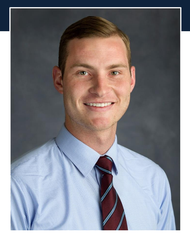
In Vitro Muscular Atrophy Model With Suspended Muscle Fibers



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Abstract: The development of relevant tissue models has been increasingly important when understanding disease within the human body. During space travel, the human body experiences dramatic changes in the surrounding environment. These changes may result in significant damage, especially to the musculoskeletal system. In this work, C2C12 mouse myoblasts were cultured *in vitro* on suspended spider and silkworm silks. This model produced a fully suspended, three-dimensional structure that can be used for testing and analysis of biological changes that affect muscle tissue. It has been demonstrated that cells grow satisfactorily and unidirectionally on the silk fibers. Additionally, silk fibers maintain mechanical properties under culture conditions.

ENGINEERING VARGIS LAB

Background

Muscular atrophy, or loss of muscle mass, is prevalent when muscle use is limited by immobilization or irregular loading. For astronauts, oxidative stress, a leading cause of muscular atrophy, increases with limited muscle use and with increased exposure to ionizing radiation during space travel. Increased oxidative stress kills muscles cells and damages DNA, increasing astronauts' cancer risk. On average, astronauts experience a 40% decrease in skeletal muscle mass over a 180-day mission in microgravity. We propose that growing muscle fibers in a suspended environment will produce an *in vitro* culture device that bears a stronger resemblance to native tissue than a monolayer culture.

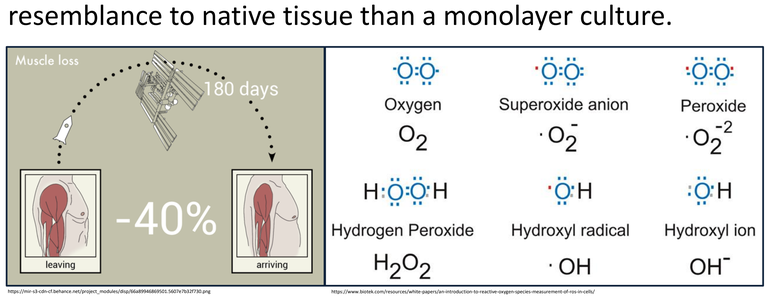


Figure 1. Left Diagram of skeletal muscle decrease in microgravity. **Right** Reactive oxygen species responsible for oxidative stress.

Materials & Methods

Cell Culture

- C2C12 mouse myoblasts
- Dulbecco's Eagle Modified Medium (DMEM)
- Fetal bovine serum (FBS)

Device Analysis

- MTT metabolic assay
- Myosin, actin, nuclei staining
- Genetic and protein analysis

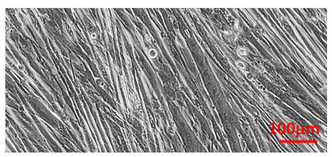


Figure 2. Striation of C2C12 myotubes in culture flask.

Device Construction

- Polymethylmethacrylate (PMMA)
- Transgenic spider silk and natural silkworm silk
- UV-curable adhesive

Materials & Methods (continued)

Device Assembly

1. Device cut using laser engraver
2. Silkworm or transgenic spider silk woven onto device

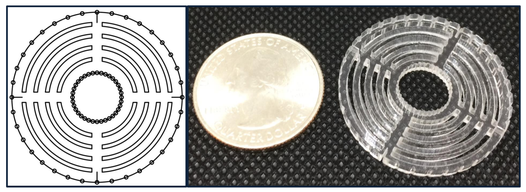


Figure 3. **Left** Device design. **Right** Fabricated device.

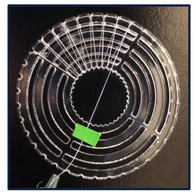
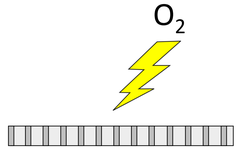
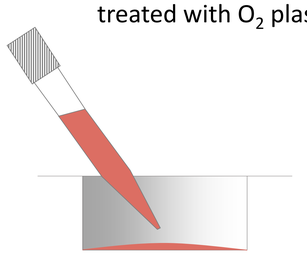


Figure 4. Partially-woven device.

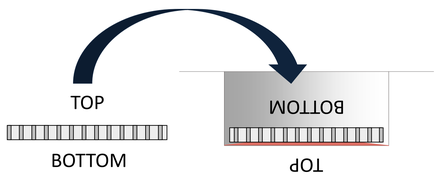
Seeding Device With Cells

1. Autoclaved, assembled device treated with O_2 plasma

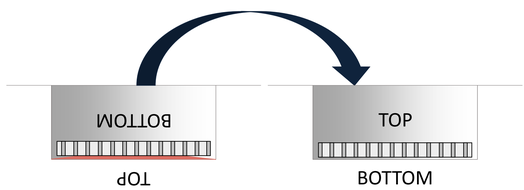




2. $1.5x10^6$ cells added to non-tissue culture-treated 6-well plate



3. Device flipped upside-down into cell-seeded well



4. After 4-6 hours, cell-seeded device is flipped into fresh well and medium is added

Results

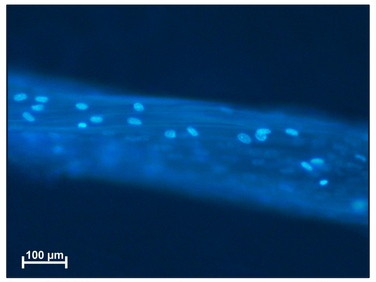


Figure 5. C2C12 myotubes growing along suspended multiplexed spider silk fiber.

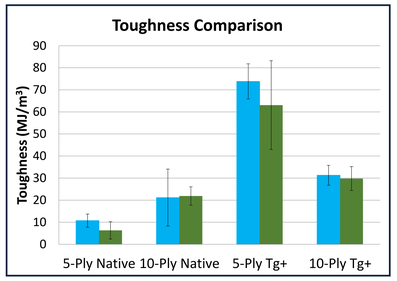


Figure 6. Comparison of silk toughness. Blue: dry; Green: soaked in DMEM for 24 hours. No significant difference in silk fiber toughness.

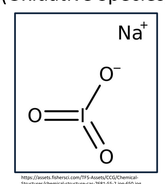
Conclusions

- Silkworm and spider silk fibers are suitable platform for C2C12 myoblast growth and differentiation
- This device seeding method produces repeatable, consistent distribution of cells on acrylic and suspended silk fibers alike
- Silk fibers maintain mechanical properties even when hydrated with DMEM

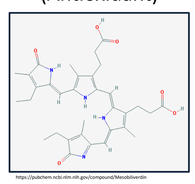
Ongoing Work

- Analysis of Muscle Tissue in Device
  1. Metabolism
  2. Immunocytochemistry
  3. Genetic expression
- Atrophy Induction and Potential Reduction

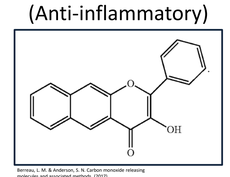
Sodium Iodate (Oxidative Species)



Mesobiliverdin IX-α (Antioxidant)



Carbon monoxide-releasing molecule (Anti-inflammatory)



- Simulated Microgravity
  - Custom rotary cell culture system

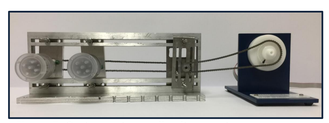


Figure 7. Custom rotary cell culture system.

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