

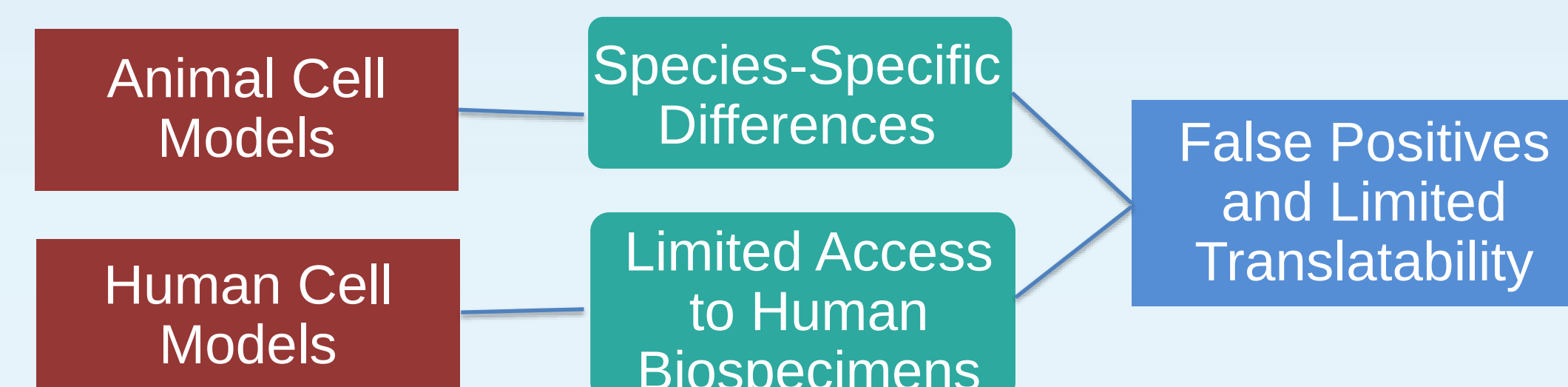
Advancing Military Health Research: Acute, traumatic injury to joints causes mechanical damage to cartilage as well as an inflammatory response associated with the synovium lining the joint, which can lead to post-traumatic osteoarthritis (PTOA), a painful chronic condition. The incidence of PTOA is more prevalent in military troops than the civilian population, due to the increased repetitive and load bearing activities on the joints that troops endure. The current treatments for OA/PTOA deal mostly with the immediate alleviation of pain or, ultimately, joint replacement procedures, rather than the regeneration of healthy cartilage or prevention of tissue degeneration. More effective experimental approaches and model systems are needed to advance the field.

Key Objectives:

1. Identify challenges for preclinical investigators developing and validating new PTOA therapies
2. Describe the advantages of a human co-culture system to replicate the damage associated with PTOA
3. Recommend an experimental approach suitable to develop and validate PTOA therapies



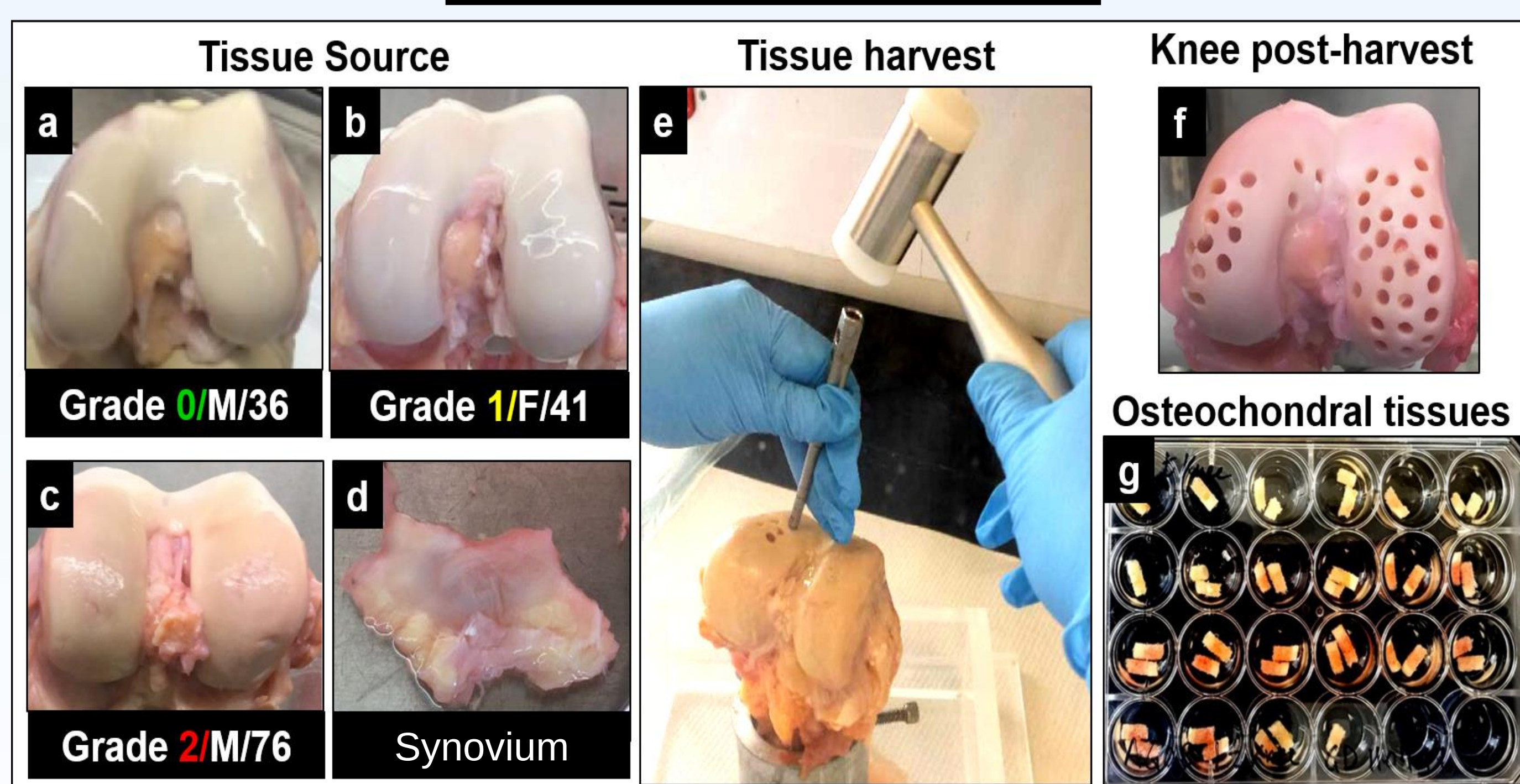
Challenges Associated with Translational Research



Evaluation of Experimental Models for PTOA Research: The evaluation of effective therapies requires validation in human cells/tissues. While animal models provide an easily accessible model system for investigators, species-specific functional properties of animal versus human cells can negatively impact advances in joint regeneration.

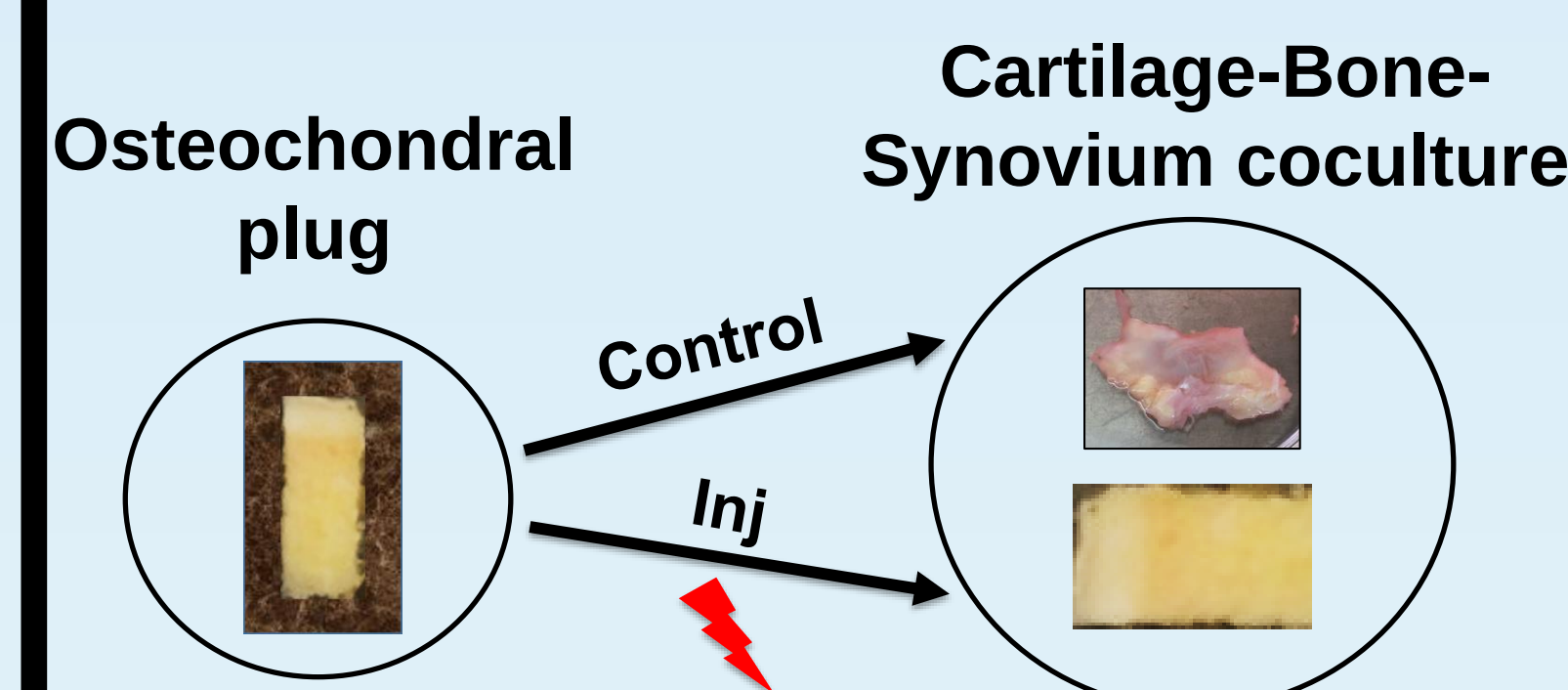
A Human Cartilage-Bone-Synovium Co-culture Model to Simulate Acute Joint Injury

Model System Methods

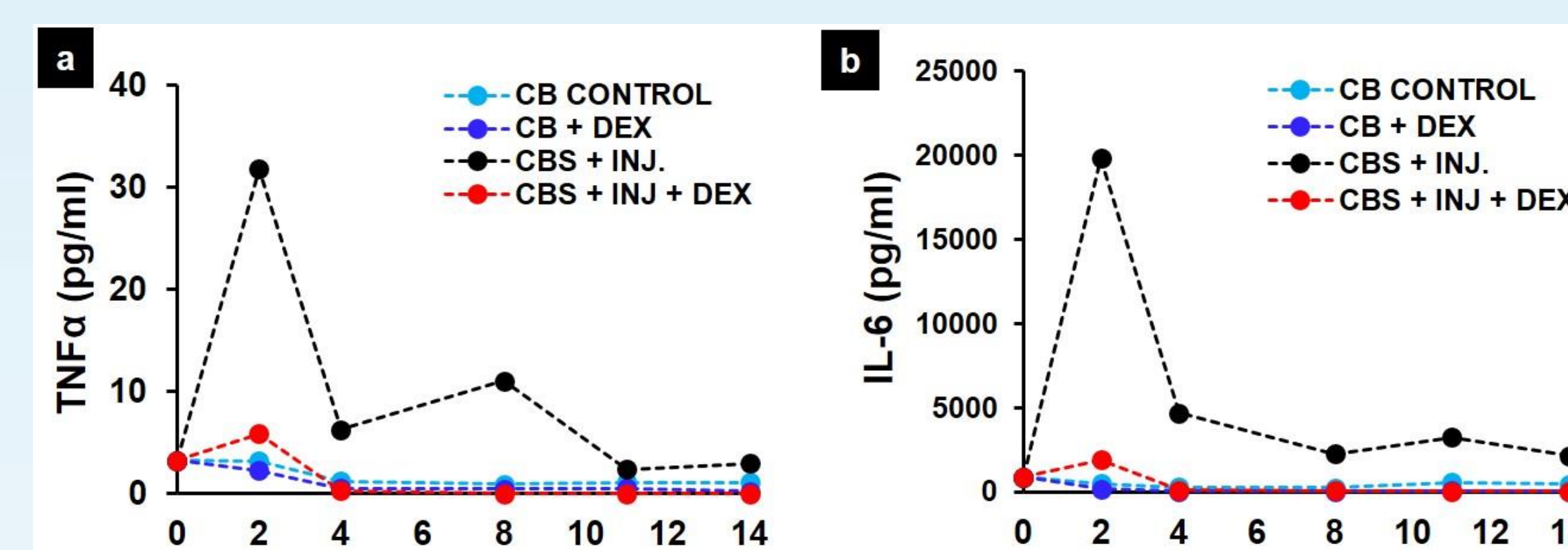


Cadaveric human distal femurs serve as a viable source of cells for experimental analysis: (a-d) Human distal femurs and synovium were recovered from cadaveric tissue donors within 16 hours post mortem in 10% FBS/DMEM with antibiotics. Cartilage was graded to assess degree of cartilage degeneration using the Collins scale. (e-g) Osteochondral plugs (3.5mm diameter and 4mm thick, including 1.5-2mm thick cartilage) harvested from the condyles and trochlear grooves of the distal femurs (n=8 knees, Collins grade 1/2, 64-82 yr) were co-cultured with 4-5mm diameter synovium explants harvested from the synovial joint capsule of the same knees.

Human CBS-MPS Replicates Damage Associated with PTOA

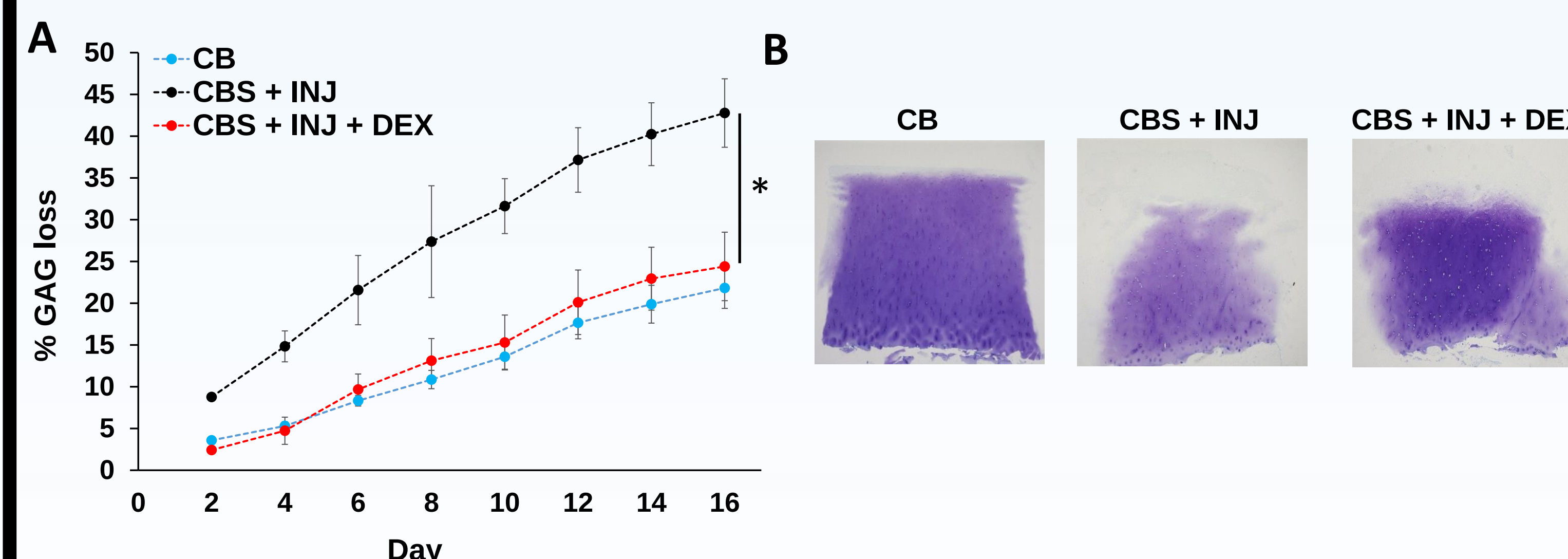
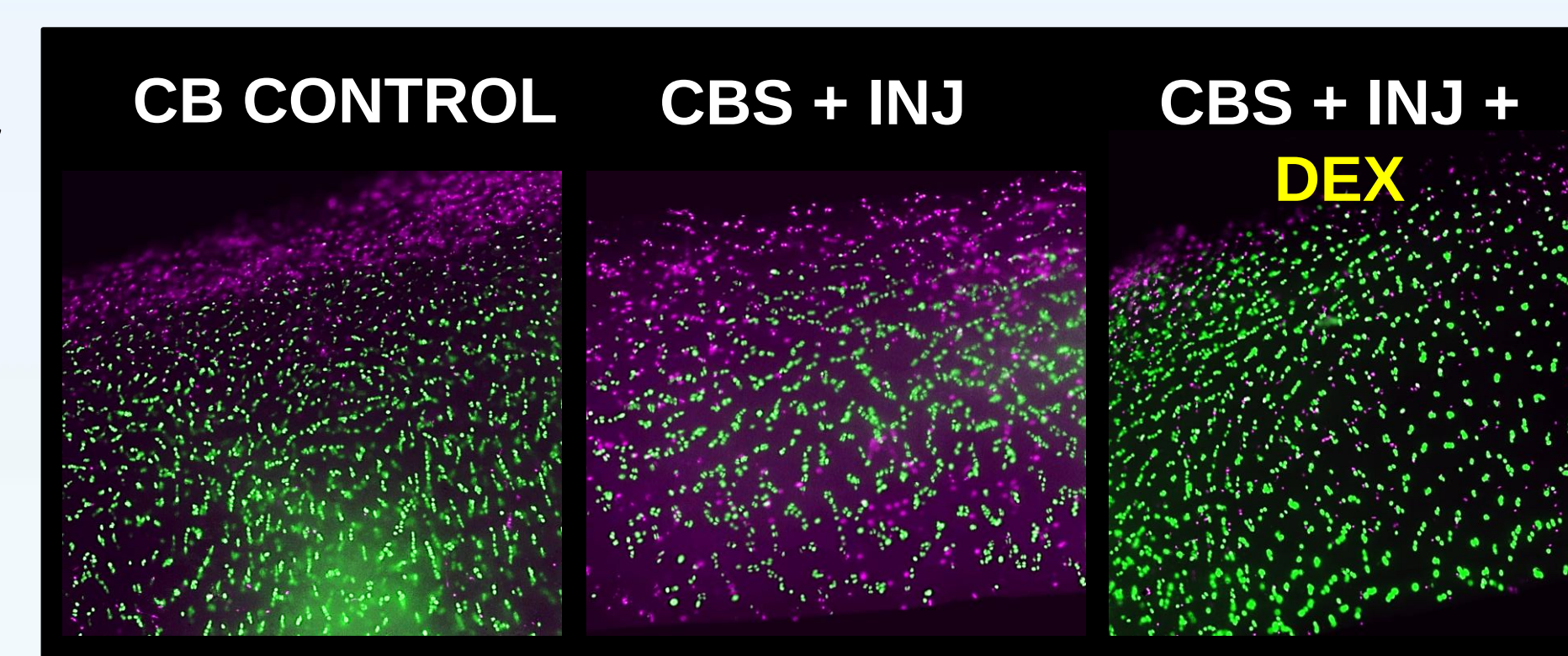


Cartilage-Bone-Synovium co-culture model (CBS-MPS) simulates acute joint injury: PTOA-like mechanical trauma was simulated by subjecting the cartilage surface of the osteochondral plugs to a single injurious unconstrained compression impact of 60% strain rate to a peak stress of 5MPa using an incubator-housed loading apparatus. Control or injured osteochondral plugs were cultured with synovium explants.



CBS-MPS subjected to injury induces pro-inflammatory cytokine release by synovium: Levels of TNFα (a) and IL-6 (b) were measured in control and injured. Both pro-inflammatory cytokines were elevated acutely within the first week following injury, which was blocked by treatment with 100nM Dex.

CBS-MPS subjected to injury exhibit chondrocyte cell death: In the cultures subjected to injury, cartilage tissue showed widespread cell death. This effect was rescued by treatment with Dex.



CBS-MPS subjected to injury exhibit glycosaminoglycan (GAG) loss: Biochemical analysis showed aggravated release of the matrix component, GAG, into the medium as shown by an DMMB assay (A). Addition of Dex significantly reduced the injury-induced GAG loss by week 2. Reduction in toluidine blue staining in injured cartilage indicates the GAG loss is associated with cartilage degeneration (B), which was blocked by Dex.

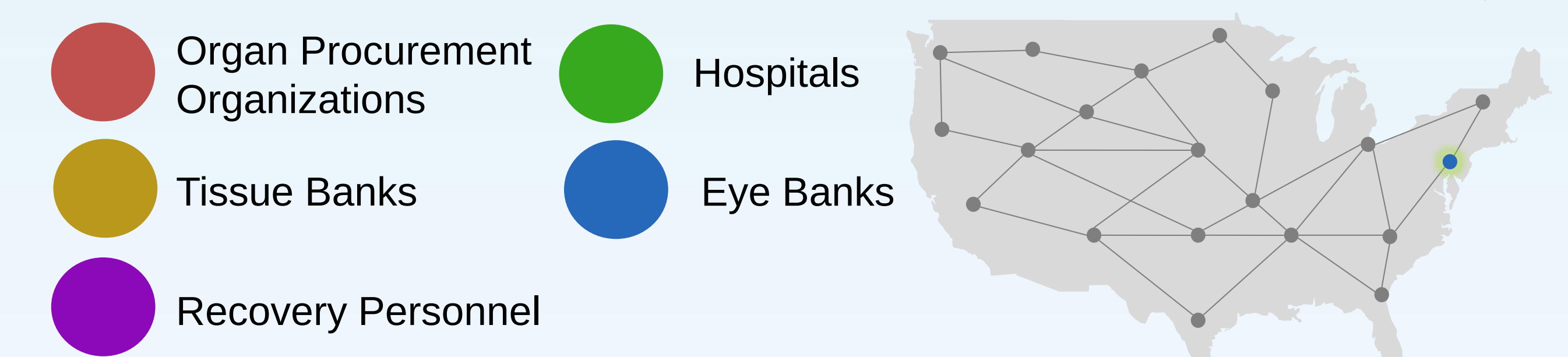
Advancing PTOA Therapeutic Research Requires Human Biospecimens

Summary

- **Human CBS-MPS Subjected to Mechanical Injury Exhibit PTOA-Like Damage**
 - Pro-inflammatory cytokines are released by synovium
 - Chondrocytes exhibit cell death
 - Aggravated release of extracellular matrix
- **Dexamethasone Reverses Damage in Human CBS-MPS, Highlighting the Effectiveness of Reducing Inflammation**

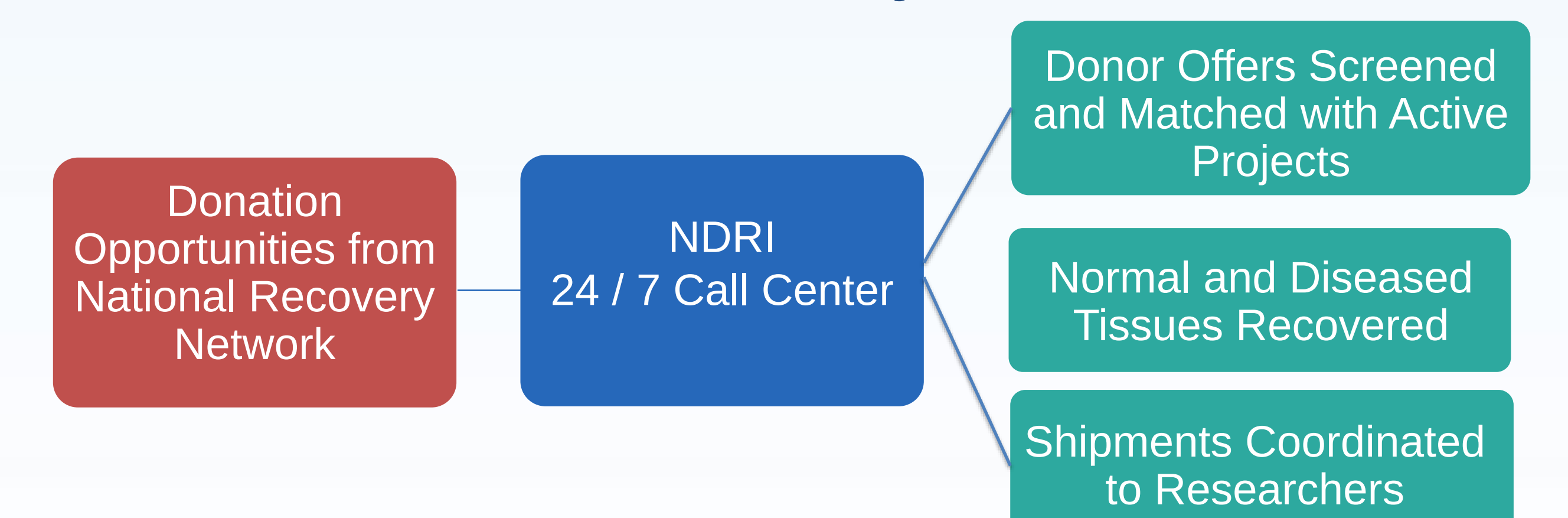
Conclusion: Here we demonstrate the proof-of-concept of using human joint explant cultures to accelerate the development of more effective therapies focused on repair of the injured tissue. These results show the value of including cartilage, bone, and synovium to a culture model system in order to accurately reflect both the direct tissue damage and inflammation associated with the injury

NDRI Provides Superior Access to Human Biospecimens



NDRI's Recovery Network Enables Biospecimen Diversity: The Centers for Medicare and Medicaid Services (CMS) mandate that US medical centers refer all acute deaths to organ, tissue, and eye donation organizations. NDRI partners with this nationwide network to screen donors for biomedical research use, allowing for the authorization and collection of tissues from individuals that match authorized research donors to specific research protocols.

NDRI: A Valued Partner for Military Medical Research



Donation to Discovery: NDRI receives biospecimen donor profiles from deceased, living, and pre-registered research donors. Our 24/7 fulfillment staff matches potential donors with active researcher protocols to allow NDRI to place multiple tissues with a wide range of investigators. This approach yields highly suitable biospecimens for accelerating the development of new medical therapies and treatments for wounded service members and civilians.