Novel Skeletal Muscle Models for Space Exploration and Medical Applications

Michele Marquette,
The University of Texas Medical Branch
Diane Byerly, *Johnson Space Center*

Marguerite Sognier,
Universities Space Research Association

Alleviating space-induced muscle atrophy in astronauts will greatly facilitate the success of future long-duration Exploration missions. Muscle atrophy, the progressive loss of muscle mass, results in diminished contraction strength and endurance. This can have deleterious affects on astronaut health and, consequently, mission success. Current countermeasures to address these effects include the performance of specific exercise protocols by astronauts in flight. These countermeasures reduce, but do not eliminate, muscle atrophy. By elucidating the alterations occurring at the cellular level in muscle under microgravity conditions, different countermeasures may be found. Studying muscle atrophy on a cellular basis is challenging due to the limited number of astronauts who have flown on long-duration space flights (e.g., International Space Station). Although human bedrest and rodent hind limb suspension models are used as equivalent models for muscle atrophy research, these models can be costly and time-consuming.

Cell-based models are a cost-effective, rapid method to study human disorders, yet tissue-equivalent skeletal muscle models are very difficult to generate due to the complex structure of the tissue. This is because skeletal muscle is unique among human body tissues in that it is composed of very long, multinucleated cells that must be aligned to simultaneously contract and exert maximum force. Immature muscle cells (myoblasts) must proliferate, align, fuse to form myotubes (elongated, multinucleated cells), and differentiate to enable muscle contraction. Duplicating these processes in the laboratory is difficult, time-consuming, and costly. Two steps are currently used to generate skeletal muscle models: (1) the introduction of various growth factors to induce fusion and differentiation; and (2) the use of complex micropatterned substrates that force myoblasts and subsequent myotubes into parallel alignment. Neither of these steps, either alone or in combination, produces the quantity of contracting muscle cells required for mechanistic studies.

To overcome these issues, we developed two new methodologies that produced novel skeletal muscle model testbeds. These methods are more tissue-equivalent; that is, capable of contraction. This work will facilitate the discovery of novel approaches to prevent and/or alleviate muscle atrophy, and will lead to an increased understanding of the complex processes necessary for skeletal muscle generation and repair.

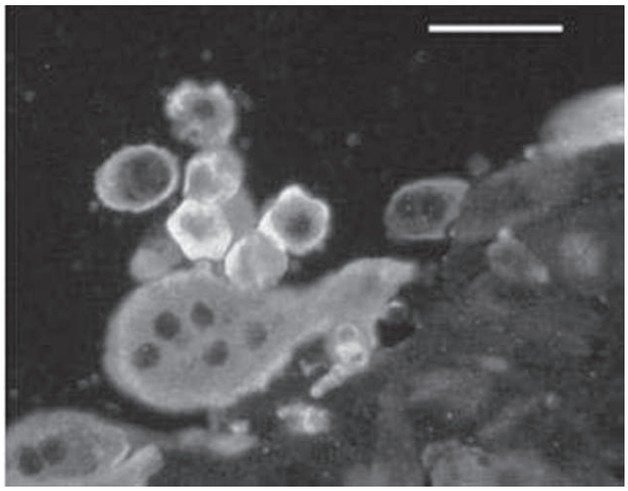

Fig. 1. *Thin section of 3D skeletal muscle aggregate.*

The first new methodology involves generating three-dimensional (3D) skeletal muscle models from C2C12 mouse myoblasts (figure 1). The C2C12 mouse cells are easily cultured, grow quickly (compared to human cell lines), are readily available, and perform a similar function (again compared to human cell lines) of restoration and repair of myoblasts. We cultured single-cell suspensions of myoblasts – without exogenous support structures/substrates and in the absence of differentiation factors – using the NASA-designed bioreactor, which is used to model some aspects of the microgravity environment. Within 12 hours, cell aggregation occurred. The myoblasts then fused and differentiated into 3D constructs without the use of serum alterations or other stimulants. Examination of thin sections of the aggregates revealed that a muscle differentiation marker, sarcomeric myosin heavy chain, was expressed in areas of synctia formation. Experiments with this model demonstrated that myoblasts could differentiate in modeled microgravity conditions. This model test system is particularly useful for investigating initial cell-binding events, myoblast fusion, and differentiation processes, which are required for muscle generation and repair. A better understanding of these processes will improve skeletal muscle tissue engineering approaches

(figure 2), current myoblast transplantation methodologies, regenerative medicine applications, and the development of cell-based countermeasures for muscle atrophy.

The second new methodology represents a major advance in tissue engineering in that, for the first time, precursor cells spontaneously fused and differentiated into masses of perfectly aligned, contracting myotubes. This cost-effective, multistep approach does not require the use of the serum alterations or micropatterned substrates thought to be essential to obtain similar constructs. Myoblasts are seeded in Petri dishes under very specific conditions (patent pending, Marquette and Sognier) that culminate in aligned, contracting myotubes as early as day 5 in culture. Numerous myotubes exhibit spontaneous contraction by the second week of culture. Additional time in culture results in sheets of aligned, synchronously contracting myotubes. This method enables (1) the construction of improved two-dimensional (monolayer) skeletal muscle testbeds; (2) the development of contracting 3D models; and (3) improved transplantable muscle tissue for biomedical and regenerative medicine applications. With adaptation, this method could be applied to produce other tissue types (i.e., bone and cardiac) from corresponding precursor cells.

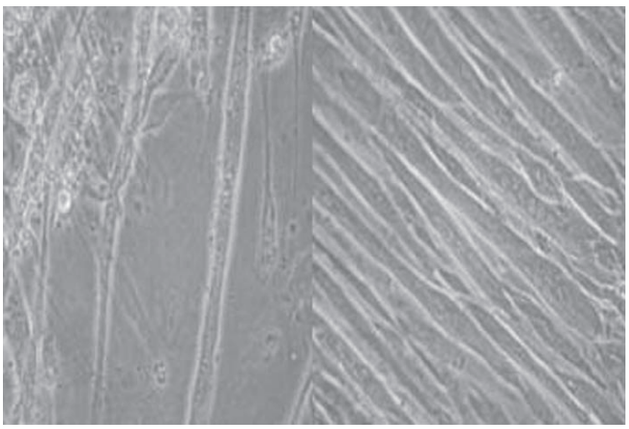

Fig. 2. *Comparison of skeletal muscle engineered using different methods.*

In summary, the two novel testbeds advance the current state-of-the art and are suitable for determining the cellular alterations involved in muscle atrophy. Such knowledge can be used to develop unique countermeasures. These models are valuable for elucidating the mechanisms of muscle diseases and atrophy associated with aging, immobilization, and denervation. Ultimately this elucidation is expected to culminate in the development of more effective therapies for use both in space and here on Earth.

Click here for next report