Sustained Delivery of Insulin via PLGA Microspheres Maintains Synovium Biochemical Properties under High Glucose Culture Conditions Towards a Model of Diabetic Osteoarthritis

Ratna Sharma[1], Neeraj Sakhrani[1], Jack Rogot[1], Clark T. Hung[1,2]

[1]Department of Biomedical Engineering, [2]Department of Orthopedic Surgery, Columbia University, New York, NY; rs4241@columbia.edu

Disclosures: RS (N), NS (N), JR (N), CTH (1; MTF. 8; Associate Editor of Journal of Orthopedic Research, Editor for Orthopedic Research and Reviews)

INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by structural and functional changes to the synovium.[1] Type 2 diabetes mellitus (DM) is a common comorbidity among patients with OA, contributing to high infection rates and poor rates of healing.[2] DM is characterized by chronic hyperglycemia caused by insulin resistance or a deficiency of insulin secretion.[3] In the joint, high glucose environments due to DM may inhibit cellular proliferation, alter intracellular signaling processes, contribute to extracellular matrix (ECM) remodeling, and increase inflammation, contributing to OA pathogenesis.[2, 4-6] Clinically prescribed insulin therapies via subcutaneous injections and oral administration have been shown to regulate blood glucose levels in a variety of tissues including connective, skeletal, and adipose.[7-9] However, the direct anabolic effects of insulin delivery on regulating synovial tissue inflammation and catabolism in patients with DM have not been thoroughly investigated.[10] Therefore, we sought to develop a targeted insulin release system to synovium using poly(d,l-lactide-co-glycolide) (PLGA) microspheres. We hypothesized that sustained insulin delivery can maintain the structural properties of synovial tissue under DM-induced hyperglycemic conditions, ultimately delaying OA progression. Study 1 characterized the drug release profile of insulin-loaded microspheres (ILMS) following polymer hydrolysis. Study 2 assessed the biochemical composition of synovium and surrounding media following hyperglycemic and ILMS exposure, with parallel untreated controls. Immunohistochemical (IHC) staining was performed in Study 3 to further characterize synovial cell distribution, collagen (COL) content, glycosaminoglycan (GAG) levels, and a specific glucose transporter implicated in insulin signaling.

METHODS: Study 1: Human recombinant insulin was encapsulated in PLGA microspheres (75:25 lactide:glycolide, MW: 66,000–107,000). Insulin was dissolved in 5 mL DMSO and added to a polymer solution consisting of 200 mg PLGA and 4.5 mL DCM. The polymer-drug mixture was added dropwise to 1% (w/v) PVA in distilled water and stirred at 400 rpm for 4h to evaporate organic solvents. ILMS were purified with three deionized water wash cycles and lyophilized before use. The loading capacity was determined by dissolving 50 mg of the ILMS in DCM and spectrophotometrically quantifying the absorbance at 270 nm. Values were compared to a standard curve of known insulin concentrations until a steady release profile was observed. Tissue Harvest: Healthy human synovium was obtained from the Musculoskeletal Transplant Foundation (N=3 donors). Specimens were cut into 1 cm x 1 cm pieces and cultured in varying glucose concentrations for 4 days. Samples were separated into euglycemic (EG; 5 mM D-glucose DMEM, 10% FBS) and hyperglycemic (HG; 100 mM D-glucose DMEM, 10% FBS) groups. Conditioned media containing ILMS were added to HG groups. Study 2: Synovial explants were digested and solubilized to obtain DNA, COL, and GAG content. Media samples were assayed for glucose uptake, nitric oxide (NO), and GAG release. Study 3: Samples were fixed in 4% PFA and sectioned into 4 µm slices. Paraffin sections were stained with Hematoxylin and Eosin (H&E), Picrosirius Red, and Safranin O. Samples were stained for glucose transporter type 4 (GLUT4; 1:250 rabbit monoclonal) using a 3,3′Diaminobenzidine rabbit substrate kit and counterstained with Mayer's hematoxylin. Statistics: Data was analyzed by one-way ANOVA with Tukey HSD post-hoc tests (α=0.05) to determine significant differences between glycemic groups and ILMS treatment. Synovial tissue biochemical composition and media analysis were normalized to average day 0 values.

RESULTS: After polymer hydrolysis, loading capacity of the ILMS (**Fig. 1A**) revealed an initial bolus release within 1h, followed by an average sustained insulin release of approximately 2.3 µg/mL **(Fig. 1B)**. Synovial biochemical analysis resulted in significantly lower DNA in HG samples compared to EG controls (**Fig. 2A**; p=0.003). ILMS treatment recovered DNA levels across HG groups (**Fig. 2A**; p=0.025). COL content was significantly attenuated in HG tissue (**Fig. 2B**; p=0.012), while no significant changes were observed with conditioned media containing ILMS. GAG levels were also significantly decreased in HG groups compared to EG samples (**Fig. 2C**; p<0.0001), while ILMS treatment increased synovial tissue GAG (p=0.041). Media analysis confirmed glucose uptake for both HG groups, where ILMS treated synovium resulted in greater uptake compared to HG controls (**Fig. 2D**; p=0.022). NO release was significantly elevated in non-microsphere treated HG explants (**Fig. 2E**; p<0.0001), while ILMS exposure recovered NO levels comparable to EG treated synovium (p=0.043). Media GAG was slightly elevated in HG samples compared to EG controls (**Fig. 2E**; p=0.049). Deeper histological staining of Picrosirius Red and Safranin O was observed in EG controls compared to HG groups (**Fig. 3E-L**). HG+ILMS samples displayed higher intensity staining, with similar staining profiles as EG explants. GLUT4 staining was significantly elevated in ILMS treated HG groups compared to EG synovium controls (**Fig. 3M-P**).

DISCUSSION: Sustained insulin delivery via microspheres maintains ECM composition of synovial tissue under DM-induced hyperglycemic environments. Conditioned media containing ILMS was able to recover DNA and GAG levels of HG treated explants to values comparable to EG controls, indicative of ECM remodeling of synovial tissue. Lower media glucose concentrations in HG media containing ILMS compared to HG controls confirmed that insulin may promote greater glucose regulation and uptake. ILMS treatment also recovered NO levels in HG media, suggesting decreased inflammation with insulin exposure. Deeper staining of Picrosirius Red and Safranin O in EG groups and ILMS exposed HG groups further confirmed the differences observed in COL and GAG levels across both glycemic and treatment conditions. High intensity staining of GLUT4 in ILMS treated explants suggests that insulin exposure may promote greater glucose metabolism by regulating transporter translocation to the cell membrane, thus facilitating glucose uptake into the synovium.[11-12]

SIGNIFICANCE: By exposing synovium to sustained low-dose insulin release using ILMS under hyperglycemic culture conditions, we attempt to develop an *in vitro* system to model the biochemical changes of synovial tissue in DM patients. In all, this study attempts to further the use of ILMS as a potential therapeutic strategy to reduce DM-induced inflammation and preserve overall joint health, thus preventing the onset of OA development and progression.

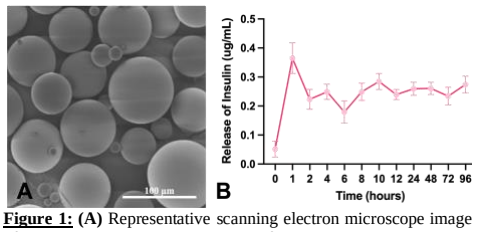

Figure 1: **(A)** Representative scanning electron microscope image of ILMS. **(B)** Microsphere release profile in DCM across 96 hours.

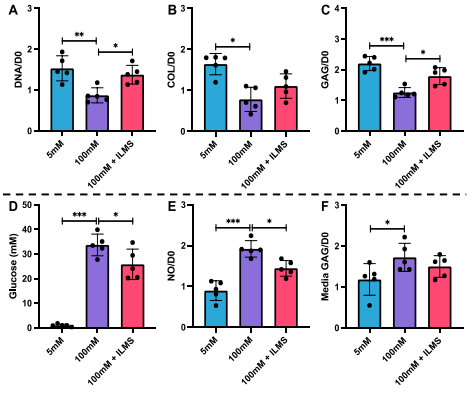

Figure 2: Synovium explants biochemical analysis of **(A)** DNA, **(B)** COL, and **(C)** GAG levels. Media analysis of **(D)** glucose uptake, **(E)** nitric oxide, and **(F)** GAG release under hyperglycemic and ILMS treatment (*p<0.01, **p<0.001, and ***p<0.0001).

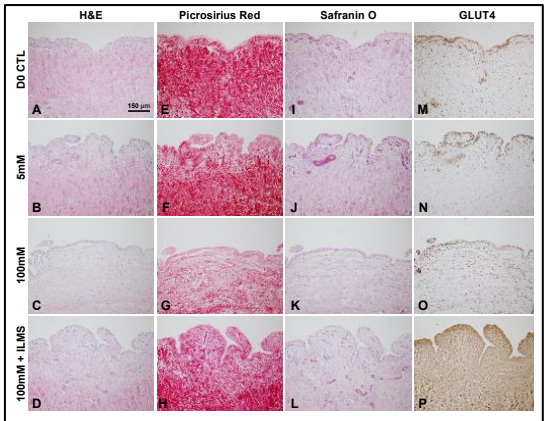

Figure 3: **(A-D)** H&E, **(E-H)** Picrosirius Red, **(I-L)** Safranin O, and **(M-P)** GLUT4 staining of synovium under hyperglycemic and ILMS treatment.

REFERENCES: 1. Estell+ *J Orthop Res* 2019. 2. Veronese+ *Semin Arthritis Rheum* 2020. 3. Röder+ *Expt & Mol Med* 2016. 4. Zahan+ *Med and Pharm Rep* 2020. 5. Buranasin+ *PLOS One* 2018 6. Lepestos+ *Mol Basis of Disease* 2016. 7. Rahman+ *Int J Mol Sci* 2021. 8. Wilcox+ *Clin Biochem Rev* 2005. 9. Weiss+ *Am J Physiol.* 1980. 10. Hamada+ Arthritis Rheumatol 2016. 11. Wang+ *Int J Pharm.* 2018. 12. Sakhrani+ *Front Bioeng Biotechnol.* 2022.

ACKNOWLEDGEMENTS: MTF Biologics, OSRF, NIH, Columbia Summer@SEAS (RS), Columbia University Egleston Scholar Program (RS), NSF GRFP (NS)