Response of Stem Cells to High Oxidative Environment

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Background

Background:

- Stem cells are known for their self-renewal abilities, which change over time [3]
- Eventually they stop replicating by becoming senescent
- One of the reasons for stem cell senescence is high oxidative stress. Overall cellular aging in many cell types is caused by oxidative stress.
- Oxidative stress can damage telomeres. Telomerase is an enzyme that extends telomeres, so oxidative stress should affect the activity of telomerase as well.

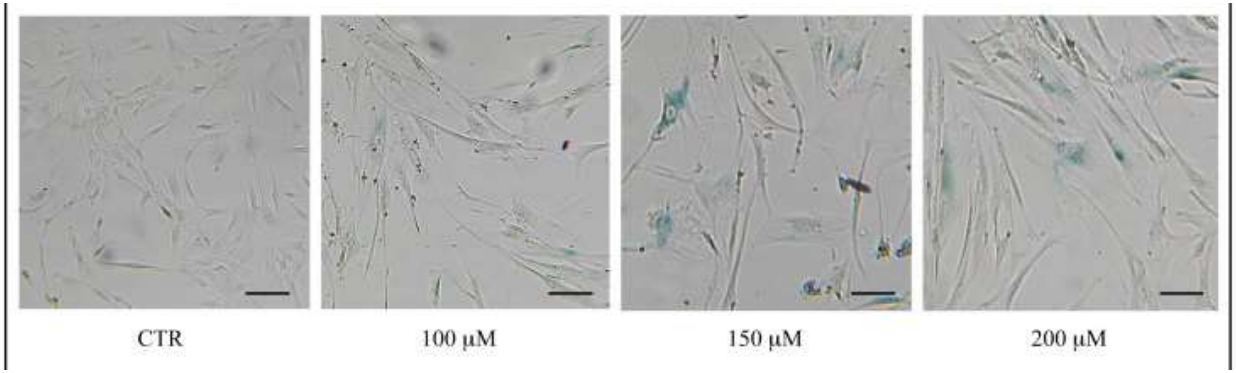


Figure 1. β-gal stained MSCs at different concentrations of hydrogen peroxide. β-gal is a marker for senescence in cells. [1]

Background

Objective:

- Develop an assay to look at the cellular response to different levels of oxidative stress
  - Staining the stressed cells with CellROX is the best way to see what happens inside the cell. CellROX Green stains the reactive oxygen species both in the cytoplasm and the nucleus.
- Create a mathematical model to predict the cellular response based on inputs of time and oxidative stress

Motivation:

- Completion of this project will provide the framework to understand the aging of stem cells, as well as other cell types

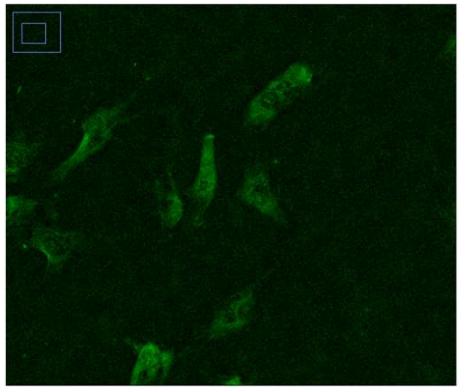
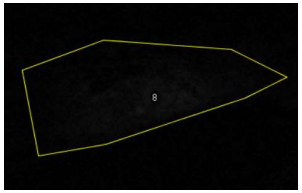


Figure 2. Example of a CellROX Green-stained MSC looks like after the application of oxidative stress

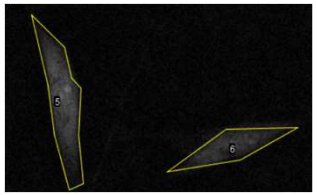
Methods/Experimental Setup

For the experiment:

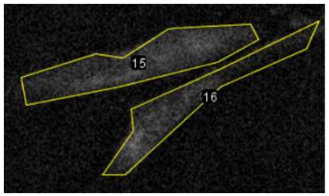
1. Culture Mesenchymal Stem Cells (MSCs) on glass slides until 70% confluency
2. Apply oxidative stress to the cells by adding set amounts of hydrogen peroxide to the medium. Discard the medium after 24 hours.
3. Stain the cells using CellROX Green for 30 minutes, fix cells with 4% paraformaldehyde and counterstain with DAPI (DNA stain)
4. Image the cells with a confocal microscope and do image processing in ImageJ for each image to quantify the intensity
5. Student's t-test was used to compare different groups. *p value of < 0.01 represents statistically significant difference.



0 uM Cells



100 uM Cells



200 uM Cells

Figure 3. To quantify the pixel intensity of the cells, cells must be individually outlined. After outlining about 20 cells, then measure the intensity using Image J functionality.

Methods/Experimental Setup

For the modelling:

1. Gather possible equations that could be used in the actual model

$$k_c(x) = \frac{\delta}{1 + \exp(\frac{\alpha - x}{\beta})}$$

$$\frac{F+M+\frac{K_r}{K_f} \pm \sqrt{(-F-M-\frac{K_r}{K_f})^2 - 4\,FM}}{2}.$$

$$n_{\text{mean}} = \frac{e^{\tilde{\mu}+\sigma^2/2}}{y_1} = \left(Q, +, \frac{y_0}{y_1}\right) e^{-Lg/2+L^2g/8} - \frac{y_0}{y_1},$$

Equation 1. Describes the rate of telomere capping based on x, the number of base pairs of a telomere, and other constants [2]

Equation 2. Describes the total concentration of bound telomeric repeats, based on the concentration of TRF2 dimers M, the forward and the reverse binding rates kf and kr, each with their own functions that are based on temperature and energy states [1]

Equation 3. Describes the mean telomere length based on the max telomere length Q, the generation number g, the fraction of telomere lost per generation L, and various rates of loss [4]

Results

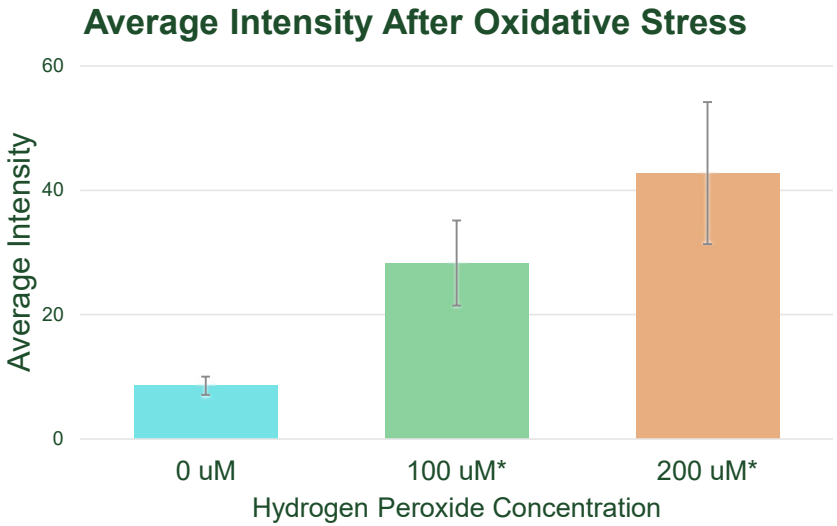
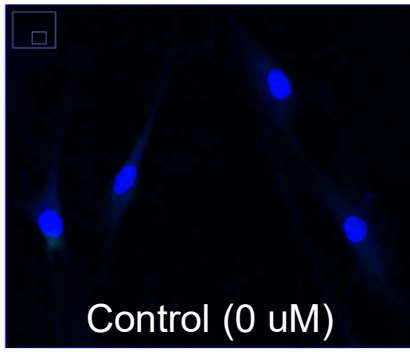
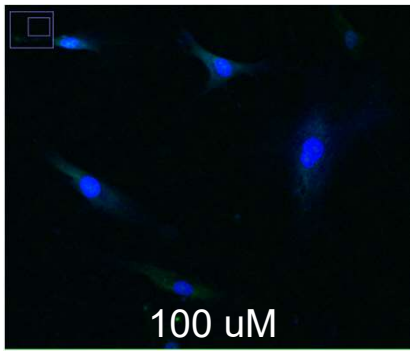


Figure 4. Graph showing the relative amounts of oxygen within each cell depending on how much hydrogen peroxide was initially added to the culture. Asterisk means that the data was statistically significant as determined through Student's T-Test.





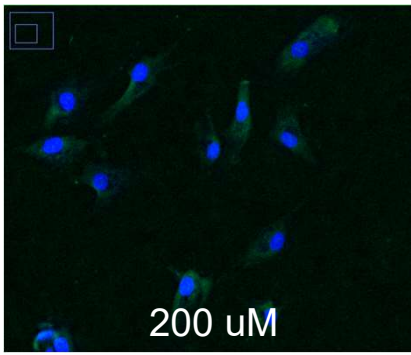


Figure 5. Comparison of the relative amounts of oxygen, where blue is the nucleus and green shows reactive oxygen species.

Discussion/Next Steps

In the future, more experiments will be run to start investigating the pathways between varying oxidative stress and higher telomerase activity and senescence.

The next steps for the modelling work is to combine all the equations to create the equation that will predict the cellular response based on the inputs of time and oxidative stress. With this model, the pathways of oxidative stress can start to be developed.

A hydrogen peroxide-releasing light-sensitive polymer-based delivery platform will be created for exposing the cells to oxidative stress in complex environment

Conclusions

The experiments show that oxygen (as formed from hydrogen peroxide) diffuses into MSCs and is present in high concentrations in the cytoplasm as well as the nucleus. Higher concentrations of hydrogen peroxide result in higher concentrations of oxygen within the cell.

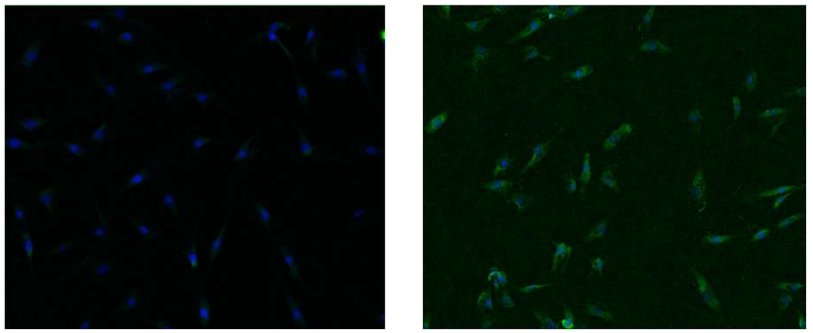


Figure 6. Colorized images of 0 uM cells (left) and 200 uM cells (right), blue shows nucleus and green shows oxygen. Images were brightened to show differences.

What benefits did you get from you SURE experience?

Thanks to the SURE Program, I was able to have an interesting introduction to what research is like. More specifically, I got experiences such as:

- ❑ Touring the TMI building and spending some time working with cell cultures
- ❑ Learning more about the biology behind the stem cells my research was focused on
- ❑ Learning to use Image J for image processing
- ❑ Doing the literature review and understanding a technical framework to expose cells to oxidative stress using a light-sensitive polymer-based hydrogen peroxide delivery platform

Unfortunately, with COVID this semester I couldn't be as involved in the lab as I would've hoped; however, thanks to the program I have a better idea of what it is like being in a research lab. The program helped me get started with research at CSU and ultimately got me interested in continuing to be in a research lab.

References & Acknowledgements

[1] N. Arkus, A mathematical model of cellular apoptosis and senescence through the dynamics of telomere loss, *Journal of Theoretical Biology*, Volume 235, Issue 1, 2005, Pages 13-32, doi.org/10.1016/j.jtbi.2004.12.016.

[2] B.V. Hirt et. al, Modelling the regulation of telomere length: the effects of telomerase and G-quadruplex stabilising drugs. *Journal of Mathematical Biology* vol. 68,6 (2014): 1521-52. doi:10.1007/s00285-013-0678-2

[3] F. Facchin et al, Comparison of Oxidative Stress Effects on Senescence Patterning of Human Adult and Perinatal Tissue-Derived Stem Cells in Short and Long-term Cultures. *International Journal of Medical Sciences* vol. 15,13 1486-1501. doi:10.7150/ijms.27181

[4] J. Wattis et al. Mathematical modelling of telomere length dynamics. *Journal of Mathematical Biology* vol. 80,4 (2020): 1039-1076. doi:10.1007/s00285-019-01448-y

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Thank you

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