MODELING MUSCLE REGENERATION IN MICROGRAVITY

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Abstract

Skeletal muscle atrophy and reductions in regenerative capacity due to microgravity exposure remain a limiting factor to astronaut health and performance on long-term space missions.[1] Missions to Mars and the Moon pose risks for muscle injury through reloading strain or trauma. Computational modeling, specifically agent-based modeling (ABM) offers a cost- and resource-effective method through which to investigate muscle regeneration in microgravity. We are developing an ABM of a murine muscle cross-section undergoing injury regeneration in microgravity to better understand the mechanisms underlying impaired regenerative capacity. The agents include muscle fibers, immune cells, fibroblasts, capillaries, and satellite stems cells that are capable of growth, migration, secretion, and proliferation, and differentiation. Calibration and validation will be performed through comparison to literature-derived experimental benchmarks. The anticipated results will demonstrate what changes in cell behaviors and cell signaling dynamics are responsible for impaired regeneration and will propose pharmacological countermeasures that have the potential to counteract deficits. Overall, this project aims to provide a computational tool with which to investigate muscle adaptations to microgravity and screen countermeasures for the maintenance of astronaut health and performance during spaceflight.

*This paper is a partial report for this study which is currently in progress with anticipated completion by December 2025.

Introduction

Skeletal muscle plays a central role in locomotion, thermogenesis, posture, and metabolism. Characterized by robust plasticity, skeletal muscles readily hypertrophy in response to mechanical overloading and atrophy under conditions of unloading. In microgravity, atrophy primarily affects postural muscles, including the gastrocnemius and soleus[2], which normally counteract gravitational forces while walking or standing. The resulting loss in muscle mass is accompanied by significant reductions in strength[3–5] and regenerative capacity[6], impairing functional performance and increasing the risk of injury. Despite the implementation of several iterations of nutrition and exercise strategies, the complete preservation of skeletal muscle quality in microgravity has yet to be achieved.[7,8]

Spaceflight is physically demanding[9], often requiring high workloads in restrictive and unfamiliar environments. Maintaining muscle function is essential for mission success and astronaut health, particularly in extended deep-space missions. A Mars-bound mission, for example, would require astronauts to endure approximately 6-9 months in microgravity (0g) before encountering the partial gravity of Mars (0.38g). These gravitational transitions pose a substantial risk, as atrophied muscles may be unable to withstand the sudden increase in mechanical loading, leading to structural damage from strain.[10,11] This is already a prevalent issue following return to Earth from missions, with astronauts frequently reporting muscle

soreness, weakness, and difficulty walking.[12] Moreover, the reduced regenerative capacity observed in microgravity could prolong or impair the healing process, increasing the likelihood of incomplete recovery and functional deficits. Given the limited availability of medical intervention in space, a deeper understanding of how microgravity disrupts muscle regeneration is crucial for countermeasure development.

Muscle regeneration is a highly coordinated, multi-step process encompassing destruction, repair, and remodeling.[13] Following injury, muscle fibers and capillaries undergo necrosis, triggering immune cell infiltration and debris clearance. Satellite stem cells (SSCs) then activate and proliferate, while fibroblasts remodel the extracellular matrix (ECM) to restore functional capacity. Early-stage angiogenesis facilitates nutrient and growth factor delivery, further supporting regeneration. Each of these processes is influenced by mechanical cues[14–19], underscoring the role of mechanical loading in supporting recovery[20]. Conversely, unloading impairs regeneration,[6,21,22] but the precise mechanisms underlying these deficits are not understood. While prior studies have examined individual cellular responses to unloading, they often fail to capture the interdependent nature of these processes, necessitating a systems-level approach to make sense of the interactions between cell behaviors and microgravity in muscle regeneration.

Traditional experimental approaches face inherent challenges in investigating muscle regeneration in microgravity. *In vivo* studies are costly and resource-intensive, while *in vitro* models lack full physiological complexity. Agent-based modeling (ABM), offers a computational approach to explore these complex dynamics, allowing for the systematic evaluation of muscle tissue behavior under microgravity conditions. ABMs are developed by assigning "rules" to "agents," where rules are observed biological behaviors and agents are cells. An example is "satellite stem cells are activated by hepatocyte growth factor (HGF). Additional examples of agent rules can be found in Tab.1.

By integrating biologically informed rules into a shared environment, ABMs can reveal emergent properties and system-level behaviors that are not apparent from isolated studies. Furthermore, ABMs can facilitate hypothesis generation and refinement by predicting outcomes of different countermeasure strategies, such as pharmacological interventions, to optimize *in vivo* experiments. As a result, ABMs serve as a valuable tool for bridging gaps in knowledge and supporting the accelerated development of protective measures to preserve muscle quality and regenerative capacity during long-duration spaceflight.

In this study, we present the development of an ABM of skeletal muscle regeneration that simulates post-injury healing under microgravity, based on murine data derived from the literature. By systematically varying cellular behaviors and regenerative signaling *in silico,* this model aims to identify key bottlenecks in the regenerative process and evaluate potential therapeutic interventions. A high-level overview of the model is provided in Fig. 1.

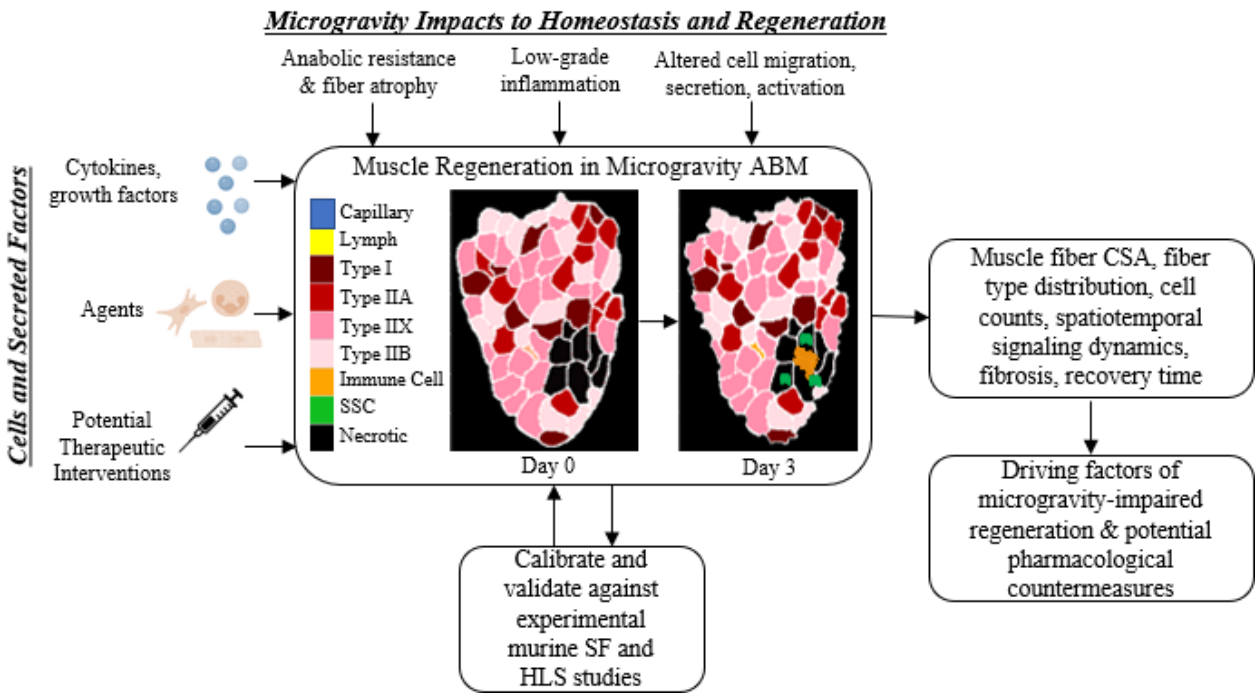

Figure 1. ABM Development. Agent and cytokine behaviors are inputs to the model and their behaviors are guided by agent rules. Agent rules are altered depending on the gravitational environment. Outputs include spatiotemporal dynamics for cells and signaling factors as well as recovery time. Simulation example displays muscle atrophy and the initiation of the regenerative response after three days. At day 3, the atrophic process is underway. Macrophages and neutrophils have been recruited to the necrotic area and activated satellite stem cells to begin regeneration.

Methods

An ABM of muscle regeneration after injury[23] was adapted to simulate the effects of microgravity. The model is built in CompuCell3D (CC3D), a Python-based biological modeling platform.[24] CC3D is based on the Cellular-Potts model framework, using logic-based representation for modeling cell behaviors and interactions. The model lattice was constructed by manually segmenting an immunohistological cross-section of muscle tissue using ImageJ[25] based on a prior myofiber imaging study[26] (Fig. 2a-b). The segmented image was converted into a PIFF file through an initialization script in CC3D.

This model includes multiple interacting cellular agents, including muscle fibers, SSCs, capillaries, macrophages, neutrophils, fibroblasts, and a lymphatic vessel. Additionally, the model incorporates several biochemical signaling factors, including IGF-1, HGF, IL-10, myostatin, TGF-β1, TNF-α, MCP-1, VEGF, and MMP. These molecules play central roles in regulating muscle fiber atrophy, hypertrophy, and regeneration after injury.[23] Agent rules and associated parameters guiding growth, migration, proliferation, secretion, apoptosis, and differentiation were developed based on experimental data from the literature (examples seen in Tab. 1).

The lattice consists of approximately 60 muscle fibers, reflecting the fiber-type distribution observed in the murine gastrocnemius[27] (Fig. 2c-d). Capillaries, fibroblasts, resident macrophages, and SSCs are initialized in proportions matching published experimental data.[28–32] This initialization ensures a biologically relevant baseline for simulating both homeostasis and perturbations due to unloading and injury.

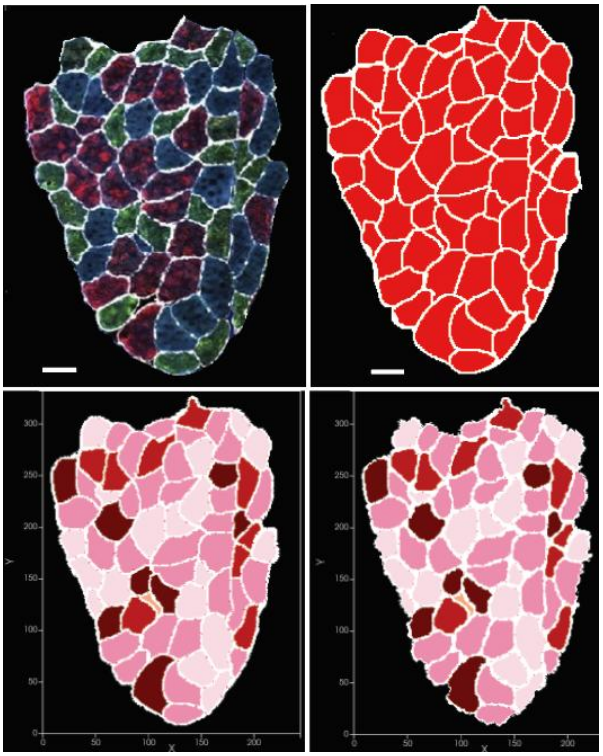

Figure 2*. (a) Top left. Original histological image. (b) Top right. ImageJ segmentation delineating fibers and ECM. Scale bar is 100um. (c) Bottom left. Initialized CC3D lattice with different fiber types, capillaries, lymphatic vessel, and ECM. (d) Bottom right. Atrophy simulation in CC3D demonstrating muscle fiber loss in cross-sectional area and increased ECM area fraction.*

The model is inherently stochastic; variability arises from both probabilistic cell behaviors and randomized spatial initialization. While the overall proportion of muscle fiber types is preserved across simulations, the exact spatial arrangement of type I, IIA, IIX, and IIB fibers varies with each run. This variability enables exploration of how local tissue architecture influences regenerative outcomes and response to unloading

Unloading

To simulate the effects of microgravity, the model calculates the growth rate of each muscle fiber by integrating anabolic (IGF-1, loading) and catabolic (myostatin, TNF- α, TGF- β1) signals. This process is further modulated by an anabolic sensitivity scaling factor, which represents the fiber's ability to respond to growth stimuli. Changes in the scaling factor over time capture the progressive anabolic resistance observed in muscle fibers under unloaded conditions[33], where sensitivity to growth-promoting signals diminishes over time. Since different muscle fiber types exhibit varying sensitivities to these biochemical and mechanical cues, the model assigns fiber-type specific equations that govern growth dynamics. Immune cell behaviors are adapted at the implementation of microgravity in the model. Activation and recruitment thresholds for immune cells are increased to simulate the delayed immune infiltration observed *in vivo* in unloaded environments.[6,34]

Injury

The simulation progresses over a series of Monte Carlo Steps (MCS), with each step corresponding to 15 minutes of real-world time. The model runs for a total of four weeks, representing the average time for complete muscle regeneration in mice.

At MCS = 1, muscle injury is introduced by inducing necrosis in a pre-specified percentage of muscle fibers. These necrotic fibers immediately begin secreting HGF and TGF-β, which initiate the activation of SSCs and fibroblasts, the primary cellular mediators of muscle regeneration and ECM remodeling. The necrotic event also triggers the recruitment of neutrophils, which are attracted in proportion to the severity of the injury.

Neutrophils function as the first responders, phagocytosing necrotic debris while secreting TNF-α and MCP-1, which act as pro-inflammatory signals to recruit resident macrophages. Once activated by these cytokines, macrophages proliferate, migrate, and participate in the clearance of apoptotic neutrophils and remaining necrotic muscle tissue. As the inflammatory phase transitions to the regenerative phase, macrophages shift toward an anti-inflammatory phenotype,

secreting IGF-1 to promote SSC proliferation and differentiation, thereby facilitating muscle repair.

Table 1. *Examples of ABM rules for skeletal muscle cells during microgravity exposure and regeneration.*

Agent	**Rule**
Muscle Fiber	Muscle fiber cross-sectional area decreases by ~40% after 13-35 days of spaceflight[35,36]
Capillaries	Capillary-to-fiber ratio in gastrocnemius is ~1.5:1[28]
Muscle Fiber	In a normal murine gastrocnemius, fiber type distribution is Type I: 15%; Type IIA: 20%; Type IIX: 38%; Type IIB: 27% [27]
Neutrophil	Neutrophils secrete TNF- α during phagocytosis. [37]
Macrophage	Macrophage recruitment to site of injury is delayed with unloading. [34]
Fibroblast	Unloading delays fibroblast migration. [38]
SSC	SSCs are activated by HGF and IGF. [39,40]

Calibration and Validation

To ensure biological accuracy, calibration will be conducted through Latin Hypercube Sampling (LHS) and CaliPro[41], a parameter density estimation technique that systematically refines model parameters based on experimental data. Calibration is performed by tuning key model parameters to match observed biological phenomena, such as changes in muscle fiber CSA[21,22,35], SSC activation[42,43], and fiber type transitions following unloading.[44] Experimental data from spaceflight studies, hindlimb suspension models, and muscle injury experiments serve as benchmarks to refine the model's predictive accuracy. By adjusting parameters within biologically plausible ranges, the model is optimized to reproduce *in vivo* muscle adaptations under unloading and regeneration conditions.

A sensitivity analysis will also be performed to gauge which parameters exert the greatest influence on muscle atrophy and regeneration. By systematically varying key parameters, such as the scaling of anabolic resistance, the recruitment thresholds for immune cells, or the magnitude of IGF-1 signaling, we will identify the relative impact of variables driving muscle recovery in microgravity. This analysis will inform subsequent *in silico* experiments, guiding the prioritization of therapeutic targets for intervention screening.

For validation, the model's outputs will be compared against independent perturbation studies of muscle regeneration under disuse conditions.[6,34] Validation criteria include accurate reproduction of atrophy kinetics, muscle fiber CSA and distribution, immune cell infiltration, and the time course of SSC activation and differentiation. If discrepancies arise, parameters will be refined iteratively to improve alignment with experimental observations. A successfully validated model will provide a robust framework for testing therapeutic countermeasures and predicting muscle responses to vasrious unloading conditions, making it a valuable tool for both spaceflight research and clinical applications in muscle-wasting conditions.

In silico experiments

Following validation, the model will be used to systematically evaluate potential therapeutic countermeasures aimed at mitigating regenerative impairments in microgravity. These interventions will be tested in isolation and in combination, with a focus on strategic timing to optimize their effects.

For example, early-phase interventions may include anti-inflammatory agents (e.g., IL-10 delivery) to modulate macrophage polarization and reduce excessive TNF-α signaling, which is known

to prevent muscle fiber growth. Mid-phase interventions could involve IGF-1 supplementation or mechanical stimulation mimetics to counteract anabolic resistance and enhance muscle fiber regrowth. Late-phase strategies may focus on TGF-β1 inhibition, preventing excessive fibrosis and promoting a more regenerative ECM environment.

By leveraging sensitivity analysis results, the model will identify the most promising intervention targets and optimal intervention windows, allowing for a prioritized approach to countermeasure testing. This will improve the efficiency of future experimental and clinical studies by narrowing the range of candidate treatments.

Anticipated Results

Following model validation and sensitivity analyses, we anticipate that the simulation will reproduce key features of muscle adaptation under unloading conditions. The model will produce quantitative outputs including time-series data of muscle fiber CSA and type, SSC and immune cell population dynamics, cytokine dynamics, and ECM area fraction and fibroblast activity metrics.

The model is expected to replicate muscle fiber atrophy trends observed in experimental studies, including fiber type-specific reductions in cross-sectional area and altered SSC activation dynamics. Type I fibers are predicted to exhibit greater anabolic resistance and atrophy due to impaired mechanotransduction, while type II fibers may be more protected due to compensatory mechanisms. We also expect the model to capture delayed immune responses characteristic of microgravity exposure. These include disrupted macrophage polarization and impaired coordination with fibroblasts, leading to inefficient ECM remodeling and delayed tissue repair. Such outcomes are consistent with observed regenerative impairments and are expected to emerge from modeled changes in cell signaling, motility, and responsiveness to biochemical cues under unloading conditions.

Simulation of cytokine and growth factor-based cocktail treatments is anticipated to attenuate regenerative deficits by enhancing SSC activation, improving fibroblast-mediated ECM remodeling, and supporting timely immune cell recruitment. These interventions are expected to promote partial recovery of muscle fiber size and organization following injury in the unloaded environment.

The insights generated from these simulations will inform future experimental designs and support the development of targeted countermeasures to preserve muscle regenerative capacity in spaceflight and other disuse contexts.

Future Work

Building upon this framework, future iterations of the model will integrate a micromechanical finite element model (FEM) of skeletal muscle to enable physiologically relevant force transmission and mechanical feedback loops. This coupling will allow for a more detailed representation of how fiber geometry, costamere signaling, and ECM mechanics influence muscle adaptation under loading and unloading conditions.

Further work will also focus on personalizing the model to different physiological conditions, including aging-related sarcopenia and myopathies. By integrating patient-specific data, the model could be adapted to predict individualized muscle degeneration trajectories and optimize rehabilitation strategies for clinical populations. The ultimate goal is to create a computational framework that bridges molecular signaling with tissue-scale biomechanics, providing a powerful tool for predicting, preventing, and treating muscle degeneration across diverse conditions.

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