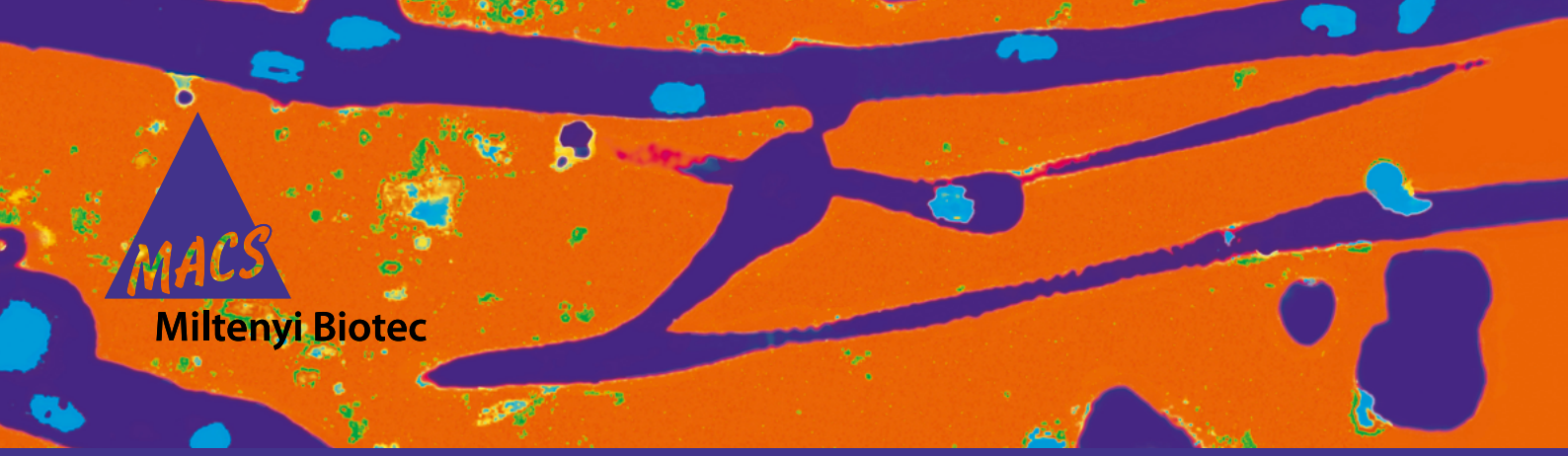

Isolation and separation

Satellite cells from skeletal muscles

Isolation of satellite cells from skeletal muscles using the gentleMACS™ Octo Dissociator with Heaters and magnetic cell separation

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Background

Skeletal muscles are constituted of long multinucleated myofibers that possess the contractile machinery to generate movement. Skeletal muscles also contain numerous small mononuclear cells (2×10^5 to 1×10^6 cells per gram of muscle) named satellite cells, based on their anatomical proximity to the myofibers. Satellite cells are responsible for muscle regeneration. In resting muscle, satellite cells are quiescent but are quickly activated following an injury to generate myogenic progenitors and to self-renew. Isolation of satellite cells is a very useful technique to study their behavior *in vitro* (primary cell culture) or *in vivo* (transplantation assay). Isolation of satellite cells by flow cytometry is achievable using negative selection against lineage markers CD11b, CD31, CD45, and Sca-1, and positive selection for alpha7-integrin and CD34.[1] To avoid excessive satellite cell commitment during isolation it is crucial to collect satellite cells as fast as possible. The gentleMACS™ Octo Dissociator with Heaters allows the fast and efficient dissociation of skeletal muscle from multiple mice with reduced hands-on steps. Moreover, magnetic cell separation using the Satellite Cell Isolation Kit is a fast and convenient way to purify satellite cells for cell culture or to decrease flow cytometry time.
This note describes the standard procedure used by the Rudnicki lab to isolate satellite cells from mice hindlimb skeletal muscles using the gentleMACS Octo Dissociator with Heaters, followed by magnetic cell separation using the Satellite Cell Isolation Kit.

Materials and methods

Materials

- gentleMACS Octo Dissociator with Heaters
- gentleMACS C Tubes
- Digestion enzymes: collagenase/dispase solution diluted in HAM-F10 medium (2.5 U/mL)
- Satellite Cell Isolation Kit, mouse
- MACS® SmartStrainer (70 µm)
- MACS Separators and MS Columns
- (Optional, to achieve highest purity) Anti-Integrin α-7 MicroBeads, mouse

Methods

Muscle dissociation

1. Collect the hindlimb muscles from mice skin. Typically, 8 week old mice provide approximately 1 g of muscle.
2. Transfer up to 1 g of muscles per gentleMACS C Tubes containing 5 mL of digestion enzymes (2.5 U/mL) and roughly cut the muscles with scissors to obtain smaller chunks (2–4 mm^2).
3. Place the C Tubes and heaters on the gentleMACS Octo Dissociator with Heaters and select the skeletal muscle dissociation program **37C_mr_SMDK_1** (figure 2). For flow cytometric analysis it is crucial to perform isolation as fast as possible. For this reason, the following protocol has been developed to lead to rapid muscle dissociation for cell isolation (total time ~25 minutes):
   - 3 minutes at 60 rpm for further dissociation of chunks
   - 9 minutes at –30 rpm for digestion using the heating function
   - 6 cycles of 5 seconds at +/–360 rpm for trituration
   - 12 minutes at –30 rpm for secondary digestion using the heating function
4. After digestion, muscles should be in homogeneous slurry without chunks. Pass the homogenate through a MACS SmartStrainer (70 µm) and proceed with flow cytometry or MACS Cell Separation.

Satellite cell isolation
Satellite cells were isolated according to the protocol of the Satellite Cell Isolation Kit.
For further purification, satellite cells have been labeled with Anti-Integrin α-7 MicroBeads and isolated using MS Columns and a MACS Separator. The flow-through fraction was discarded, and satellite cells were collected in the eluted fraction.

Results

Satellite cells were isolated from 6 week old *Pax7-zsGreen* reporter mice, in which all satellite cells are labeled with zsGreen fluorescent protein. Satellite cell purity was evaluated by flow cytometry from muscles digested with the gentleMACS Octo Dissociator with Heaters with or without the Satellite Cell Isolation Kit and Anti-Integrin α-7 MicroBeads (figure 1). Results show a 46-fold enrichment in satellite cell content with the Satellite Cell Isolation Kit in combination with positive selection using the Anti-Integrin α-7 MicroBeads.

Satellite cells were also isolated from wildtype or *mdx* mice (dystrophin-deficient mice) using the gentleMACS Octo Dissociator with Heaters, followed by magnetic cell separation with the Satellite Cell Isolation Kit. *Mdx* muscles contain high density of inflammatory cells and fibroblasts, and satellite cells are typically more difficult to isolate and purify from these muscles. After isolation with the Satellite Cell Isolation Kit cells were plated and immunostained for the satellite cell marker Pax7. A high level of purity was obtained from both wildtype and *mdx* muscles using the Satellite Cell Isolation Kit (figure 2).

The gentleMACS Octo Dissociator with Heaters was also used to dissociate muscles to isolate satellite cells for transplantation assay. Muscles from *Pax7-zsgreen* reporter mice were dissociated using a short customized program (~25 minutes) and satellite cells were isolated by flow cytometry through gating for zsGreen and Hoechst. Ten thousand satellite cells were resuspended in 0.9% NaCl and immediately transplanted into the *tibialis anterior* muscles of FK506-immunosupressed mice that had been injured two days before[2]. The rapid cell isolation minimized satellite cell commitment and allowed long-term engraftment of satellite cells in the niche (figure 3).

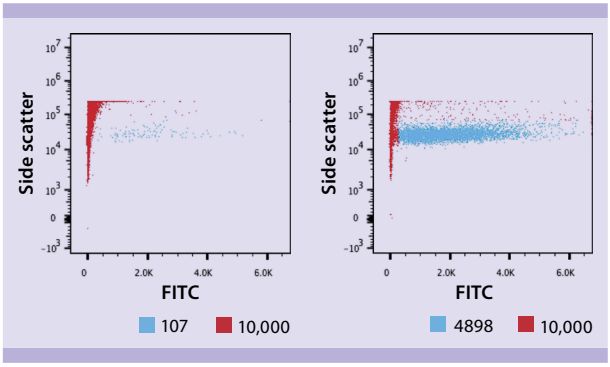

Figure 1: Satellite cell enrichment with the Satellite Cell Isolation Kit. Hindlimb muscles from *Pax7-zsgreen* reporter mice were digested using the gentleMACS Octo Dissociator with Heaters using a customized program to reduce digestion time. Satellite cell population is marked by zsGreen expression (FITC). Events in blue represent satellite cells, while events in red are other cell types and debris. Left plot shows a very small population of satellite cells in the eluted (lineage-positive) fraction of the Satellite Cell Isolation Kit. Right plot shows a 46-fold enrichment in satellite cell population after MACS Cell Separation with the Satellite Cell Isolation Kit and Anti-Integrin α-7 MicroBeads.

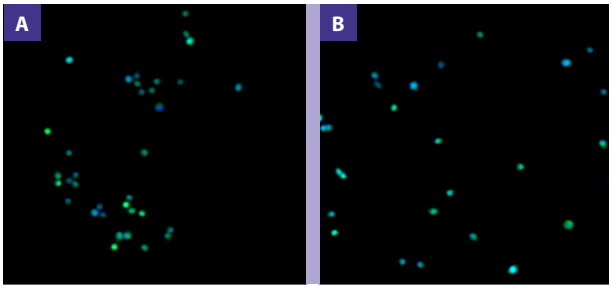

Figure 2: Satellite cell purity for *in vitro* cell culture. Satellite cells from wildtype and *mdx* (dystrophin-deficient) mice were isolated using the gentleMACS Octo Dissociator with Heaters with the 37C_mr_SMDK_1 program, followed by magnetic cell separation with the Satellite Cell Isolation Kit and the Anti-Integrin α-7 MicroBeads. Cells were plated and stained for Pax7 (green) and DAPI (blue). Images show that cells isolated from wildtype (A) and *mdx* (B) muscles are almost all positive for Pax7, which indicates high satellite cell purity.

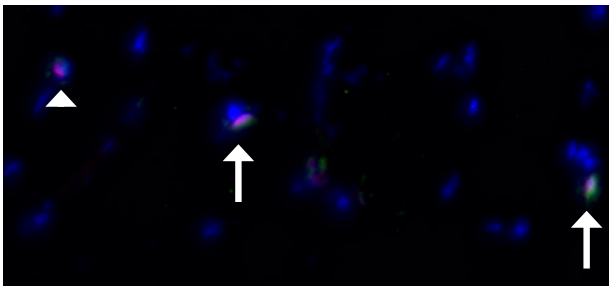

Figure 3: Satellite cell transplantation. Hindlimb muscles from *Pax7-zsgreen* reporter mice were digested using the gentleMACS Octo Dissociator with Heaters using a customized program to reduce digestion time. Satellite cells were sorted by flow cytometry through gating for zsGreen and Hoechst. Ten thousand satellite cells were resuspended in 0.9% NaCl and immediately transplanted into the *tibialis anterior* muscles of FK506-immunosupressed mice that had been injured 2 days before. Three weeks post-injection, *tibialis anterior* muscle is removed and muscle sections are immunostained for Pax7 (red), zsGreen (green), and DAPI. Picture show transplanted satellite cells expressing zsGreen (arrows) that engrafted into the host muscle together with endogenous satellite cells (arrowhead).

Conclusion

Satellite cell isolation is useful technique to study muscle stem cell biology. The gentleMACS Octo Dissociator with Heaters allows the rapid digestion of multiple samples at the same time with limited hands-on steps. Moreover, magnetic cell separation using the Satellite Cell Isolation Kit is a convenient way to quickly purify satellite cell for cell culture or to decrease flow cytometry sorting time.

References

1. Pasut, A. *et al.* (2012) Isolation of muscle stem cells by fluorescence activated cell sorting cytometry. Methods Mol. Biol. Clifton NJ 798: 53–64.
2. Bentzinger, C. F. *et al.* (2014) Wnt7a stimulates myogenic stem cell motility and engraftment resulting in improved muscle strength. J. Cell Biol. 205: 97–111.

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