Role of Advanced Glycation End-Products in Age-Related Osteoarthritis
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ACKNOWLEDGEMENTS:

BACKGROUND
- Osteoarthritis (OA) is a degenerative joint disease characterized by the breakdown of cartilage and underlying bone.
- Articular cartilage is composed of an extracellular matrix (ECM) comprised of collagen fibrils and aggrecan glycosaminoglycans (GAGs) that create a dense network around chondrocytes, the cells responsible for maintaining the ECM.
- Over time, biologically abundant sugars like ribose react spontaneously with the amino acids in collagen molecules to create crosslinks, called advanced glycation end-products (AGEs) [1].
- These crosslinks accumulate in the cartilage, preventing chondrocytes from functioning properly, reducing ECM stability, and contributing to the stiffening and inflammation characteristic of OA [2].

OBJECTIVES
- Harvest and Culture of Cartilage Explants
  - Cartilage explants were harvested from juvenile bovine knee joints and cultured in serum-free media. The explants were randomized into one of four treatment groups, then incubated in control media, 100 mM, 300 mM, or 900 mM ribose for 8 days. Media was collected and replenished every two days.

METHODS
- B. Biochemical and Biomechanical Assays
  - Numerous assays were performed to assess the biochemical and biomechanical tissue properties of the treatment groups:

RESULTS
- The tissue properties of the 100 mM ribose condition accurately mimic the tissue properties of age-related OA, while remaining viable in vitro:
  - After 8 days of treatment, the 100 mM condition remained viable, while the 300 mM and 900 mM conditions experienced widespread cell death (Fig. 2).
  - Cellular metabolism dropped to about 40% of the healthy control in the 100 mM condition, while dropping to approximately 20% in the 300 and 900 mM conditions (Fig. 2).
  - The elastic moduli of the 100 mM and 300 mM conditions were twice that of control, while reaching nearly three times the control in the 900 mM condition (Fig. 2).
  - AGE fluorescence increased significantly in all conditions, at 2.8x, 6.5x, and 13.5x the control for the 100 mM, 300 mM, and 900 mM conditions, respectively (Fig. 2).
  - Cumulative GAG loss was less than control in ribose conditions, significant at day 8 (Fig. 2).

DISCUSSION
- The data shows that the 100 mM ribose condition accurately mimics the tissue properties of osteoarthritic tissue, thereby establishing a live, in vitro model of this condition.
- Most importantly, the 100 mM ribose condition showed similar chondrocyte viability to control. This requirement is essential for creating a living in vitro model, as the response of living cells is measured.
- In addition to remaining viable, the 100 mM ribose condition showed a significant increase in AGE crosslinking and tissue stiffness, two of the key tissue properties of OA.
- GAG loss was decreased in all ribose treatment groups, likely due to AGE crosslinks preventing the release of these molecules into the ECM.
- Based on the data, it is concluded that the 100 mM ribose condition will be used to model live, age-related OA in vitro and to examine the effectiveness of proposed OA therapies.

FUTURE WORK
There are numerous proposed OA therapies for which this established model could help test:
1. Interfering with the formation of AGEs – Since the formation of AGEs contributes to tissue stiffness and inflammation, it is hypothesized that blocking the formation of these molecules would remediate these symptoms. The efficacy of these AGE-blocking drugs could be examined by incubating cartilage explants in 100 mM ribose and an experimental therapy, then measuring the tissue properties.
2. Activating the AMPK pathway – The AMPK pathway is a metabolic pathway that has been shown to promote cellular homeostasis [4]. By stimulating this process, it is hypothesized that the negative tissue properties of OA would be reversed. The response of the established model to treatment could be used to determine the effectiveness of AMPK activator drugs.

ACKNOWLEDGEMENTS: Northeastern University Global Resilience Fund, Northeastern University Office of Undergraduate Research and Fellowships.