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Lori Caldwell
Utah State University

Charles Harding
Utah State University

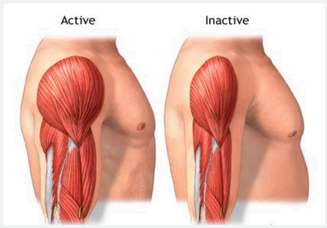
JR Dennison
Utah State Univesity

Elizabeth Vargis
Utah State University

Characterization of the Effects of Radiation on Skeletal and Smooth Muscle Cells

Lori Caldwell[1], Charles Harding[1], Eryn Hansen[1], Dr. JR Dennison[2], Dr. Elizabeth Vargis[1] | [1]*Department of Biological Engineering,* [2]*Department of Physics*

Introduction



Muscular atrophy is a serious issue for extended spaceflight. Understanding and preventing the role of ionizing radiation in skeletal muscle loss would preserve the strength and endurance of astronauts and enable longer duration space travel and exploration. Irradiation was performed in the USU material physics group's Space Survivability Test Chamber. C2C12 and CRL-1999 cells were exposed to dosages ranging from 0.5 – 36.8 Gy. Cell viability and growth rate were measured immediately following irradiation.

Irradiation

Cell Culture

C2C12 skeletal muscle cells were differentiated in DMEM F-12 10% FBS for 6 days then reduced to 2% FBS for 9 days. CRL-1999 aortic smooth muscle cells were differentiated in DMEM F-12K with ascorbic acid, insulin, HEPES, TES, and 10% FBS for 15 days.

Suspended Cell Irradiation

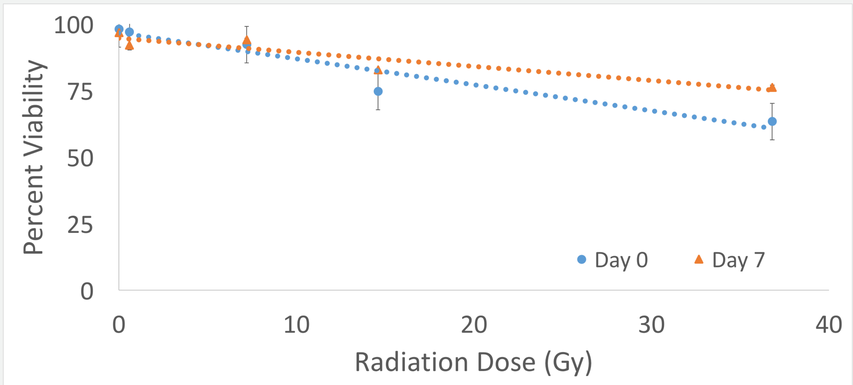
C2C12 cells were suspended in 150 µl of DMEM 10% FBS medium and sealed in a 1 atm chamber. Cells were irradiated with ^{90}Sr at a dosage rate of 7 Gy/hour to achieve a total dosage of 0.6, 7.2, 14.6, and 36.8 Gy.

Cell Monolayer Irradiation

C2C12 and CRL-1999 cells were differentiated in 24-well plates and irradiated at a rate of 1.13 Gy/hr to achieve a total dosage of 0.5, 1, 2, and 4 Gy.

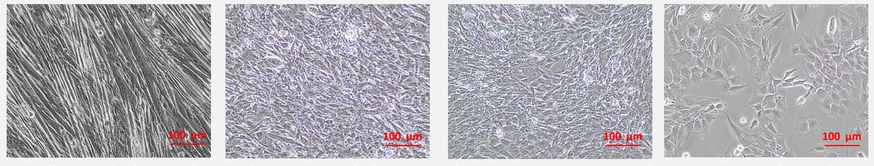
Results

Suspended C2C12 Cell Viability



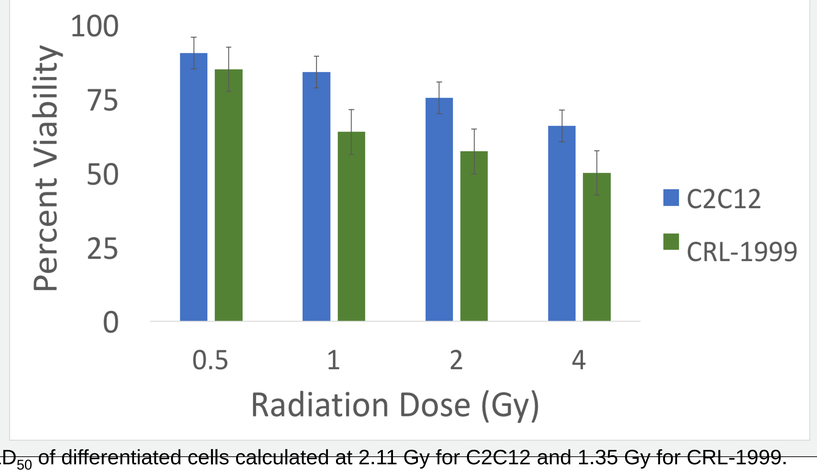
LD_{50} of undifferentiated C2C12 cells calculated at 47 Gy. Human LD_{50} is approximately 4.5 Gy [1]. N = 4.

Suspended C2C12 Cell Morphology



Cell morphology after 7 days. From left: Control, 7.2 Gy – overgrowth, 14.6 Gy – slowed growth, did not differentiate, 36.8 Gy – severely slowed growth, did not differentiate.

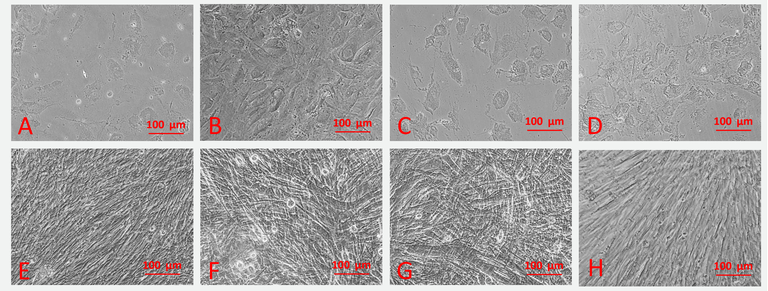
Cellular Monolayer Viability



LD_{50} of differentiated cells calculated at 2.11 Gy for C2C12 and 1.35 Gy for CRL-1999.

Morphology

Cellular Monolayer Morphology



Cell morphology following irradiation. Top – CRL-1999 A) 0.5 Gy B) 1.0 Gy C) 2.0 Gy D) 4.0 Gy. Bottom – C2C12 E) 0.5 Gy F) 1.0 Gy G) 2.0 Gy H) 4.0 Gy

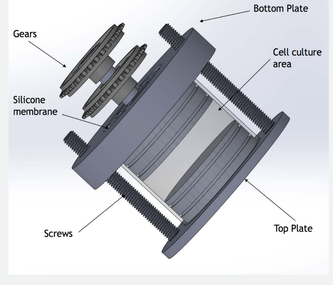
Conclusions and Ongoing Work

Conclusions

- Cell viability decreased substantially with increased accumulated radiation
- Following a 7-day recovery period, undifferentiated cell viability increased compared to Day 0
- Differentiated monolayers have a lower LD_{50} than undifferentiated cells
- Vascular smooth muscle cells are more sensitive to radiation than skeletal muscle cells

Ongoing Work

- Irradiation of differentiated C2C12 and CRL-1999 cells in a rotary cell culture system to simulate microgravity
- Fluorescent staining for H2AX to visualize double stranded DNA damage



Lori Caldwell
Utah State University
Department of Biological Engineering
12lcaldwell@gmail.com

[1] Mole, R. H. "The LD50 for uniform low LET irradiation of man." The British journal of radiology 57.677 (1984): 355-369.