of their regenerative capacity using bioluminescence imaging[6] and assays of twitch and tetanic force[15,16]. The Blau laboratory developed a strategy for prospective isolation of primary murine MuSCs from manually dissociated skeletal muscle tissue. Cells expressing the lineage markers CD31, CD45, CD11b, and Sca-1 were depleted from the resulting single-cell suspension using biotinylated antibodies in combination with Streptavidin MicroBeads. Flow sorting was then used to positively select for MuSCs based on simultaneous expression of α7 integrin and CD34. The negative selection step performed using the MACS® Technology system greatly reduced the sorting time and resulted in a higher yield of viable cells compared to non-depleted tissue.

This note describes the isolation of MuSCs used by Dr. Ermelinda Porpiglia in the laboratory of Professor Helen Blau at Stanford University.

The technology consists of a standardized, automated tissue dissociation protocol using the gentleMACS™ Octo Dissociator with Heaters in combination with the Skeletal Muscle Dissociation Kit that efficiently produces a viable single-cell suspension while preserving the lineage cell surface markers as well as α7 integrin and CD34. Dissociation is followed by magnetic depletion of lineage (CD31, CD45, CD11b, and Sca−1)-positive cells using the Satellite Cell Isolation Kit, which contains magnetic particles that are directly conjugated to lineage markers, therefore providing a fast and reliable assay for depletion of lineage-positive cells with broad utility.

Materials and methods

Materials

- gentleMACS Octo Dissociator with Heaters
- Skeletal Muscle Dissociation Kit, mouse and rat
- MACS SmartStrainers (70 µm)
- Red Blood Cell Lysis Solution
- Satellite Cell Isolation Kit
- Cell separation buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

For a detailed protocol, please refer to the respective data sheet.

Methods

Skeletal muscle dissociation

Tibialis anterior muscles were dissected from C57BL/6 mice, cut into small pieces of 2–4 mm, and digested to single-cell suspension:

1. Dissociate the samples using the Skeletal Muscle Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters according to the protocol. To further increase the yield of satellite cells, run program **37C_mr_SMDK_2** in step 4 and continue with step 12 of the protocol.
2. Pellet cells and resuspend in 1× Red Blood Cell Lysis Solution for 2 minutes before pelleting again in cell separation buffer.

Satellite cell isolation

Label cell suspensions rapidly with the Satellite Cell Isolation Kit according to the data sheet. Perform lineage depletion by using an LS Column placed in a MidiMACS™ Separator on a MACS MultiStand. Isolate satellite cells by flow sorting based on expression of α7 integrin and CD34 and using the gating strategy as shown in figure 1.

Results

Tibialis anterior and *gastrocnemius* muscle tissues from a single mouse were dissociated on the gentleMACS Octo Dissociator with Heaters using the Skeletal Muscle Dissociation Kit and then treated with the Satellite Cell Isolation Kit. The gating strategy of MuSCs is shown (fig. 1). Cells were gated based on forward and side scatter (fig. 1, panel A). Viable cells were identified as DAPI negative (fig. 1, panel B) and lineage positive cells were excluded (fig. 1, panel C). MuSCs were identified as double positive for the cell surface markers CD34 and α7 integrin (fig. 1, panel D). MACS Column-based negative selection of lineage$^+$ cells prior to sorting allowed enrichment of MuSCs from 1% to 80% of total (fig. 2, panels A and B). Cells were further purified via flow sorting to a final purity of 95% (fig. 2, panel C). Isolated cells (fig. 3, panel A) were cultured and differentiated to yield fusion-competent myoblasts using standard differentiation protocols (fig. 3, panel B).

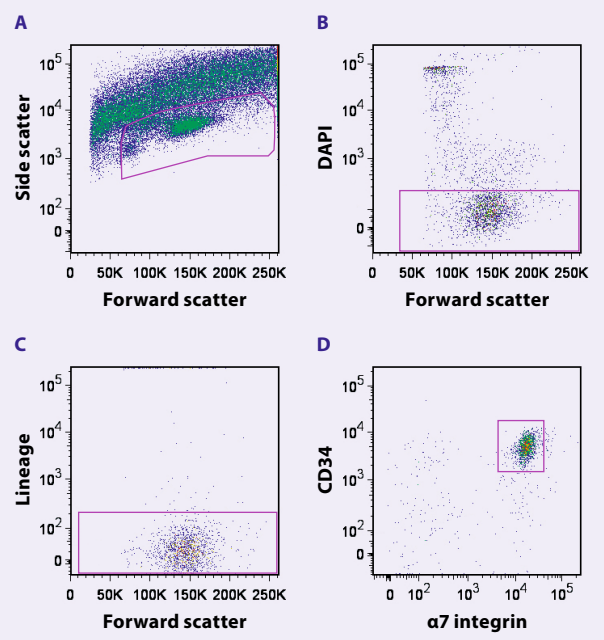

Figure 1: Gating strategy of MuSCs. Cells were gated based on forward and side scatter (A) and viable cells were gated as DAPI negative (B). Lineage positive cells were excluded (C) and MuSCs were identified as α7 integrin and CD34 double positive (D).

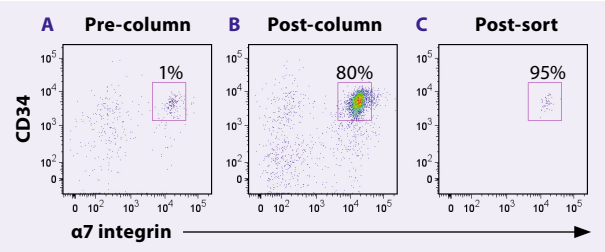

Figure 2: Isolation of MuSCs from *Tibialis anterior* and *gastrocnemius* muscles from C57BL/6 mice. Muscle tissue was dissociated using the gentleMACS Octo Dissociator with Heaters and the Skeletal Muscle Dissociation Kit. Muscle cells were analyzed by flow cytometry using the gating strategy as described above after dissociation (A), after lineage depletion with the Satellite Cell Isolation Kit (B), and following isolation of MuSCs by flow sorting using antibodies against α7 integrin and CD34 (C).

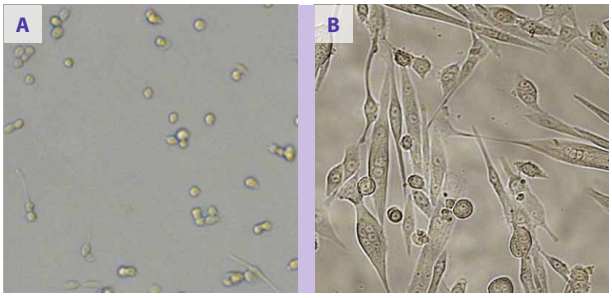

Figure 3: Freshly isolated muscle stem cells (A) were cultured and differentiated using standard protocols to yield fusion-competent myoblasts (B).

Conclusion

The data show that dissociation using the Skeletal Muscle Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters is suitable for obtaining viable single-cell suspensions. Further, it is shown that the Satellite Cell Isolation Kit represents a rapid (<30 minutes) and reliable method for lineage depletion of muscle tissue preparations prior to flow sorting purification of muscle stem cells. This lineage depletion method greatly reduces sorting times and increases cell viability. Data further show that these stem cells can give rise to fusion-competent myoblasts upon standard differentiation culture conditions.

References

1. Charge, S. B. and Rudnicki, M. A. (2204) Cellular and molecular regulation of muscle regeneration. Physiol. Rev. 84: 209–238.
2. Montarras, D. *et al.* (2005) Direct isolation of satellite cells for skeletal muscle regeneration. Science 309: 2064–2067.
3. Collins, C. A. *et al.* (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122: 289–301.
4. Asakura, A. *et al.* (2007) Increased survival of muscle stem cells lacking the MyoD gene after transplantation into regenerating skeletal muscle. Proc. Natl. Acad. Sci. U. S. A. 104: 16552–16557.
5. Cerletti, M. *et al.* (2008) Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. Cell 134: 37–47.
6. Sacco, A. *et al.* (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456: 502–506.
7. Lepper, C. and Fan, C. M. (2010) Inducible lineage tracing of Pax7-descendant cells reveals embryonic origin of adult satellite cells. Genesis 48: 424–436.
8. Sambasivan, R. and Tajbakhsh, S. (2015) Adult skeletal muscle stem cells. Results Probl. Cell Differ. 56: 191–213.
9. Mauro, A. (1961) Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9: 493–495.
10. Muir, A. R. *et al.* (1965) The structure of the satellite cells in skeletal muscle. J. Anat. 99: 435–444.
11. Snow, M. H. (1977) Myogenic cell formation in regenerating rat skeletal muscle injured by mincing. II. An autoradiographic study. Anat. Rec. 188: 201–217.
12. Grounds, M. D. and McGeachie, J. K. (1987) A model of myogenesis *in vivo*, derived from detailed autoradiographic studies of regenerating skeletal muscle, challenges the concept of quantal mitosis. Cell Tissue Res. 250: 563–569.
13. Jacobs, S. C. *et al.* (1995) Satellite cell activation after muscle damage in young and adult rats. Anat. Rec. 242. 329–336.
14. Rocheteau, P. *et al.* (2015) Dormancy and quiescence of skeletal muscle stem cells. Results Probl. Cell Differ. 56: 215–235.
15. Sacco, A. *et al.* (2010) Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. Cell 143. 1059–1071.
16. Cosgrove, B. D. *et al.* (2014) Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat. Med. 20: 255–264.

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