Cancer Cell Scaffold for C13 MRI Hyperpolarization Studies

Josh Kolz, Vanessa Grosskopf, Sarah Czaplewski, Sarah Sandock, Vivian Chen
Client: Sean Fain, Ph.D **Advisor:** Brenda Ogle, Ph.D

Abstract

3D cell scaffolds promote cell growth to a high density in bioreactors, which are used to maintain the viability of cells. The client desires high cell density in order to obtain clear MRI signals to measure cancer metabolic rate. The objective of this project is to develop a scaffold that promotes high density cell growth to $5x10^7$ cells/mL and maintains viability throughout the experiment. Collagen-coated and non-coated polystyrene microcarriers were chosen as the cell scaffold. T47D cells were seeded on the microcarriers and developed a peak density of $3.4x10^7$ cells/mL with the collagen coated microcarriers after 4 days. This is about 70% of the desired cell density. The results demonstrate that cells were able to attach and proliferate on the microcarriers, rendering them useful for MRI studies. By optimizing the microcarrier culture protocol, a higher cell density may be achieved.

Background

- Provide site for cell attachment in 3D space
- Have a greater surface area which leads to a high cell density
- Allow for better diffusion of nutrients to cells
- Can be coated with extracellular matrix (ECM) components that promote adhesion and proliferation [1]

Competition

Many commercial options & techniques:

- Calcium alginate encapsulation
- Other microcarriers designs (GE, Sigma Aldrich, etc.)
- ECM protein scaffolds (GE, Sigma Aldrich, etc.)
- Hollow fibers cassettes (Spectrum Labs, Fiber Cell)

Final Design

Design Criteria

- Large surface area : volume ratio
- Promote high density cell growth > $5x10^7$ cells/mL [3]
- Maintain cell viability for up to 5 days
- Allow perfusion of media to cells
- Ensure proper inoculation for different cell lines
- Does not contain ferrous metal for use with MRI
- Test both T47D breast cancer cells and 99T glioblastoma cells

Microcarriers

- SoloHill cross-linked polystyrene [2]
  - Animal protein-free (plain)
  - Coated with type 1 porcine collagen
- 125 – 212 micron diameter
- 360 cm^2/g surface area

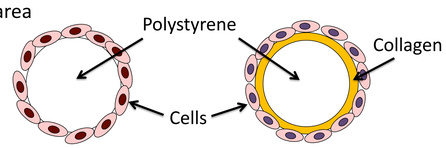

Figure 1 Schematic of both non-coated and collagen-coated microcarrier designs

Protocol

- Place 1.45 g of beads in 500 mL in autoclaved, silanized glass bottle
- Add 35 mL of media and approximately 8.1 x 10^6 cells
- Put culture bottle(s) on incubated shaker plate at 37 °C
- Spin at 80 rpm or as slow as possible while stirring beads evenly
- Alternate spinning 3 minutes on and 30 minutes off for 3-8 hours
- Spin constantly after alternating cycle
- Add 65 mL of media after 24 hours
- Increase speed to 120 rpm to continue even spinning
- Count cells daily
- Replace about 1/2 to 2/3 medium every day

Testing

Two discrete assays were needed for testing. The first test was intended to show that the microcarriers are able to maintain sufficient quantities of attached cells. The second test was to ensure that the microcarriers are unable to pass through a filter, leaving the bioreactor cartridge. Both tests are vital to integration with the MRI compatible bioreactor.

Growth Curves on Microcarriers

To access the density of the cells, a sample was taken from each microcarrier culture once a day. The sample was subsequently trypsinized and multiple counts were performed with a hemocytometer.

1st culture attempt: 99T

- Culture to bead ratio according to SoloHill protocol
- Stir bar and magnetic stir plate

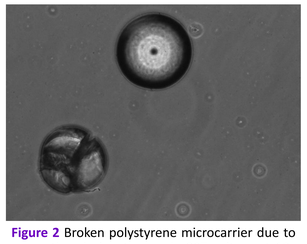

Figure 2 Broken polystyrene microcarrier due to magnetic spin bar trauma in 99T culture at 10x magnification.

- Attachment was slow
- ~40% of microcarriers broke by day 7 as seen in figure 2
- Peak density of $2.11x10^7$ cells/mL
- Lack of media may have stunted cell growth

2nd culture attempt: T47D

- Lower media to bead and cell ratio in culture during attachment period
- Absence of stir bar; shaker plate used to keep cells in suspension
- Cells reached a peak density of $3.4x10^7$ cells/mL with the collagen-coated microcarriers after 4 days, which is 70% of the desired cell density

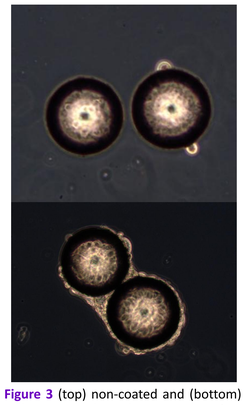

Figure 3 (top) non-coated and (bottom) collagen-coated polystyrene carriers seeded with T47D cells at day 3 at 20x magnification.

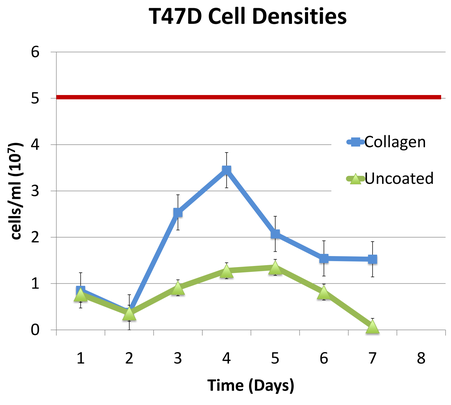

Figure 4 Calculated viable densities of collagen-coated and non-coated polystyrene microcarriers with T47D cells over a week

Filter

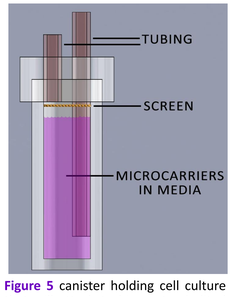

Figure 5 canister holding cell culture that will attach to bioreactor (edited image that was created in SolidWorks by Bioreactor team)

The flow out of the cartridge will be strong enough to pick up the microcarriers. To prevent the microcarriers from entering the flow system, a filter screen will be placed at the top of the cartridge

- high density microcarrier stock solution
- mesh size of 106 um filter

- Flow through contained no microcarriers
- Figure 6 shows a filter examined under magnification:
  - Showed no signs of clogging
  - Beads where lightly on the surface

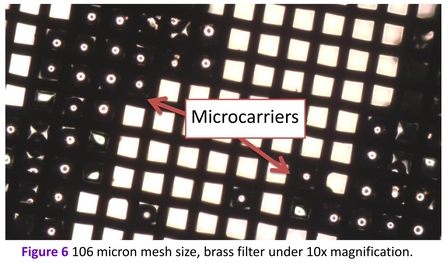

Figure 6 106 micron mesh size, brass filter under 10x magnification.

Conclusion

✓ Microcarriers promote cell attachment and proliferation in a 3D space

✓ Collagen-coated microcarriers promotes quicker attachment and more proliferation than non-coated microcarriers

✓ The developed protocol for using microcarriers as a scaffold in MRI research can save time and money for researchers

✗ The culture protocol along with insufficient equipment allowed us to reach only about 70% of the client-specified cell density

The current protocol shows promise in allowing cells to attach and proliferate on the microcarriers. However, improved techniques, equipment, and experience are necessary to optimize cell densities.

Future

Immediate Future

- Test the microcarriers in the bioreactor cartridge
- Determine the minimum T47D cell density required to detect a clear MRI signal, which may be significantly lower than $5x10^7$ cells/mL

Future Semester(s)

- Test different cell culturing protocols to promote efficient cell attachment
- Culture other cell lines on microcarriers to determine individual cell line attachment and proliferation characteristics while trying to develop a general growth curve for microcarrier cultures

Acknowledgements

- Brenda Ogle, Ph.D
- Sean Fain, Ph.D
- Jeremy Gordon
- Matt Smith
- Jim Molenda
- Dave Niles
- Sara Montanez-Sauri
- John Puccinelli, Ph.D

References
[1] Cell Culture Bioreactors.University of Minnesota. Retrieved February 9, 2011, from http://hugroup.cems.umn.edu/Cell_Technology/Notes/Cell%20Culture%20Bioreactors.pdf
[2] Nunclon Microcarriers. Milian USA. Retrieved April 25, 2011, from http://www.milian.com/Milian_USA/en/Catalog/Cell_culture/Microcarriers/Nunc_2D_MicroHex_Nunclon_microcarriers
[3] Harris, T., Eliyahu, G., Frydman, L., &Degani, H. Kinetics of hyperpolarized 13C1-pyruvate transport and metabolism in living human breast cancer cells. PNAS, 106(43), 18131-18136.

BME Design 301
Spring 2011