<i>In Vitro</i> Simulation of Microgravity Induced Muscle Loss Successfully Increases Expression of Key <i>In Vivo</i> Atrophy Markers | Biological Engineering

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Full Abstract

Muscular atrophy, defined as the loss of muscle tissue, is a serious issue for immobilized patients on Earth and in human spaceflight, where microgravity prevents normal muscle loading. Developing countermeasures for atrophy in spaceflight will require extensive screening of pharmaceuticals for efficacy, safety, contraindications, and dosage schedule. Due to the cost of spaceflight and limited crew time aboard the International Space Station, high throughput screening of pharmaceuticals under real microgravity conditions is not feasible. While traditional ground-based atrophy studies using the rodent hind-limb unloading model are effective at inducing physiological changes similar to spaceflight, they are not suited for first round screening of novel therapeutics due to resource and regulatory challenges. Here, we present a protocol for maximizing the expression of atrophy-related mRNAs using an <i>in vitro</i> ground-based model of microgravity with a rotary cell culture system.

To model the microgravity conditions on the International Space Station, murine C2C12 myoblasts were cultured in a rotary cell culture system. Alginate hydrogel encapsulation was compared against polystyrene microcarrier beads as a substrate for C2C12 muscle cells. We hypothesize that alginate encapsulation with pre-differentiated tissue will provide a more accurate 3D model of mature muscle and improve mRNA expression similarity to <i>in vivo</i> atrophic conditions. Microcarrier beads, commonly used for suspension culture of adherent cell lines, require starting with undifferentiated cells, thereby reducing biosimilarity to <i>in vivo</i> conditions. Changes after culture under simulated microgravity conditions were characterized by assessing the atrophy markers MuRF1 and MAFbx using qRT-PCR. Cell morphology and substrate structure were evaluated with fluorescent and brightfield imaging.

Contrary to expectations, both undifferentiated and differentiated cells encapsulated in alginate failed to display significant changes in MuRF1 and MAFbx expression when cultured in simulated microgravity. On the other hand, simulated microgravity cultures with polystyrene microcarriers resulted in significantly increased expression of both MuRF1 and MAFbx, relative to normal-gravity controls. Ground-based simulation of microgravity will provide a valuable platform for drug discovery and an understanding of the multiple mechanisms underlying muscular atrophy.