Hydrogen peroxide releasing biomaterials for vascularization in bone tissue regeneration

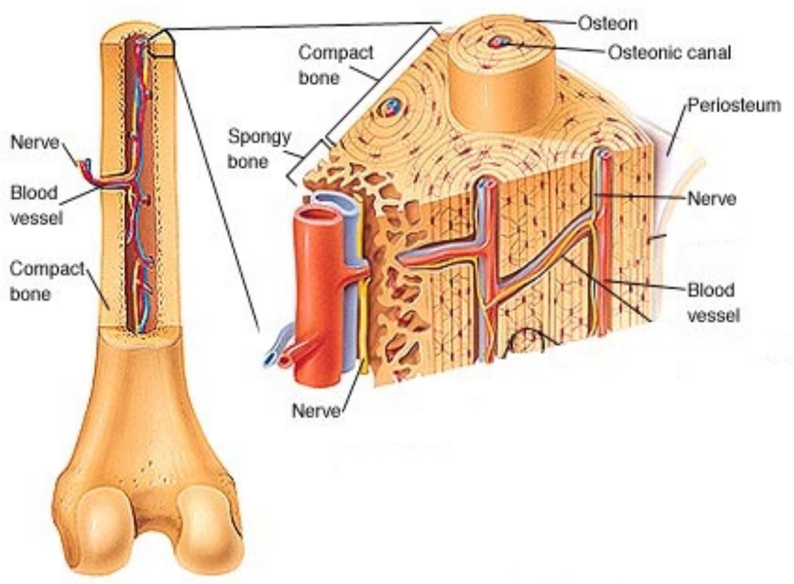
Mollie J. Harrison, Brittany N. Allen, and Bret D. Ulery

Department of Biomedical, Biological, and Chemical Engineering
University of Missouri

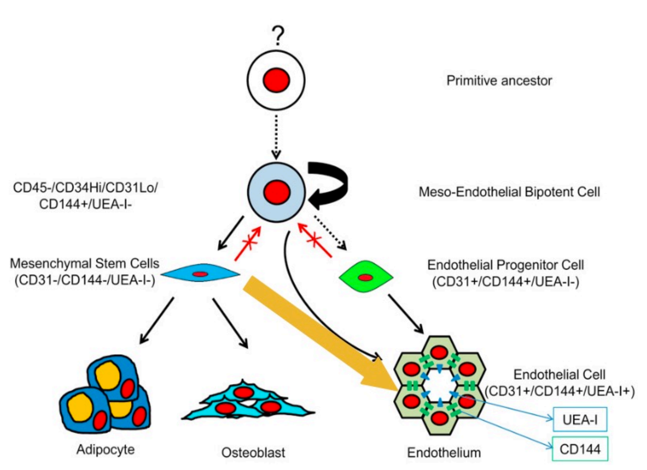
BioMEL

Introduction

The Biomodulatory Materials Engineering Laboratory has been working to develop a cheaper and more widely applicable solution to nonunion bone fractures than currently available. Restricted supply and possible immune response to bone grafts limit their applications[1], and other new regenerative engineering treatments have proven too expensive and patient-specific for broad clinical implementation[2].



Bone tissue regeneration is complex because the tissue contains not only osteoblasts, but a blood vessel and nerve network as well[3]. Each of these cell types can be encouraged to grow by a different simple signaling molecule[4,5]. This project focuses on the use of hydrogen peroxide (H_2O_2) for the vascularization of regenerated bone tissue.

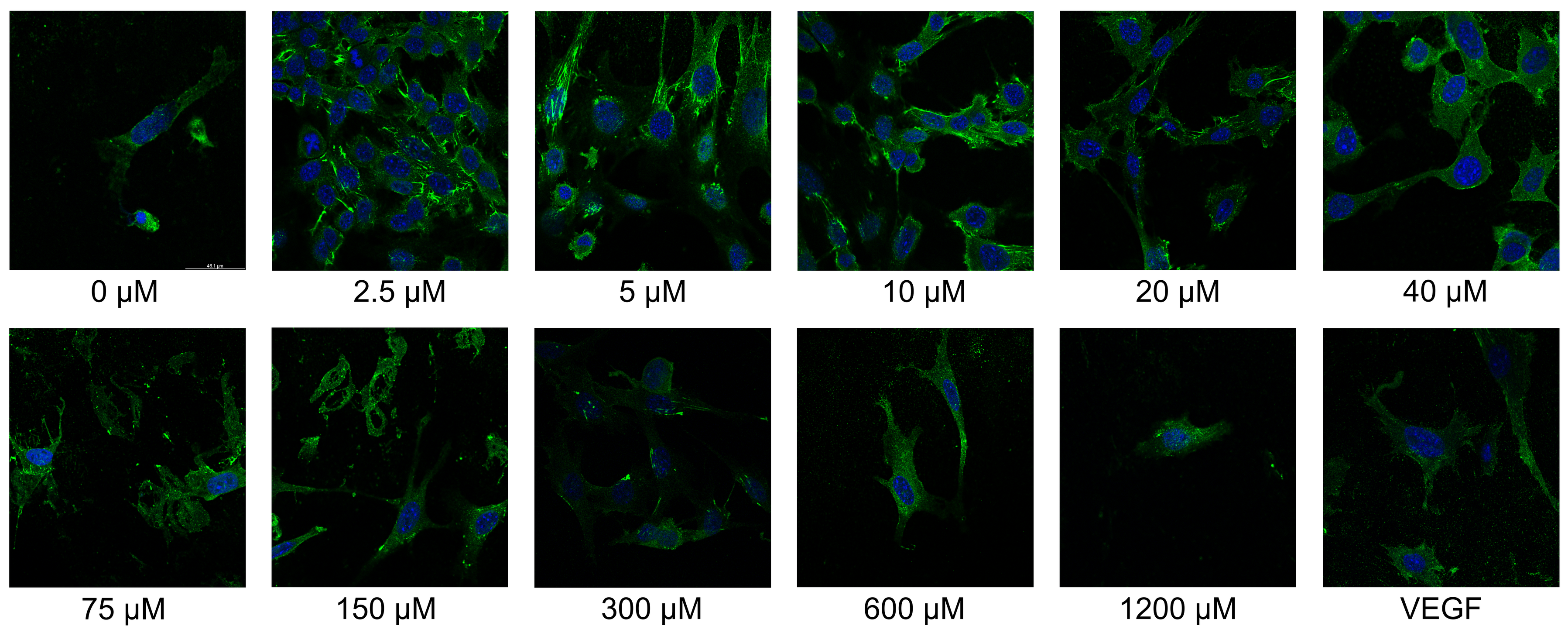


Objectives

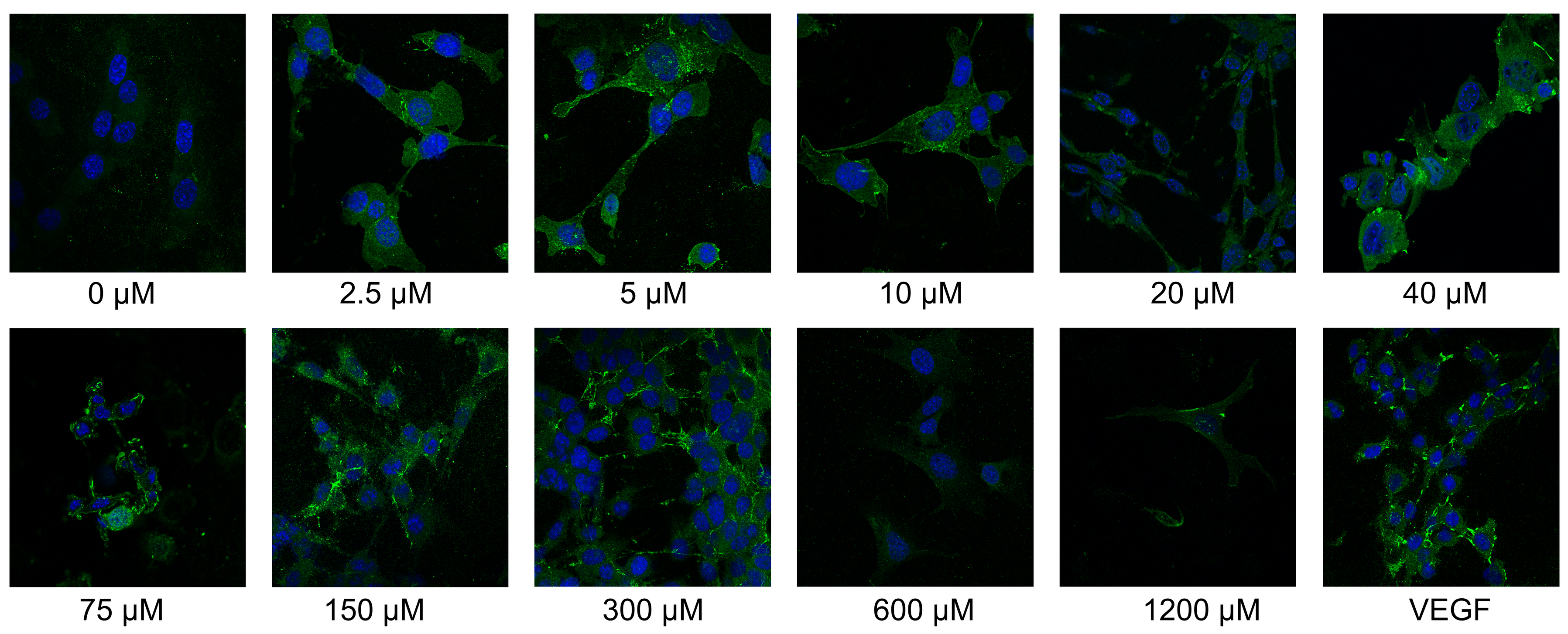
- Determine the therapeutic window of hydrogen peroxide for the differentiation of mesenchymal stem cells into endothelial cells using
- Synthesize a novel biomaterial which releases hydrogen peroxide within the determined therapeutic window

H_2O_2 Angiogenic Inductivity

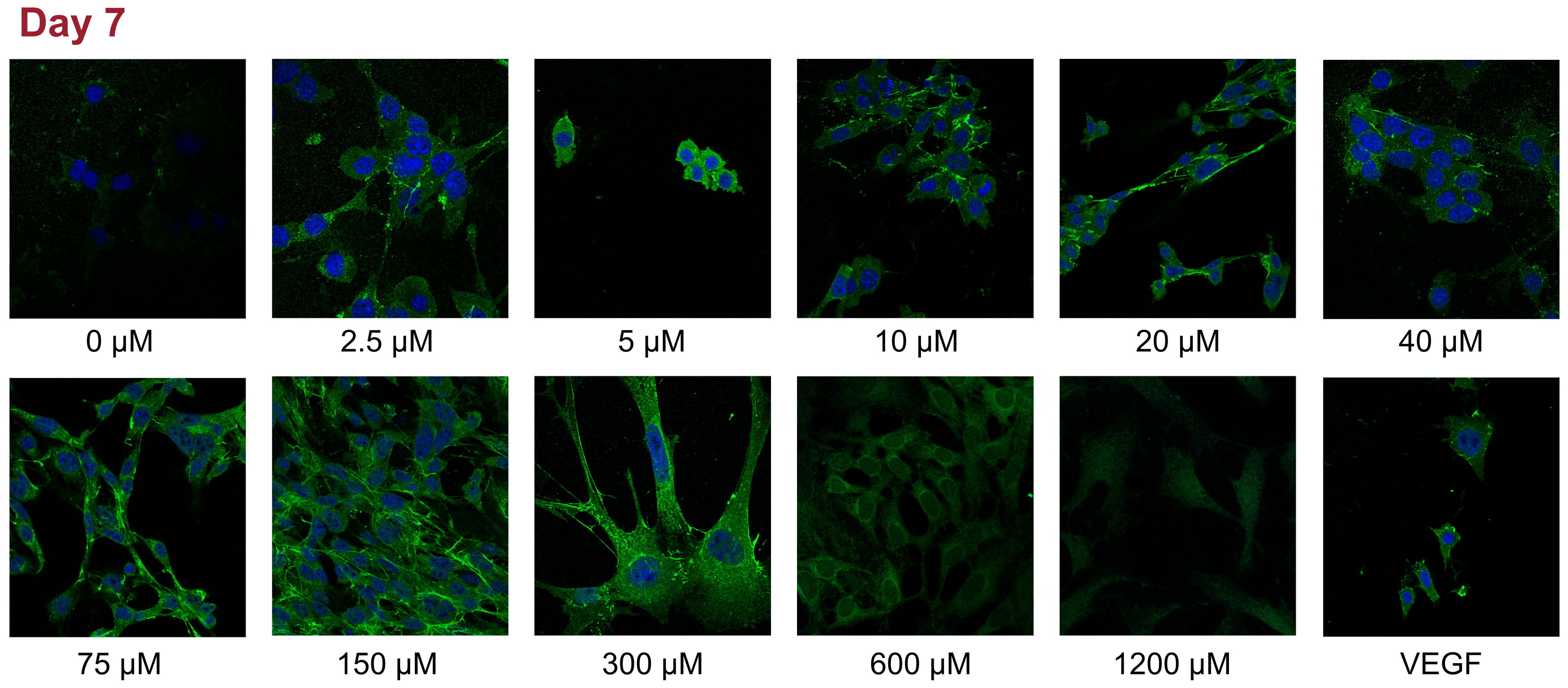
Day 1



Day 3



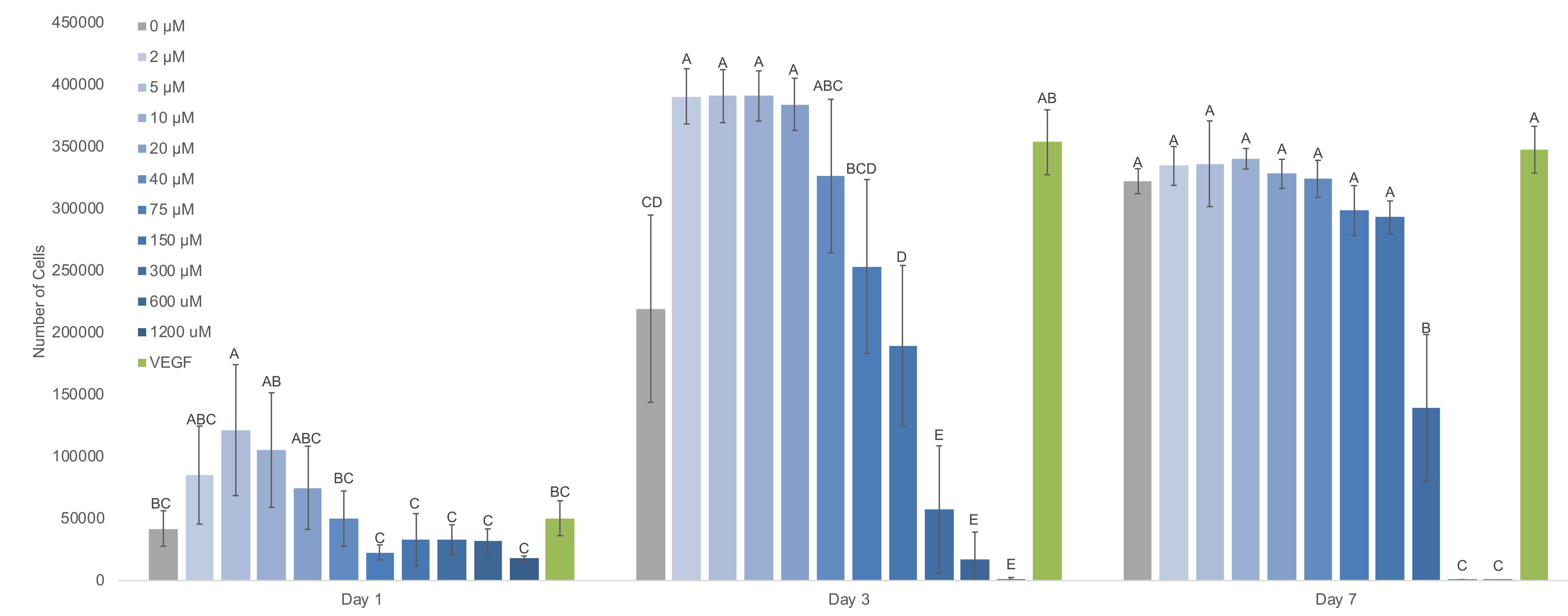
Day 7



- Cells were tested for angiogenesis by fluorescent staining for von Willebrand Factor, a protein expressed by endothelial cells. Images are representative of all N=4 experimental groups.
- DAPI, which stains the nucleus of the cell, is falsely colored blue and FITC, the fluorescent marker for von Willebrand Factor, is falsely colored green

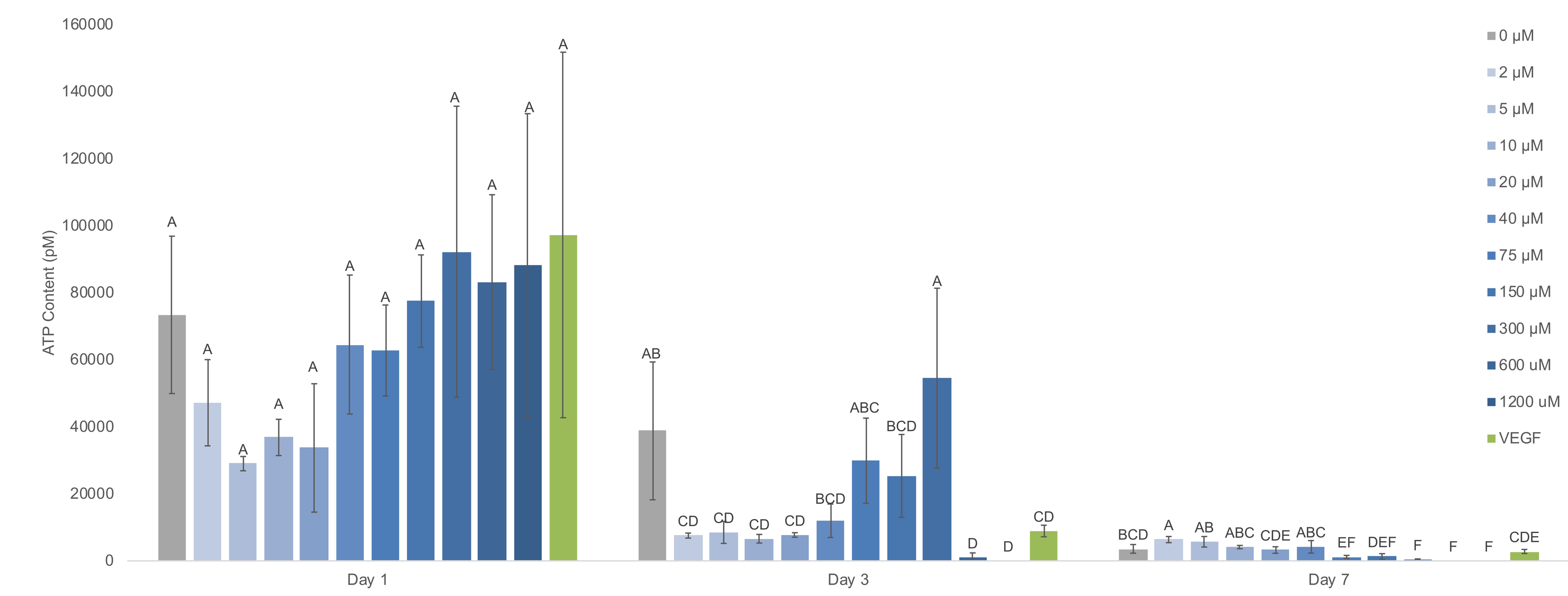
H_2O_2 Cytotoxicity

DNA Assay



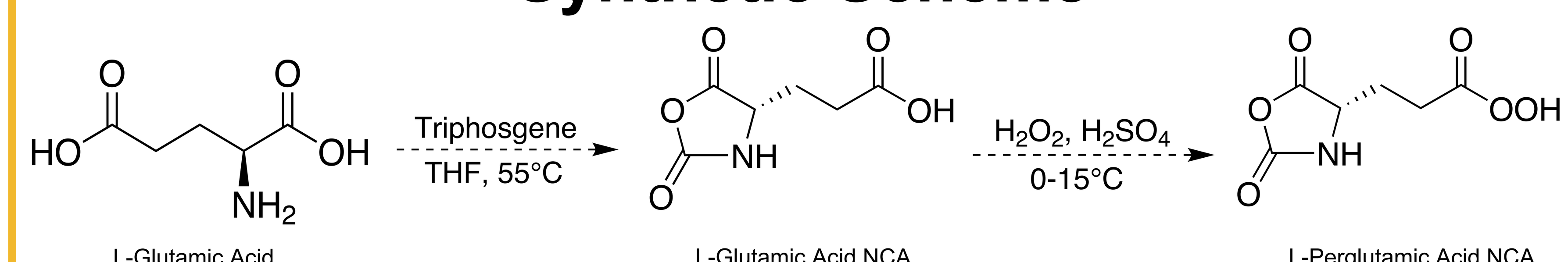
- PicoGreen assay fluorometrically measures the amount of DNA in a sample, from which we can infer cell count. N=4 for all groups. Groups that possess different letters have statistically significant differences ($p < 0.05$) in mean whereas those that possess the same letter are statistically similar.
- Cells exposed to 300 µM and higher concentrations of hydrogen peroxide displayed significant cell death after 7 days

ATP Assay



- CellTiter-Glo assay measures the amount of ATP in a sample using luminescent signals caused by a chemical reaction. N=4 for all groups. Groups that possess different letters have statistically significant differences ($p < 0.05$) in mean whereas those that possess the same letter are statistically similar.
- Cells exposed to 600 µM and higher concentrations of hydrogen peroxide displayed a significant decrease in ATP production after 7 days

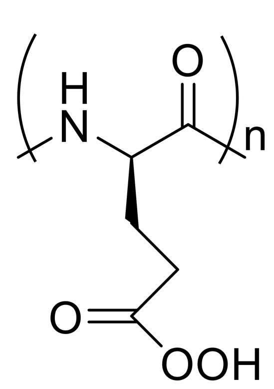
Synthetic Scheme



- An n-carboxy anhydride (NCA) synthesis was chosen due to the versatility of NCAs to be polymerized into many varieties of polymers for varying potential future applications
- Glutamic acid was chosen due to its common presence in the body

Conclusions & Future Work

Based on results from both the inductivity and cytotoxicity tests, the therapeutic window of hydrogen peroxide for the differentiation of endothelial cells from mesenchymal stem cells has an upper limit of 300 µM. Concentrations of hydrogen peroxide as low as 2 µM are still angioinductive.



In the future, we would like to determine the low end of therapeutic window and complete the synthesis of the novel biomaterial. Then we will perform release tests on the biomaterial to determine its hydrogen peroxide release profile, test its biocompatibility and inductivity, and explore polymerization.

Acknowledgements & References

This research was partially supported by the Cherng Summer Scholars Program. We would like to thank Andrew and Peggy Cherng and the Panda Charitable Foundation for their gift to this program.

Images:
1. "Structure of Bone Tissue."*Structure of Bone Tissue | SEER Training*, U.S. Department of Health and Human Services.
2. "Stem Cell Pathways."*R&D Systems*, R&D Systems.

References:
1. Vang & Day, "Advan. and Disadvan. Allograft vs. Autograft". Wichita State University, 2006.
2. Quan et al. *Cell. Phys. and Biochem.* 46, no. 1 (2018): 401-17.
3. Marrella et al. *Materials Today.* 21, no. 4 (2018): 362-76.
4. Cushnie et al. *PLOS ONE.* 9, no. 7 (2014).
5. Yasuda et al. *Life Sciences.* 64, no. 4 (1999): 249-58.