Characterisation of Polymeric Nanoparticle-Protein Interactions

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Background:

Nanoparticles (NPs) are colloidal particles sized 1-100 nm and are increasingly used for the delivery of a diverse molecule portfolio

Upon administration biological media, nanoparticles spontaneously interact with proteins resulting in protein surface-adsorption and the formation of the ‘’ protein corona”.

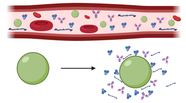

Fig 1. Protein corona formation on nanoparticle surface following introduction to vascular conditions (i.v. injection).

The protein corona alters the physical (size, charge) and chemical properties of nanoparticles, which subsequently impacts nanoparticle biological fate (cellular interactions and uptake, organ distribution).

Objective:

To develop a robust pipeline for the reproducible characterisation of nanoparticle size changes following protein corona formation.

Methods:

Unmodified and amine-modified Latex nanoparticles were dispersed treated with 10% v/v FBS at 37°C for 24 hours and subsequently isolated using the centrifugation-wash protocol. Quantification of surface-adsorbed protein was performed using the 660 nm protein assay. Proteins were eluted from isolated NPs and SDS-PAGE was performed as a preliminary analysis for fingerprinting proteins.
Isolated nanoparticles were characterised using Dynamic Light Scattering (size and zeta potential) and Nanoparticle Tracking Analysis (size and concentration). Parallel *in situ* Nanoparticle Tracking Analysis measurements were performed for protein treated/untreated particles.

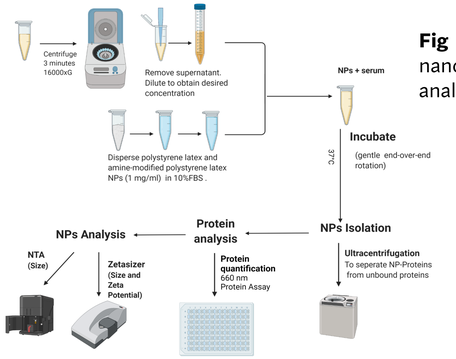

Fig 2. Pipeline used for nanoparticle recovery and analysis.

Results and Discussion:

Baseline Characterisation of Latex Nanoparticles

Latex nanoparticles were sized under baseline formulation conditions and following treatment with protein-containing media.

Table 1. DLS measurements of unmodified and amine-modified latex nanoparticles following protein corona formation measured using Zetasizer Nano ZS. Nanoparticles incubated within protein containing media were redispersed in PBS following isolation.

Parameters	Unmodified Latex Baseline	Unmodified Latex Treated	Amine-modified Latex Baseline	Amine-modified Latex Treated
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

Baseline DLS measurements for unmodified and amine-modified latex nanoparticles show a unimodal size distribution with a mean z-average of 116 nm for unmodified and 82 nm for amine-modified particles, respectively. Changes in latex nanoparticle size following protein corona formation were measured using DLS (**Table 1**). An increase in size and polydispersity for both unmodified and amine-modified latex NPs was observed following protein corona formation.

Total surface-adsorbed protein concentrations for isolated nanoparticles was **111.2** and **386.6** µg/ml for unmodified and amine-modified particles, respectively.

These results show that positively charged amine-modified latex particles adsorb a larger concentration of (negatively-charged) proteins compared to unmodified latex nanoparticles. This explains the shift in zeta potential following treatment with protein-containing media. Furthermore, a larger increase in the mean z-average of the amine-modified latex nanoparticles was observed, consistent with the higher measured surface-adsorbed protein concentration.

Nanoparticle Tracking Analysis of Latex Nanoparticles *In Situ* and Following Isolation

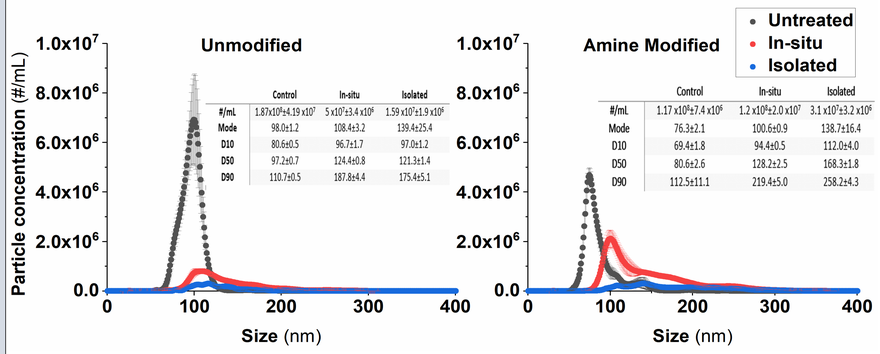

Fig 3. Changes in size distribution of unmodified (left) and amine-modified (right) latex nanoparticles following treatment with FBS.

NTA results show the size distribution of NPs following treatment with protein-containing media. Following protein treatment, both nanoparticles show a broader size distribution with multiple sub-populations emerging at larger size ranges. These sub-populations are caused by nanoparticle-protein and nanoparticle-nanoparticle interactions leading to agglomeration.
Particle concentration is significantly reduced relative to in situ measurements of samples containing the same latex content, potentially indicating particle loss during centrifugation-wash isolation steps.

Conclusions:

Treatment with protein-containing media leads to increased particle size due to protein surface-adsorption and nanoparticle agglomeration.

Nanoparticle surface chemistry plays a crucial role in nanoparticle-protein interactions as observed with the differences between amine-modified and unmodified latex nanoparticles.

Comparisons between NTA measurements for isolated particles and *in-situ* particles highlight the need for *in-situ* measurement techniques owing to changes induced in nanoparticle parameters following isolation, which render the analysis of protein adsorption effects on particle size challenging. These include nanoparticle loss during each centrifugation-wash step and nanoparticle agglomeration.

For future measurements, we will use asymmetric field flow fractionation (AF4) coupled with light scattering, allowing the gentle recovery of treated nanoparticles and their *in situ* analysis. We will perform a comparison between various isolation methods in combination with proteomics analysis.

Reference(s):

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