Detection of Elevated CTXII Levels within the Knee Joint of a Rat Monoiodoacetate OA Model using a Novel Magnetic Capture Technique

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Introduction: Analysis of OA biomarkers in human urine and serum has demonstrated potential to diagnose osteoarthritis (OA) at an earlier stage than traditional radiographic scans. Moreover, recent studies in horses suggest biomarker analysis in synovial fluid may actually detect OA prior to the serum and urine analysis of the same biomarker. However, biomarker analysis in small joints is technologically limited by an inability to aspirate sufficient synovial fluid volumes, including small animal models of knee OA, limiting our ability to test and develop new, powerful OA biomarkers through small animal OA models.

To address this limitation, our group has developed a magnetic nanoparticle-based technology to collect OA biomarkers from synovial fluid (Figure 1). First, a targeting molecule for an OA biomarker is conjugated to the surface of magnetic particles. These functionalized particles are injected into a joint to bind biomarkers in the joint space. A magnetic probe is then used to collect a percentage of the particle-biomarker conjugate. Following removal from the joint, biomarker is released from particles, followed by release of the particles from the probe. By quantifying biomarker collected per particle, the amount of biomarker in the joint space can be estimated as long as particles are not saturated by the biomarker. The purpose of this study is to demonstrate the utility of this technology to detect elevated levels of an OA biomarker in a rat monoiodoacetate (MIA) model of knee OA.

Methods: Antibodies against the C-terminus telopeptide of type II collagen (CTXII, Immunodiagnostic Systems) were conjugated to 1 μm diameter polystyrene particles containing 10-20 nm diameter superparamagnetic iron oxide nanoparticles (SPIONS) within the particle core. To demonstrate the technique in vitro, 1.9 billion anti-CTXII particles were mixed with 300 µL of either hyaluronidase-treated or natural bovine synovial fluid. Samples were incubated for 1 hr, then divided into 25 µL samples. Cylindrically shaped NdFeB magnetic probes (1 mm diameter by 1 mm length) were inserted in natural synovial fluid for 30 s, 1, 2, 4, 8, 15, 30, 60, or 120 min or in hyaluronidase-treated synovial fluid for 0.5, 1, 2, 4, 8, 15, 30, 60, or 120 s, then removed. Following particle collection, CTXII was released using 3 min of 85°C heat. Particles collected by the probe were released using mechanical agitation and sonication. Both particles collected and particles remaining in synovial fluid were quantified via fluorescence. CTXII was quantified using a CTXII ELISA (Immunodiagnostic Systems).

To demonstrate the technique in a small animal model, twelve Sprague-Dawley rats were first euthanized and then received an intra-articular injection of 180, 360, or 720 million anti-CTXII particles (n=4 per groups). Particles were allowed to incubate in the joint for at least 2 hrs, then a 16 gauge catheter was then inserted into the femoral groove. A magnetic probe was inserted into the joint space through the catheter, and particles were collected on the probe for 10 minutes. The probe was removed and processed as described above. Following this feasibility experiment, eight Sprague-Dawley rats received an intra-articular injection of MIA (3 mg in 25 µL of saline) with OA allowed to develop over 4
weeks; an additional 8 age-matched animals were acquired as naïve controls. All animals were first humanely euthanized and serum was also collected for biomarker analysis. Then, 720 million anti-CTXII particles were then injected into the knee joint, with particles incubated, collected and processed as described above. All studies were conducted under IACUC-approved protocols at the University of Florida.

**Results:** Time to collect magnetic particles on an NdFeB magnet was strongly influenced by synovial fluid viscosity (Figure 2 left, compare white and black shapes), where magnetic collection was much faster in hyaluronidase-treated synovial fluid (white) relative to natural synovial fluid (black). More importantly, the percentage of particles collected (black squares) compares favorably with the percentage of CTXII collected (white squares) at each collection time (Figure 2 center). Using the ratio of CTXII to particles, the amount of CTXII in the sample can be accurately predicted, as long as sufficient material is collected on the probe (Figure 2 right). Extending the technology to the rat knee, different concentrations of anti-CTXII particles were injected into naïve rat knee joints, then collected on a magnetic probe. Following processing, the predicted amount of biomarker in the joint space was similar regardless of the amount of particles injected into the joint, demonstrating that, under proper conditions, magnetic capture can yield a stable prediction of biomarker load in a rat joint (Figure 3 left). Magnetic capture was also used to assay the amount of CTXII in rat knee OA model. Elevated levels of CTXII were detected in OA-affected joints, significantly higher than both contralateral knee and naïve controls (Figure 3 center). Moreover, assessments of CTXII in the serum of these same animals did not yield statistically significant difference between MIA-affected and naïve animals (Figure 3 right). These data demonstrate the utility of biomarker analysis within the OA-affected knee and magnetic captures ability to facilitate these analyses.

**Discussion:** These data demonstrate the ability to magnetically collect and assess biomarkers from small volumes of synovial fluid, both in vitro and within a rat knee joint. The critical variable in magnetic capture is biomarker per particle, where it is essential that particles are not saturated with biomarker. Under these conditions, the ratio of biomarker per particle along with information about the particle binding kinetics can be used to estimate the total amount of biomarker within the joint. This prediction is not affected by synovial fluid viscosity, and while it should be noted that the final measure for magnetic concentration is total biomarker and not biomarker concentration, total biomarker is preferred relative to concentration in most instances, since total biomarker in the joint space is not affected by joint effusion.

OA diagnosis may require the assessment of multiple biomarkers, and while assessment of multiple biomarkers was not conducted in this experiment, magnetic capture could be adapted for multiplex analysis. The purpose of this study was to demonstrate the utility of magnetic capture for biomarker analysis in a rat knee, and the detection of a single biomarker was advantageous for experiment design and planning. Similarly, while biomarker collection was done post-euthanasia in this study, magnetic capture can be adapted for in vivo use and we are currently developing the technology for these purposes. However, prior to the development of magnetic capture for in vivo use, it was ethically essential to demonstrate feasibility of magnetic collection in the unique geometry and environment of the rat knee. These data provide these critical proof-of-concept work for the development of magnetic capture as a method to assess joint-level biomarker in vivo.
**Significance:** A novel magnetic particle technology for the assessment of biomarkers (magnetic capture) can be used to detect elevated levels of an OA-biomarker, CTXII, in the knee joints of the rat MIA model of OA.
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