Validation of a novel organoid model of osteoarthritis for high-throughput drug screening

Max L. Lee[1], Glen Liau[1], Mayu Morita[1], Yasemin Sude Ergul[1], Chao Ma[1], Kuan-Lin Chen[1], Simon Kwoon-Ho Chow[1], Hang Lin[2], Bruce A. Bunnell[3], Qi Gao[1], Stuart B. Goodman[1]

[1]Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, CA, USA; [2]Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15219, USA; [3]Department of Microbiology, Immunology and Genetics, University of North Texas Health Science Center, Fort Worth, TX, 76107, USA

Email of Presenting Author: maxlee12@stanford.edu

Disclosures: None

INTRODUCTION: Osteoarthritis (OA) is the most common joint disease among adults, causing significant pain and morbidity. Inflammation is a major contributor to the development and progression of OA, particularly through pro-inflammatory cytokines such as IL-1β and TNF-α as well as the NF-κB signaling pathway.[1] Conventional in vivo and in vitro models for studying OA are limited by differences in anatomy and physiology among species and the inability to adequately replicate the spatial organization and cell-cell interactions of the native joint.[2] Organoid models, or self-organizing 3D tissue models derived from multiple cell types, have grown in popularity for applications such as tissue regeneration and cancer biology. However, organoids have not yet been widely adopted to study musculoskeletal diseases such as OA.[3] We report the generation and validation of a novel OA organoid model that mimics key features of the human knee joint and the inflammatory pathogenesis of OA for future high-throughput drug screening (HTS) applications.

METHODS: Primary cells were obtained from cartilage and subchondral bone tissue following total joint arthroplasty for OA. The tissue was digested, and primary chondrocytes and mesenchymal stem cells (MSCs) were isolated and collected. Our OA organoids were designed to have two connected yet distinct layers: one layer of chondrocytes representing articular cartilage, separated by a layer of MSCs representing subchondral bone (Figure 1A through 1B). To create the organoid, we seeded 5000 MSCs and 5000 chondrocytes separately into separate wells on Corning® low adhesion culture plates, allowing cells to self-assemble into small spheroids over 24 and 48 hours, respectively. After confirming spheroid formation, the chondrocyte spheroids were removed from the well with a 200 µL pipette and placed individually into the wells containing the MSC spheroids, allowing them to combine. Prior to seeding, a subset of MSCs was stained with Invitrogen® DeepRed cell tracker. OA organoids were then stained with DAPI immediately prior to imaging and analyzed under a confocal microscope (Leica® SP8) to visualize the distribution and migration of different cell subtypes over time. Cells in the treatment group were exposed to 40 ng/mL of IL-1β to induce acute inflammation and simulate the pathophysiology of OA. Cell viability was assessed using the Invitrogen® Live/Dead viability assay kit. 3D images were taken using confocal microscopy, average intensity was calculated across the Z-stack, and the average percentage of dead and live cells as a proportion of total cells was calculated daily. Comparisons were made using Welch's t-test. Organoid width and diameter were also tracked over time. This project was approved by the Institutional Review Board of Stanford University (IRB #51386). Statistical analysis was performed using RStudio (Posit, Boston, MA, USA).

RESULTS: By Day 1, the organoids had merged and demonstrated a strong layer of separation between the chondrocyte and MSC layers, depicted with blue DAPI and red DeepRed stain, respectively (Figure 1A through 1C). Subsequently, the organoids demonstrated minor cell migration and a blurring of the junction between MSCs and chondrocytes. However, even at Day 9, there was still a clear separation between the chondrocyte and MSC layers (Figure 1D through 1G). The average width and height of our organoids (N=3, daily) were 525µm x 393µm on Day 1, reflecting an ovoid shape immediately following the combination of chondrocyte and MSC spheroids. By Days 6-9, the organoids became more compact and spherical, and both width and height equalized in size to between 300µm- 350µm (Figure 2). For cell viability, on Day 1, there were few dead cells in both control and treatment groups (N=3 for each group, on all days studied). By Day 3, the average proportion of dead cells to total cells in the IL-1β treatment group increased to 0.11, while nearly none of the cells had died in the control group, although this difference was not significant. By Day 6, the average proportion of dead cells to total cells in the treatment was 0.55, which was significantly higher than in the control group, where the ratio was less than 0.01 (p=0.02, Figure 3).

DISCUSSION: Our multicellular OA organoid reproduced key structural features of the osteochondral unit and demonstrated IL-1β–induced inflammatory injury, as evidenced by progressive, significant reductions in cell viability. The preservation of a layered organization, together with measurable inflammatory responses, supports the utility of this platform for the investigation of the pathophysiology of OA and for examining the effects of drug interventions in a 3D human-based system.

SIGNIFICANCE/CLINICAL RELEVANCE: This OA organoid model provides a physiologically relevant platform for studying the disease mechanisms of OA with potential applications in precision medicine and high-throughput drug screening.

ACKNOWLEDGEMENTS: This work was supported by NIH grants UG3TR002136, R01 AR063713, R01 AR073145, and the Ellenburg Chair in Surgery at Stanford University.

REFERENCES: 1. Chen D, Shen J, Zhao W, et al. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res*. 2017
2. Zeng D, Chen Y, Liao Z, et al. Cartilage organoids and osteoarthritis research: a narrative review. *Front Bioeng Biotechnol*. 2023
3. Zhao Z, Chen X, Dowbaj AM, et al. Organoids. *Nat Rev Methods Primers*. 2022

IMAGES AND TABLES:

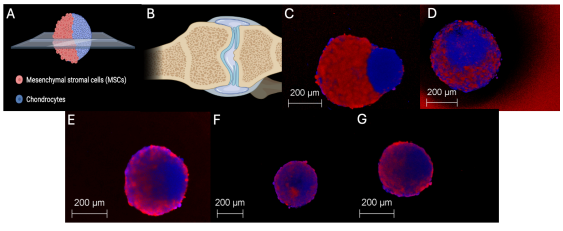

Figure 1: A) "Ideal" OA organoid and depiction of cross-section taken using confocal microscope. All images are viewed from inside the organoid, looking upwards. MSCs are stained red, and chondrocytes are stained blue. B) Normal knee joint, serving as an anatomical model for our OA organoid. Created using BioRender. C) through F): Cell distribution on Days 1, 3, 5, 7, and 9, respectively.

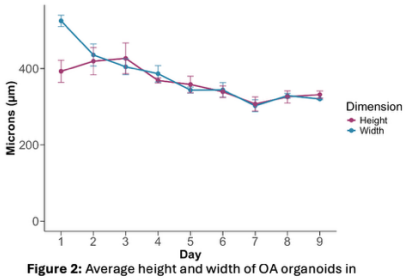

Figure 2: Average height and width of OA organoids in micrometers, by day

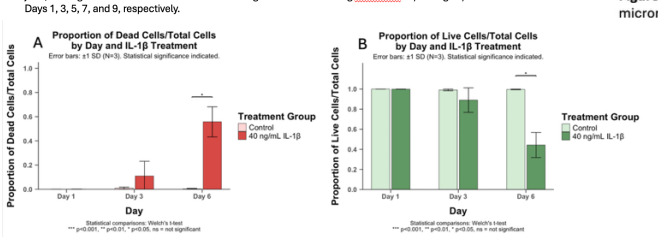

Figure 3: A) Average Proportion of Dead Cells/Total Cells by Day and Treatment with 40 ng/mL IL-1β B) Average Proportion of Live Cells/Total Cells by Day and Treatment with 40 ng/mL IL-1β