

Development of a Blood-Joint Spheroid System to Investigate the Effect of Hyperglycemic Culture Conditions in a Model of Diabetic Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease marked by synovial inflammation and cartilage breakdown.¹ The prevalence of OA has been associated with type 2 diabetes mellitus (DM), a chronic metabolic disorder characterized by elevated blood glucose levels.² The connection between both diseases has been attributed to increasing age and obesity-induced joint loading.³⁻⁴ However, underlying pathophysiological mechanisms implicated in DM and OA have not been thoroughly investigated due to the associated comorbidity involved with treating this patient population.⁵ Therefore, the complex interplay between blood vessels, synovium, and cartilage necessitates the development of *in vitro* models that recapitulate physiological conditions of the joint. In this study, we present a novel blood-joint spheroid model composed of human umbilical vein endothelial cells (hUVECs), fibroblast-like synoviocytes (FLS), and articular chondrocytes (ACs), aiming to replicate cellular crosstalk within the joint and characterize the effects of DM-induced hyperglycemia in the bloodstream on OA-associated degradation. Study 1 characterized cell viability under varying blood glucose levels. In Study 2, spheroid dissociation followed by cell sorting and gene expression of extracellular matrix, proinflammatory cytokines, oxidative stress, and glucose regulation markers were assessed in all cells. Study 3 characterized the release of nitric oxide (NO), glycosaminoglycan (GAG), and glucose uptake in media following blood glucose treatment. **METHODS:** Cell Isolation: Healthy human synovium and cartilage grafts were obtained from the Musculoskeletal Transplant Foundation (Edison, NJ). Explants were digested to isolate FLS and ACs. Primary hUVECs were purchased from Angio-Proteomic. Red Blood Cell (RBC) Extraction: O-positive human blood was obtained from NYBC and RBCs were isolated via Ficoll-Paque technique. Spheroid Culture: Spheroids were formed with a chondrocyte core and layered with FLS and hUVECs (**Fig. 1**). Using a 96-well low-adhesion plate, ACs were seeded at 8,000 cells per well and centrifuged at 200g for 10 min to form the spheroid core (**Fig. 2A**). ACs were incubated overnight in DMEM containing 10% FBS, 1% antibiotic/antimycotic (AA), 5 ng/mL FGF-2 and 1 ng/mL TGF-β1 to allow the cells to coalesce. The same procedure was repeated to develop the FLS layer on day 2 (**Fig. 2B**) with aMEM supplemented with 10% FBS, 1% AA, and 5 ng/mL FGF-2 followed by the hUVEC layer on day 3 (**Fig. 2C**) using EGM-2 (Lonza). The multi-cell spheroids were separated into euglycemic (EG; 5 mM D-glucose) or hyperglycemic (HG; 100 mM D-glucose) treated RBCs (+BLD; 40% v/v in DMEM), with parallel no blood controls for 2 days (N=5). Study 1: Live/dead staining was performed to assess cell viability using Calcein AM and Ethidium Homodimer. Study 2: Following blood glucose exposure, spheroids were labeled with FITC-CD31, PE-CD90, and APC-CD151 antibodies (1:20, Biolegend) to separate hUVECs, FLS, and ACs respectively. DAPI was used as the viability stain (1:1000, Life Technologies) and cells were isolated using a MA900 Cell Sorter (Sony). RNA was extracted from each cell type and gene expression was analyzed using qPCR for matrix degradation (MMP1, MMP2, MMP3, MMP13), matrix synthesis (ACAN, COL1A1, COL2A1, FN1), proinflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α), oxidative stress (AGER, ROMO1), and glucose regulation (GLUT1, INSR). Study 3: Media was assayed for NO, GAG, and glucose uptake. Statistics: For qPCR, target genes were normalized to GAPDH, and further normalized to day 0 measurements across all cell types. Comparisons of media constituents were made using a one-way ANOVA with Tukey HSD post-hoc tests (α=0.05). **RESULTS:** Cell viability slightly decreased under blood and glucose exposure toward the inner regions of the spheroid (**Fig. 2D-I**), while dead cells were not observed in DMEM controls (**Fig. 2A-C**). Gene expression of high blood glucose treated spheroids showed significant differences across all three cell types and treatment conditions. Markers of matrix degradation, inflammatory cytokines, and oxidative stress were mostly upregulated with blood exposure compared to controls, with significantly higher expression in HG treated spheroids (**Fig. 3D**). Matrix synthesis genes showed an opposite trend with elevated expression in DMEM controls compared to downregulation under blood and glucose culture conditions. Media analysis confirmed glucose uptake in HG blood treated spheroids (**Fig. 3E**). NO and GAG release was significantly elevated with EG treatment compared to DMEM controls (p = 0.005 and p=0.028 respectively; **Fig. 3F-G**). NO levels were also elevated in EG and HG spheroids compared to non-blood treated samples (p = 0.021 and p=0.019 respectively; **Fig. 3F**).

DISCUSSION: Spheroids exposed to high blood glucose had overall lower viability near the core compared to DMEM controls, suggesting hyperglycemic conditions can promote downstream cell death. Elevated expression of glucose transport proteins (GLUT1) and decreased insulin receptor activity (INSR) was observed in HG blood groups, potentially indicative of DM-induced insulin resistance. Gene expression across all cell types confirmed that elevated blood glucose promotes ECM degradation, joint inflammation, and oxidative stress characteristic of OA.⁶ Media analysis demonstrated increased inflammation (NO) and glucose uptake under hyperglycemic conditions, reflecting features of the OA and DM disease states. **SIGNIFICANCE:** Our blood-joint spheroid model offers a promising platform to study the effects of high blood glucose on endothelial dysfunction, synovial inflammation, and cartilage degradation, allowing for future tests that explore therapies to reverse or mitigate DM-induced hyperglycemia and OA development.

REFERENCES: 1. Loeser+ *Arthritis Rheum* 2012. 2. Hamada+ *Arthritis Rheumatol* 2016. 3. King+ *OAC* 2015. 4. Piva+ *Clin Geriatr Med* 2015. 5. Sakhrani+ *Front Bioeng Biotechnol* 2022. 6. Li+ *Exp Mol Med* 2021.
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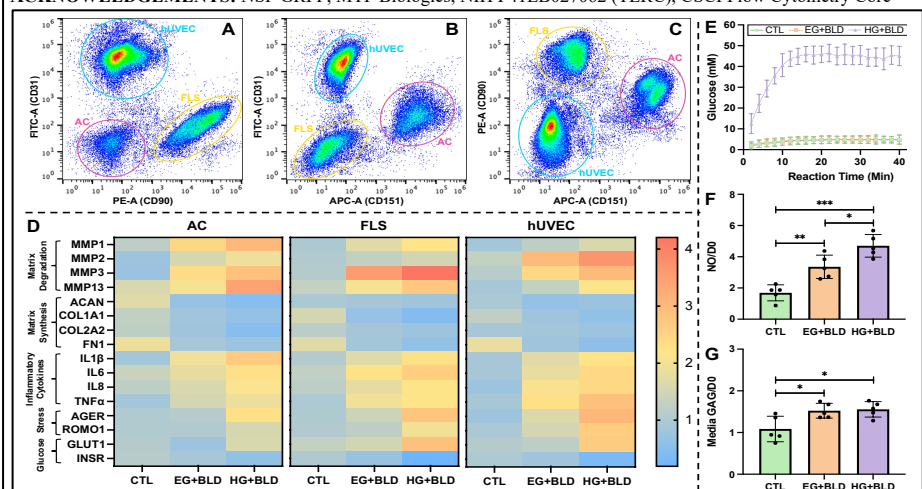
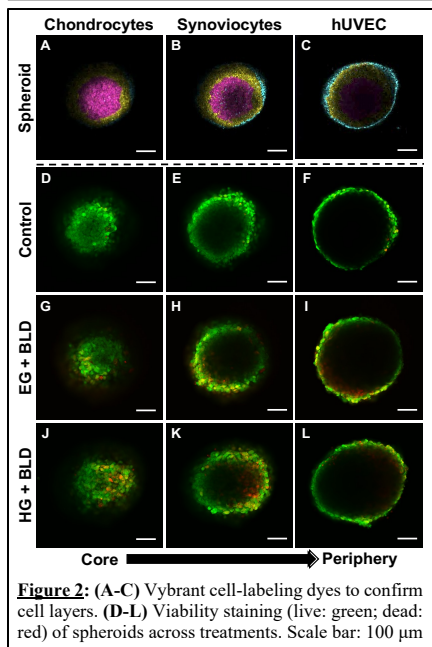
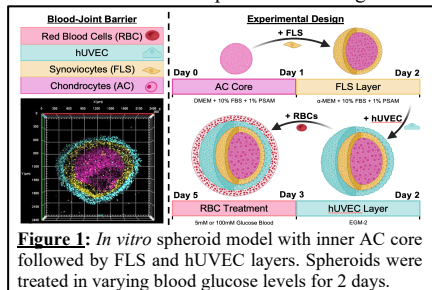


Figure 3: (A-C) Flow cytometry scatterplots to sort spheroids using CD31, CD151, and CD90. (D) Gene expression of matrix degradation, matrix synthesis, proinflammatory cytokines, oxidative stress, and glucose regulation across all cell types and treatment groups. Spheroid media analysis to assess (E) glucose levels, (F) nitric oxide, and (G) GAG release.