Nanoparticles are under investigation as diagnostic and therapeutic agents for joint diseases, such as osteoarthritis. However, there is incomplete understanding of nanoparticle diffusion in synovial fluid, the fluid inside the joint, which consists of a mixture of the polyelectrolyte hyaluronic acid, proteins, and other components. Here, we show that rotational and translational diffusion of polymer-coated nanoparticles in quiescent synovial fluid and in hyaluronic acid solutions is well described by the Stokes-Einstein relationship, albeit with an effective medium viscosity that is much smaller than the macroscopic low shear viscosity of the fluid. This effective medium viscosity is well described by an equation for the viscosity of dilute polymer chains, where the additional viscous dissipation arises because of the presence of the polymer segments. These results shed light on the diffusive behavior of polymer-coated inorganic nanoparticles in complex and crowded biological environments, such as in the joint.

INTRODUCTION

The application of nanoparticles as diagnostic and therapeutic agents has been of great interest over the past few decades. Understanding the diffusion of nanoparticles in biological environments is critical in their design and eventual clinical application. For example, nanoparticles are being engineered to monitor and treat osteoarthritis (1–3), yet systematic studies of the diffusion of nanoparticles in the synovial fluid within the joint space are lacking. Synovial fluid is a modulator of nanoparticle fate in the joint and is thought to impede transport of some nanoparticles and their ability to reach intended target tissues (2, 4). However, these inferences are often made without actual measurements of particle diffusion in synovial fluid and could be confounded by many factors, such as nanoparticle stability and charge-mediated interactions between the nanoparticles and synovial fluid components. We approach this problem from the point of view of studying the translational and rotational diffusion of colloidal stable and neutral nanoparticles in bovine synovial fluid and in solutions of hyaluronic acid (HA), a major constituent of synovial fluid.

Transport of particulates in a fluid occurs by convection and diffusion. Colloidal particles suspended in solution undergo diffusion due to random thermal fluctuations, which cause the random translation and rotation of the particles. This is phenomenologically described through the translational and rotational diffusivity of the particles, which is a function of particle and fluid properties. The diffusion of spherical colloids in simple fluids is related to the thermal energy $k_B T$ and hydrodynamic drag $f$ by the Sutherland-Einstein equation, $D = k_B T f / 6 \pi \eta r_p$. An expression for the translational diffusion coefficient is obtained by introducing the Stokes friction factor (7), $f_R = 6 \pi \eta r_p$, in the Sutherland-Einstein equation. Similarly, an expression for the rotational diffusion coefficient is obtained by introducing Debye’s rotational friction factor, $D_{R-SE} = k_B T / 8 \pi \eta r_p^2$.

$D_{T-SE} = k_B T / 6 \pi \eta r_p$ Diffusivities of nanoparticles are accurately described by Eq. 1 when the assumptions defining the Stokes and Einstein components hold (9). However, in polymer melts and polymer solutions, noncontinuum effects come into play, and deviations from the predictions of the Stokes-Einstein relations have been found in both translational (10–13) and rotational (14–16) diffusivities of nanoparticles in solution. Most past studies of small particle diffusion in polymer solutions have focused on translational diffusion of small dye molecules, proteins, or bare spherical nanoparticles (e.g., gold, polystyrene, or silica) in neutral polymer solutions. It is not immediately clear whether results observed for bare nanoparticles will carry over to the behavior of polymer-grafted nanoparticles in biological fluids or polymer solutions. For nanoparticles smaller than the radius of gyration of the polymer or when comparable to its correlation length, differences between the experimental translational diffusivity and that predicted from the Stoke-Einstein equation using the low shear viscosity have been reported (10, 11, 17–19).

Similarly, rotational diffusion of nanoparticles smaller than the radius of gyration of the polymer has also shown deviations from the Stokes-Einstein relations (14, 20, 21). Studies have shown how polymer grafting on the nanoparticles can also notably affect their diffusion in brain tissue (22) and in polymer solutions (23, 24).

Most biological fluids consist of crowded solutions of polyelectrolytes with varying ionic strengths. The structure and dynamics of polyelectrolyte solutions are known to be different from those of neutral polymer solutions and vary with ionic strength (25). Most of
these nanoparticle diffusion studies have been done using flexible, neutral polymers or moderately flexible polymers like DNA (26). Comparatively fewer studies have been performed using polyelectrolyte solutions (20, 27, 28). Models based on obstruction effects, hydrodynamic theories, free volume theory, and length scale–dependent viscosity have been proposed to explain deviations from the Stokes-Einstein equation for nanoparticles undergoing diffusion in complex liquids (29, 30). Theoretical and experimental work on the diffusive motion of nanoparticles in model polymer solutions has helped build a foundation for understanding their transport in complex fluids. Nonetheless, a fundamental understanding of nanoparticle diffusion in biological and polyelectrolyte solutions is lacking and is essential to guide the design of nanoparticles for biomedical applications, including diagnosis and therapy of joint diseases.

Synovial fluid, a viscous non-Newtonian fluid within synovial joints, is essential for shock absorption, lubrication, and modulation of transport of various molecules to different components of the joint. HA, a major component of synovial fluid (31), contributes substantially to its viscoelastic properties (32). The molecular weight of HA present in synovial fluid can range from 0.5 to 7 MDa depending on disease state, and its concentration can range between 3 and 4 mg/ml (33). The solution conformation of this high–molecular weight negatively charged polysaccharide is influenced by the hydrophilic and semiflexible character of its backbone and its ionizability (34). In deionized water under salt-free conditions, HA adopts an extended rod-like conformation due to electrostatic repulsion between the carboxylate groups of the linear polymer made of N-acetyl-d-glucosamine and d-glucuronic acid. In contrast, under physiological ionic strength, HA chains behave closer to random coils due to screening of the negative charges present on the polymer backbone by ions in solution (32). The translational diffusivity of nanoparticles in a narrow range of HA solutions at 0.2 N NaCl was studied previously using sedimentation of polystyrene latex spheres with diameters ranging between 88 and 365 nm (35). Related studies in model polyelectrolyte systems, such as xanthan solutions and ribonuclease, suggest the significance of probing both translational and rotational diffusion of nanoparticles (20, 36, 37). However, there is a lack of systematic studies of the diffusion of nanoparticles in synovial fluid and its components, although this understanding could guide the design of nanoparticle-based intraarticular drug delivery strategies.

Here, we study the translational and rotational diffusion of polymer-grafted nanoparticles of two different hydrodynamic sizes in quiescent synovial fluid and quiescent HA solutions using x-ray photon correlation spectroscopy (XPCS) measurements and dynamic magnetic susceptibility (DMS) measurements, respectively. The nanoparticles were coated with poly(ethylene glycol) (PEG), a neutral hydrophilic polymer that confers colloidal stability while avoiding specific interactions with HA and other synovial fluid components. We find that the translational and rotational diffusion of nanoparticles in quiescent synovial fluid and quiescent HA solutions are well described by the Stokes-Einstein relations but with a nanoscale viscosity that is much smaller than the low shear viscosity of the fluid. Systematic studies in HA solutions suggest that this nanoscale viscosity is slightly higher than the solvent viscosity and is well described by a theory proposed by Huggins (38) in 1942. These findings provide insight into the diffusion of polymer-coated nanoparticles in biological environments, such as those found in the joint.

**RESULTS**

**Diffusion of nanoparticles in synovial fluid**

Polymer-coated cobalt ferrite nanoparticles of different hydrodynamic sizes were used in this study. The smaller nanoparticles were synthesized by thermal decomposition and grafted with a layer of 5-kDa PEG. These nanoparticles had an inorganic core diameter of $15 \pm 2$ nm and a hydrodynamic diameter of $44.1 \pm 9$ nm [denoted as PEG NP, see Fig. 1 (A and C) for transmission electron microscopy and size distributions]. Larger composite nanoparticles coated with the block copolymer PEG$_{5\text{k}}$-PLA$_{48}$ were prepared via flash nanoprecipitation, resulting in a hydrodynamic diameter of $220.5 \pm 43$ nm [denoted as Composite NP, see Fig. 1 (B and C), for transmission electron microscopy and size distributions]. Translational and rotational diffusivities of these polymer-coated nanoparticles were measured in bovine synovial fluid. Rheological characterization of the synovial fluid showed shear-thinning behavior with a low shear viscosity of 0.71 Pa·s (Fig. 1D).

Small-angle x-ray scattering (SAXS) measurements were performed to evaluate the structure and aggregation state of the nanoparticles in the synovial fluid. For PEG-coated nanoparticles in water and synovial fluid (Fig. 1E), the Guinier peak at $Q > 0.05$ Å$^{-1}$ and the Bessel function oscillation observed in the SAXS profile are strongly suggestive of individual inorganic cores coated with PEG. For the larger composite nanoparticles in water and synovial fluid (Fig. 1F), two Guinier peaks were observed. The first Guinier peak at $Q > 0.02$ Å$^{-1}$ and the Bessel function oscillation observed in the SAXS profile are strongly suggestive of individual inorganic cores that constitute the composite nanoparticles. The second peak between 0.001 $< Q < 0.01$ Å$^{-1}$ corresponds to the composite nanoparticles in solution. Neat synovial fluid had a distinct scattering signal, also shown in Fig. 1 (E and F). Particle sizes and the power-law slopes obtained from the fits for PEG and composite nanoparticles, along with analysis of background-subtracted SAXS data and comparisons to models for solid and fractal spheres (Supplementary Materials), suggest individual cores dispersed with no aggregation in synovial fluid.

XPCS measurements were used to study the translational diffusion of the nanoparticles in synovial fluid. Figure 2 (A and B) shows the representative intensity-intensity autocorrelations as a function of delay time for the PEG and composite nanoparticles in synovial fluid. The characteristic time obtained from a simple exponential fit showed linear dependence when plotted against wave vector, $q$ [see Fig. 2(C and D) for representative results], suggesting Brownian diffusion of the nanoparticles. The corresponding translational diffusion coefficient was extracted from the slope of these curves.

The nanoparticles respond to alternating magnetic fields by physical particle rotation induced by oscillating magnetic torques. This is called Brownian relaxation (39), and for these magnetically blocked cobalt ferrite nanoparticles, it follows the Debye model (40, 41). DMS measurements for both nanoparticles in synovial fluid were fit to the Debye model to obtain estimates of the rotational diffusion coefficient. DMS measurements for the PEG-coated nanoparticles in synovial fluid showed characteristic Debye behavior with the in-phase susceptibility decreasing monotonically and the out-of-phase susceptibility showing a single peak (Fig. 2E). In contrast, for the larger composite nanoparticles in synovial fluid, a monotonic decrease in the in-phase susceptibility was observed but not a clear peak of the out-of-phase susceptibility (Fig. 2F). From this observation, we infer that rotation of these larger nanoparticles is more substantially restricted in synovial fluid.
Table 1 summarizes measured diffusivities and those calculated from the Stokes-Einstein relations in synovial fluid. The translational diffusivity of the PEG nanoparticles and of the composite nanoparticles was ~270 times and ~50 times higher, respectively, than expected based on the low shear rate viscosity of the synovial fluid. Similarly, the rotational diffusivity of the PEG-coated nanoparticles and of the composite nanoparticles was ~450 times and ~100 times higher, respectively, than expected based on the low shear rate viscosity of the synovial fluid. The corresponding nanoscale viscosities experienced by the nanoparticles in synovial fluid were calculated using the measured rotational and translational diffusion coefficients, the measured hydrodynamic diameters determined from dynamic light scattering (DLS) in water for each nanoparticle, and the Stokes-Einstein relations. The resulting nanoscale viscosity for both types of nanoparticle was much lower than the low shear viscosity determined from rheometry for the synovial fluid. These measurements suggest that the diffusion of polymer-coated nanoparticles in quiescent synovial fluid is well described by the Stokes-Einstein relationship, albeit with an effective medium viscosity that is much smaller than the macroscopic low shear viscosity of the fluid.

For validation, we obtained XPCS and DMS measurements for both nanoparticles in Newtonian glycerol solutions with viscosities of 13.3 to 155 mPa·s (fig. S3). We observed close agreement between experimental values for the diffusivities and the corresponding predictions of the Stokes-Einstein relations using the hydrodynamic radius of the nanoparticles determined from independent DLS measurements in water and the viscosity of the glycerol solutions determined using a rheometer. To further understand these observations of nanoparticle diffusion in synovial fluid, we studied the diffusion of nanoparticles in HA solutions, the major component of synovial fluid.

**Diffusion of nanoparticles in HA solutions**

While proteins and other components in synovial fluid can influence the diffusion of the polymer-grafted nanoparticles, here, we limit the attention to the role of HA on influencing nanoparticle diffusion. In these studies, we used HA with a molecular mass of 1150 kDa and polydispersity index of 1.17, determined using gel permeation chromatography. The concentration range for the HA solutions was chosen to span the semidilute unentangled to semidilute entangled regime, as a way of probing nanoparticle diffusion in these systems. The lower limit of HA solution concentrations was set by our ability to distinguish bulk viscosity with available instrumentation. The upper limit was chosen to be above the physiological concentration of HA in synovial fluid. We studied diffusion in HA solutions with no salt and with added salt at a concentration representative of physiological ionic strength in order because changing ionic strength would lead to changes in HA solution conformation. HA solutions with 0 M NaCl were prepared at HA concentrations ranging from 0.5 to 9.77 mg/ml in deionized water. HA solutions at 0.15 M NaCl were prepared with HA concentrations ranging from 0.04 to 10 mg/ml. The HA solution with 0 M NaCl should correspond to highly extended chains, while the HA solution with 0.15 M NaCl was prepared to mimic the typical physiologic ionic strength. The intrinsic viscosity of HA solutions with 0.15 M NaCl was calculated to be 2046 cm³/g, and the radius of gyration was calculated to be 170 nm (42).
HA solutions were characterized by rheometry. Steady shear measurements for HA solutions with 0 M NaCl display shear-thinning non-Newtonian behavior for all concentrations studied (Fig. 3A). In contrast, for HA solutions with 0.15 M NaCl, shear-thinning non-Newtonian behavior was observed for concentrations of 2, 5, and 10 mg/ml HA (Fig. 3B). Apparent Newtonian behavior was observed for HA solutions below 1 mg/ml. However, the shear rate range for these measurements was reduced because of instrument limitations. A low shear Newtonian plateau could be measured directly only for the solutions with the highest concentrations of HA molecules.

For HA solutions showing shear-thinning behavior, the experimental rheology data were fit using the Cross model to obtain low shear and high shear viscosities. For solutions with 0 M NaCl, the low shear viscosities were found to range from 7.0 to 8.2 Pa·s, and the high shear viscosities were found to range from 1.4 to 12 mPa·s. For HA solutions with 0.15 M NaCl, the low shear viscosities were found to range from 1.1 to 2.0 Pa·s, and the high shear viscosities were found to range from 1.1 to 7.5 mPa·s.

Specific viscosity as a function of concentration was evaluated for the HA solutions prepared at 0 and 0.15 M NaCl, suggesting that solutions spanned the semidilute unentangled and entangled regimes (Fig. 3C). The entanglement concentration ($C_e$) corresponds to the transition between the unentangled and entangled semidilute regimes and marks the solution concentration at which chains overlap sufficiently to form topologically constrained entanglements (25). Consistent with theoretical predictions for polyelectrolytes with 0 M NaCl or minimal residual salt, we found that $\eta_p$ scales as

![Fig. 2. XPCS and DMS measurements of nanoparticles in synovial fluid.](image-url)
$C^{0.5}$ in the semidilute unentangled regime and scales as $C^2$ in the semidilute entangled regime (43). The scaling laws of polyelectrolytes with added salt vary with the degree of salt addition (44). HA solutions with 0.15 M NaCl have $\eta_s$ that scales as $C^{1.3}$ in the semidilute unentangled region and as $C^{3.2}$ in the semidilute entangled region at high concentrations, consistent with observations for xanthan and sodium carboxymethylcellulose in aqueous NaCl solutions (45, 46). Polyelectrolyte solutions without salt are expected to entangle at lower concentrations because chains that are extended in solution occupy a larger hydrodynamic volume relative to random coil chains (47). The chains adopt an extended rod-like confirmation due to electrostatic repulsion between charges on the side chains. For HA solutions with 0 M NaCl, we found $C_e$ to be 1.2 mg/ml, and for the HA solutions with 0.15 M NaCl, $C_e$ was estimated to be 1.4 mg/ml. At 0.15 M NaCl, the charges are screened, and the HA molecules tend to coil (48). This results in a smaller volume being occupied by the coil (25) and contributing to lower viscosity at a given concentration. More polymer chains are required in solution to obtain the same amount of intermolecular interaction seen in HA solutions with no salt added (49). Our observation that the entanglement concentrations for both solutions are relatively close is consistent with other studies showing that low salt content has small effects on the $C_e$ of polyelectrolyte solutions (44).

SAXS measurements were performed to evaluate the structure and aggregation state of the nanoparticles in the HA solutions with 0.15 M NaCl. Measurements were performed in water and HA solutions with 0.15 M NaCl at two concentrations of 1 and 10 mg/ml for both PEG and composite nanoparticles, and the results are shown in Fig. 3 (D and E). For PEG$_{4.9k}$-PLA$_{6k}$-coated composite nanoparticles, two hydrodynamic diameters were used in these studies, 220-nm nanoparticles were used for HA solutions with 0 M salt and 180-nm nanoparticles for HA solutions with 0.15 M NaCl. Particle sizes and the power-law slopes obtained from the fits for PEG-coated nanoparticles, along with analysis of background-subtracted SAXS data and comparisons to models for solid and fractal spheres (Supplementary Materials), suggest nanoparticles dispersed with no aggregation in HA. The nanoparticle sizes and power-law slopes obtained from the fits for composite nanoparticles, along with analysis of background-subtracted SAXS data and comparisons to models for solid and fractal spheres (Supplementary Materials), suggest that the composite nanoparticles remain intact in water, but there is evidence of broader polydispersity for nanoparticles in HA solutions with 1 and 10 mg/ml.

Translational diffusivities for the nanoparticles in HA solutions with 0 M NaCl obtained from XPCS measurements are plotted as a function of HA concentration in Fig. 4A. The corresponding rotational

| $D_h$ [nm] | $D_T$ ($\times 10^{-2}$ um$^2$/s) | $D_R$ ($\times 10^2$ rad/s) | $\eta$ ($\times 10^{-2}$ Pa·s) |
|------------|-----------------|-----------------|-----------------|
| DLS | XPCS | SE | DMS | SE | XPCS | DMS | Rheology |
| PEG NP | 44 | 374 | 1.5 | 99.5 | 0.23 | 0.17 | 0.002 | 1.38 | 0.72 |
| Composite NP | 220 | 14.4 | 0.3 | 0.17 | 0.002 | 1.38 | 0.16 | 66 |

Table 1. Nanoparticle diffusion coefficients and macro- and nanoscale viscosities in synovial fluid.
diffusivities obtained from DMS measurements are shown in Fig. 4B. Both diffusivities decrease with increasing HA concentration, as expected from the dependence of diffusivity on viscosity and the increasing viscosity of HA solutions with increasing HA concentration. However, the experimental translational and rotational diffusivities in HA solutions were up to two orders of magnitude higher than expected based on the Stokes–Einstein predictions using the low shear viscosity obtained from rheological measurements. The autocorrelation functions, q-vector ranges and corresponding length scales, plots of characteristic time versus wave vector, and corresponding scaling of characteristic time versus q for each of the measurements and the DMS spectra with fits to the Debye model may be found in the Supplementary Materials.

Translational and rotational diffusivities for the nanoparticles in HA solutions with 0.15 M NaCl obtained from XPCS and DMS measurements are plotted as a function of the concentration of HA in Fig. 4 (D and E), respectively. As with the 0 M NaCl solutions, there is a concentration-dependent decrease in diffusivity for both nanoparticle sizes. However, here, we observe the agreement between experimental diffusivities and the prediction of the Stokes–Einstein relation for HA solutions in the unentangled semidilute regime. On the other hand, for HA solutions in the entangled semidilute regime, the diffusivities were up to two orders of magnitude higher than expected based on the Stokes–Einstein prediction using the low shear viscosity.

According to the Stokes–Einstein relations for the translational and rotational diffusivities, their ratio should be proportional to \(\frac{4}{3} r_p^2\), where \(r_p\) is the nanoparticle’s hydrodynamic radius. Figure 4 (C and F) shows the hydrodynamic radii determined from this relation and the ratio of the experimental translational and rotational diffusivities as a function of HA concentration in comparison to the hydrodynamic radii determined independently from DLS measurements in water with and without salt for each nanoparticle. The agreement between the two, particularly for the nanoparticles in 0 M NaCl HA solutions and for the composite nanoparticles in 0.15 M NaCl HA solutions, suggests that the nanoparticles diffuse in the HA solutions as in a simple fluid with translational and rotational diffusivities described by the functional form of the Stokes–Einstein equations. However, the nanoscale viscosity is distinct from the macroscopic low shear viscosity determined from rheometry.

The experimental translational and rotational diffusivities and corresponding hydrodynamic diameters of the nanoparticles determined from DLS measurements in water were used along with the Stokes–Einstein relations to calculate the corresponding nanoscale
viscosity experienced by the nanoparticles. As seen in Fig. 5, the nanoscale viscosity experienced by both nanoparticles in HA solutions with 0 M NaCl is similar, despite their different hydrodynamic radii, and increases with increasing concentration of HA in solution. Over the entire HA concentration range studied, the nanoscale viscosity is substantially smaller than the macroscale low shear viscosity determined from rheometry. In contrast, for the nanoparticles in HA solutions with 0.15 M NaCl, we observe that nanoscale and macroscale viscosities are of the same order of magnitude for HA concentrations below ~1 mg/ml and are several orders of magnitude smaller for HA concentrations >1 mg/ml. This coincides with the transition between unentangled and entangled semidilute regimes for that solution. Furthermore, for the smaller PEG-coated nanoparticles, the nanoscale viscosity is insensitive to HA concentration except at the highest HA concentrations, whereas it increases with increasing HA concentration for the composite nanoparticles. The nanoscale viscosities obtained from rotational diffusion coefficient measurements in 0.15 M NaCl HA solutions were different for the two nanoparticles, with the values for the smaller PEG-coated nanoparticles being smaller than those for the larger composite nanoparticles.

For polymers in solution in the dilute regime, a model by Huggins (38) describes their viscosity based on the concentration of the polymer molecules. We compared the nanoscale viscosities determined from both techniques and for both nanoparticle sizes with the concentration-dependent viscosity of dilute polymer solutions according to the model by Huggins for the entire range of HA concentrations with 0.15 M NaCl. According to Fig. 5, there is reasonable agreement between the nanoscale viscosities and viscosities determined from the model by Huggins, except for nanoscale viscosities determined from rotational diffusion measurements of the smaller PEG-coated nanoparticles in 0.15 M NaCl solutions. A comparison is provided only for the 0.15 M NaCl solution because the Huggins model requires the intrinsic viscosity, which could not be determined for the 0 M NaCl solutions. HA solutions in 0 M NaCl at concentrations as low as 0.04% (w/v) were still shear thinning in nature. Hence, we could not obtain reliable viscosity measurements in the dilute regime for HA solutions with 0 M NaCl.

DISCUSSION

Nanoparticles intended for therapeutic or diagnostic purposes are often polymer-grafted and have hydrodynamic diameters ranging between 10 and 1000 nm (50). Understanding the diffusion of nanoparticles in biological environments is critical in their design and eventual clinical application. For example, nanoparticles are being developed for intraarticular drug delivery to treat conditions such as arthritis (1–3). Depending on the therapeutic of interest, common targets in the joint include the cartilage, synovium, and the cells within those tissues and nanoparticles must navigate a
complex fluid environment to reach these targets. Synovial fluid is a
modulator of nanoparticle fate in the joint, influencing nanoparticle
stability, hydrodynamic size and surface nature, and transport
because of its highly viscous nature, the presence of entangled net-
works of high–molecular weight polyelectrolytes such as HA, and
its complex mixture of proteins and other biomacromolecules (2, 4).
However, there are no prior studies reporting direct measurements of
nanoparticle diffusion in synovial fluid or in HA solutions.

Here, we studied translational and rotational diffusion of single
and composite nanoparticles of two different hydrodynamic sizes
coated with the neutral polymer PEG. PEG is a widely used coating
for nanoparticles intended for biomedical applications. It was
chosen for this study because it should prevent specific interactions
with components in synovial fluid. However, we note that there are
a wide range of polymers with different charge and chemical groups
that can mediate interactions with synovial fluid components and
that are being evaluated for intrarticular drug delivery applications
(1–3). The studies reported here serve as a baseline case of diffusion
of neutral noninteracting nanoparticles, against which future stud­
ies with charged and interacting nanoparticles can be compared.
Previous studies have demonstrated that PEG-coated particles
obtained with similar methods as used here are colloidally stable in
biological fluids because of a dense PEG brush with dimensions con­
sistent with a density distribution model (51–53). Similarly, a pre­
vious study with composite particles obtained with similar methods
as used here suggest that the particles are colloidally stable and do
not change in hydrodynamic size over a period of 14 days (54).

Measurements of the translational and rotational diffusivity were
made in synovial fluid and in HA solutions using XPCS and DMS
measurements, respectively. HA is a major component of synovial
fluid and is a major contributor to the bulk rheology of synovial
fluid because of its high–molecular weight, polyelectrolyte nature,
and typical concentrations that lead to entanglements. SAXS mea­
surements suggested that the PEG-coated and composite particles
were well dispersed and unaggregated in synovial fluid and in HA
solutions. In quiescent synovial fluid and HA solutions, the transla­
tional and rotational diffusion coefficients of the nanoparticles
were found to follow the predictions of the Stokes-Einstein equa­
tions, albeit with a viscosity that was much lower than the low shear
macrosopic viscosity. This so-called nanoscale viscosity was found to
be similar for both particle sizes and when obtained from transla­
tional and rotational diffusion measurements, with the exception of
the viscosity obtained from rotational diffusivities of the PEG-coated
particles in HA solutions with 0.15 M NaCl. Consistent observations
in synovial fluid, which contains proteins, and in HA solutions,
which is devoid of proteins, suggest that protein adsorption does
not substantially influence the diffusion of the tested nanoparticles.

To address potential concerns with particle aggregation, we note
that because XPCS measures translational diffusivity of the diffusive
entities, if the nanoparticles were aggregated, then the translational
diffusivity would be lower than that predicted by the Stokes-Einstein
equation based on the hydrodynamic diameter in water. Instead,
our XPCS measurements show much faster diffusion than predicted
by the Stokes-Einstein relation. Similar arguments apply for rotational
diffusivity measurements using DMS. Thus, we believe that these
SAXS measurements, coupled with the low translational and rota­
tional diffusivities, and the agreement in nanoscale viscosity ob­
tained from rotational and translational diffusivity measurements
strongly suggest that the composite particles are not aggregated.

We hypothesize that for a nanoparticle that is much larger than
the monomer units but that is small enough to be unaffected by
polymer entanglements, the surrounding medium viscosity will be
larger than the solvent viscosity due to the additional viscous dissi­
pation caused by the presence of the polymer repeat units. For a
suspension of hard spheres in dilute solution, Einstein attributed
the increase in viscosity of the suspension to the volume fraction of
spheres in solution (55). For polymers in solution, Huggins (38)
modified Einstein’s model for hard spheres to obtain a model de­
scribing the viscosity of polymeric solutions based on the concen­
tration of the polymer molecules in the dilute regime. In that case,
Huggins argued, the increase in viscosity arises because of the addi­
tional viscous dissipation resulting from the presence of the polymer
segments and could be determined by estimating the hydrodynamic
volume occupied by the polymer segments. The resulting model is
based on the value of the intrinsic viscosity [n] of the polymer in
solution. We found that the nanoscale viscosity obtained from
translational and rotational diffusion measurements for nanoparti­
cles in HA solutions with 0.15 M NaCl was similar in magnitude
and concentration dependence to those predicted by the Huggins
equation, supporting our hypothesis.

Fast diffusion of nanoparticles in solutions of neutral polymers
have been reported and explained in terms of relative length scales
of the particles and the polymer solution system. However, it is not
clear that understanding of nanoparticle diffusion in neutral polymer
solutions will translate directly to nanoparticle diffusion in polyelec­
trolyte solutions, such as in the HA solutions, or to nanoparticle
diffusion in complex biological fluids, such as synovial fluid. In
solutions of charged polymers, electrostatic repulsion between mono­
mers alters the structure and chain flexibility (25). The size of the
charged group and its recurrence within the monomers results in
conformations ranging from a rigid rod to a semiflexible chain. As
a result of these structural differences, the onset of entanglements in
charged polymers is shifted to much higher concentrations than for
neutral chains. It is expected that these pronounced differences in
structure in charged polymers would have varying effects on nano­
particle transport, yet there are few studies of nanoparticle diffusion
in polyelectrolyte solutions.

We are aware of two prior studies of nanoparticle diffusion in
polyelectrolyte solutions. Both studies considered translational diffu­
sion of electrostatically stabilized nanoparticles in model polyelec­
trolyte solutions. Senanayake and co-authors (27) studied translational
diffusion of electrostatically stabilized anionic tannic acid–coated
gold nanoparticles (sizes of 5, 20, and 40 nm) in polyacrylic acid
(1000 kDa) solutions in distilled water (no salt). The authors reported
correlation lengths of 34 to 69 nm; therefore, their studies
considered regimes where the particles are smaller or approximately
the same size as the polymer correlation length. Their studies sug­
gest that particle diffusion is decoupled from polymer network
dynamics for this system. However, the ionic strength in their study
was not physiologically relevant and is expected to affect polyelec­
trolyte chain conformation substantially and therefore nanoparticle
diffusion through the solution. In addition, because both the nanopar­
ticle and polymer are ionic, it is possible that their observations are due
to repulsive charge interactions. Furthermore, note that the stability
and aggregation state of the nanoparticles were not evaluated in their
study. Slim and co-authors (56) showed that, in solutions of low
ionic strength, particle translational diffusion deviates nonmono­
tonically from bulk predictions as polymer concentration increases

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and are not accurately predicted by available models. In that study, translational diffusion of anionic polystyrene (2200 kDa) nanoparticles with diameters of 100 to 790 nm was studied in dilute and semidilute solutions of a model-flexible polyelectrolyte, sodium polystyrene sulfonate (a semiflexible polyelectrolyte), at three different ionic strengths. However, although the authors report deviations from Stokes-Einstein behavior, these were typically modest, with the ratio of measured diffusivity to Stokes-Einstein diffusivity ranging from ~1 to ~2. In contrast to these studies, here, we report translational and rotational diffusivities that are up to ~3 orders of magnitude larger than expected from the Stokes-Einstein relations for polymer-grafted nanoparticles in a biological fluid (synovial fluid) and in solutions of a biologically relevant polyelectrolyte (HA) spanning the semidilute untangled to semidilute entangled regimes. Furthermore, our measurements clearly show that translational and rotational diffusivities are well described by the functional form of the Stokes-Einstein relations, albeit with a nanoscale viscosity that varies with HA concentration and that appears to be well described by a simple model for viscosity of dilute polymer solutions reported by Huggins (38).

Cai and co-authors (57) reported scaling models for the mean square displacement (MSD) and diffusion of hard-sphere nanoparticles in neutral polymer solutions. Because XPCS and DMS measurements do not directly measure the MSD, we cannot test their theory directly. However, we do observe diffusive transport in our experiments, with diffusivity that is much higher than expected based on the Stokes-Einstein relation and the bulk viscosity of the solution. Hence, we calculated the relevant length scales of correlation length ($\xi$) radius of gyration $R_g$, and tube diameter $a$ for the HA solutions containing 0.15 M NaCl. As can be seen in table S5, our experiments, with diffusivity that is much higher than expected from the Stokes-Einstein relation and the bulk viscosity of the solution. Hence, we calculated the relevant length scales of correlation length ($\xi$) radius of gyration $R_g$, and tube diameter $a$ for the HA solutions containing 0.15 M NaCl. As can be seen in table S5, our experimental translational and rotational diffusion measurements agree with this, and instead, we see very fast diffusion. This would seem to suggest that the model by Cai and co-authors (57) does not adequately describe our measurements. Furthermore, in their model, the diffusivity for small nanoparticles scales with the solvent viscosity, whereas the nanoscale viscosities calculated from our experimental translational and rotational diffusion measurements suggest that diffusivity scales with an effective medium viscosity that is described by the simple model by Huggins (38) and that accounts for the viscous dissipation because of the presence of the polymer repeat units. However, it must be recalled that the model by Cai and co-authors (57) was derived for neutral polymer solutions and not for polyelectrolyte solutions.

Although our eventual goal is to understand the transport of nanoparticles in the crowded and confined space of the joint, here, we have taken a reductionist approach by studying the diffusion of nanoparticles in synovial fluid, which bathes the joint, and in HA solutions, since HA is one of the main components in synovial fluid. The composition and rheological properties of synovial fluid are known to vary with disease and age, and this will likely influence nanoparticle diffusion in those contexts. Furthermore, our studies were limited to nanoparticles of two distinct hydrodynamic diameters and coated with the neutral polymer PEG, which should prevent specific interactions between the nanoparticles and components of synovial fluid, including HA. Nanoparticles of a broad range of sizes and surface charges and with coatings that mediate nonspecific and specific interactions with synovial fluid components and other biological entities in the joint are being actively studied for intraarticular drug delivery and diagnostic applications (1–3). Our results should serve as a baseline case for comparison in future studies that directly measure diffusion of such nanoparticles in synovial fluid. Last, our results suggest that PEG-coated nanoparticles should be able to rapidly diffuse throughout the synovial fluid, potentially reaching targets such as cartilage, synovium, and the cells within those tissues. However, additional studies are needed to evaluate transport of these nanoparticles in the porous cartilage matrix (58) and in the multilayered synovium (59) and to evaluate uptake of these nanoparticles by cells in these tissues.

We have reported measurements of translational and rotational diffusivities for nanoparticles in bovine synovial fluid and HA solutions. The diffusion coefficient of polymer-coated nanoparticles in quiescent synovial fluid and HA with and without salt is well described by the Stokes-Einstein relationship, albeit with an effective medium viscosity that is much smaller than the macroscopic low shear viscosity of the fluid. This effective medium viscosity was well described by a model due to Huggins for the viscosity of dilute polymer chains, where the additional viscous dissipation arises because of the presence of the polymer segments. These studies contribute to understanding diffusive behavior of polymer-coated nanoparticles in biological fluid and their constituents and can help guide their design for biomedical applications.

**MATERIALS AND METHODS**

**Materials**

Cobalt chloride (II) hexahydrate (ACS reagent, 98%), iron chloride (III) hexahydrate (ACS reagent, 97%), oleic acid (90%), hexane (ACS reagent, ≥98.5%), diethyl ether (anhydrous, ACS reagent, ≥99%), toluene (ACS reagent, ≥99.5%), and chloroform (for high-performance liquid chromatography, gas chromatography, and residue analysis, ≥99.9%) were purchased from Sigma-Aldrich. 1-Octadecene (90%) was purchased from Acros Organics, and sodium oleate (>97%) was purchased from TCI America. PEG, 5 kDa for ligand exchange, was procured from Sigma-Aldrich, and block copolymer polyethylene glycol-b-poly(lactic acid) PEG$_{40}$kDa-b-PLA$_{60}$kDa was purchased from Evonik Industries. Tetrahydrofuran (THF; 99.9% Acros Organics) was dried using sodium sulfate before use in Flash Nanoprecipitation (FNP). HA sodium salt from *Streptococcus equi* was purchased from Sigma-Aldrich. Bovine synovial fluid was obtained from Animal Technologies. Water was used from Milli-Q grade (Millipore, Bedford, MA).

**Nanoparticle synthesis**

Cobalt ferrite nanoparticles were synthesized by thermal decomposition of the organometallic precursor at 320°C in the presence of oleic acid using 1-octadecene as the solvent (60). In a typical synthesis, a metal-oleate was prepared by reacting 1 mmol of cobalt chloride (II) hexahydrate, 2 mmol of iron chloride (III) hexahydrate, and 8 mmol of sodium oleate in a mixture of 50 ml of deionized
water, 100 ml of hexane, and 50 ml of ethanol at 67°C under reflux for 4 hours. The resulting oleate was washed with 25 ml of water and aged for 15 days in a vacuum oven at 70°C. To synthesize the cobalt ferrite nanoparticles, 25 g of metal-oleate, 100 ml of 1-octadecene, and 2 g of oleic acid were initially heated to 200°C for 3.3°C/min under an inert atmosphere. The reaction was left to proceed under reflux by ramping the temperature to 320°C, while the inert gas (N₂) flow was removed. The reaction was left to proceed at 320°C for 3 hours. The unreacted reagents were washed by solvent-antisolvent precipitation where the product was initially suspended in chloroform and then precipitated with acetone in a 1:2 volume ratio. We used magnetic separation to recover the nanoparticles.

**Surface modification of cobalt ferrite nanoparticles**

PEG₅₀kDa-coated nanoparticles: To covalently attach PEG-silane with a molecular weight of 5000 g/mol onto the nanoparticle surface, 1.50 g of the silanated PEG (61) was dissolved in 200 ml of toluene and mixed with 200 mg of oleic acid–coated cobalt ferrite nanoparticles suspended in 200 ml of toluene and 88 μl of acetic acid. It was placed on a shaker at room temperature for 72 hours. Afterward, the polymer-coated nanoparticles were precipitated by adding diethyl ether to the mixture in a 1:2 volume ratio (toluene: diethyl ether). The PEG-coated nanoparticles were magnetically separated from the solution and dried in a vacuum oven for 12 hours. The PEG-coated nanoparticles were dissolved in water, and the solution was filtered using a 0.22-μm nylon filter to remove any nanoparticle aggregate before storing at 4°C.

PEG₄₅kDa-PLA₆₅kDa-coated nanoparticles: Cobalt ferrite composite nanoclusters were prepared using flash nanoprecipitation (62) in a confined impinging jet (CIJ) mixer. Oleic acid cobalt ferrite nanoparticle suspension in THF at 2.5 mg/ml was mixed with PEG₄₅kDa-PLA₆₅kDa dissolved in THF at a nanoparticle to polymer mass ratio of 1:1.25. THF stream with the solutes were rapidly mixed in the CIJ against equal volumes of deionized water using KD Scientific syringe pump at 52 ml/min and precipitated into reservoir of water. (1:4 THF:water was the dilution factor used). The precipitated composite particles were passed through a Miltenyi column to remove free polymer and small unencapsulated nanoparticles. The nanoparticles were recovered by flushing the column with water and stored in 4°C.

**Characterization of the nanoparticles**

Dynamic light scattering: The hydrodynamic radius of the nanoparticles in water was measured through DLS using a Brookhaven Instruments ZetaPALS operating at room temperature. All measurements were performed at a scattering angle of 90°.

Transmission electron microscopy: Physical diameters ($D_p$) of the nanoparticles were obtained by imaging using a Hitachi H 7000 transmission electron microscope at 100 keV. Images of nanoparticles in water sampled on an ultrathin nickel type B, 3- to 4-nm carbon, and 5- to 6-nm formvar (from Electron Microscopy Sciences) were acquired using a Veleta charge-coupled device side-mount camera and analyzed using ImageJ.

**Solution preparation and dispersion of nanoparticles**

Bovine synovial fluid stored at −20°C was thawed in a 4°C refrigerator and then warmed to room temperature before addition of particles. HA solutions were studied because HA is a major component in synovial fluid and is a major contributor to the bulk rheological properties of synovial fluid. Furthermore, HA solutions can be prepared without proteins, which eliminates concerns of protein adsorption on the nanoparticles. Because HA is a polyelectrolyte, it also poses an interesting system to study nanoparticle transport in. The concentration range for HA solutions was chosen to span the semidilute unentangled to semidilute entangled regime, as a way of probing nanoparticle diffusion in these systems. The lower limit of HA solution concentrations was set by our ability to distinguish bulk viscosity with available instrumentation. The upper limit was chosen to be above the physiological concentration of HA in synovial fluid. We studied diffusion in HA solutions with no salt and with added salt at a concentration representative of physiological ionic strength in order because changing ionic strength would lead to changes in HA solution conformation. This is evident in the difference in bulk rheology. These solutions were prepared by dissolving HA in deionized water and 0.15 M NaCl continuously stirring at 4°C for at least 24 hours. Glycerol solutions were prepared by dissolving glycerol in deionized water. The solutions were then stored at 4°C before use. Nanoparticles were added to the biopolymer and glycerol solutions at concentrations less than 0.1 weight % and mixed by pipetting with Gilson MICROMAN pipettes for both DMS and XPCS measurements.

**Rheological measurements**

Shear viscosity of the HA solutions and bovine synovial fluid was measured at 25°C using an Anton Paar MCR 702 twin drive rheometer. Measurements were made on 0.5 to 1 ml of sample in a parallel plate measuring system with gaps maintained between 0.25 and 1 mm. The shear viscosity of the sample was measured by increasing the shear rate from 0.1 to 10,000 s⁻¹. Except for the most concentrated samples, the low shear Newtonian plateau could not be measured because of torque sensitivity limits in the instrument. The Cross model (63), given by Eq. 2, was used to characterize the shear rate dependence of the viscosity and to calculate the zero shear, $\eta_0$, and infinite shear, $\eta_\infty$, viscosities

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (C\gamma)^m}$$

where, $m$ is the Cross rate constant and $γ$ is the Cross time constant.

The glycerol solutions were measured in an Anton Paar MCR 302 in a Couette cell double-gap measuring device using 8 ml of the sample with shear rates between 10 and 1000 s⁻¹.

**Huggins model for concentration dependent viscosity of dilute polymer solutions**

The concentration-dependent viscosity of dilute polymer solutions with long-chain molecules was calculated according to (38)

$$\eta = \eta_0(1 + [\eta]C + k_b[\eta]^2C^2 + \ldots)$$

where the intrinsic viscosity was calculated using $[\eta] = 0.029M^{0.80}$ (42, 47).

**Translational diffusion coefficients from XPCS measurements**

XPCS measurements were performed in the small-angle scattering geometry at beamline 8-ID-1 at the Advanced Photon Source, Argonne National Laboratory (ANL). XPCS measurements reported
in this work were carried out over two-beam times at a photon energy of 7.4 and 10.85 keV, respectively. Partially coherent x-rays of transverse dimensions 15 μm by 15 μm were selected using precision slits with a coherent flux of 5 × 10^10 photons/s.

To capture the dynamic time scales for the different samples with a wide range of viscosities, two different photon counting x-ray detectors were used. A large area medipix based detector array (LAMBDa) (64) detector with a pixel size of 55 μm and 516 × 1556 pixels operating at a maximum frame rate of 2000 frames/s was used to capture time scales in the range of 0.0005 to several seconds. To access even faster dynamic time scales, an ultrafast x-ray camera (LAMBa) (65, 66) with a pixel size of 75 μm and 128 × 256 pixels operating at a maximum frame rate of 50,000 frames/s was used to capture time scales in the range of 20 μs⁻¹. The detectors were positioned at a distance of 4 m from the sample covering a transverse dimensions 15 μm by 15 μm were selected using precise beam times at a photon energy of 7.4 and 10.85 keV, respectively. Partially coherent x-rays of transverse dimensions 15 μm by 15 μm were selected using precision slits with a coherent flux of 5 × 10^10 photons/s.

The nanoparticles were suspended in HA solution and glycerol at <0.1 volume percent and loaded into 1 or 2 mm (based on the photon energy used) quartz capillary tubes procured from Charles Supper, using a syringe with 25.5-gauge needle and sealed with glue to prevent evaporation.

The intensity recorded as a function of time for each pixel was correlated in time. The autocorrelation function (g₂) was generated by averaging the correlation functions from the group of pixels spanning the wave vector q (67). The correlation functions obtained were fitted using a simple exponential model for diffusive motion using Eq. 4 to yield characteristic relaxation time tau (T) that in turn gives diffusion coefficient according to Eq. 5

\[
g_2(q, t) = B + C \exp(-2t/T)
\]

\[
T = D_T q^2
\]

In Eq. 4, B is the baseline and C is a contrast factor.

**Rotational diffusion coefficient measurements from DMS**

DMS measurements of nanoparticles (at n = 3, using 200 μl) in water, HA solutions, glycerol, and synovial fluid were performed in an Acreo DynoMag AC Susceptometer with an excitation field amplitude of 5 Oe and evaluated between the frequency of 10 Hz and 160 kHz. The frequency-dependent response of PEG-PLA–coated composite particles (at n = 3, using 100 μl) in polymeric solutions and glycerol was measured using Quantum Design MPMS-3 superconducting quantum interference device magnetometer equipped with an alternating current (AC) susceptometer with an excitation field amplitude of 1.5 to 2 Oe and were evaluated between 1 and 1000 Hz. All measurements were performed at room temperature.

The dynamic response of the nanoparticles was described using the Debye model as given by Eq. 6

\[
\chi = \chi' - i\chi'' = C + \frac{\chi_0 - \chi_\infty}{1 + \Omega^2 \tau^2} \exp(-\frac{T}{\tau})
\]

where \(\chi'\) and \(\chi''\) are the real and imaginary components of the DMS, \(\Omega\) is the alternating magnetic field frequency, \(\chi_0\) is the initial susceptibility, \(\chi_\infty\) is the infinite frequency susceptibility, and \(\tau\) is the magnetic relaxation time of the nanoparticles in the suspension.

To estimate the rotational diffusivity, \(D_R\), nonlinear regression of the experimental data was fit to the Debye model in Eq. 7 weighted by the lognormal size distribution of the nanoparticles (68), \(n(D_h)\), using Eq. 8 in MATLAB. In the fits, the viscosity determined using a rheometer for HA, glycerol, and bovine synovial fluids was used, and the hydrodynamic diameter from DLS measurements was used.

\[
\chi'' = \int_0^\infty n_h(y) \chi_{0,h'} \Omega^2 \tau_{hv} y^3 \ln^2 \left( \frac{D_h}{D_{hv}} \right) \exp(-\frac{y^2}{2 \ln^2 \sigma_y}) dy
\]

where, \(D_h\) is the volume-weighted mean diameter, \(\ln \sigma_y\) is the geometric deviation, and \(\chi_{0,h'}\) and \(\tau_{hv}\) are the volume-weighted average initial susceptibility and relaxation time, respectively.

**Nanoscale viscosity calculation from the measurements**

The translational \(\eta_T\) and rotational \(\eta_R\) viscosity were calculated using

\[
\eta_T = \frac{k_B T}{6\pi r_P D_T}
\]

\[
\eta_R = \frac{k_B T}{8\pi r_P^3 D_R}
\]

where \(k_B\) is the Boltzmann constant, \(T\) is the temperature, \(r_P\) is the hydrodynamic radius of the particle, \(D_T\) is the translational diffusion coefficient from XPCS, and \(D_R\) is the rotational diffusion coefficient from DMS measurements.

**Estimation of errors and error propagation**

Errors associated with the translational \((D_{T,\text{error}})\) and rotational diffusion \((D_{R,\text{error}})\) coefficients and calculated nanoscale translational \((\eta_{T,\text{error}})\) and rotational \((\eta_{R,\text{error}})\) viscosities were estimated as follows. Errors in the translational diffusion coefficients obtained from XPCS measurements were calculated using

\[
D_{T,\text{error}} = \frac{T_{\text{error}}}{q^2}
\]

where \(T_{\text{error}}\) is the error at each of the characteristic times obtained from the simple exponential fits, as described above. \(D_{T,\text{error}}\) was averaged over at least 10 wave vectors.

Errors in the rotational diffusion coefficient, \(D_{R,\text{error}}\), from DMS measurements were calculated using the nonlinear regression parameter confidence intervals (nlparci) function in MATLAB for the
data fitted using the lsqcurvefit function with confidence interval set at 95%.

Errors in the radii, $r_{p,\text{error}}$, calculated from the translational and rotational diffusion coefficients were obtained using

$$r_{p,\text{error}} = \frac{1}{2} r_p \sqrt{\left( \frac{D_T}{D_T} \right)^2 + \left( \frac{D_R}{D_R} \right)^2}$$

(12)

Errors in the translational and rotational nanoscale viscosity were calculated according to the formulas

$$\eta_{T,\text{error}} = \eta_T \sqrt{\left( \frac{D_{T,\text{error}}}{D_T} \right)^2 + \left( \frac{r_{p,\text{error}}}{r_p} \right)^2}$$

(13)

$$\eta_{R,\text{error}} = \eta_R \sqrt{\left( \frac{D_{R,\text{error}}}{D_R} \right)^2 + 9 \left( \frac{r_{p,\text{error}}}{r_p} \right)^2}$$

(14)

where the error in the radius of the nanoparticle $r_{p,\text{error}}$ was extracted by least-square fitting of the lognormal distribution of the diameters using the Levenberg-Marquardt algorithm.

The error in the low shear viscosity obtained from fitting rheological measurements to the Cross model, $\eta_{T,\text{error}}$, was determined using the curve fitting tool in MATLAB. The fitting parameters were obtained using the nonlinear least-square method, with the least absolute residuals and trust region optimization as fit options.

Errors associated with the translational ($D_T$) and rotational ($D_R$ – $\text{SE}_{\text{error}}$) diffusion coefficients obtained from the Stokes-Einstein relation predictions were obtained using the formulas

$$D_T - \text{SE}_{\text{error}} = D_T - \text{SE} \sqrt{\left( \frac{\eta_{\text{error}}}{\eta} \right)^2 + \left( \frac{r_{p,\text{error}}}{r_p} \right)^2}$$

(15)

$$D_R - \text{SE}_{\text{error}} = D_R - \text{SE} \sqrt{\left( \frac{\eta_{\text{error}}}{\eta} \right)^2 + 9 \left( \frac{r_{p,\text{error}}}{r_p} \right)^2}$$

(16)

SAXS measurements

SAXS measurements were performed on PEG and composite nanoparticles in water, HA with salt at 1 and 10 mg/ml, and in synovial fluid loaded in Nuclear magnetic resonance (NMR) tubes. All solutions without any nanoparticles was also measured as background. Representative samples were chosen for the measurements to check for aggregation and structure.

SAXS experiments were performed at the Advanced Photon Source, ANL (Argonne, IL) using the 9-ID-C beamline (69). It was operated using a synchrotron radiation of 21 keV. The scattered intensity from the specimens was measured in the range of 0.001 Å$^{-1}$ ≤ q ≤ 0.1 Å$^{-1}$, traversing two decades in size. Irena packages for Igor Pro were used to reduce and merge the datasets and to perform the analysis (70).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/27/eabf8467/DC1

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Fast nanoparticle rotational and translational diffusion in synovial fluid and hyaluronic acid solutions

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