Microfluidic Platform of the Blood-Joint Barrier to Assess the Effects of High Blood Glucose on Synovium-Cartilage Crosstalk: Toward a Model of Diabetic Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by synovial inflammation and cartilage damage.[1] Its prevalence has been associated with type 2 diabetes mellitus (DM), a metabolic disorder marked by chronic hyperglycemia.[1] While increasing age and joint loading due to obesity are known risk factors contributing to both conditions, underlying pathophysiological mechanisms remain poorly understood due to the complexity of treating these comorbidities.[2-3] In this study, we develop a custom multi-cellular microfluidic chip that models the blood-joint barrier by incorporating human umbilical vein endothelial cells (hUVECs), fibroblast-like synoviocytes (FLS), and articular chondrocytes (ACs). The design features three vertically assembled compartments that enable directional media flow and paracrine signaling (**Fig. 1A-B**), recapitulating cellular crosstalk across the joint to investigate the effects of DM-induced hyperglycemia in the bloodstream on OA-associated cartilage degradation. Using this platform, Study 1 characterized glucose uptake, inflammatory signaling, and nitric oxide (NO) release into the synovial fluid (SF) media in response to high blood glucose exposure. In Study 2, underlying AC gene expression was assessed for markers of matrix synthesis, degradation, oxidative stress, and glucose regulation under hyperglycemic culture conditions.
METHODS: Cell Isolation: Healthy human synovium and cartilage grafts were obtained from MTF Biologics (N=4; only male cadaver samples were used due to donor availability). Explants were digested to isolate FLS and ACs. Primary hUVECs were sourced from Angio-Proteomie. Red Blood Cell (RBC) Isolation: O-positive human blood was obtained from NYBC, and RBCs were isolated via Ficoll-Paque. Microfluidic Setup: The chip was 3D-printed using BioMed Clear Resin (**Fig. 1C**). Cells were cultured on laser cut nylon mesh inserts (8 µm pores), fabricated via overmolding in polypropylene and secured with an O-ring to provide a fluid-tight seal. Multichannel peristaltic pump maintained continuous media recirculation. The top compartment was perfused with either euglycemic (EG; 5 mM glucose) or hyperglycemic (HG; 100 mM glucose) treated RBCs (+BLD; 40% v/v in DMEM), with parallel no blood controls for 48h (CTL). RBC settling was minimized by stirring at 300 rpm, which maintained a homogeneous solution without excessive hemoglobin release. Cell seeding densities were optimized to ensure barrier integrity, confirmed by TEER measurements. Blood-treated media was perfused across a confluent hUVEC monolayer (1×10^5 cells/insert), positioned under a silicone gasket to simulate vascular exposure and endothelial barrier characteristics. Low-glucose DMEM was circulated below the hUVEC layer to support nutrient exchange. Beneath this compartment, confluent FLS (1×10^5 cells/insert) were cultured on a second insert and positioned above a monolayer of ACs (1×10^5 cells/slide), separated by 50% v/v SF in DMEM to mimic the native interstitial environment. Shear stress in both blood and SF compartments was optimized using viscosity, flow rate, and channel dimensions. Parameters were validated computationally with SOLIDWORKS and COMSOL simulations (**Fig. 1D-E**). Study 1: SF media was assayed for glucose uptake, NO release, and inflammatory analytes using a LEGENDplex assay for IL-1β, IL-6, and TNF-α. Study 2: RNA was isolated from ACs, and gene expression was assessed using qPCR for markers of matrix degradation (MMP1, MMP3), matrix synthesis (ACAN, COL2), oxidative stress (AGER, ROMO), and glucose regulation (GLUT1, INSR). Statistics: Media constituents were compared via one-way ANOVA with Tukey HSD post-hoc tests (α=0.05). For qPCR, genes were normalized to GAPDH and day 0 controls.
RESULTS: SF media analysis confirmed glucose uptake in HG blood-treated systems (**Fig. 2A**). NO release was significantly elevated in both EG and HG groups compared to non-blood controls ($p = 0.0042$ and $p=0.0003$ respectively; **Fig. 2B**). All three inflammatory analytes increased with HG blood exposure compared to controls, with IL-6 release significantly elevated in HG treated cells compared to EG ($p = 0.035$; **Fig. 2C**). Gene expression analysis showed markers of matrix degradation, oxidative stress, and glucose transport were upregulated with blood exposure, with most significant effects observed under HG conditions (**Fig. 3**). Conversely, matrix synthesis and insulin receptor markers exhibited decreased expression under both blood and HG treatment (**Fig. 3**).
DISCUSSION: Media analysis demonstrated HG and blood exposure increased inflammatory release, reflecting features of both OA and DM disease states.[3] Gene expression of ACs showed high blood glucose promotes ECM degradation and oxidative stress, characteristic of OA.[4] Upregulation of GLUT1 and reduced INSR expression in HG blood groups suggest enhanced glucose transport and insulin resistance respectively, consistent with DM pathology.[4]
SIGNIFICANCE: This blood-joint microfluidic system provides a physiologic *in vitro* model of hyperglycemia-driven joint degeneration, tracing high blood glucose levels to hUVEC dysfunction, FLS inflammation, and AC breakdown, and provides a promising platform to test future therapies for DM-induced OA.
REFERENCES: 1. Hamada+ *Arthritis Rheumatol* 2016. 2. King+ *OAC* 2015. 3. Sakhrani+ *Front Bioeng Biotechnol* 2022. 4. Li+ *Exp Mol Med* 2021.
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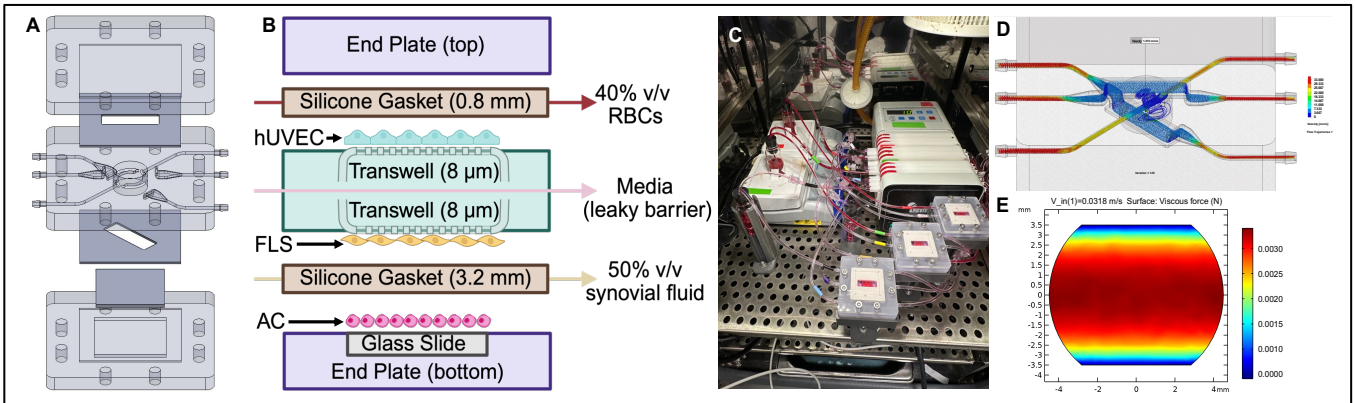

Figure 1: (A-C) Custom microfluidic chip to model flow dynamics across the blood-joint barrier, with wall shear stress across RBC and SF layers validated by **(D–E)** simulations

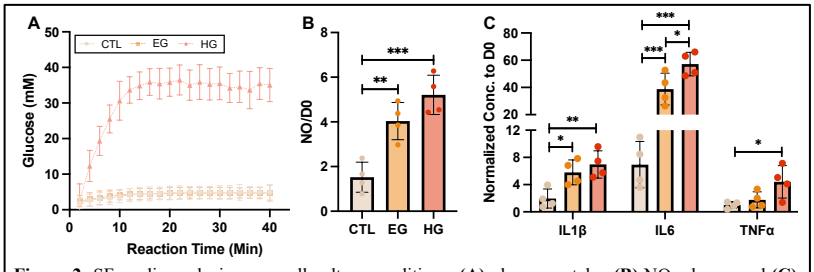

Figure 2: SF media analysis across all culture conditions: **(A)** glucose uptake, **(B)** NO release, and **(C)** inflammatory cytokine production under high blood glucose exposure. *p<0.05, **p<0.01, ***p<0.001

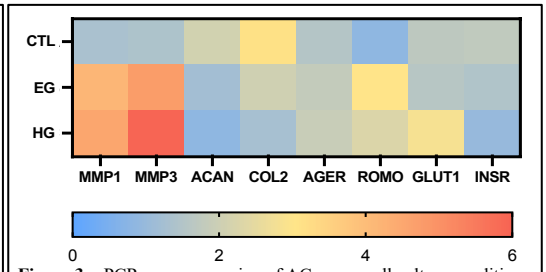

Figure 3: qPCR gene expression of ACs across all culture conditions for ECM structure, oxidative stress, and glucose regulation markers