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The Development of an AF4 Characterization Pipeline for Polymeric Nanoparticle-Protein Interactions

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SUMMARY

The centrifugation-wash method is currently the standard technique used for the recovery of nanoparticle-protein complexes from protein-containing serum. However, this technique is disruptive, and leads to changes in nanoparticle parameters and the surface-bound protein composition. In this study, we explore the use of asymmetric flow field flow fractionation (AF4) for the development of a robust pipeline for the characterization of nanoparticle parameters following protein corona formation under physiologically-relevant conditions. Model surface-modified polystyrene latex nanoparticles were treated with protein-containing medium mimicking cell-culture conditions under physiologically-relevant conditions. Following incubation, nanoparticle-protein samples were then fractionated using the AF4 system and characterized using in-line detectors. Conventional nanoparticle-protein studies typically use the highly invasive centrifugation-wash method which leads to changes in measured particle parameters. Our studies show that AF4 allows for high resolution and characterization of *in-situ* nanoparticle-protein complexes. This work will allow for the development of a more harmonized approach for characterizing nanoparticle-protein interactions under biologically-relevant conditions.

INTRODUCTION

Polymeric nanoparticles are routinely explored for the development of novel medicines for unmet clinical need. However, there is currently a high attrition rate of nanoparticles from the bench to clinic. This is in part caused due to a lack of understanding of nanoparticle-protein interactions under physiologically-relevant conditions. Upon exposure to protein-containing medium, nanoparticles spontaneously interact and adsorb proteins onto their surface forming a 'protein corona'. Protein corona formation leads to changes in nanoparticle parameters which subsequently alters their biological fate. Hence, it is crucial to characterize how nanoparticle parameters change following protein corona formation. The current standard for nanoparticle-protein isolation is the centrifugation-wash method, which is a highly invasive protocol and leads to changes in the physical and chemical parameters of nanoparticles (size, concentration) and alters the composition of surface-bound proteins due to protein loss or the formation of protein aggregates. Asymmetric flow field-flow fractionation (AF4) is an increasingly popular high-resolution separation technique that allows for the gentle separation of complex nanoparticle-protein mixtures. AF4 can be coupled with multiple inline detectors including

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multiangle light scattering (MALS), dynamic light scattering (DLS), and refractive index (RI) detectors as examples. Each of these detectors can be used to generate additional quantitative information about a sample. Despite the numerous advantages of AF4 for nanoparticle characterization this technique has seldom been used to study nanoparticle biological interactions. The aim of this project is to develop a robust AF4 pipeline for nanoparticle characterization following incubation with protein containing media under physiologically relevant conditions.

MATERIALS AND METHODS

Model polystyrene latex nanoparticles with different surface chemistries including unmodified, amine-, and carboxylate-modified nanoparticles were incubated for (2 and 24 hours) at 37 ˚C in the presence of 10 vol% fetal bovine serum (FBS) in phosphate-buffered saline using gentle agitation to mimic physiologically-relevant conditions. The nanoparticles were resolved using (frit-inlet) AF4 fractionation, with a power cross flow gradient (2.5 mL/min), and a corresponding exponent of 0.2 over a 50 min run duration. 0.2% NovaChem was used as the carrier liquid across all measurements. Particle parameters were analysed using ultraviolet (UV), multiangle light scattering (MALS) and dynamic light scattering (DLS) in-line detection. Multiple models of fit including sphere, zimm, and random coil were used for AF4-MALS data analysis.

RESULTS AND DISCUSSION

Corresponding fractograms of UV absorbance (280 nm) and MALS (90˚) for unmodified nanoparticles at baseline and following (24 hr) incubation are shown in (Figure 1) below.

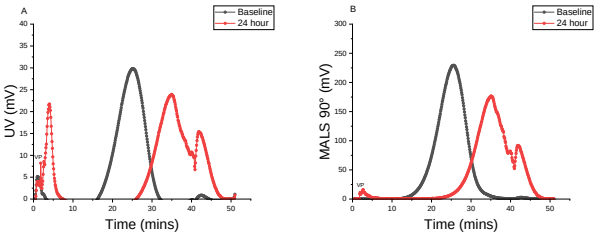

Fig. 1. *An AF4 fractionation profile using UV (280 nm), and MALS (90˚) for unmodified polystyrene latex nanoparticles at A) baseline and following a B) 24 hour incubation in 10% FBS in PBS.*

Baseline particle characterization show a single peak which elutes between (16-32 mins) in both UV and MALS fractograms. Samples incubated for (24 hr) showed an increase in elution time with two separate peaks at (35 mins) and (45 mins). Furthermore, a peak was observed between (1-6 mins) for the UV fractogram and this shows that nanoparticle-proteins were separated from the unbound serum proteins.

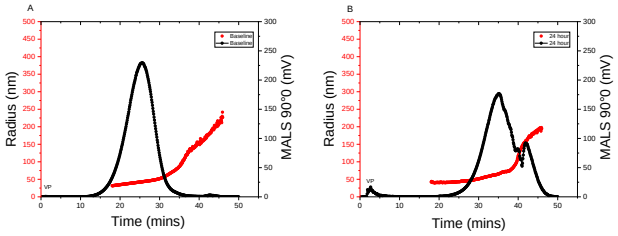

Fig. 2. *Unmodified polystyrene latex nanoparticle size characterization using (AF4-MALS) for samples at A) baseline and following B) a 24 hour incubation.*

The AF4 separation of polystyrene nanoparticles incubated for (24 hr) allowed for the gentle resolution of sample sub-populations. We observed an increase in mean particle radius from baseline (44 nm) to (51 nm) at for particles eluted at 35 mins which further increases to (156 nm) for the peak at 45 mins. These results correlate with previous studies by (Miller *et al.*, 2012) which shows an increase in mean particle size following incubation within protein-containing medium.

CONCLUSIONS

Protein corona studies typically employ highly invasive isolation methods prior to characterization. AF4 is a gentle method which allows for the *in-situ* analysis of nanoparticle-protein samples at higher resolution than current techniques.

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