Inducing Inflammation in Bovine Chondrocytes

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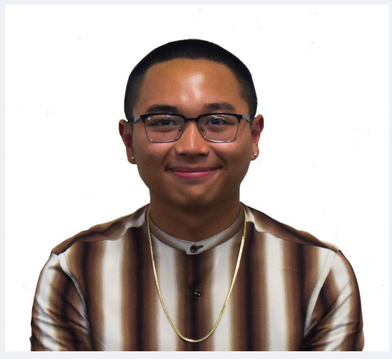

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WeARE Research Area

- Tissue engineering
- Bacterial adhesions for environmental and medical applications
- Bioreactors

Motivation or Background

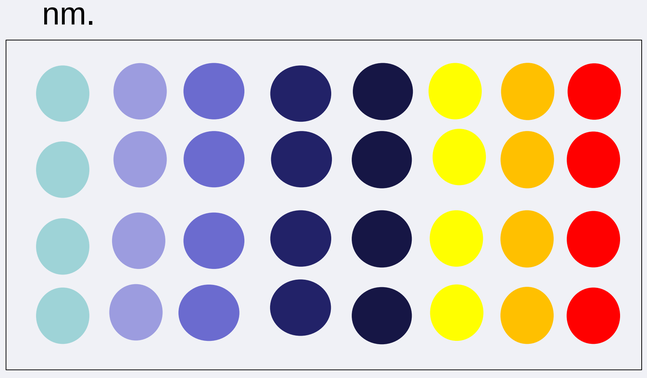
Osteoarthritis (OA) is a disease that affects articulating joints and is marked by the degeneration of articular cartilage tissue and inflammation in the joint, marked by an elevation in nitric oxide (NO) levels. Symptoms of OA include joint stiffness, chronic pain and loss of mobility. OA affects at least 30 million adults in the U.S. and is an increasing problem while current treatments only manage OA symptoms with a total knee replacement surgery needed as a final resort. Inflammation in OA contributes to further AC damage and chronic pain. As such we aim to understand the inflammatory nature of OA by studying the diseased state *invitro.* We will induce a diseased state in vitro with various induction methods and measure the NO that is formed as a marker of the inflammation.

Objectives

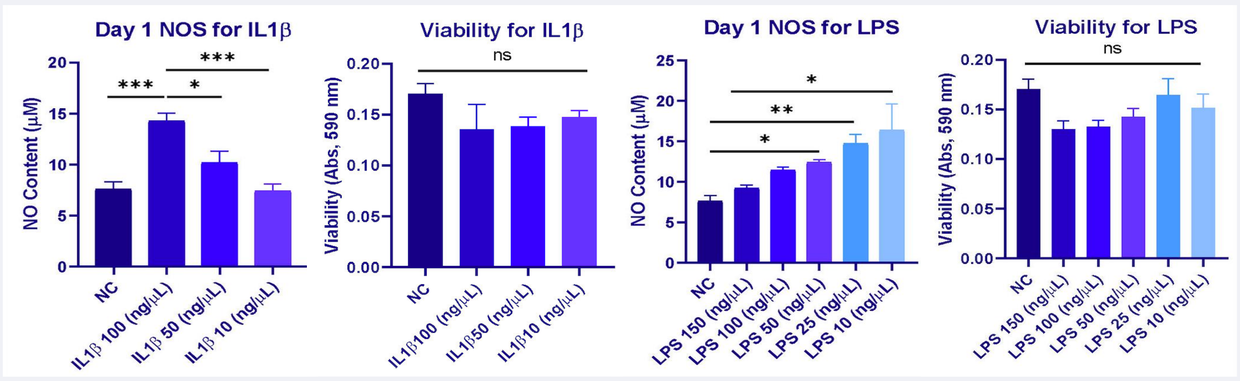
- The objective of this experiment was to identify an induction method to model the diseased state of Osteoarthritis which is marked by high inflammatory markers such as Nitric Oxide (NO), that can be used for further experiments to induce a diseased state.
- As such we wanted to explore the use interleukin-1-beta (IL1ß) and Lipopolysaccharides (LPS) separately on healthy bovine chondrocytes and measured cells viability and NO content after the induction period.

Methodology

1) Isolation of healthy bovine chondrocytes.
2) Chondrocytes were pooled and cultured in monolayer at a seeding density of 20 K cells/cm^2
3) Cells were induced with either IL1ß or LPS at the concentrations shown below, for 24 hours.
4) After the induction period, a sample of media was taken for the NO quantification using the Griess Assay.
5) For viability of cells, the PrestoBlue dye was added to the well at 1/10 the media volume and incubated for 15 mins. After the incubation, absorbance was read at 590 nm.



Results



- IL1ß - NO is most present when the concentration is the most (100 ng/mL). While also having the least viability. The most presence of viability and least amount of NOS is at a concentration of 10 ng/mL.
- LPS – NO has an increasing trend as the concentration is decreasing. NO is most present when the concentration is at 10 ng/mL. At this concentration though, it does not have the most viability. Instead it is when the concentration is at 25 ng/mL is when viability is at its most.

Skills and Experience

I was able to apply the techniques and skills that I have experienced during my undergraduate courses of chemistry labs at the University of Texas at San Antonio to my research. In addition, I was trained on safe and bio-safe practices in biological labs. I also learned about ethical performance of research. Experimentally, I learned how to prepare solutions, isolate cells from tissues, change media and use the microplate reader. I also learned how to do a literature search and summarize findings from a paper. I also learned how to communicate my findings during group meetings.

What I Learned

I learned about the inflammatory environment of osteoarthritis and the various induction methods we can use. With that, its affects and application to the cells. In addition I also learned many cell culture techniques such as seeding cells, maintaining a cell line and various techniques such as biochemical assays, histology, and viability assays to assess our results.

Future Plans

As I look forward, I plan to expand on the research that had been conducted. I look to apply more methods that will decrease the inflammation that occurs within our bodies. This can possibly be done through changing the concentration of the inducers used. Also, I would like to quantify the charge and hydrophobicity of the chondrocytes using zeta sizer and contact angle measurements, respectively.

Acknowledgments

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