Optimization of Mammalian Cell Culture Media for In Vitro Photosynthetic Co-Culture with Cyanobacteria *Synechococcus elongatus*

Athena J. Chien[1, 2], Hanjay Wang[1], Matthew A. Wu[1], Camille E. Hironaka[1], Justin M. Farry[1], Kiah M. Williams[1], Michael J. Paulsen[1], Lyndsay M. Stapleton[1,3], Amanda N. Steele[1,3], Y. Joseph Woo[1,3]

[1]Department of Cardiothoracic Surgery Stanford University, Palo Alto, CA, [2] Department of Bioengineering Rice University, Houston, TX, [3]Department of Bioengineering Stanford University, Palo Alto, CA

Motivations

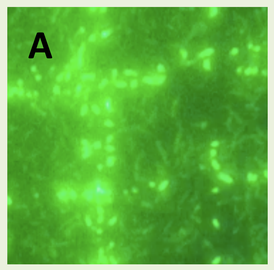
- *S. elongatus* is capable of generating O_2 in the presence of light, via photosynthesis
- When injected into infarcted ventricle in rats *in vivo*, *S. elongatus* enhances oxygenation and cardiac output leading to improved cardiac function after heart attack[1]
- *S. elongatus* has no significant immune response in rats[2]

Current Limitations

- *S. elongatus* cultured in specialized BG11 media
- Cardiomyocytes and other mammalian cells are typically cultured in Dulbecco's Modified Eagle Medium (DMEM)
- A suitable co-culture media is needed to study the symbiotic relationship *in vitro*
- **Hypothesis:** Mammalian cell culture media (DMEM) can be altered to improve *S. elongatus* viability, thereby enabling study & optimization of the biologic mechanisms to develop the symbiotic therapy

Culturing *S. elongatus*

- Cultured in glass 25 mL Erlenmeyer flasks in a rotating incubator under fluorescent lighting 34 °C and 125 rpm
- Purity checks were performed either by ensuring no growth when the liquid cultures were plated on Luria broth agar, or by ensuring no heterogeneous bacterial subpopulations by microscopy
- Grow to early stationary phase in BG11 cyanobacteria culture media, then aliquot 10 mL and resuspend in the desired media
- S. elongatus monitored for 7 days via spectrophotometry (min. n=6)

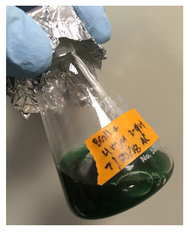


(A)S. elongatus auto fluorescent, no contaminant present

(B) 10 mL control flask, covered with aluminum foil cap

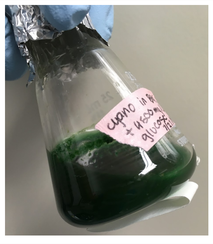
Preliminary Experimentation

- Cultured in cyanobacteria media, BG11, with individual DMEM additives: L-glutamine, fetal bovine serum (FBS), glucose, & penicillin-streptomycin.

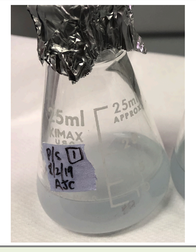


BG11 + L-Glutamine

BG11 + Fetal Bovine Serum (FBS)



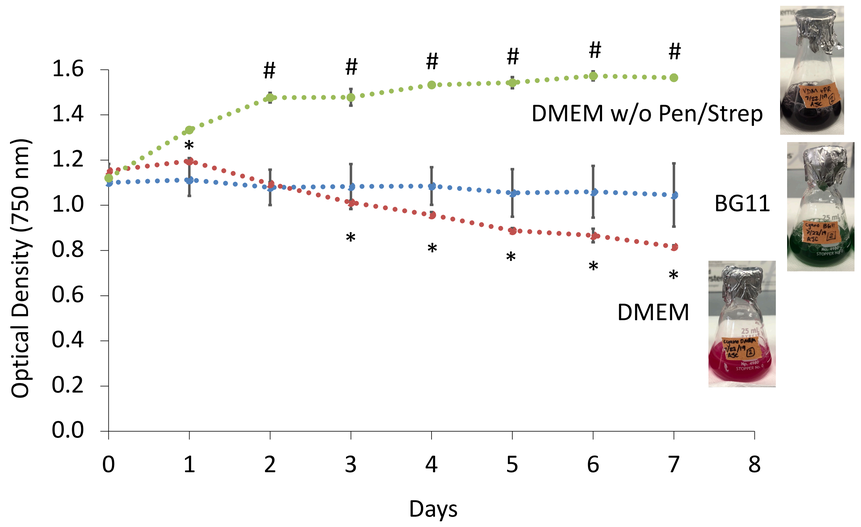
BG11 + Glucose



BG11 + Penicillin /Streptomycin

S. elongatus grew well for 7 days except in the presence of penicillin-streptomycin antibiotics.

Modifying Mammalian Cell Media for *S. elongatus*

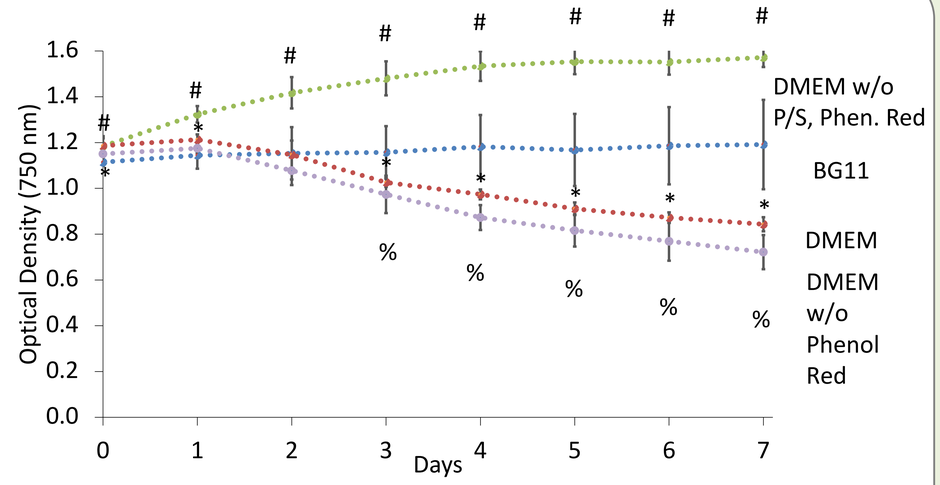


* DMEM is significantly different from BG11 (ANOVA and Tukey's HSD, p< 0.05)
# DMEM without antibiotics is significantly different from BG11

Figure 1. S. elongatus in DMEM without Penicillin/Streptomycin compared to positive and negative controls (BG11, DMEM)

S. elongatus grew significantly better after 7 days in DMEM without antibiotics compared to BG11 and DMEM with all additives.

Removing Phenol Red for Optical Observation



DMEM w/o P/S, Phenol Red is significantly different from BG11 (ANOVA and Tukey's HSD, p< 0.05)
* DMEM statistically different from BG11 control (p<0.05)
% DMEM w/o Phenol Red statistically different from BG11 control (p<0.05)

Figure 2. Removing phenol red to enable visual estimation of S. elongatus viability visually (green).

S. elongatus grew significantly better after 7 days in DMEM without phenol red and antibiotics compared to BG11, DMEM without Phenol Red, and DMEM with all additives.

Conclusions

- *S. elongatus* is capable of maintaining in early stationary phase for 7 days in **DMEM without pen/strep, & DMEM without pen/strep, phenol red**
- This result enables the possibility of studying a co-culture of *S. elongatus* with mammalian cells *in vitro* over 7 days – for development of **a symbiotic therapy leveraging the photosynthetic capabilities of cyanobacteria**

Future Directions

- Measuring **oxygen production** of *S. elongatus* in DMEM w/o pen/strep, w/o phenol red to ensure cyanobacteria maintains therapeutic oxygen production in the new media
- Viability assessment of **co-culturing** cyanobacteria and neonatal rat cardiomyocytes

References & Acknowledgements

1. Cohen, J. E., A. B. Goldstone, M. J. Paulsen, et al. An innovative biological system for photon-powered myocardium in the ischemic heart. Science Advances, 3(6), 2017.
2. Williams, K. M., H. Wang, M. J. Paulsen, A. D. Thakore, et al. Safety of photosynthetic *synechococcus elongatus* for *in vivo* cyanobacteria-mammalian symbiotic therapeutics. Microb. Biotechnol. 0(0), 1-13, 2020.

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