Sterility and C2C12 Cell Growth Potential of Polycarbonate

BIOLOGICAL ENGINEERING
UtahStateUniversity

Morgan Bishop, Tessa Burrows, Eryn Hanson, Luke Lemmon, Phillip Simmons, and Nathan Sperry *Utah State University* | Elizabeth Vargis PhD, Lori Caldwell BS, *Utah State University*

I. Introduction

Background

- Research on muscle atrophy in space depends on simulating microgravity and radiation experienced during space travel in Earth conditions
- Polystyrene is commercially available but is non-reusable and cannot be modified to fit novel simulation measurements in lab
- Polycarbonate (PC) is more resilient and potentially reusable, but there is no data on its ability to be sterilized or cytotoxicity to C2C12 cells

Objectives

- Determine hydrophobicity of PC to ensure cells can attach to hydrophilic surface
- Determine if sterilization or cell growth affect tensile strength of PC
- Determine sterilization technique that eliminates contamination and cytotoxicity from: ethanol, UV light, dry heat, and wet heat; and compare to control sample

II. Methods

Polycarbonate Preparation

- Cut two polycarbonate sheets into 15 squares and 15 “dogbones”

Hydrophobicity

- Performed water contact angle tests

Tensile Strength Tests

- Placed “dogbone” samples in Instron 5542 to perform tensile and compressive tests

Sterilization

- **Ethanol**: submerged samples in 500 mL of the 70% ethanol for 30 seconds and dried by waving vigorously for 10 seconds within a sterile environment and then wrapped samples in treated foil
- **UV**: samples left under a UV light for 30 minutes in sterile conditions and then wrapped in treated foil
- **Dry Heat**: wrapped samples in treated foil then placed in oven at 101.67 °C for one hour and left in the oven to cool down to room temperature
- **Wet Heat**: wrapped samples in treated foil and placed into an autoclave at 15 psi and 110 °C for 15 minutes and cycling dry heat for 30 min and let cool to room temperature in autoclave
- **Control**: wrapped control samples in treated foil until seeding

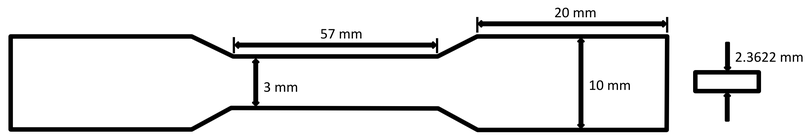
Cell Growth and Passaging

- Passaged 3 T75 plates with cell growth into 15 petri dishes
- Split samples into three groups (A, B, C) of 5 petri dishes
- Dishes received ~2 mL of passaged cells, 4 mL of DMEM 10% FBS growth medium
- Changed media every 3 days and replaced in incubator with 5% CO_2

Cell Destruction

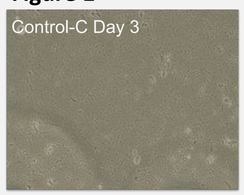
- Removed media and rinsed with 2 mL of 10X PBS
- Added 2 mL of 0.25% Trypsin and incubated at 37 °C for 7 minutes
- Sterilized plates and squares with 70% Ethanol

Study conducted with lab assistance from the USU Department of Biological Engineering, Dr. Yu Huang, and Dr. Elizabeth Vargis.



“Dogbone” tensile strength sample with dimensions adhering to ASTM standard for polycarbonate tensile testing.

Figure 1



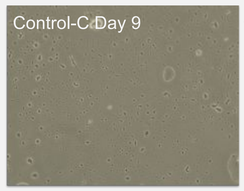


Figure 1: Control sample C.
Day 3: minimal cell attachment to the plate and minimal cell growth
Day 9: the attached cells did not differentiate and some detached

Figure 2

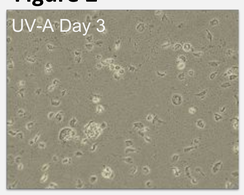
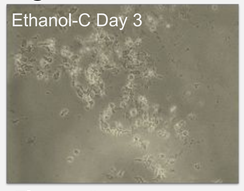


Figure 2: UV treated sample A.
Day 3: minimal cell attachment and beginning of cell growth
Day 9: all cells detached and died

Figure 3



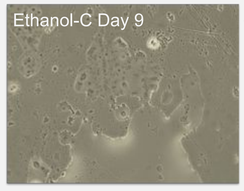


Figure 3: Ethanol treated sample C.
Day 3: minimal cell attachment and beginning of cell growth
Day 9: all cells detached and died

Figure 4

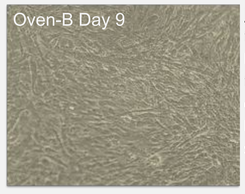


Figure 4: Dry heat (Oven) treated sample B.
Day 3: cells attached and began to differentiate, small amounts of cell death
Day 9: cells differentiated and showed increased cell growth

Figure 5

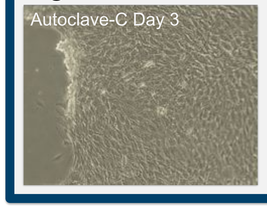


Figure 5: Wet heat (Autoclave) treated sample C.
Day 3: cell differentiation was apparent and cells were confluent
Day 9: cells differentiated and increased cell growth

Morgan Bishop, Tessa Burrows, Eryn Hanson*
Utah State University
Department of Engineering
eryn.hanson@aggiemail.usu.edu

IV. Results

Hydrophobicity

- The majority of polycarbonate samples had water contact angles <90°, indicating hydrophilicity

Tensile and Compressive Testing

- Proved to be unsuccessful; the Instron machine did not have a high enough force load to break or stretch the polycarbonate

Sterilization and Growth

- **Ethanol:** after 9 days, all plates were uncontaminated but had little to no cell growth
- **UV:** 2 plates were contaminated by bacteria and the remaining plate had no cell growth after 9 days
- **Dry Heat:** 1 plate was contaminated by bacteria, remaining plates had both cell growth and differentiation after 9 days
- **Wet Heat:** 1 plate was contaminated by bacteria, while the other two plates had cell growth and differentiation after 9 days
- **Control:** 2 plates were contaminated by bacteria, and the final plate had no growth by day 9

V. Conclusions

- The polycarbonate used in this experiment was already hydrophilic, and there was no need for treatment to make it compatible for cell growth
- Testing of mechanical strength was inconclusive; the potential development of mechanical defects from sterilization or contact with cells and media requires more research
- 9 samples were contaminated during the cell culture process, more testing is needed to determine the source of the contamination
- Ethanol treated samples and the control samples did not allow attachment and showed signs of an unknown live organism, possibly a protozoa
- The UV treated sample did not allow cell attachment, and thus had no growth but remained uncontaminated
- The wet heat treated samples had significant cell growth and differentiation
- The dry heat treated samples had significant cell growth and differentiation
- **The result of the uncontaminated samples indicate that polycarbonate is not cytotoxic and can be sterilized to limit contamination. Thus, polycarbonate is a viable option as a replacement for the polystyrene tubes with options available for the cells to attach and detach as needed to simulate muscle atrophy in space travel.**

VI. References

Instron Series 5500 Load Frames Reference Manual – Equipment, **2005** ; Kempf, M.; Kimble, R. M.; Cuttle, L. “Cytotoxicity testing of burn wound dressings, ointments and creams: a method using polycarbonate cell culture inserts on a cell culture system.” *ScienceDirect* **2011**, *37* (6), 994–1000 ; Lee, J. H.; Lee, S. J.; Khang, G.; Lee, H. B. “Interaction of fibroblasts on polycarbonate membrane surfaces with different micropore sizes and hydrophilicity.” *J. of Biomats. Sci.* [Online] **1999**, *10*, 3, doi.org/10.1163/156856299X00351 ; *Lexan® 9034 Sheet*; SABIC Innovative Plastics: Pittsfield, MA; Jan 20, 2008, Typical Property Values, https://www.usplastic.com/catalog/files/specsheets/Lexan%209034%20-%20Sabic.pdf ; *Plastics Sterilization Compatibility*; ISM, https://www.industrialspec.com/resources/plastics-sterilization-compatibility Parsons, E.; Boyce, M.; Parks, D. “An Experimental Investigation of the Large Strain Tensile Behavior of Neat and Rubber-Modified Polycarbonate.” *Polymer* **2004**, *45* (8), 2665–2684. Polycarbonate. https://www.curbellplastics.com/Research-Solutions/Materials/Polycarbonate (accessed Oct 5, 2017) . ; Polycarbonate Chemical Compatibility Chart. https://www.calpaclab.com/polycarbonate-chemical-compatibility-chart/ (accessed Oct 5, 2017) . ; Riss, T. L.; Moravec, R. A.; Niles, A. L.; Duellman, S.; Benink, H. A.; Worzella, T. J.; Minor, L. “Cell Viability Assays.” May 1, 2013 . ; Scholz, W. K. “Cell Adhesion and Growth on Coated or Modified Glass or Plastic Surfaces.” Technical Bulletin No. 13; Thermo Fisher Scientific: 2010 . ; Slentz, D. H.; Truskey, G., A.; Kraus, W. E. “Effects of chronic exposure to simulated microgravity on skeletal muscle cell proliferation and differentiation.” *In Vitro Cell. Dev. Biol. - Animal* **2001**, *37*, 148-156.