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Research Statement

Calcium (Ca^{2+}) and Reactive Oxygen Species (ROS) crosstalk signaling in 3D cell culture to control inflammatory response at multiple length scales

Specific Aims

Calcium (Ca^{2+}), an intracellular second messenger, plays a significant role in various physiological functions including cell growth, development and signaling, autophagy and apoptosis. Reactive oxygen species (ROS) also serve as signaling molecules in apoptosis, cell signal transduction and gene expression. Dysregulation in these second messengers can cause cellular damage and death. An example of this can be seen in the degenerative joint disease osteoarthritis (OA). OA is characterized by the breakdown of the articular cartilage, synovial inflammation, and biochemical changes in chondrocyte extracellular matrix (ECM) production under oxidative stress. Under these conditions, the transcription factor protein, NF-κB involved in inflammatory responses and cell growth is upregulated. The NF-κB pathway can be activated by ROS, pro-inflammatory cytokines, ECM degradation, and Ca^{2+} signaling. Thus, it is important to further understand Ca^{2+} and ROS crosstalk in inflammatory response. Previous studies have shown that inhibiting NF-κB signaling via regulation of the MAPK and PI3K/Akt signaling pathways prevents apoptosis by suppression of ROS production. Current treatments for OA include medications, lifestyle interventions and surgery. However, there is no effective treatment for reducing the damage caused by ROS and Ca^{2+} dysregulation at various scale lengths. Therefore, there is a need to better understand the interplay between these two second messengers that control inflammatory response. Previous studies from our lab investigates the effect of hypoxia on chondrocyte metabolism in thermosensitive injectable hydrogels composed of poly (N- vinylcaprolactam) (PVCL) and methacrylated hyaluronic acid (meHA). PVCL hydrogels showed promise as new materials for cartilage tissue engineering to improve chondrocyte viability and biochemical synthesis of ECM proteins under hypoxic conditions We propose that regulation of Ca^{2+} and ROS can reduce damage caused by inflammatory response in mammalian chondrocyte cell lines. This work aims to ascertain the mechanism that facilities the crosstalk between the second messengers, Ca^{2+} and ROS, that can mitigate inflammatory response under oxidative stress at the cellular and nanoscale levels.

Hypothesis: Regulation of Ca^{2+} and ROS can reduce damage caused by inflammatory response by reducing NF-κB signaling under hypoxic conditions.

Specific Aim 1: Determine the role of Ca^{2+} and ROS signaling in static culture under normoxic/hyperoxic ($20^{\%}$ O_2) and hypoxic/physioxic (1-5% O_2) conditions in an OA model.

Approach: Ca^{2+} and NF-κB will be inhibited in chondrocyte cells in 2D static culture to determine the role of Ca^{2+} and ROS in inflammatory responses. This will be followed by protein quantification and gene expression of key players involved in the NF- **κ**B pathway. Also, quantification of ROS, Nitric Oxide (NO and $[Ca^{2+}]$ will be performed. This aim will also utilize biochemical assays to further understand the role of $[Ca^{2+}]$ and ROS.

Specific Aim 2: Elucidate the cellular response and nano biomechanics of $[Ca^{2+}]$ and ROS signaling in 3D culture under under normoxic/hyperoxic ($20^{\%}$ O_2) and hypoxic/physioxic (1-5% O_2) conditions.

Approach: C28/I2 human chondrocyte cells will be cultured under normoxic and hypoxic conditions in 3D culture: hydrogels and biomaterial scaffolds**.** Ca^{2+} and NF-κB will be inhibited in chondrocyte cells to determine the role of $Ca^{2+}$and ROS on mechanical properties using atomic force microscopy (AFM) under the influence of different extracellular $[Ca^{2+}]$ concentrations.

Specific Aim 3: Evaluate chemo mechanical response of Ca^{2+} and ROS signaling in 3D culture under normoxic/hyperoxic (20% O_2) and hypoxic/physioxic (1-5% O_2) conditions.

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