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Magnetic Particle Translation as a Surrogate Measure for Synovial Fluid Mechanics

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Abstract

The mechanics of synovial fluid vary with disease progression, but are difficult to quantify quickly in a clinical setting due to small sample volumes. In this study, a novel technique to measure synovial fluid mechanics using magnetic nanoparticles is introduced. Briefly, microspheres embedded with superparamagnetic iron oxide nanoparticles, termed magnetic particles, are distributed through a 100 μ L synovial fluid sample. Then, a permanent magnet inside a protective sheath is inserted into the synovial fluid sample. Magnetic particles translate toward the permanent magnet and the percentage of magnetic particles collected by the magnet in a given time can be related to synovial fluid viscosity. To validate this relationship, magnetic particle translation was demonstrated in three phases. First, magnetic particle translation was assessed in glycerol solutions with known viscosities, demonstrating that as fluid viscosity increased, magnetic particle translation decreased. Next, the relationship between magnetic particle translation and synovial fluid viscosity was assessed using bovine synovial fluid that was progressively degenerated via ultrasonication. Here, particle collection in a given amount of time increased as fluid degenerated, demonstrating that the relationship between particle collection and fluid mechanics holds in non-Newtonian synovial fluid. Finally, magnetic particle translation was used to assess differences between healthy and OA affected joints in equine synovial fluid. Here, particle collection in a given time was higher in OA joints relative to healthy horses ($p < 0.001$). Combined, these data

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Author Contributions: YYS, LM, and NSP collected all the data in this study. The experiments were designed by YYS and EGY, under the guidance and mentorship of CR, JD, and KDA. AHB collected all equine samples of synovial fluid and provided clinical expertise. YYS and KDA analyzed the data and drafted the manuscript. All authors assisted in the editing of the manuscript. Additionally, all authors have approved the final version of the manuscript and figures.

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demonstrate potential viability of magnetic particle translation in a clinical setting to evaluate synovial fluid mechanics in limited volumes of synovial fluid sample.

Keywords

Synovial Fluid; Magnetic Nanoparticles; Superparamagnetic Iron Oxide; Viscosity; Osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative joint disease affecting entire articular joints. As a result, the joint experiences a continuum of physiological changes across the cartilage, bone, ligaments, and synovium (Buckland-Wright 1999). In the clinic, OA is typically diagnosed using radiographic techniques. However, these methods tend to focus on structural changes in bone and cartilage and have limited ability to assess molecular or mechanical changes within the joint. As such, there is a need of new techniques to quantify and diagnose functional changes in the entire joint, beyond cartilage and bone assessments.

Synovial fluid viscosity may act as a mechanical biomarker for OA. Correlative studies between synovial fluid viscosity and OA progression were carried out as early as the 1920s (Schneider 1925). In the 1950s, Ogston and Stanier further investigated the correlation of synovial fluid viscosity to OA (Ogston and Stanier 1950; 1951; 1953). These studies were technologically limited by the rudimentary viscometers of the 1950s, which were unable to account for the non-Newtonian behavior of synovial fluid. Modern rheometers can account for the non-Newtonian behavior exhibited by synovial fluid, however such assessment typically requires sample volumes over 10 mL (Jeong et al. 2016). Unfortunately, obtaining this volume of synovial fluid is impractical for human joints and most preclinical animal models. Hence, methods that allow for quick and accurate quantification of synovial fluid mechanics with smaller sample volumes than traditional rheology may facilitate the development of mechanical biomarkers of joint disease.

Magnetic particle translation, a novel technique presented in this work, can be used to quantify synovial fluid degradation by measuring the translational motion of magnetic particles through synovial fluid. Superparamagnetic iron oxide nanoparticles are embedded in a polystyrene microsphere, hereafter referred to as magnetic particles. In the absence of a magnetic field, these magnetic particles can be dispersed throughout the sample with minimal aggregation. However, in the presence of an external magnetic field, particles become magnetized and experience a translational force toward the magnetic field source due to the field gradient. Our prior work predicts changes in magnetic particle collection rates in glycerol as a function of viscosity and time (Garraud et al. 2016). While our prior work assumes the fluid is Newtonian, it is reasonable that an empirical relationship can still be constructed that differentiates non-Newtonian synovial fluids at various degrees of degradation.

In the present work, the possibility of applying the framework of Garraud et al.'s multiphysics simulations to construct empirical correlations between particle capture and fluid mechanical properties is explored in three separate but related studies. In the first study,

magnetic particle translation is examined in glycerol:water solutions to confirm the predictions of Garraud et al. 2016. In the second study, changes in magnetic particle translation due to synovial fluid breakdown are examined using bovine synovial fluid degenerated via serial ultrasonication. In the third study, the clinical relevance of magnetic particle translation is examined using equine synovial fluid acquired from healthy and OA-affected joints. Together, these data confirm the relationship between magnetic particle translation and fluid viscosity, demonstrate the sensitivity of the technique for measuring HA breakdown in synovial fluid despite its non-Newtonian fluid behavior, and provide clinical evidence of the technique's potential for detection of disease-related mechanical changes in synovial fluid.

Experimental Methods

Magnetic Particle Translation Technique

A conceptual schematic for magnetic particle translation measurements in synovial fluid is presented in Figure 1. First, magnetic particles are thoroughly mixed in each sample (Figure 1A); in our studies, particle concentration was 2 mg/mL and sample volume was 100 μ L. A collection tool is then placed in the center of each tube using a micromanipulator (Figure 1B); for our studies, this tool contained a 1 mm diameter NdFeB magnet on a soft magnetic rod placed inside a protective catheter sheath. While the tool is submerged, particles translate toward the collection tool and aggregate on the surface of the sheath (Figure 1C). The magnet and sheath are then removed from the sample, and the magnet is removed from the sheath (Figure 1D). The sheath is then placed into a PCR tube containing a release buffer; our buffer consisted of phosphate buffered saline, 0.05% tween-20, and 2% bovine serum albumin. Using an external magnet, particles can be removed from the buffer submerged sheath (Figure 1E); in this study, the sheath was submerged for one hour. Particles are then briefly sonicated, resuspended in buffer (Figure 1F), and quantified via fluorescence intensity or optical density; in our studies, particles were quantified via fluorescence intensity at 530/590 nm in a 5 μ L aliquot using a Synergy 2 Multi-Mode Microplate Reader spectrophotometer. Samples of known particle content were used for calibration.

Magnetic Particles

All experiments described herein used polystyrene microparticles embedded with superparamagnetic iron oxide nanoparticles and a fluorescent dye (Corpuscular, Inc., Cold Spring, NY, USA). The particle iron oxide content was 20% by weight per manufacturer's specification.

To confirm the manufacturer's specifications, particle size distribution was analyzed using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Magnetic properties were analyzed using a superconducting quantum interference device (SQUID) magnetometer. From DLS, the particle diameter was 772 nm with geometric deviation of 0.04 (Figure 2A). From SEM, particles had an average diameter of 936 nm with geometric deviation of 0.08 (Figure 2A). The monodisperse size distribution (Figure 2B), suggested the effects of drag would be similar for particles translating at a similar velocity. Additionally,

according to measurements in the SQUID magnetometer, the remanent magnetization of the particles was near 0 Am²/kg (Figure 2C). The lack of remanent magnetization confirmed the superparamagnetic character of the particles. Finally, the average magnetic diameter of the superparamagnetic iron oxide nanoparticles embedded within the polystyrene matrix was 10 nm.

Assessing Magnetic Particle Translation in Glycerol:Water Solutions

Glycerol was pipetted into a tube of deionized water and vigorously pipetted up and down to mix the solutions. In this manner, 0%, 20%, 40%, 60%, 80%, and 100% aqueous glycerol solutions were produced (Fisher Scientific International, Inc., Pittsburgh, PA, USA). Magnetic particle translation was then evaluated for each glycerol solution in triplicate. The viscosity of each glycerol solution was calculated as described in the literature (Cheng 2008).

Assessing Magnetic Particle Translation After Simulated Degradation of Synovial Fluid

Bovine synovial fluid was purchased commercially (Animal Technologies, Inc., Tyler, TX, USA) and centrifuged at 3000 rpm for 10 minutes to remove cellular debris, fat, and extraneous tissues. While on ice, 200 mL of centrifuged synovial fluid was mechanically degraded using a Qsonica Q500 ultrasonicator (38% amplitude) in 10-second increments. After each increment, a 12 mL aliquot was collected. Using this serial sonication technique, aliquots were generated at time points ranging from 10 seconds to 200 seconds, each with a progressively degraded polysaccharide network (Orvisky 1993). To mimic the extremes of synovial fluid degradation, native synovial fluid and enzymatically degraded synovial fluid were included as controls. For enzymatic degradation, samples were treated with 0.01 mg/mL hyaluronidase for 30 minutes, thereby reducing the synovial fluid viscosity to approximately three times that of water (Nijenhuis et al. 2008) (Type I-S, Sigma-Aldrich Corporation, St. Louis, MO, USA). After treatment, all samples were stored at 4 °C for 1-7 days. Synovial fluid viscosity was then assessed using magnetic particle translation, as described previously, with the magnetic collection tool submerged in synovial fluid samples for 5 minutes.

In addition to magnetic particle translation measurements, degradation of synovial fluid due to ultrasonication was confirmed via rheology. Here, synovial fluid viscosity was measured using an Anton Paar Physica MCR-301 series Rheometer (Anton Paar, Ashland, VA, USA) with a cup and bob geometry using the remaining 8 mL of synovial fluid from each sonication time point. Furthermore, the shear rate dependent viscosity was measured at 25 °C. At each time point, the viscosity was measured while the shear rate was changed in three cycles: increased from 10 s⁻¹ to 1000 s⁻¹, decreased from 1000 s⁻¹ to 10 s⁻¹, and increased from 10 s⁻¹ to 1000 s⁻¹.

Verifying Viscosity Changes in Clinical Equine Samples

All synovial fluid samples were drawn under IACUC-approved protocols at the University of Florida. In a local veterinary clinic, synovial fluid was drawn from the metatarsophalangeal and metacarpophalangeal joints of 12 horses to assess polyarthropathy. Excess synovial fluid not used by the veterinary clinic was donated for this study. The

investigators of this study had no role in the clinical decision to withdraw synovial fluid. To prevent unnecessary burden to the horse, samples were only drawn from the arthritis diagnosed limb. Arthritis was diagnosed by a board certified equine surgeon via lameness and radiographic assessments.

Control synovial fluid was donated from horses that were euthanized for unrelated medical complications at the University of Florida Large Animal Hospital. The investigators of this study had no role in the decision to euthanize these animals. Veterinary records of control horses stated no history of OA, which was confirmed by a board certified surgeon via lameness examinations and normal radiographic scans. From the control horses, synovial fluid samples were collected from multiple metatarsophalangeal and metacarpophalangeal joints.

For both healthy and OA samples, the viscosity was assessed using magnetic particle translation, as described previously. Samples were measured in triplicate for the control animals and in duplicate for the OA subjects, due to volumetric constraints. The magnetic collection tool was placed in the equine synovial fluid for 3 minutes.

Statistical Analysis

Differences in average magnetic particle collection from OA and healthy equine synovial fluid were assessed using student's t-test, and differences in magnetic particle variability between OA and healthy equine synovial fluid samples were assessed using an F-test. P-values less than 0.05 were accepted as significant for both tests.

Results

Assessing Magnetic Particle Translation in Glycerol:Water Solutions

Magnetic particle translation in glycerol solutions demonstrated an inverse relationship between the percent of particles collected and the viscosity of the solution (Figure 3). Magnetic particle translation followed a logarithmic fit between 0.9 mPa·s and 75 mPa·s ($R^2=0.951$).

Assessing Magnetic Particle Translation After Simulated Degradation of Synovial Fluid

Rheometry confirmed the decrease in bovine synovial fluid viscosity with increasing sonication time (Figure 4A). Additionally, as sonication time increased, the percentage of magnetic particles collected by the probe also increased (Figure 4B). Combining the data from rheometry and magnetic particle translation, a strong correlation can be observed between the percentage of particles collected during magnetic particle translation and synovial fluid viscosity at each shear rate (Figure 5).

Verifying Viscosity Changes in Clinical Equine Samples

The percent of particles collected from healthy metatarsophalangeal and metacarpophalangeal joint samples ranged from 0-14.7%. Particle collection in samples from horses diagnosed with OA ranged from 1-78.2%, indicating synovial fluid from OA samples was less viscous ($p<0.001$) but had high variability ($p<0.001$) (Figure 6). Despite

high inter-sample variability, magnetic particle translation measurements were highly repeatable within a given sample, indicating variability was due to differences between samples and not due to the reproducibility of the measurement.

Discussion

OA is a chronic disease which leads to the destruction of articular joints. The macroscale effects of OA on diseased joints are well known, including the development of cartilage lesions, osteophyte formation, and subchondral bone thickening. However, the underlying molecular changes that drive OA pathogenesis are not yet completely understood (Lorenz and Richter 2006). As such, the quantification of potential biomarkers may produce more sensitive measures of OA severity and progression (Hunter et al. 2014). In this study, magnetic particle translation is presented as a simple technique to assess mechanical changes within the articular joint by evaluating changes in synovial fluid viscosity. Importantly, magnetic particle translation only requires a small sample volume.

In this work, the potential of magnetic particle translation to assess synovial fluid mechanics was tested in three studies. First, the relationship between particle translation and medium viscosity was tested using glycerol:water solutions, a Newtonian fluid with well-characterized rheological behavior. An increase in viscosity resulted in a decrease in particle translation through the fluid in a given amount of time. These data agree with the simulations presented previously, which predicted that an increase in viscosity would result in a decrease in particle collection in a given amount of time (Garraud et al. 2016). Additionally, the percentage of magnetic particles collected in pure water (viscosity = 0.9 mPa·s) was nearly 100%. Conversely, the percentage of magnetic particles collected in 80% glycerol (viscosity = 75.7 mPa·s) was nearly 10%. As a result, a logarithmic calibration curve was demonstrated from 0.9 mPa·s to 75.7 mPa·s. This range encompasses several orders of magnitude and is representative of the physiological range of synovial fluid viscosity (Al-Assaf et al. 1996; Palfrey and Davies 1968; Schurz and Ribitsch 1987).

Magnetic particle translation was assessed in bovine synovial fluid samples degraded via ultrasonication. Here, ultrasonication was used to mechanically break down the hyaluronan network within synovial fluid in a controlled manner (Orvisky 1993). As sonication time increased, synovial fluid viscosity decreased. Hyaluronidase treated synovial fluid and healthy naïve synovial fluid were included as controls; as such, a complete spectrum of physiologic synovial fluid viscosities was examined. By combining data from rheology and magnetic particle translation, an empirical correlation between the percent of particles collected and fluid viscosity could be made.

This study also compared magnetic particle translation to traditional rheological measures of synovial fluid. These properties are most likely related to the hyaluronan network (Cowman 2005; Fam, Bryant, and Kontopoulou 2007; Martin-Alarcon and Schmidt 2016). However, it should be noted that the tribology of synovial fluid is related to lubricative glycoproteins such as lubricin, and, these properties are partially retained even after destruction of the hyaluronan network (Jay and Waller 2014). We did not measure synovial fluid lubrication, and at this point, it is unknown how sonication affects synovial fluid lubrication. Assessing

the relationship between magnetic particle translation and synovial fluid lubrication could be the subject of future studies. Nonetheless, from this study, we were able to demonstrate that the relationship observed using glycerol solutions was maintained in synovial fluid despite its complex non-Newtonian properties.

In the third study, magnetic particle translation was used to quantify the percent of magnetic particles collected in clinical equine samples. The variability of magnetic particle translation (Figure 6) in OA synovial fluid samples reflects the known variability in viscosity of synovial fluid samples reported in the literature [Al-Assaf et al. 1996; Palfrey and Davies 1968; Schurz and Ribitsch 1987]. As such, the wide range of particle collections was to be expected. This observed variability may have been associated, in part, with varying degrees of OA progression in each tested joint. Future studies may use the magnetic particle translation technique to correlate loss of viscosity to various stages of disease pathogenesis.

This study tested synovial fluid drawn from both the metacarpophalangeal and metatarsophalangeal joints. Previous studies have demonstrated the viscosity may differ between different joints from the same animal (Panizza 1931; Davies 1944). However, the disease state has a larger effect on viscosity than viscosity differences between the two differing joints (Figure 6).

Current techniques to examine synovial fluid viscosity from patients in the clinic are often qualitative and highly subjective. The standard practice, known as the “string test”, qualitatively evaluates how a droplet of synovial fluid falls from the end of a syringe onto a glass slide approximately 1-2 inches below the syringe (Brannan and Jerrard 2006). If the fluid leaves a thin strand between the syringe and the slide, the fluid is assumed to have a healthy viscosity. This rudimentary technique is often implemented because it is quick and requires small volumes. Since magnetic translation does not require large volumes and can be conducted within a few minutes, the technique has the potential to be used as a rapid, quantitative clinical test of synovial fluid viscosity. Analyzing these changes can provide additional mechanical assessments of joint health, complementary with traditional radiographs and other emerging biomarkers of joint health.

Tracking magnetic particle translation due to magnetic forces has been used to estimate fluid viscous properties for almost a century. In 1950, Crick and collaborators used this technique to estimate the viscosity of cell cytoplasm [Crick and Hughes 1950]. More recently, magnetic beads have been used to assess the microrheology of complex fluids (MacKintosh and Schmidt 1999; Squires and Mason 2010). These approaches primarily include magnetophoresis, whereby magnetic bead translation caused by a magnetic field gradient is tracked using laser or video microscopy (Amblard et al. 1996; Schmidt et al. 1996; Ziemann et al. 1994). To estimate viscosity from the particle's velocity and applied magnetic force, these magnetophoresis techniques rely on accurate tracking of particle positions in well-characterized magnetic field gradients. The technique presented in this study also relies on a magnetic field gradient to translate magnetic beads across a complex fluid; however, our magnetic particle translation technique does not use specialized instrumentation to make active measurements during particle translation. Instead, the mass of magnetic particles captured by a magnet during a specified period is quantified, which can then be correlated to

fluid viscosity by comparison to standard curves. As such, magnetic particle translation can be developed to make *in situ* measurements without highly specialized equipment. Currently, magnetic particle translation cannot assess synovial fluid as a function of various shear rates, nor can the technique provide more sensitive or precise measurements than current microrheology methods. However, at this stage of development, it is not clear whether this level of sophistication is necessary in a clinical setting. Instead, the rapid quantitative characterization of synovial fluid mechanics may have a higher clinical utility as a rapid assessment of synovial fluid health, even if this assessment is simplified to a single shear rate.

Finally, the rate of particle collection is a function of particle diameter and magnetic properties. In this study, these properties were verified by dynamic light scattering and SQUID magnetometry. However, it is essential that particle properties be as consistent as possible between samples to reduce these effects during the comparison of different fluid samples.

In this study, a novel technique to quantify synovial fluid viscosity in small volumes, termed magnetic particle translation, was presented. Magnetic particle translation was demonstrated in glycerol solutions with known viscosities, mechanically-degraded bovine synovial fluid, and osteoarthritic equine synovial fluid. Through these studies, an empirical relationship between the percent of magnetic particles collected in a given amount of time and the viscosity of the synovial fluid was constructed. Furthermore, proof-of-concept data demonstrate magnetic particle translation can be used to evaluate differences in synovial fluid mechanics between OA and healthy equine joints. Future work will continue to develop magnetic particle translation as an *in situ* evaluation of synovial fluid mechanics in clinical settings.

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References

- Al-Assaf S, Meadows J, Phillips GO, Williams PA. The application of shear and extensional viscosity measurements to assess the potential of hyaluronan in viscosupplementation. *Biorheology*. 1996; 33(4-5): 319–32. [PubMed: 8977658]
- Amblard F, Maggs AC, Yurke B, Pargellis AN, Leibler S. Subdiffusion and anomalous local viscoelasticity in actin networks. *Physical Review Letters*. 1996; 77:4470–3. [PubMed: 10062546]
- Brannan SR, Jerrard DA. Synovial fluid analysis. *Journal of Emergency Medicine*. 2006; 30(3):331–9. [PubMed: 16677989]
- Buckland-Wright, C. Radiographic imaging of osteoarthritis. In: Reginster, JY, Pelletier, JP, Martel-Pelletier, J, Henrotin, Y., Crasborn, L., editors. *Osteoarthritis*. Springer Berlin Heidelberg; Berlin, Germany: 1999. p. 246–68.
- Cheng NS. Formula for the viscosity of a glycerol-water mixture. *Industrial and Engineering Chemistry Research*. 2008; 47(9):3285–8.
- Cowman MK, Matsuoka S. Experimental approaches to hyaluronan structure. *Carbohydrate Research*. 2005; 340(5):791–809. [PubMed: 15780246]

- Crick FHC, Hughes AFW. The physical properties of cytoplasm: a study by means of the magnetic particle method. 1. Experimental. *Experimental Cell Research*. 1950; 1:37–80.
- Davies DV. Observations on the volume, viscosity and nitrogen content of synovial fluid, with a note on the histological appearance of the synovial membrane. *Journal of Anatomy*. 1944; 78(Pt 3):68–78. [PubMed: 17104946]
- Fam H, Bryant JT, Kontopoulou M. Rheological properties of synovial fluids. *Biorheology*. 2007; 44(2):59–74. [PubMed: 17538199]
- Garraud A, Velez C, Shah Y, Garraud N, Kozissnik B, Yarmola EG, Allen KD, Dobson J, Arnold DP. Investigation of the capture of magnetic particles from high-viscosity fluids using permanent magnets. *IEEE Transactions on Biomedical Engineering*. 2016; 63(2):372–8. [PubMed: 26208261]
- Hunter DJ, Nevitt M, Losina E, Kraus V. Biomarkers for osteoarthritis: Current position and steps towards further validation. *Best Practices in Research: Clinical Rheumatology*. 2014; 28(1):61–71.
- Jay GD, Waller KA. The biology of lubricin: Near frictionless joint motion. *Matrix Biology*. 2014; 39:17–24. [PubMed: 25172828]
- Jeong HH, Mark AG, Lee TC, Alarcon-Correa M, Eslami S, Qiu T, Gibbs JG, Fischer P. Active nanorheology with plasmonics. *Nano Letters*. 2016; 16(8):4887–94. [PubMed: 27367304]
- Lorenz H, Richter W. Osteoarthritis: Cellular and molecular changes in degenerating cartilage. *Progress in Histochemistry and Cytochemistry*. 2006; 40(3):135–63. [PubMed: 16759941]
- MacKintosh FC, Schmidt CF. Microrheology. *Current Opinion in Colloid & Interface Science*. 1999; 4(4):300–7.
- Martin-Alarcon L, Schmidt TA. Rheological effects of macromolecular interactions in synovial fluid. *Biorheology*. 2016; 53(2):49–67. [PubMed: 27472842]
- Nijenhuis N, Mizuno D, Schmidt CF, Vink H, Spaan JAE. Microrheology of hyaluronan solutions: implications for the endothelial glycocalyx. *Biomacromolecules*. 2008; 9(9):2390–8. [PubMed: 18700796]
- Ogston AG, Stanier JE. On the state of hyaluronic acid in synovial fluid. *Biochemical Journal*. 1950; 46(3):364–76. [PubMed: 16748691]
- Ogston AG, Stanier JE. The dimensions of the particle of hyaluronic acid complex in synovial fluid. *Biochemical Journal*. 1951; 49(5):585–90. [PubMed: 14886349]
- Ogston AG, Stanier JE. The physiological function of hyaluronic acid in synovial fluid; viscous, elastic and lubricant properities. *Journal of Physiology*. 1953; 119(2-3):244–52. [PubMed: 13035748]
- Orvisky E, Soltes L, Chabreck P, Novak I, Stancikova M. Size exclusion chromatographic characterization of sodium hyaluronate fractions prepared by high energetic sonication. *Chromatographia*. 1993; 37(1):20–22.
- Palfrey AJ, Davies DV. Immediate viscosity of synovial fluid. *Journal of Applied Physiology*. 1968; 25(6):672–8.
- Panizza B. *Archivio Di Fisiologia*. 1931; 29:576.
- Schmidt FG, Ziemann F, Sackmann E. Shear field mapping in actin networks by using magnetic tweezers. *European Biophysics Journal with Biophysics Letters*. 1996; 24:348–53. [PubMed: 8766693]
- Schneider J. *Biochemische Zeitschrift*. 1925; 160(325)
- Schurz J, Ribitsch V. Rheology of synovial fluid. *Biorheology*. 1987; 24(4):385–99. [PubMed: 3663897]
- Squires TM, Mason TG. Fluid mechanics of microrheology. *Annual Review of Fluid Mechanics*. 2010; 42:413–38.
- Ziemann F, Radler J, Sackmann E. Local measurements of viscoelastic moduli of entangled actin networks using an oscillating magnetic bead micro-rheometer. *Biophysics Journal*. 1994; 66:2210–6.

Figure 1.

An illustration of the magnetic particle translation technique. **A)** Superparamagnetic iron oxide nanoparticle embedded microspheres are distributed evenly throughout a sample of synovial fluid. **B)** A magnetic collection tool (a 1 mm by 1 mm cylindrical NdFeB magnet placed inside a catheter sheath) is inserted into the fluid using a micromanipulator. **C)** Particles collect on the surface of the tool. **D)** The tool is removed from the tube and the NdFeB magnet is removed from the tool. **E)** The sheath is placed in a fresh tube containing buffer. Then, an external magnetic field is applied to remove particles from the tool. **F)** The particles are resuspended, sonicated to break any aggregates, and quantified via a spectrophotometer.

Figure 2.

Physical and magnetic characterization of the magnetic particles. **A)** The SEM size fit to a lognormal size distribution yields a diameter of 936 nm with geometric deviation of 0.08. The DLS sizes fit to a bimodal lognormal size distribution yields a diameter of 772 nm with geometric deviation of 0.04 for 91% of the particles (by volume), and a diameter of 2.3 μm with geometric deviation of 0.03 for the rest of the particles. **B)** SEM image of magnetic particles shows a uniform size distribution. **C)** The magnetization of particles as measured by SQUID. The absence of remnant magnetization once the field is removed confirms iron oxide nanoparticles are superparamagnetic. The box indicates axis scaled near zero.

Figure 3.

Empirical correlation between particle collection and viscosity of water:glycerol solutions.

As the fraction of glycerol in aqueous solution increased, the viscosity increased.

Subsequently, the increase in viscosity resulted in a decrease in the percent of particle collected. Magnetic particle translation was measured in triplicate (n=3). All data are presented as raw values.

Figure 4.

Change in synovial fluid rheological properties and percentage of particle collection due to progressive sonication. **A)** Progressive sonication of the synovial fluid decreases the viscosity as measured by a rheometer. **B)** As sonication time increases, the same samples show an increase in the percentage of particles collected. All data are presented as raw values ($n=3$) per measurement.

Figure 5.

Correlation between particle collection and representative synovial fluid viscosity. Combining data from Figure 4A and 4B shows the percentage of particles collected could serve as a surrogate measure for the viscosity of the fluid. All data are presented as raw values (n=3) per measurement.

Figure 6.

Magnetic particle collection in healthy and diseased equine synovial fluid samples. Synovial fluid was collected from 7 horses diagnosed with OA and 5 control horses. The percentage of particle collection in the OA group is significantly different to the control group ($p < 0.001$). Additionally, measurement of magnetic particle translation within a sample is highly repeatable. The wide range of magnetic particles collected in OA joints can be attributed to high inter-sample variation. Magnetic particle translation in control samples were measured in triplicate ($n=3$) and all OA samples were measured in duplicate ($n=2$). All data are presented as raw values.