Preparation of mouse quadriceps muscle slices for immunohistochemistry investigations.

Corinne BARREAU, Maxime MATHIEU, Amandine GIROUSSE, Coralie SENGENES

RESTORE, Team STROMAGICS, 4bis Avenue Hubert Curien, Bâtiment INCERE, 31100 Toulouse, France

Introduction

Skeletal muscles represent 40% of the body mass. It is formed by contractile multinucleated muscle fibres, resulting from the fusion of myoblasts. The main function of skeletal muscles is to generate the forces required to maintain posture and produce movement. After a trauma, skeletal muscles exhibit this amazing capacity to regenerate and repair. However, this regenerative capacity is dramatically impaired as we age leading to muscle mass decline, associated with physical frailty and increased risk of morbidity.

It has been shown that adipose tissue mass is correlated with skeletal muscle function and mobility, such that adipose tissue deposition in muscle and loss of muscle mass occur simultaneously with aging and in other muscle-related diseases [1-5]. Furthermore, muscle regeneration after injury is also associated with a transient remodeling of the stromal and immune cell component of muscular cells [6]. Thus, a better characterization of both adipocyte accumulation and cellular composition in skeletal muscle after injury is mandatory to increase our knowledge of the events occurring during muscle regeneration post-damage.

Tissue clearing techniques, especially those using organic solvents have enabled three-dimensional imaging of whole tissues by transforming thick tissues into optically transparent thick tissues. But these techniques present some drawbacks since direct lipid staining is impossible because of their complete extraction, making it impossible to visualize adipocytes by using probes staining lipids (bodipy), Furthermore, some tissues such as large skeletal muscles are difficult to clear entirely. An intermediate solution for quantification of both adipocyte content and cellular composition post-injury resides in pseudo 3D imaging on thick muscle slices.

Procedures

Sample preparation: For this purpose, we routinely perform quadriceps femoris muscle sampling in mice. Our objective is to obtain a global vision of the events present in the muscle at a given time. Longitudinal sections are the most pertinent to answer this question.

The muscle is fixed in neutral buffered 4% (w/v) paraformaldehyde (24h at room temperature). Note, if samples are fluorescent, it is important to keep them away from light during fixation. Then, the muscle is rinsed in PBS and embedded in 3% agarose gel to perform longitudinal sectioning. In order to make parallel sections with the muscle fibres, the muscle must be embedded as shown in **Figure 1**.

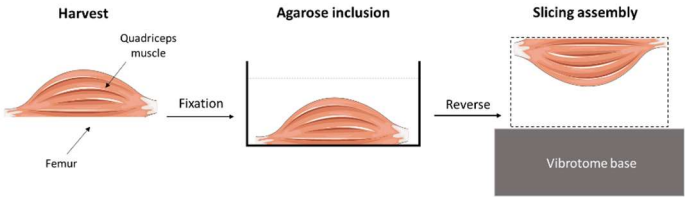

Figure 1: Schematic representation of the quadriceps femoris muscle mounting

Sample sectioning: The agarose block containing the muscle is then turned over and attached with glue to the metal tissue mount of the 5100mz vibrotome. Then, the whole muscle is immersed in a pre-cooled PBS solution making sure to keep the temperature between 3 and 4°C to improve the sectioning efficiency. The cutting speed is a key parameter to preserve the integrity of the tissue. The amplitude, frequency and the section thickness remain fixed. We use a speed of 0.25mm/s for young mice muscles and 0.10mm/s for the injured/old ones. Using a paintbrush, the floating muscle thick section is then transferred to a culture dish previously filled with PBS containing antibiotics and antifungals, for longer preservation.

Specific examples

Fat accumulation in the form of adipocytes located between muscle fibres in response to diet challenge [4] or after muscle injury was investigated. BODIPY, a marker of neutral lipids, was used to stain the lipid droplets of mature adipocytes. Then, using confocal microscopy on whole muscle section we quantified the surface occupied by adipocytes within the muscle.

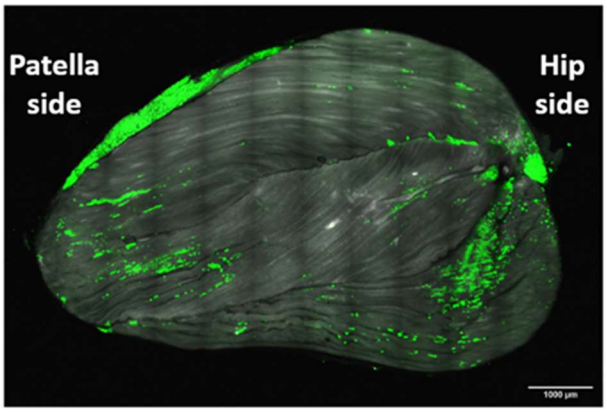

Figure 2: Mouse quadriceps muscle section stained with bodipy (green). (Scale 1000 µm)

This type of muscle sectioning combined with the immunofluorescence technique has also allowed us to observe both muscle-derived stromal and immune components. Using a CD45 antibody, a leukocyte marker, and PDGFRα antibody, a marker of muscle mesenchymal stromal cells, we were able to monitor the evolution of these cell types in the muscle over time after injury.

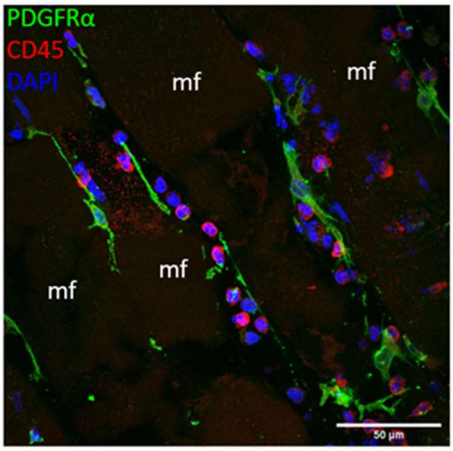

Figure 3: Mouse quadriceps muscle section stained with PDGFRα (green), CD45 (red) antibody and DAPI (blue) 1 day after muscle injury. (mf: muscle fibers; Scale 50 µm)

References

1. **Batsis, J.A.** Obesity in the Older Adult: Special Issue. J. Nutr. Gerontol. Geriatr. 2019, 38, 1–5. https://doi.org/10.1080/21551197.2018.1564197
2. **Newman, A.B.; Haggerty, C.L.; Goodpaster, B.; Harris, T.; Kritchevsky, S.; Nevitt, M.; Miles, T.P.; Visser, M.; Health, A.** Body Composition Research Group. Strength and muscle quality in a well-functioning cohort of older adults: The Health, Aging and Body Composition Study. J. Am. Geriatr. Soc. 2003, 51, 323–330. https://doi.org/10.1046/j.1532-5415.2003.51105.x
3. **Newman, A.B.; Kupelian, V.; Visser, M.; Simonsick, E.; Goodpaster, B.; Nevitt, M.; Kritchevsky, S.B.; Tylavsky, F.A.; Rubin, S.M.; Harris, T.B.**; et al. Sarcopenia: Alternative definitions and associations with lower extremity function. J. Am. Geriatr. Soc. 2003, 51, 1602–1609. https://doi.org/10.1046/j.1532-5415.2003.51534.x
4. **Girousse A, Gil-Ortega M, Bourlier V, Bergeaud C, Sastourné-Arrey Q, Moro C, Barreau C, Guissard C, Vion J, Arnaud E, Pradère JP, Juin N, Casteilla L, Sengenès C.** The Release of Adipose Stromal Cells from Subcutaneous Adipose Tissue Regulates Ectopic Intramuscular Adipocyte Deposition. Cell Rep. 2019 Apr 9;27(2):323-333.e5. https://doi.org/10.1016/j.celrep.2019.03.038
5. **Amandine Girousse, Maxime Mathieu, Quentin Sastourné-Arrey, Sylvie Monferran, Louis Casteilla, Coralie Sengenès** Endogenous Mobilization of Mesenchymal Stromal Cells: A Pathway for Interorgan Communication? Front Cell Dev Biol. 2021 Jan 8 ;8. https://doi.org/10.3389/fcell.2020.598520
6. **Julier Z., Park A. J., Briquez P. S.,Martino M. M.** Promoting tissue regeneration by modulating the immune system. Acta Biomaterialia 2017, 53, 13-28 https://doi.org/10.1016/j.actbio.2017.01.056