The Development of a Novel Pipeline for Polymeric Nanoparticle Characterization following Incubation Under Shear Flow Conditions

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Introduction

Nanoparticles are small colloidal particles with a dimension between 1-100 nm in size. Polymeric nanoparticles are routinely explored for the development of novel medicines for unmet clinical need due to their unique properties. However, there is currently a high attrition rate for bench-clinic translation, and this may be due to a lack of understanding of the behaviour of nanoparticles under physiologically-relevant conditions.

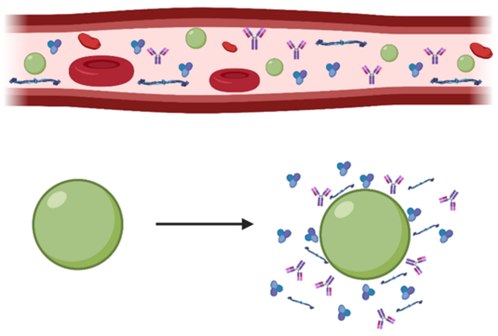


Figure 1. Schematic showing protein corona formation on nanoparticle surface following injection.

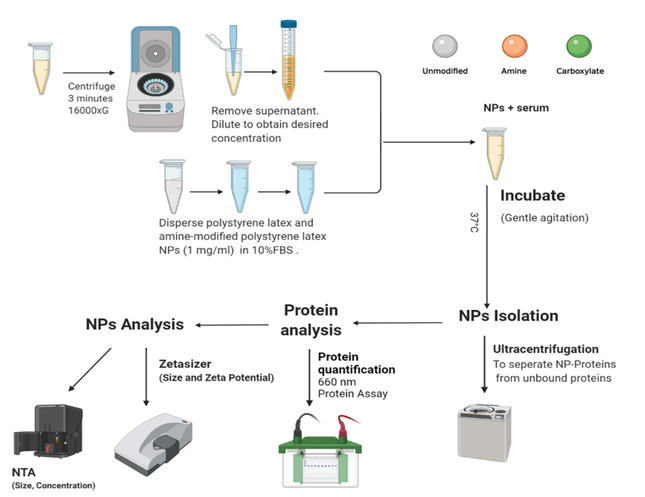
Upon administration to protein-containing medium, nanoparticles will spontaneously adsorb proteins onto their surface and form what is known as the 'protein corona' (figure 1) . Protein corona formation leads to changes in the physical and chemical parameters of nanoparticles, which subsequently alters their biological fate (cellular uptake, biodistribution). With most nanoparticles intended for intravenous administration, it is therefore crucial to characterize the impact of biological shear flow conditions on nanoparticle-protein interactions and how this impact their colloidal stability.

Aims & Objectives

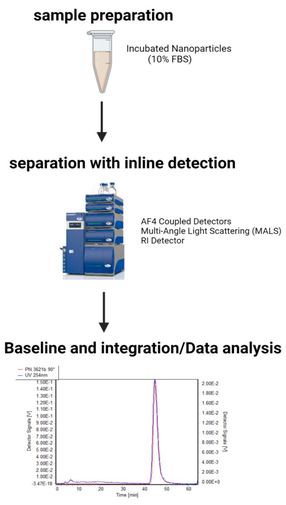
- To develop a robust pipeline for the reproducible characterization of nanoparticles following protein corona formation under physiologically relevant shear flow conditions.
- To use a range of nanoparticle isolation and analytical techniques to measure the impact of sample handling conditions on particle parameters using model nanoparticles (polystyrene latex)

Methods

Pipeline for comparison between different surface functionalization



In situ asymmetric Flow Field-Flow Fractionation (FFF) Pipeline



Results and Discussion

1) The impact of shear flow on polystyrene latex nanoparticle size

Table 1. Nanoparticle parameters measured by PTA and DLS before (baseline, static) and after incubation with Phosphate-buffered saline (PBS) containing 10% v/v fetal bovine serum (FBS) under conditions mimicking shear flow in the median cubital vein (0.85 cm/s), and arteries (8.5 cm/s).

Flow rate	PTA-measured Diameter (nm)	Z-average (nm)	PDI	ζ-Potential (mV)	Protein content (μg)
Unmodified					
before incubation	94.3 ± 0.6	118.1 ± 0.5	0.035 ± 0.003	-33.5 ± 0.8	N/A
0.85 cm/s	123 ± 2	141.4 ± 0.4	0.072 ± 0.005	-25.1 ± 0.2	70 ± 2
8.5 cm/s	140.0 ± 0.3	179 ± 1	0.152 ± 0.007	-23.9 ± 0.2	68 ± 1
Amine					
before incubation	84.0 ± 0.5	82.4 ± 0.2	0.041 ± 0.007	50.4 ± 0.8	N/A
0.85 cm/s	181 ± 4	375.4 ± 0.4	0.28 ± 0.02	-25 ± 2	79 ± 3
8.5 cm/s	178 ± 5	333 ± 6	0.26 ± 0.03	-7.6 ± 0.3	78 ± 5
Carboxylate					
before incubation	91.1 ± 0.6	94.9 ± 0.2	0.017 ± 0.002	-34.0 ± 0.5	N/A
0.85 cm/s	112.9 ± 0.4	122.1 ± 0.5	0.015 ± 0.001	-22.3 ± 0.4	45 ± 4
8.5 cm/s	110.2 ± 0.5	119.9 ± 0.4	0.015 ± 0.002	-23.1 ± 0.4	49 ± 3

An increase in mean (unmodified, amine, and carboxylate) particle diameter is observed following incubation within treatment media at 0.85 cm/s. A further increase in mean particle size was observed for unmodified particles (8.5 cm/s) this was likely due to an increase in nanoparticle-protein and protein-protein interactions.

We see an increase in mean particle size for samples characterized using DLS as opposed to PTA

2) Further analysis for PTA-measured nanoparticle diameter

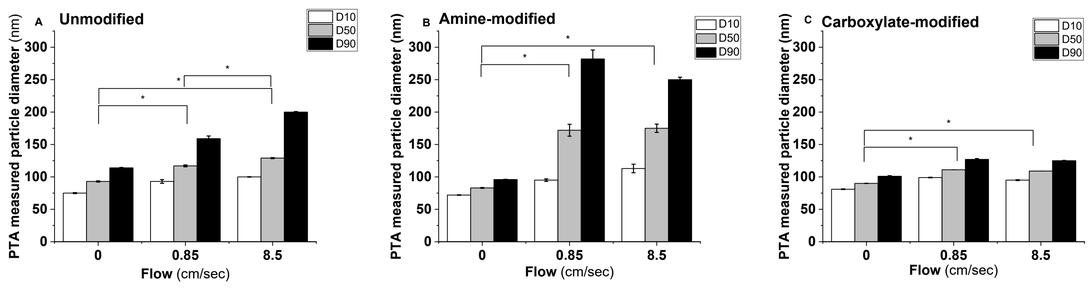


Figure 4. Shear flow impacts nanoparticle parameters following incubation with protein containing media. Polystyrene latex nanoparticles treated with PBS containing 10% v/v FBS under (0.85 cm/s, 8.5 cm/s) shear flow conditions for (2 hours) and isolated using three cycles of centrifugation wash isolation. P<0.05 deemed as statistically significant for a paired t-test. Independent replicated (n=3) with (n=5) measurements per replicate.

Unmodified and carboxylate-modified polystyrene latex nanoparticles were incubated for (2 hours) at (0.85 cm/s, 8.5 cm/s), mimicking biological shear flow conditions in the median cubital vein and arteries. PTA analysis following isolation shows a significant increase in mean particle size when incubated under shear flow conditions.

3) The impact of the centrifugation-wash method on particle parameters

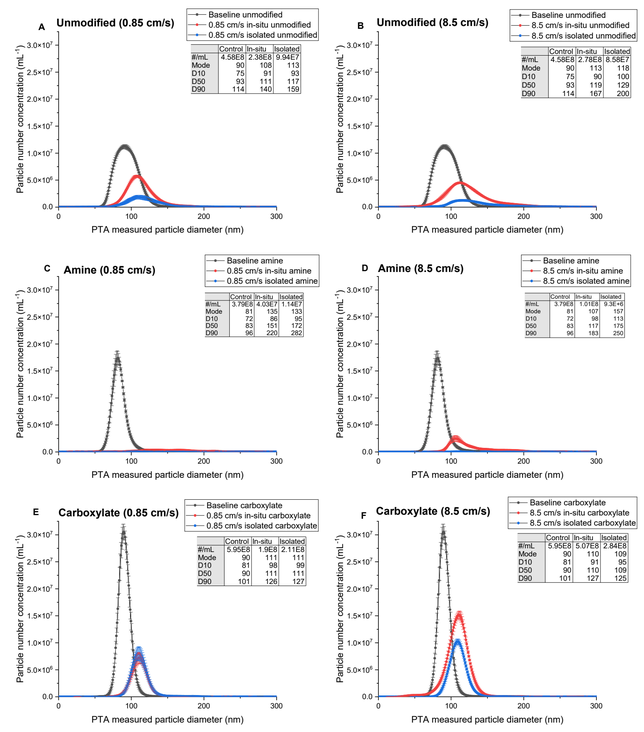


Figure 5. Sample preparation techniques influence nanoparticle parameters following treatment with PBS containing 10% v/v FBS. Unmodified and carboxylate-modified polystyrene latex polystyrene nanoparticles incubated at (0.85 cm/s, 8.5 cm/s) in protein-containing media for 2 hours, measured at baseline and following incubation using (NTA). Baseline traces are represented by (--), in situ analysis with NTA following 2 hour incubation by (--), and centrifugation-resuspension isolated particles by (--), n=3 independent replicates.

Unmodified, amine-, and carboxylate-modified polystyrene latex nanoparticles were incubated for (2 hours) under various shear flow conditions (0.85 cm/s, 8.5 cm/s). NTA analysis was performed as in line in-situ analysis and on nanoparticles isolated using the centrifugation-resuspension technique. We see an increase in mean nanoparticle size following incubation under physiologically relevant flow conditions (0.85 cm/s-median cubital vein) and a further increase at (8.5 cm/s- arterial). **Isolation via the centrifugation-wash protocol is highly invasive leading to an increase in mean nanoparticle size due to the centrifugation-resuspension steps, and sample loss at each step of centrifugation-resuspension.**

4) A comparison of protein composition with SDS-PAGE

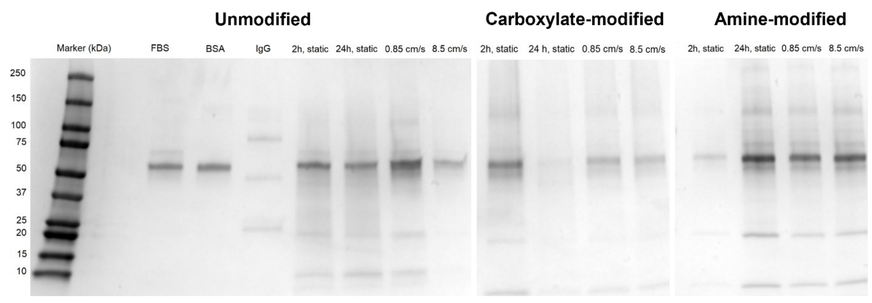


Figure 6. SDS-PAGE shows different protein composition profile for proteins isolated from unmodified, amine- and carboxylate-modified polystyrene latex nanoparticles treated with media containing protein under shear versus static conditions. The total amount of sample loaded was normalized to the sample protein content (20 μg per lane).

- The protein composition profile differed between polystyrene nanoparticles with different surface chemistries (~15 kDa band present with carboxylate-modified nanoparticles, but absent with unmodified nanoparticles).
- The identity and relative quantity of proteins differed in composition as a function of shear flow conditions to which the particles were subjected versus static conditions.

5) In situ analysis of changes in nanoparticle parameters with AF4-MALS-UV

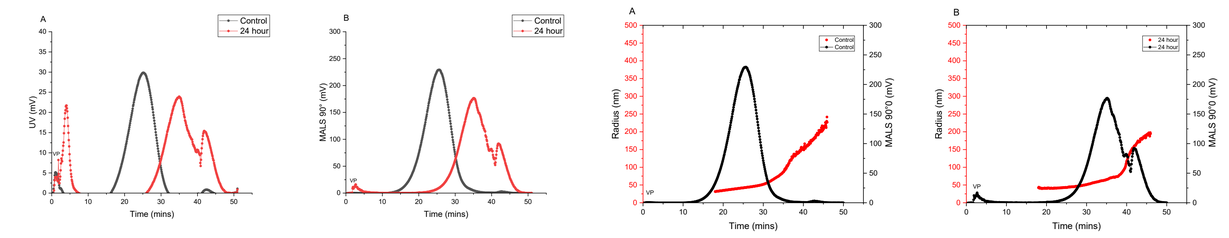


Figure 7. AF4 Fractograms showing the elution profiles and radius for unmodified nanoparticles at baseline and following (24 hr) incubation. Spacer thickness 350 μm, amphiphilic regenerated cellulose 10 kDa membrane and elution buffer 0.2% NovaChem

Following incubation within protein-containing medium a shift in the elution profile was observed accompanied by an increase in size and the gentle separation of nanoparticle-protein complexes **AF4 is a gentle separation technique for studying the impact of protein corona formation on nanoparticle parameters.**

Conclusions & Future Work

- The centrifugation-wash isolation method is highly invasive and leads to an increase in mean particle size and sample loss, limiting the relevance of this approach in studying the protein corona.
- There is an increase in mean particle size when nanoparticles are incubated under shear flow conditions (0.85 cm/s, 8.5 cm/s) when compared to static incubations.
- AF4 allows for the gentle separation of nanoparticle-protein samples from protein-containing medium giving us a more accurate representation of nanoparticle size within the biological system.

References

Jayaram, D.T. *et al.* (2018) *Biophys J,* 115 (2), pp. 209-216

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