Development of a Cell Culture System to Examine the Acclimation, Contraction, and Cytoskeletal Remodeling of A7r5 Smooth Muscle Cells to Microgravity

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Abstract

Few studies have examined the acclimation and contraction of smooth muscle cells in microgravity. In addition, the cytoskeleton in smooth muscle has not been thoroughly examined habituated to microgravity. Although previous studies have noted that the phenotype of smooth muscle may be gravity dependent, those that have been performed have utilized simulated microgravity. The cytoskeleton has been implicated by several investigators as allowing single cells in vitro to respond to changes in gravity and may serve as a gravity sensor (reviewed in Vorselen et al., 2016) at the individual cell level. This investigation will focus on the effect of microgravity on the alpha-actin, beta-actin, and myosin components of the cytoskeleton in resting and contracting A7r5 smooth muscle cells. We have provided evidence of differential remodeling of the alpha-actin, beta-actin, and myosin cytoskeletal domains in smooth muscle (Brown et al., 2006, Li et al., 2001, Fultz et al., 2000, Fultz and Wright, 2003, and Thatcher et al., 2011). At rest these are organized into filamentous stress cables that run the length of the cell. Upon stimulation, □-actin and myosin interact to produce force that results in shorting of the cell. This shortening applies stress on the □eta-actin filaments which then undergo subsequent remodeling into configuration that holds the cell in the shortened state. Alpha-actin and myosin then undergo disassembly/reassembly into a configuration that is optimized for further contraction and aggregate into podosomes that appear as column-like structures in the contracted cell. While we have proposed that this remodeling is essential mechanism in the development and maintenance of force in smooth muscle (Li et al., 2001). Others have proposed that pododome formation may be relevant in the invasiveness of smooth muscle that is observed in cardiovascular disease as podosomes formation correlates with cell motility and localized degradation of the extracellular matrix (Burgstaller and Gimona, 2005; Lener et al., 2006; Murphy and Courtneidge, 2011). In collaboration with SpaceTango (Lexington, KY) and the Craft Academy for Excellence in Science and Mathematics at Morehead State University, a cell culture system has been developed that will allow for the culture, visualization, stimulation, and subsequent fixation of A7r5 cells aboard the International Space Station. Upon return to Earth, components of the cytoskeleton will be examined by fluorescent microscopy to investigate if microgravity alters the characteristic remodeling observed on Earth.

Background

Smooth muscle cells exhibit several unique properties that separate it characteristically from striated muscle. The myosin content of smooth muscle may be as little as 20% that of striated muscle, yet smooth muscle is capable of generating at least as much force as striated muscle (Murphy et al., 1974). In addition to structural considerations, smooth muscle has the unique ability to slowly develop tension and then maintain this tension for extended periods. Also noteworthy is the finding that phorbol ester-induced contraction of smooth muscle can occur in the absence of a detectable increase in intracellular calcium (Nakajima et al., 1993) or concomitant increased myosin light chain phosphorylation (Singer and Baker, 1987).

We have shown that A7r5 cells contract to both the elevation of intracellular calcium and to phorbol ester (Fultz et al., 2000). Prior to contractile stimuli, alpha-actin and beta-actin form densely packed parallel arrangements of stress cables that extend across the cell body. During the interval of cell contraction, beta-actin cables shorten without visible evidence of disassembly or new cable formation. By comparison, shortly after contractile stimuli the majority of alpha-actin cables disassemble and reform into intensely fluorescing column-like structures extending vertically from the cell base at the center of clusters of randomly oriented alpha-actin filaments. The alpha-actin columns of contracting cells show strong co-localization of alpha-actin.

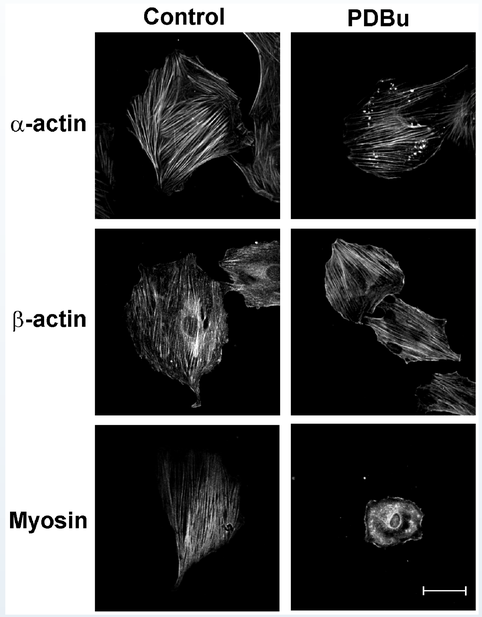


Figure 1: Comparison of alpha-actin (top row), beta-actin (middle row) and myosin (bottom row) in a relaxed cell and a contracted cell that underwent 40 minutes of PDBu stimulation.

We hypothesize that the differential remodeling of the alpha-actin and beta-actin cytoskeleton and preferential localization of myosin with alpha-actin provides further evidence of implicating the division of the actin cytoskeleton into a contractile role (alpha-actin) and a role to hold the cell in the shortened configuration (beta-actin).

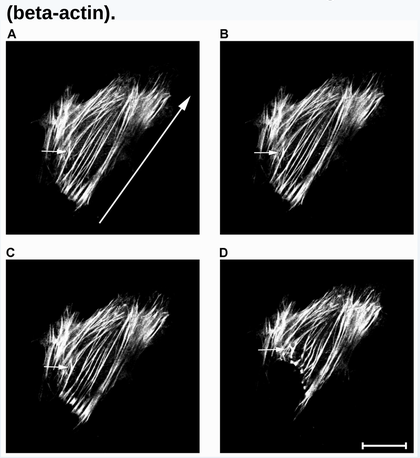


Figure 2: Shortening of beta-actin cables during stimulated contraction.

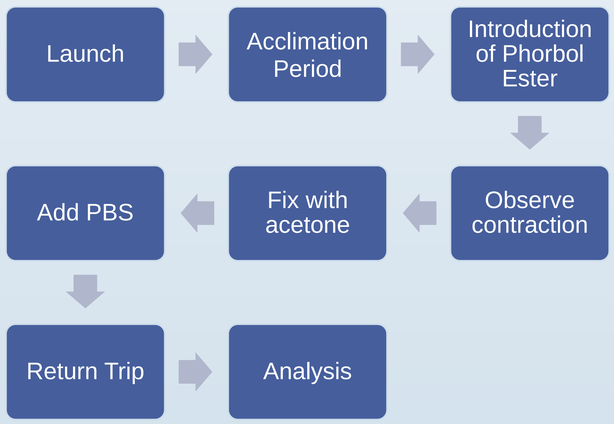
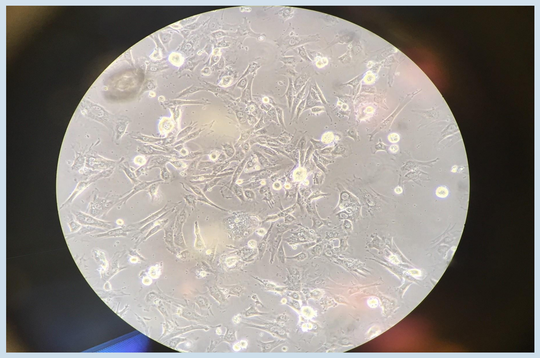


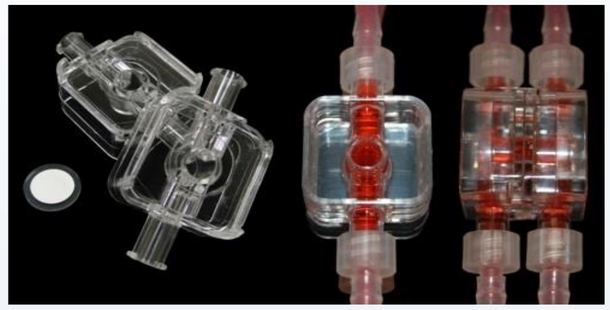
Figure 3: Flow chart illustrating the procedure that will be followed during SpaceX-10 launch and subsequent stint on the ISS.



Graphic 1: Microscopic view of the in-vitro culturing of A7r5 cells; confluent. Source: Dr. Michael Fultz.

Graphic 2: SpaceX's Falcon-9 rocket on launch pad 39A at the Kennedy Space Center, two days prior to the February 19th, 2017 launch. The Dragon module, containing the experiment, was launched to the ISS as part of CRS-10. Source: Gentry Barnett.

Model Diagram



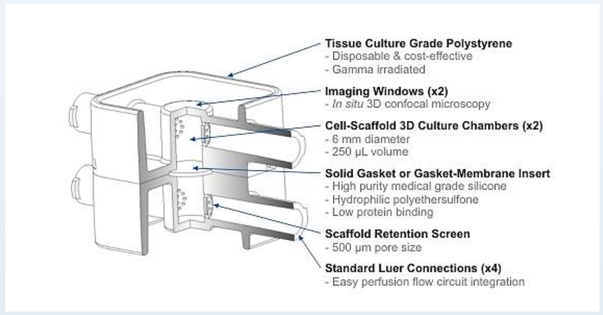
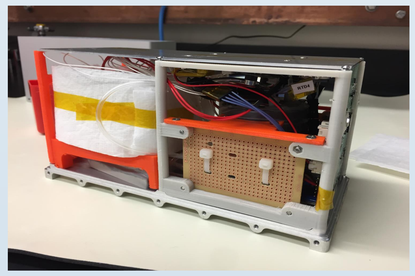


Figure 4: 3D Cell Culture Plasticware for segregated co-culture (KiYATEC Inc.). Polystyrene chambers provide a culturing substrate for cells that parallel 96-well plate technology, as well as allowing for continuous gas exchange with the environment and fluid control through the output and input tubes, shown above. Source: Kiyatec



Graphic 3: The Double CubeLab designed by SpaceTango Inc., housing the smooth muscle experiment. Some of the systems shown include the fluid exchange system, gas exchange system, and parts of the cell culturing platform. Source: SpaceTango Inc.

References

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Murphy, R.A., J.T. Herliky, and J. Meyerman. Force-generating capacity and contractile protein content of arterial smooth muscle. J. Gen. Physiol. 64:691-705, 1974.

Nakajima, S., M. Fujmoto, and M. Veda. Spatial changes of [Ca2+]i and contraction caused by phorbol esters in vascular smooth muscles. Am. J. Physiol. 265(4):C1138-C1145, 1993

Paul, R.J. Functional compartmentalization of oxidative and glycolytic metabolism in vascular smooth muscle. Am. J. Physiol. 244:C399-C409, 1983.

Singer, H.A. and K.M. Baker. Calcium dependence of phorbol 12, 13 dibutyrate-induced force and myosin light chain phosphorylation in arterial smooth muscle. J. Pharmacol. Exptl. Therapeutics. 243(3):814-821, 1987.