# 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 cartilage deterioration and synovial inflammation.[1] Previous studies have shown that type 2 diabetes mellitus (DM) and its associated hyperglycemia may increase the progression and incidence of OA.[2] The connection between both diseases has historically been attributed to increasing age and joint loading due to obesity.[3] However, the underlying pathophysiological mechanisms implicated in DM and OA have not been thoroughly investigated due to the associated comorbidity involved with treating this patient population.[4] Furthermore, the complex interactions between blood vessels, endothelial cells, synovium, and articular cartilage necessitates the development of *in vitro* models that recapitulate physiological conditions of the joint space. In this study, we develop a novel blood-joint transwell system consisting of human umbilical vein endothelial cells (hUVEC), fibroblast-like synoviocytes (FLS), and articular chondrocytes (ACs) (**Fig. 1**). The transwell system allows for cross-talk between the cell types either across the transwell membrane or via paracrine factors released into shared media. As such, the system can recapitulate signaling within the joint and model the effects of DM-induced hyperglycemia in the bloodstream on OA-associated cartilage degradation.

**METHODS:** Cell Isolation: Healthy human synovium and cartilage were obtained from MTF Biologics and digested to isolate FLS and ACs. Primary hUVECs were obtained from Angio-Proteomie. Red Blood Cell (RBC) Extraction: Human blood samples were sourced from NYBC, and RBCs were isolated via Ficoll-Paque technique. Transwell Culture: ACs were seeded onto the transwell plates at 1 million cells (DMEM, 10% FBS, TGF-β1, FGF-2) and FLS were seeded onto inverted transwell inserts at 500,000 cells (αMEM, 10% FBS, FGF-2). After 24 hours AC media was switched to low-glucose DMEM and transwell inserts were flipped and placed into the transwell plate. hUVECs were seeded onto transwell inserts at 500,000 cells (EGM-2). Blood Treatment: After 24 hours, hUVEC media was replaced with either euglycemic (EG; 5 mM D-glucose) or hyperglycemic (HG; 100 mM D-glucose) treated RBCs (+BLD; 40% v/v in DMEM) as well as a control of low-glucose DMEM (n=4). Additionally, the basal compartment was replaced with synovial fluid (50% v/v in DMEM).

**RESULTS:** Nitric oxide (NO) and lactate dehydrogenase (LDH) release into the media were significantly elevated with blood exposure compared to DMEM controls (**Fig. 2**). NO levels were further elevated under HG conditions. Gene expression analysis revealed significant differences across all cell types and treatment conditions. Markers for ECM degradation, inflammatory cytokines, oxidative stress, and glucose transport were upregulated with blood exposure compared to controls, with higher expression observed under HG treatment (**Fig. 3**). Conversely, matrix synthesis and insulin receptor genes exhibited decreased expression under both blood and HG exposure (**Fig. 3**).

**DISCUSSION:** Media analysis demonstrated increased inflammation (NO) and cytotoxicity (LDH) with HG as well as blood exposure, reflecting features of both OA and DM disease states.[5] Gene expression across all cell types confirmed that elevated blood glucose promotes ECM degradation, joint inflammation, and oxidative stress characteristic of OA.[6] 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.[7] The blood-joint transwell system offers a promising platform to investigate the effects of hyperglycemic environments. This model recapitulates cellular interactions of the joint space, allowing for future studies that explore therapies to mitigate the pathogenesis of DM and OA.

**REFERENCES:** 1. Loeser+ *Arthritis Rheum* 2012, 2. Eitner+ *Bone Joint Res* 2021, 3. King+ *OAC* 2015, 4. Sakhrani+ *Front Bioeng Biotechnol* 2022, 5. Laiguillon+ *OAC* 2015, 6. Li+ *Exp Mol Med* 2021, 7. Petersen+ *Physiol Rev* 2018.

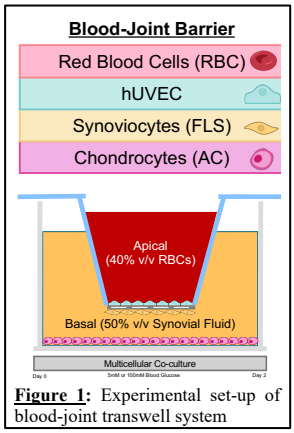


**Figure 1:** Experimental set-up of blood-joint transwell system

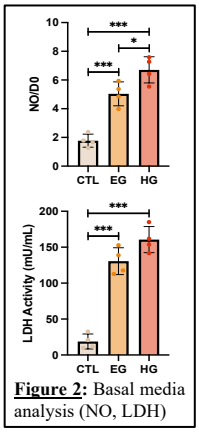


**Figure 2:** Basal media analysis (NO, LDH)

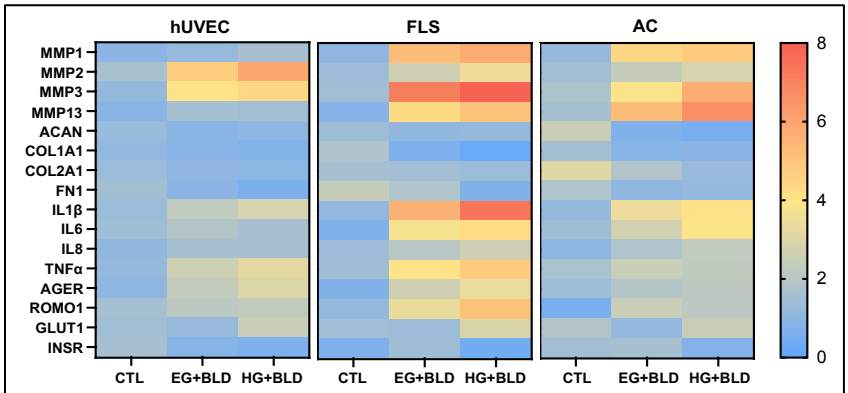


**Figure 3:** qPCR gene expression across cell types (hUVEC, FLS, AC) and treatment groups (CTL, EG, HG)