Characterization of Antioxidant MnPBuOE Encapsulated in Chondroitin Sulfate-A Microparticles for Long-term ROS Scavenging

Kayla Ney, Fei San Lee, Rebecca A. Wachs
University of Nebraska Lincoln, Department of Biological Systems Engineering

Purpose: Low back pain is a leading cause of disability worldwide (1). Current treatments for chronic pain do not directly address the cause of pain. Pharmaceutical treatments and corrective surgeries target the symptoms, not the pathways that initiate and perpetuate pain, further, these treatments can have harmful side effects (2,3). There is a need for treatments that target the source of pain. Studies have indicated that excess reactive oxygen species (ROS) play a role in degenerative diseases, including intervertebral disc disease, arthritis, cardiovascular disease, and more (4,5,6). ROS cause cellular damage and trigger inflammatory pathways (4,7). This creates a cycle of degradation and damage and can lead to pain (8). Antioxidants are being investigated to eliminate excess ROS and stop this cycle of degeneration (9). Systemically delivered antioxidants have limited efficacy due to the rapid breakdown of antioxidant and poor delivery to target tissues, and can have off-target effects (10,11). **To create an effective long-term treatment for pain, we propose encapsulating a long-lasting antioxidant in a microparticle treatment that can be delivered via injection and retained at the site of injury.** We are investigating manganese porphyrin (MnTnBuOE-2-PyP^{5+}, MnP), a SOD mimic, which can scavenge ROS, including superoxide (9). To retain the MnP at the site of degeneration and to shield the drug, chondroitin sulfate (CS), a natural biomaterial, is used to encapsulate the MnP. Here we outline the fabrication, antioxidant activity, and in vitro cytocompatibility of our antioxidant microparticle treatment.

Methods: Microparticles are fabricated via a water in oil emulsion (12). Briefly, methacrylated CS is suspended at 126.4 mg/mL in PBS with 3 mg of MnP. After dissolving, crosslinking agents are added, then the solution is added drop wise to corn oil and homogenized for 5 minutes at 2000 rpm. The resulting solution is purged with N_2 and thermally polymerized for 30 minutes. Particles are then stained with a dye that stains CS, and size was quantified. Morphology was assessed long-term over 120 days. To test the ability of our treatment to scavenge ROS long term, a superoxide dismutase (SOD) assay (BioVision K335-100) is being conducted every 4 weeks for 12 weeks. Previous SOD assays were saturated, therefore the microparticle treatment was diluted below 100% scavenging capacity so that any change in scavenging ability over time could be observed. On test days, four batches of MnP microparticles are challenged with superoxide and superoxide scavenging capacity is quantified. Between timepoints, the treatments are kept at physiological pH 7.38 in 1X phosphate buffered saline at 37ºC in an oven. Cytocompatibility of antioxidant microparticles is being assessed by culture with human nucleus pulposus (NP) cells. Alamar blue and LDH assays (Thermo Scientific 88951, Thermo Scientific C20301) are being measured over three days after NP cells are treated with plain media, or media with empty or MnP microparticles at a concentration of 100 µg CS/mL (0.5 µg MnP/mL). The assays are conducted using three separate NP cell donors and using six total batches of microparticles- three containing MnP and three that did not contain the antioxidant.

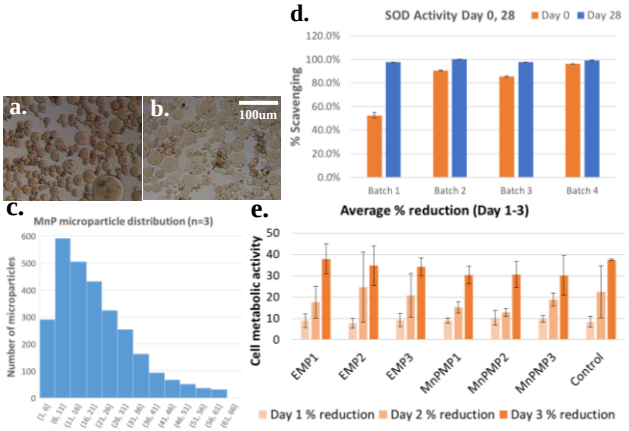

Fig 1: a) MnP microparticles on day 0 and b) day 120 to assess morphology over time (scale bar=100um). c) Size quantification of 3 MnP microparticle batches. d) Superoxide scavenging activity from day 0 and day 28. e) Cell metabolic activity measured by Alamar blue.

Results: MnP Microparticles maintained spherical morphology for the duration of the 120-day observation period (Fig 1a,b). The majority of particles created are 6-11 µm in diameter (Fig 1c). From day 0 to day 28 of the SOD activity assay, there has not been a decrease in scavenging activity (Fig 1d). Preliminary data shows that control and treated cells have similar metabolic activity, indicating the microparticle treatment is not cytotoxic to human NP cells (Fig 1e). Work is currently underway to complete the 12-week SOD assay and to further assess cytocompatibility.

Conclusions: Preliminary data shows that the microparticle treatment is structurally stable, capable of scavenging for weeks, and biocompatible. Our future work will include assessment of the treatment in vitro in an oxidative stress environment with NP cells to evaluate the treatment's ability to affect cells in a degradative environment. An animal study will be conducted using the LBP model developed by our lab to assess the treatment on pain.

References:

(1) Vos T. The Lancet. 2016;388:1545-1602. (2) Singh G. J Rheumatol. 1999;56:18-24 (3) Wang H. Medicine. 2017:96 (4) Suzuki S. Arthritis Res Ther. 2015;17:1-17 (5)Yudoh K. Arthritis Res Ther. 2005;7:1-12 (6) Brinkley TE. Hypertension. 2009;53:846-852 (7) Feng C. Oxid Med Cell Longev 2017. 2017. (8) Zheng J. Oxid Med Cell Longev 2021. 2021 (9) Schlichte SL. Redox Biology 2020;101610. (10) Kartha S. Adv Healthc Mater. 2017;6:170050 (11) Schlichte SL. The FASEB Journal. 2020;34:1. (12) Lim JJ. Acta Biomater. 2011;7:986-995.