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Characterization of Polymeric Nanoparticle-Protein Interactions

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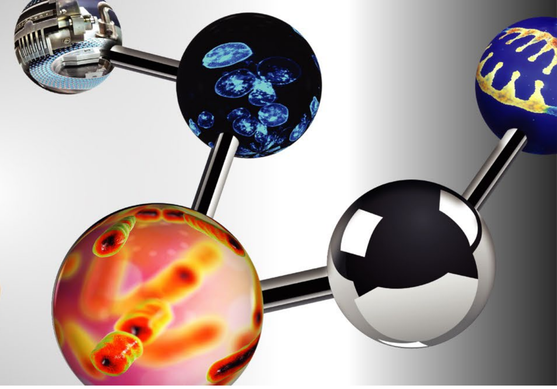


PURPOSE

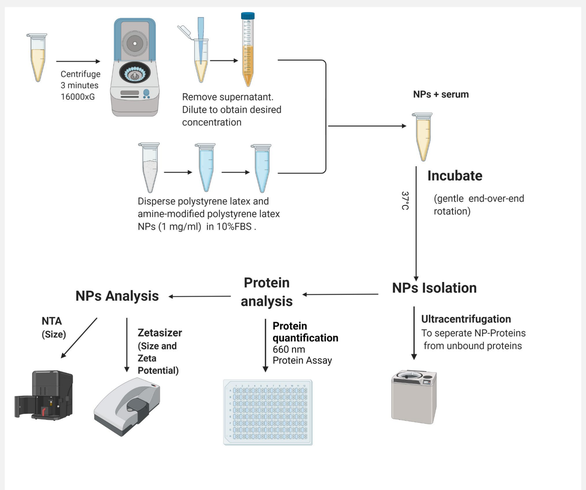
Polymeric nanoparticles have shown significant potential for the development of new medicines for unmet clinical need. Upon administration into protein-containing media, proteins will spontaneously adsorb onto the surface of nanoparticles and form a 'protein corona'. Protein corona formation alters the physiochemical properties of nanoparticles which consequently impacts their biological fate. The aim of this study was to develop a robust pipeline for the reproducible characterization of polymeric nanoparticles following protein corona formation.

OBJECTIVE(S)

- To measure the impact of protein corona formation on the physical (size and charge) properties of nanoparticles.
- To investigate the impact of the centrifugation-wash isolation protocol on nanoparticle parameters

METHOD(S)

- Latex polystyrene (1 mg/ml) and Amine-modified Latex Polystyrene NPs (1mg/ml) were dispersed in 10% v/v FBS.
- The samples were then immediately incubated at 37°C using gentle end-over-end rotation to mimic physiological conditions.
- The nanoparticles-proteins were then isolated from protein-containing media using the centrifugation-wash protocol (20 000 x G) for 30 minutes and then re-suspended in PBS.
- The 660 nm protein assay was then used to quantify the changes in protein concentration following protein corona formation.
- Isolated nanoparticles were then characterized using the Zetasizer (size and zeta potential) and NTA (size). Parallel size measurements were performed for in-situ nanoparticles.



RESULT(S)

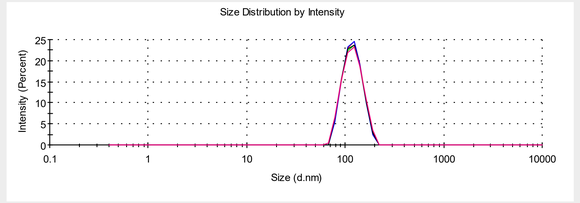


Figure 1. The intensity distribution of baseline unmodified latex polystyrene nanoparticles. Measured at (20 µg/ml) and dispersed in 0.015 M of PBS using Zetasizer Nano AS.

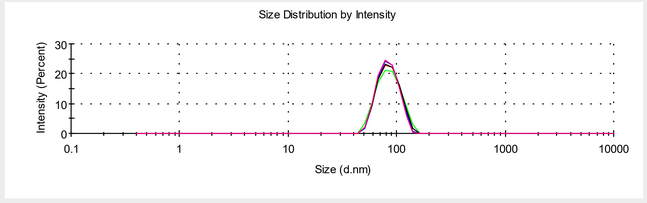


Figure 2. The intensity distribution of baseline amine-modified latex polystyrene nanoparticles. Measured at (20 µg/ml) and dispersed in 0.015 M of PBS using Zetasizer Nano AS.

- The images above show the intensity distribution for unmodified polystyrene latex **(Figure 1)** and amine-modified polystyrene latex **(Figure 2)** nanoparticles as measured using dynamic light scattering. These results show that both particles have a narrow and unimodal size distribution.

Table 1. Changes in the physiochemical properties of unmodified and amine-modified nanoparticles following protein corona formation measured using Zetasizer Nano ZS. All samples were measured at (20 µg/ml) and baseline size control samples were dispersed in 0.015 M of PBS. Nanoparticles incubated within protein containing media were suspended with 0.15 M PBS following centrifugation-wash protocol and all Zeta potential measurements were performed within 2mM KCL.

PARAMETERS	Unmodified polystyrene latex	Unmodified polystyrene latex (10% v/v FBS)	Amine-modified polystyrene latex	Amine-modified polystyrene latex (10% v/v FBS)
Z-average (d.nm ± SD)	116 ± 0.5	163 ± 5	82 ± 0.5	271 ± 5
PDI (mean ± SD)	0.02 ± 0.01	0.2 ± 0.02	0.03 ± 0.02	0.2 ± 0.01
Zeta potential (mV ± SD)	-38 ± 0.5	-31 ± 0.7	47 ± 2	-14 ± 0.8

- The results in **(Table 1)** show the changes in the properties of nanoparticles following protein corona formation. A general trend can be observed for size as this increases for both nanoparticles following protein corona formation.
- Protein quantification following protein corona formation showed that unmodified latex polystyrene nanoparticles had a protein concentration of **(111.2 µg/ml)** and amine-modified nanoparticles had a protein concentration of **(386.6 µg/ml).** The positively charged amine-modified nanoparticle interacted and adsorbed a larger concentration of negatively charged proteins compared to the unmodified nanoparticle. This explains why there was a larger increase in the size of amine-modified nanoparticles when compared to the negatively charged unmodified polystyrene latex nanoparticle.

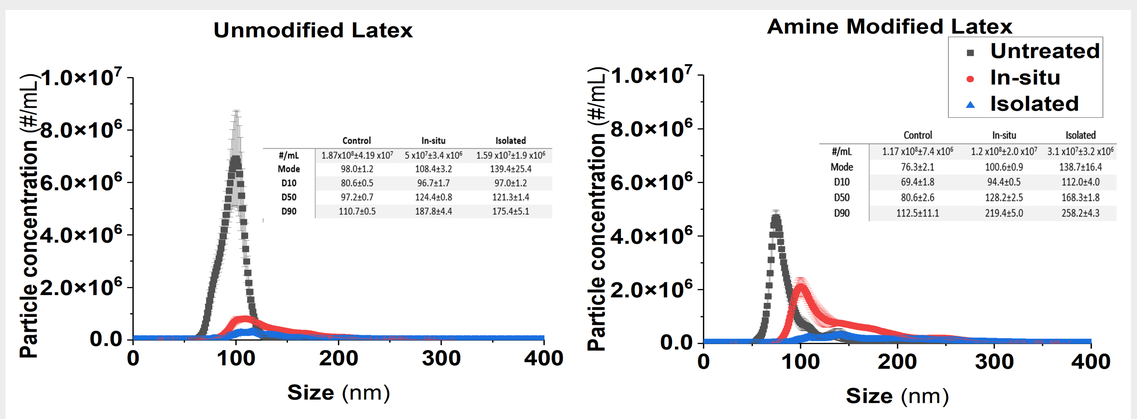


Figure 3. The changes in size distribution of unmodified and amine-modified latex polystyrene nanoparticles following protein corona formation. Unmodified and amine-modified nanoparticle size measurements were performed using the Nanosight NS300 at camera level(6) and screen gain (6) with a focus of (27). The baseline nanoparticle samples were dispersed in 0.015M of PBS, while incubated particles were dispersed in 0.15M PBS. The incubated nanoparticles were diluted and measured at (1 µg/ml).

- The images above show the size distribution of nanoparticles following protein corona formation. The baseline measurement of both nanoparticles show a narrow and unimodal size distribution which correlated with previously shows DLS data. Following protein corona formation the size distribution for these nanoparticles show a much wider size distribution with multiple sub-populations of sizes which we are now able to identify. These different populations are caused to due nanoparticle-protein and also protein-protein interactions such as aggregation.

RESULT(S)

- By comparing the isolated and in-situ nanoparticles we are able to better understand the impact of the centrifugation-wash protocol on the results. A general trend that can be observed for both particles is that following isolation there is a mean increase in the size of particles and this may have been caused by protein aggregation caused by high speed centrifugation.
- There is also a loss in the number of particles following the centrifugation-wash protocol, this is another cause for concern as this increases the margin of error for size measurements and may not be an accurate representation of the samples. Furthermore, as seen in the amine-modified particles size distribution there was a shift in the peak of isolated nanoparticles when compared with in-situ

CONCLUSION(S)

- There is an increase in the size of nanoparticles following incubation within protein-containing media due to the adsorption of proteins and protein corona formation as measured using DLS and NTA.
- The surface chemistry of nanoparticles plays a crucial role in nanoparticle-protein interactions and protein corona formation as observed with the positively charged amine-modified latex polystyrene nanoparticles adsorbing a larger number of proteins.
- The comparison of nanoparticles following isolation using the centrifugation-wash protocol with in-situ particles has shown the impact of isolation techniques on the sample with an increase in mean particle size and a loss of sample. This has highlighted the need for improved in-situ nanoparticle characterization techniques.
- This study will be followed by developing and optimizing asymmetric field flow fractionation (AF4). This technique allows for the gentle separation of protein from nanoparticles which will provide a more accurate representation of the sample following protein corona formation as this will prevent aggregate formation and sample loss.

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