Development of a Blood-Joint Transwell System to Investigate the Effect of High Blood Glucose Exposure in a Diabetic Osteoarthritis Model

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Introduction

- Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage deterioration and synovial inflammation.[1]
- Studies have shown that type 2 diabetes mellitus (DM) and its associated hyperglycemia may increase the progression and incidence of OA (Fig. 1).[2]
- The connection between both diseases has historically been attributed to increasing age and joint loading due to obesity.[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]
- The complex interplay between blood vessels, endothelial cells, synovium, and articular cartilage necessitates the development of *in vitro* models that recapitulate physiological conditions of the joint space (Fig. 2).
- Under hyperglycemic conditions, synoviocytes secrete inflammatory factors (TNFs and ILs), matrix degradation enzymes (MMPs), and oxidative stress markers (ROS and AGEs) into the articular cavity, which can further induce joint inflammation and cartilage breakdown (Fig. 3).[6-7]
- We present a blood-joint transwell system containing human umbilical vein endothelial cells (hUVECs), fibroblast-like synoviocytes (FLS), and articular chondrocytes (ACs) aiming to recapitulate cellular crosstalk and model DM-induced hyperglycemia in the bloodstream on OA-associated degradation.

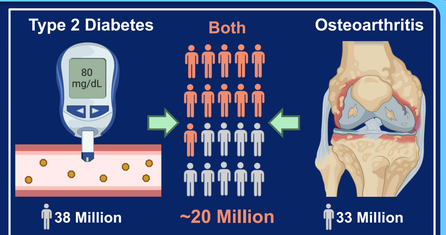

Figure 1: Prevalence and combined incidence of type 2 diabetes and osteoarthritis in the United States.

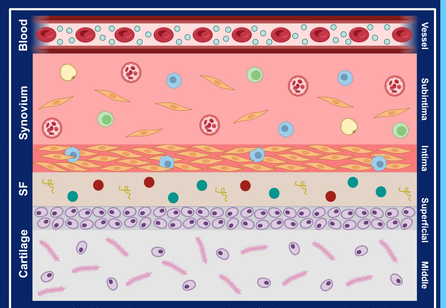

Figure 2: Cellular components of the blood-joint barrier including blood vessels lining the synovium and the underlying avascular articular cartilage surface.

Methods

Red Blood Cell (RBC) Extraction and FLS/Chondrocyte Culture:

- RBCs were isolated from O+ human blood via Ficoll-Paque technique.
- Healthy human synovium and cartilage grafts were obtained from MTF. Explants were digested to isolate FLS and ACs. Primary hUVECs were purchased from Angio-Proteomie.

Multi-cellular Transwell Set-Up:

- Cells were cultured on 24 mm transwells with 8 µm pore inserts.
- FLS were seeded on the transwell underside in αMEM and AC were cultured on the plate bottom in DMEM, supplemented with 10% FBS + 5ng/mL FGF-2
- After 24h, transwells were inverted to allow coculture of FLS and AC in low-glucose (LG) DMEM while hUVECs were seeded on the apical side in EGM-2.
- Following overnight attachment, apical media was replaced with euglycemic (EG; 5mM D-glucose) or hyperglycemic (HG; 100mM D-glucose) treated RBCs (40% v/v in LG DMEM) with parallel no blood treated controls and basal media was replaced with synovial fluid (50% v/v in LG DMEM) for 48h.

Cell Viability, Immunocytochemical (ICC) Staining, and Basal Media Analysis:

- Viability staining was performed via Calcein AM and Ethidium Homodimer.
- Transwells were stained for VE- and OB- Cadherin using confocal microscopy to identify hUVECs and FLS respectively
- Media samples from the basal compartment were assayed for nitric oxide (NO), lactate dehydrogenase (LDH), hemoglobin release, and glycosaminoglycan (GAG) content.

Gene Expression Analysis:

- qPCR for markers of matrix degradation, matrix synthesis, inflammatory cytokines, oxidative stress, and glucose regulation.

Statistics: One-way ANOVA with Tukey HSD post-hoc test at α=0.05.

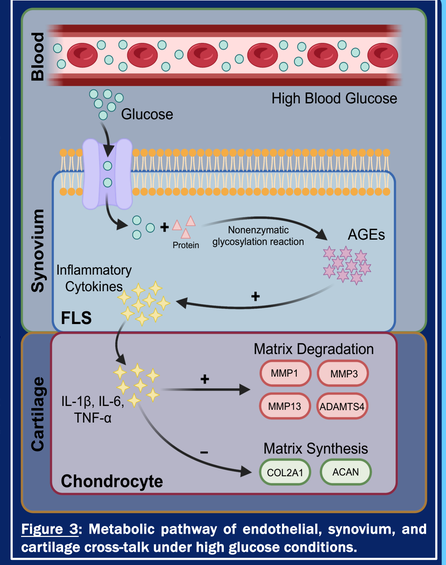

Figure 3: Metabolic pathway of endothelial, synovium, and cartilage cross-talk under high glucose conditions.

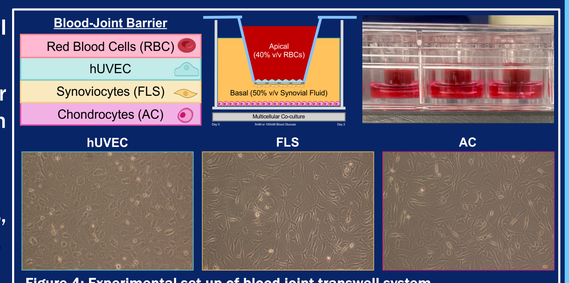

Figure 4: Experimental set-up of blood-joint transwell system

Results

Cell Viability and ICC Staining:

- Slight cell death observed in FLS group with 5mM and 100mM blood glucose exposure compared to non-blood treated DMEM controls (Fig. 5A).
- Cell viability remained consistent for hUVEC and AC across all treatment conditions (Fig. 5A).
- Clear distinction between hUVEC and FLS layers on the transwell filter observed through VE- and OB- Cadherin stains (Fig. 5B).
- Migration of hUVECs across transwell membrane into the underlying synovium layer for control and EG treated groups (Fig. 5B).

Basal Media Analysis:

- Media NO release was significantly increased with blood exposure and further elevated under HG culture conditions (Fig. 6A).
- Media LDH activity was significantly elevated with EG and HG exposure compared to non-blood treated controls (Fig. 6B).
- Media GAG was elevated with HG treatment compared to DMEM controls (Fig. 6C).
- Significant hemoglobin release into basal media for EG and HG treated groups compared to non-blood treated controls (Fig. 6D).

Gene expression:

- MMP expression was upregulated with blood exposure and further elevated under HG culture conditions (Fig. 7).
- Matrix synthesis genes were downregulated with blood exposure and HG treatment (Fig. 7).
- Significant increase in inflammatory markers with EG and HG conditions compared to non-blood treated controls for FLS and AC (Fig. 7).
- Upregulation of AGER, ROMO1, and GLUT-1 with decreased INSR expression under HG conditions across all cell types (Fig. 7).

Significance: *p<0.05, **p<0.01, ***p<0.001

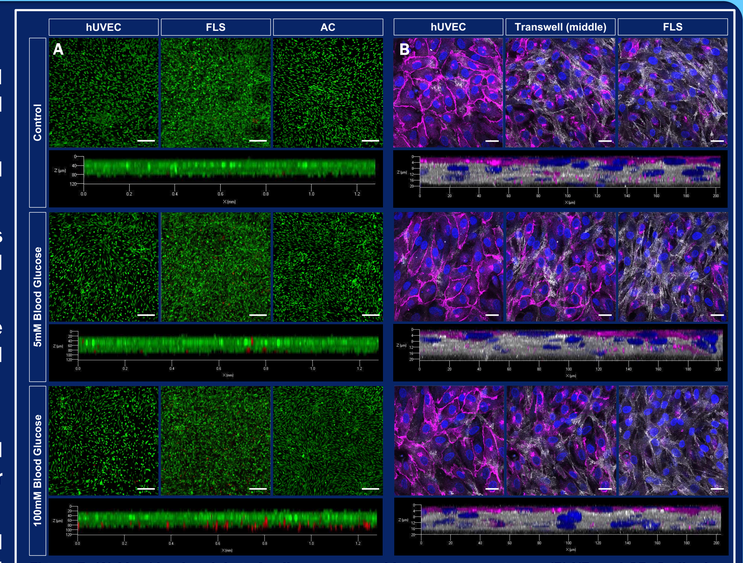

Figure 5: (A) Live/dead staining of cell types across blood treatment groups (B) VE- and OB- Cadherin staining on transwell insert across blood treatment groups. Scale bar: 200µm (left) and 25µm (right).

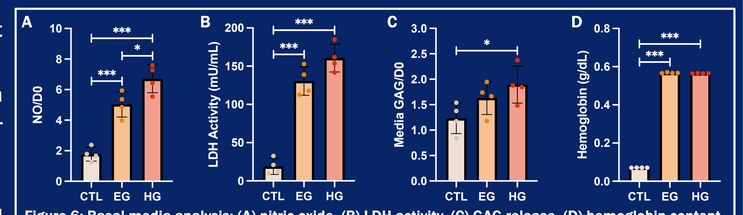

Figure 6: Basal media analysis: (A) nitric oxide, (B) LDH activity, (C) GAG release, (D) hemoglobin content.

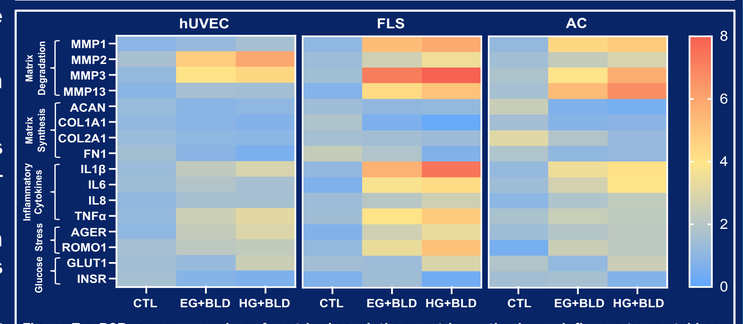

Figure 7: qPCR gene expression of matrix degradation, matrix synthesis, proinflammatory cytokines, oxidative stress, and glucose regulation markers across all cell types and treatment groups.

Conclusions

- FLS viability were decreased in groups with blood exposure, suggesting hyperglycemic conditions may promote cell death.[8]
- Media analysis demonstrated increased inflammation (NO) and cytotoxicity (LDH) with hyperglycemic as well as blood exposure, reflecting features of both OA and DM disease states.[9]
- Increased hemoglobin release into the basal compartment with blood exposure may result in excess iron deposition, reactive oxygen species (ROS) generation, pro-inflammatory cytokine production, and potentially ferroptosis.[10]
- Gene expression across all cell types confirmed that elevated blood glucose promotes ECM degradation, joint inflammation, and oxidative stress characteristic of the OA disease state.[6,11]
- Elevated expression of glucose transport proteins (GLUT1) and downregulation of insulin receptor activity (INSR) in HG blood groups may be indicative of increased glucose transport and insulin resistance, resembling the DM diseased state.[12]

References

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