Visualizing Myoblast Fusion: Cellular Coordination and Mechanisms Underlying Fiber Formation

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INTRODUCTION: Skeletal muscle growth and regeneration depend on the unique ability of muscle cells to fuse together. Unlike most cells in the body, muscle cells fuse to form large, multinucleated fibers that establish the tissue's structure and function. Myoblast fusion—a critical step in myotube formation and myogenesis—consists of five key events: migration, elongation, cell–cell recognition, adhesion, and membrane fusion[1-3]. During migration, elongated fusion-competent myocytes extend actin-rich filopodia and lamellipodia that promote cell–cell contact and recognition, while adhesion molecules such as N-cadherin, V-CAMs, and integrins stabilize these interactions and bring membranes within ~10 nm to initiate pore formation and fusion. In muscle development and repair, myoblasts arise from the proliferation and differentiation of activated muscle stem cells and fuse into multinucleated myotubes in vitro or muscle fibers in vivo through tightly regulated processes involving cell–cell interactions, protein signaling, and migration. Similar mechanisms occur during muscle regeneration following injury. Cell–cell fusion also plays essential roles in other physiological contexts, including osteoclast and macrophage giant cell formation and placental development, and is increasingly applied in regenerative medicine and cell-based therapies. Experimental models using *Drosophila* embryos or murine myoblasts remain central for identifying essential fusion molecules, though newer studies now incorporate chicken embryos and zebrafish due to their developmental accessibility and genetic malleability [4]. Advances in live-cell imaging (LCI)—including fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP), and fluorescence lifetime imaging microscopy (FLIM)—enable detailed observation of dynamic fusion events, with super-resolution predicted to further advance the field [5-6]. Emerging imaging approaches continue to deepen our understanding of myogenesis and may guide future therapies for myopathies and other applications. Here, we present new findings characterizing mouse and human myoblast fusion, focusing on pre-fusion behavior, fusion polarity, migration, and membrane fusion timing.

METHODS: Live-cell imaging of myoblast activity was performed using myoblast cell line, and primary mouse and human muscle stem cells. Myoblasts were isolated from C57BL/6J mice via established protocols. Cells were cultured in DMEM with 10–20% serum and imaged using an inverted Nikon microscope in a stage-top incubator. Both male and female muscle cells were examined, and will be presented, based on our previous reports of sex-based differences in muscle stem cells. Time-lapse images were acquired at 10x or 20x mag, and at 2min, 5 min and 10 min intervals. ImageJ software was used for semi-manual analysis of division, migration, fusion, and dedifferentiation, with particular attention to the timing and sequence of fusion events.
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RESULTS: We observe key stages of myoblast behavior during differentiation, including cell–cell contact, alignment, migration, and membrane fusion. Notably, fusion can occur in under two hours after initial contact between myoblasts. In connection with cell division, we find cell–cell fusion occurring approximately 24 hours after division; remarkably, we also observe in subset of muscle cells in which cell-cell fusion occurs between daughter cells within 2 hrs (mean) of cell division. This rapid post-division fusion could enable newly generated cells to quickly augment fiber nuclei, potentially accelerating the recovery of contractile function, and underscores the importance of further studies to clarify how post-mitotic timing influences fusion efficiency. Ongoing studies are analyzing this rapidly fusing subset. Additional findings characterizing the timing, polarity, and pre-fusion behaviors will be presented.

DISCUSSION: Despite extensive research, the cellular mechanisms governing myoblast fusion remain incompletely understood. While proteins such as Myomaker and Myomerger are known molecular drivers, the dynamic behaviors preceding fusion events have been less studied. Our live-cell imaging revealed distinct stages of cell alignment, membrane contact, and cytoplasmic mixing, as well as show that different patterns of cell fusion exist. These observations uncover new aspects of temporal coordination and fusion polarity, offering insight into how fusion efficiency and muscle architecture are regulated. Understanding these dynamics provides critical insight into how multinucleated muscle fibers form and regenerate, informing both basic science and therapeutic strategies. SIGNIFICANCE: This knowledge has particular relevance for emerging chimeric cell therapies that fuse healthy donor myoblasts with patient-derived cells in Duchenne muscular dystrophy[7], enhancing survival, migration, and metabolic function. Studies in this area have the potential to shape the development of next-generation cell-based therapies.

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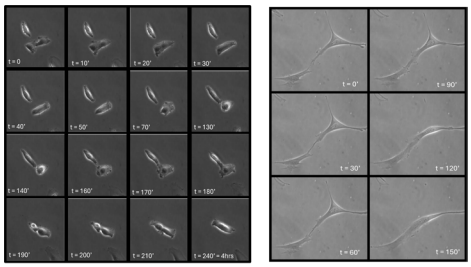

Figure 1. Cell fusion dynamics. (A) Formation of multinucleate early myotube. (B) Cell-cell fusion in primary muscle cells.