Manganese Dioxide as a chondroprotective nanozyme for osteoarthritis

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INTRODUCTION: Cartilage damage is a hallmark characteristic of osteoarthritis (OA) that can be caused or made worse by oxidative stress, which plays a central role in OA initiation and progression. Traditional treatments for oxidative stress have included antioxidant enzymes or small molecules that target reactive oxygen species (ROS) in the joint, but they have had limited clinical success due to poor stability and poor bioavailability to the joint. Intraarticular delivery of most therapies leads to rapid clearance from the joint. Cartilage targeting is a challenge for therapeutics due to the dense, charged extracellular matrix (ECM) that limits uptake before drug clearance occurs. To address the limitations of delivering conventional antioxidants to OA joints, we have designed manganese dioxide nanoparticles (MnO_2 NPs) with properties favorable for cartilage localization and retention. MnO_2 NPs scavenge ROS by catalyzing the breakdown of hydrogen peroxide into water and oxygen, functioning like antioxidant enzymes found in the joint. We have previously shown that MnO_2 NPs are retained in the joint space in a non-invasive knee injury model for over a week. Our in vitro studies also demonstrated MnO_2 protect bovine cartilage from characteristic breakdown in OA. The objectives of this work are to evaluate the effects of MnO_2 NPs on human OA cartilage and understand MnO_2 biodistribution and retention in a surgical model of OA.

METHODS: MnO_2 NPs were synthesized following previously reported methods[1]. Cartilage explants (1-2mm) were collected from patients undergoing total knee arthroplasty. Samples were washed in PBS + Pen/Strep and maintained in standard culture conditions (37°C, 5% CO_2) for up to 14 days. Cartilage explants were given a single dose of MnO_2 (20 ug/ml) and necessary groups were treated with 10 ng/ml IL1B. Media was collected and changed every 2-3 days and analyzed for nitric oxide (NO) release and GAG content. Explants were fixed in 10% neutral buffered formalin and stained with Safranin O Fast Green. Explant viability and MnO_2 penetration were evaluated via confocal microscopy. For joint retention and biodistribution, OA was induced in male Lewis rats (n = 14) via medial meniscus transection (MMT). Each joint (OA and control) was injected with a single dose of MnO_2 NPs (10 mg/ml) 3 days after the surgery. The joints were imaged daily for 14 days using an In Vivo Imaging System (IVIS) to track MnO_2 retention. Following the endpoint, biodistribution imaging was done on the joint tissues (femoral head, tibial plateau, extensor mechanism, meniscus, and tendons) (n = 8). Additional samples were used for histological analysis of the whole joint to visualize MnO_2 retention in articular surfaces. Statistical analysis was conducted on GraphPad PRISM 10.2 whereby error bars indicate standard deviations and statistical comparison of means was conducted in GraphPad *via* a Dunnett's test or one-way ANOVA with Tukey's multiple comparisons tests.

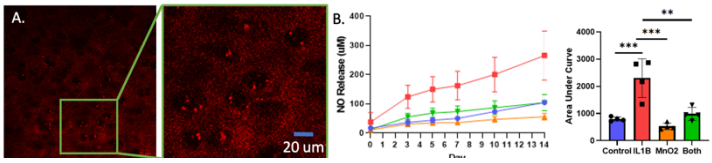

Fig 1. A) MnO_2 NPs (red) in cartilage explants are visible on the surface of human OA cartilage and localize in chondrocytes after 24hr of co-incubation. B) NO release from cartilage explants is decreased in the presence of MnO_2 NPs following a co-incubation with IL1B. NO release is lowest in control samples treated with MnO_2 NPs, indicating a chondroprotective affect for in diseased cartilage. ** $p<0.01$, *** $p<0.005$

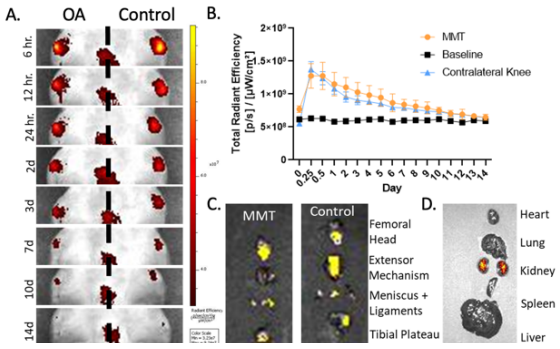

Fig 2. A) IVIS imaging of MnO_2 (10 mg/ml) in OA and control joints following a single B) Total radiant efficiency for MnO2 retention is the same in OA and control joints. C) MnO_2 is retained on cartilaginous surfaces of OA and control joints, and on the extensor mechanism of control joints. D) MnO_2 is only present in the kidneys but is not visible in other organs.

RESULTS: MnO_2 (20 ug/ml) penetrated through the ECM of human OA cartilage explants (1mm) and localize to chondrocytes within the tissue, as evidenced by punctate appearance of MnO_2 within the cross cartilage cross section (Fig 1A). Cartilage explants exposed to MnO_2 + IL1B (20 ug/ml, 10 ng/ml) had decreased nitric oxide (NO) release compared to samples treated with IL1B ($P<0.01$). Similarly, samples exposed to MnO_2 decreased NO release compared to control samples, however this difference was not statistically significant for all donors (Fig 1B). MnO_2 did not affect cartilage viability. Following the surgical induction of OA via MMT, there was no statistically significant difference in retention between the OA and the control joint (Fig 2A-B). Both joints had visible retention for at least 7 days post injection. MnO_2 was retained on the articular surfaces of the OA joint and could also be visualized in the extensor mechanism of the control joint (Fig 2C) There was minimal MnO_2 presence in the kidneys, which was expected due to clearance of the NPs through the joint space (Fig 2D).

DISCUSSION: Poor retention and poor bioavailability are hallmark limitations of small molecule therapies targeted at cartilage protection. Our results indicate that MnO_2 NPs integrate with cartilage explants and can be retained within a viable human cartilage explant for up to two weeks. It is expected that the MnO_2 NPs are taken up by chondrocytes along the superficial and middle layers of cartilage. In an *ex vivo* system of OA cartilage, the MnO_2 NPs significantly decreased the production of NO over two weeks in a system with exogenous IL1B added every 48-72 hours. There was a similar, though not significant, response between the control OA cartilage samples and those treated with only MnO_2, indicating that MnO_2 had a protective response to already-diseased cartilage samples. This response further supports the use of MnO2 NPs as a chondroprotective therapy for later stages of OA. I*n vivo* tracking of MnO_2 indicates that the NPs can be retained within a healthy or control joint for at least seven days before the fluorescent signal is no longer visible (Fig 2A). Most conventional antioxidant therapies delivered via intraarticular injection are cleared within a few hours. As such, the sustained retention is promising for the long-term effects of this therapy. MnO_2 NPs were visible on the articular surfaces of both the control and injured joints, and there was no pooling of the NPs visible around the surgical incision. This indicates that the cartilage targeting properties of this therapy were effective in supporting particle retention.

SIGNIFICANCE/CLINICAL RELEVANCE: Chondroprotection is an important target outcome for most OA-specific treatments, this work advances our understanding of MnO_2 NPs as a possible chondroprotective therapy. Prolonged retention in the joint space following surgical induction of OA is promising for use of MnO_2 as a treatment following surgery. Additionally, chondroprotective effects of MnO_2 observed on human OA samples are a step forward in moving toward translational applications.

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REFERENCES: [1] Kumar, S., et al. 2019.