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]
- The prevalence of OA has been associated with type 2 diabetes mellitus (DM), a chronic metabolic disorder characterized by elevated blood glucose levels and insulin resistance (Fig. 1).[2]
- The connection between both diseases has historically been attributed to increasing age and joint loading due to obesity.[3-4]
- However, 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 *in vitro* models that recapitulate physiological conditions of the joint (Fig. 2).
- With hyperglycemia, synoviocytes secrete inflammatory factors (TNFs and ILs), matrix degradation enzymes (MMPs), and oxidative stress markers (ROS and AGEs), 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 blood on OA-associated degradation.**

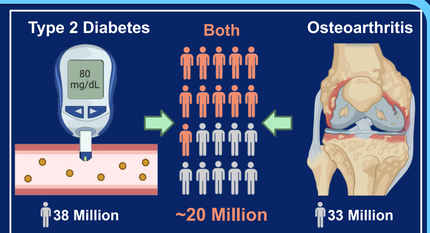

Figure 1: Prevalence and combined incidence of type 2 diabetes and osteoarthritis.

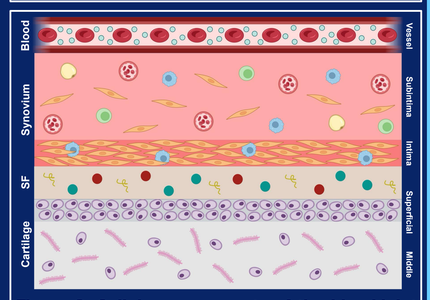

Figure 2: Cellular components of the blood-joint barrier including blood vessels, synovium, and the avascular articular cartilage surface.

Methods

Red Blood Cell (RBC) Extraction and Cell Culture:

- Human blood samples were obtained from NYBC, and RBCs were isolated using the Ficoll-Paque technique.
- Healthy human synovium and cartilage 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 24mm transwells with 8μm pores.
- 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 overnight attachment, 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.
- 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 (Fig. 4).

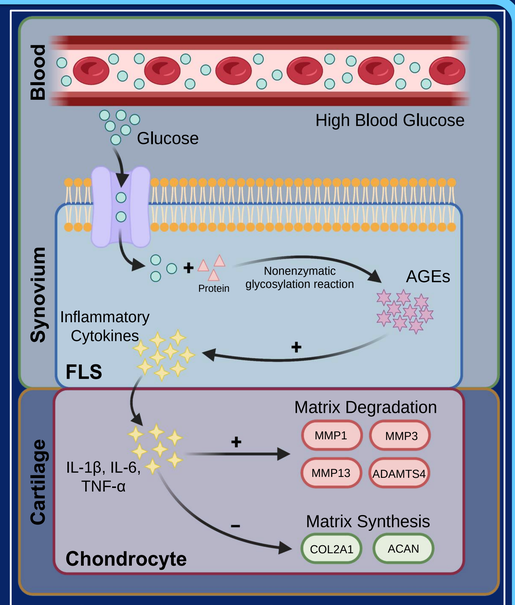

Figure 3: Metabolic pathway of endothelial, synovium, and cartilage cross-talk under high glucose conditions. Glucose uptake from blood can promote production of inflammatory cytokines in FLS that can result in downstream matrix degradation of chondrocytes.

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

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

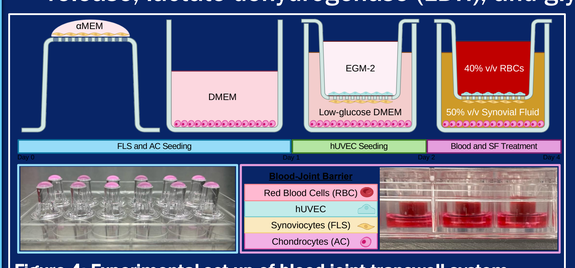

Figure 4: Experimental set-up of blood-joint transwell system.

Gene Expression Analysis:

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

Statistics:

- Genes normalized to GAPDH and day 0 controls. Media comparisons were assessed using one-way ANOVA with Tukey HSD post-hoc test at α=0.05.

Results

Cell Viability and ICC Staining:

- Slight cell death observed in FLS group with blood exposure (Fig. 5A).
- Cell viability remained consistent for hUVEC and AC (Fig. 5A).
- Distinction between hUVEC and FLS layers on transwell filter from VE- and OB- Cadherin stains (Fig. 5B).

Basal Media Analysis:

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

Gene expression:

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

Statistical significance: *p<0.05, **p<0.01, ***p<0.001

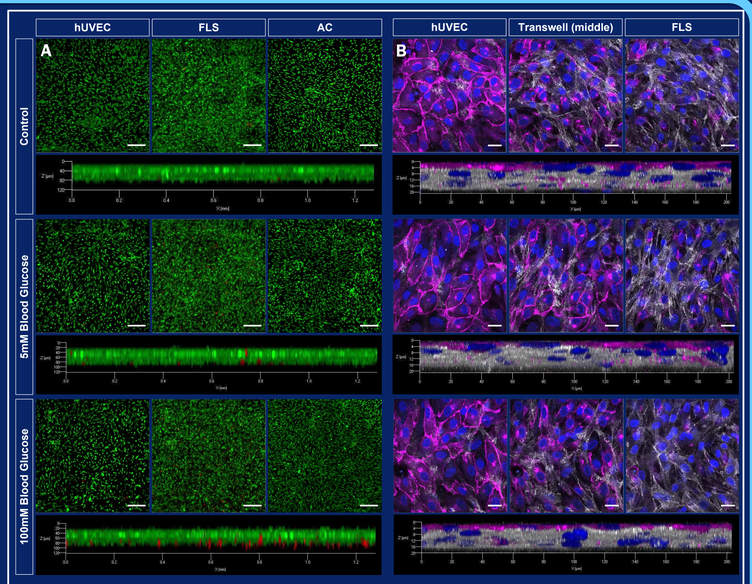

Figure 5: (A) Live/dead staining of all three cell types across blood treatment and control groups (B) VE- and OB- Cadherin staining on transwell insert for all treatment conditions and cell types. Scale bar: 200μm (left) and 25μm (right).

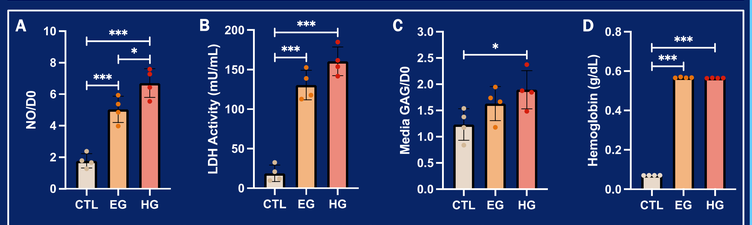

Figure 6: Basal media analysis of synovial fluid across all treatment conditions: (A) nitric oxide, (B) LDH activity, (C) GAG release, (D) hemoglobin content.

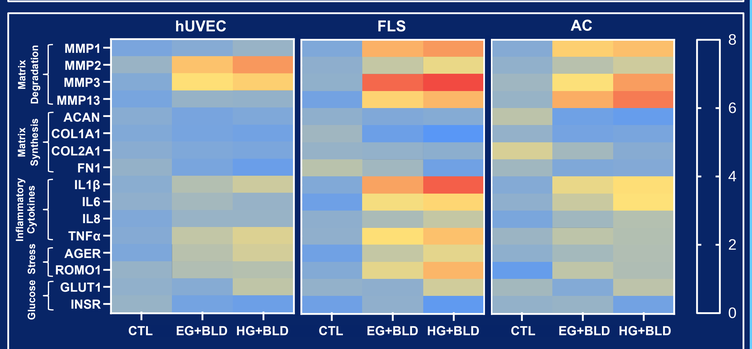

Figure 7: qPCR gene expression of matrix degradation, matrix synthesis, proinflammatory cytokines, oxidative stress, and glucose regulation markers across all three cell types and treatment conditions.

Conclusions

- FLS viability were decreased in groups with blood and glucose exposure, **suggesting hyperglycemic culture 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 (iron induced cell death pathway).**[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]
- Overall, the blood-joint transwell **recapitulates cellular interactions of the joint space.** This *in vitro* system provides a physiologic model to assess the effects of the **vascularized synovial membrane on the underlying avascular articular cartilage surface.**

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