Video Article

In vivo Micro-circulation Measurement in Skeletal Muscle by Intra-vital Microscopy

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URL: http://www.jove.com/video/210
DOI: doi:10.3791/210

Keywords: Cellular Biology, issue 4, mouse, skeletal muscle, microscopy, circulation

Date Published: 5/28/2007

Citation: Asai, A., Sahani, N., Ouchi, Y., Martyn, J., Yasuhara, S. In vivo Micro-circulation Measurement in Skeletal Muscle by Intra-vital Microscopy. *J. Vis. Exp.* (4), e210, doi:10.3791/210 (2007).

Abstract

BACKGROUND: Regulatory factors and detailed physiology of in vivo microcirculation have remained not fully clarified after many different modalities of imaging had invented. While many macroscopic parameters of blood flow reflect flow velocity, changes in blood flow velocity and red blood cell (RBC) flux does not hold linear relationship in the microscopic observations. There are reports of discrepancy between RBC velocity and RBC flux, RBC flux and plasma flow volume, and of spatial and temporal heterogeneity of flow regulation in the peripheral tissues in microscopic observations, a scientific basis for the requirement of more detailed studies in microcirculatory regulation using intravital microscopy.

METHODS: We modified Jeff Lichtman's method of in vivo microscopic observation of mouse sternomastoid muscles. Mice are anesthetized, ventilated, and injected with PKH26L-fluorescently labeled RBCs for microscopic observation.

RESULT & CONCLUSIONS: Fluorescently labeled RBCs are detected and distinguished well by a wide-field microscope. Muscle contraction evoked by electrical stimulation induced increase in RBC flux. Quantification of other parameters including RBC velocity and capillary density were feasible. Mice tolerated well the surgery, injection of stained RBCs, microscopic observation, and electrical stimulation. No muscle or blood vessel damage was observed, suggesting that our method is relatively less invasive and suited for long-term observations.

Video Link

The video component of this article can be found at http://www.jove.com/video/210/

Protocol

RBC membrane staining

1. Mouse red blood cells were corrected from the same mouse strain with heparinization.
2. Mouse RBC were stained with PKH26 dye (551/567 nm; Red fluorescent cell linker kit, Sigma, U.S.A.).
3. RBC were washed in PBS and incubated with 2μM solution of PKH26 dye for 5 minutes at room temperature. The reaction was stopped by adding plasma (heat inactivated for 1 hour at 65°C beforehand) and incubated for 1 minute.
4. The stained RBC were washed 5 times with PBS.

Mouse Preparation

1. Three to six month old male C57BL/10 mice were used in this study. We anesthetized mice by intraperitoneal injection of pentobarbital (50mg/kgBW).
2. Mice were then intubated with a 20-gage polyethylene tube, mechanically ventilated, and warmed at 37°C on a Kapton sheet heater connected to a thermocouple sensor and feedback temperature controller system.
3. The ventral side of the neck was shaved. The right and left sternomastoid muscles were exposed. The muscles were supported by a stainless steel holder and superfused with sterile Krebs Ringer solution.

Injection

1. Stained RBCs were warmed at 37°C, diluted with Krebs Ringer. 50ul of stained RBC (hematocrit 12%) was injected into mouse from the penile vein.

In vivo Microscopic Observation of Skeletal Muscles

We followed Jeff W. Lichtman's method with modification[1:]

1. Animals were placed on a heating pad on top of a hand-made iron stage of a Nikon Eclipse-800 microscope. Water-dipping objective lenses were utilized for live epi-fluorescent observation. We observed PKH26 stained RBC flowing inside of vascular under fluorescent light of a Mercury lamp. We identified primary arterioles, secondary arterioles, capillaries and veins by their RBCs flow directions and architectures.
2. An intensified SIT Camera (Hamamatsu Photonics, C2400, Japan) and video-frame grabber were installed to capture the low-intensity signal at the real-time video rate (30 frames per second). Images were recorded on DVD as video files.

Muscle Stimulation

1. An electrical pulse was generated using a Peripheral Nerve Stimulator (Innervator 252, Fisher & Paykel Healthcare, New Zealand), and delivered to muscle surface via a coated stainless probe. A minus probe end was placed on the proximal part of muscle and a plus end was placed on the distal part of muscle. Tetanus stimulations were given at the rate of 50Hz for 5 seconds. The electrical stimuli did not cause direct myofiber damage on the vicinity of contact area.

RBC flux analysis

1. Recorded DVD video images were transferred Video Savant image files through frame grabber software (Video Savant, U.S.A.). Video images were reviewed in adjusted speed in order to visualize individual stained RBC. RBC flux was measured by counting RBC, which flew through a focused primary arteriole per minute.

Discussion

Important techinical points are as follows: (1) maintenance of physiological status of the animal (ventilation, pH of the perfusative solution, body temperature), (2) injection volume of the stained RBC, and (3) conditions for observation (optimal lens selection, fluorescence intensity). Potential future applications are as follows: (a) combination with pharmacophysiological and/or molecular biological interventions, (b) long-term observation of arterio/arteriolo-sclerosis and neovascularization.

Disclosures

All the procedures related to animal experiments were reviewed and approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital.

Acknowledgements

We thank JW Lichtman for his advice in in vivo observation of muscles.

References

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