Supporting information

Formation and characterization of stable antifouling colloidal particles via Surface-Initiated-PET-RAFT for standardized biophysical measurements

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MIFlowCyt

This document aims to provide the minimum information required to reproduce the flow cytometry experiments on colloidal particles performed in the study: “Formation and characterization of stable antifouling colloidal particles via Surface-Initiated-PET-RAFT for standardized biophysical measurements”. This document is based on one published standardization frameworks and guidelines [1].

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Experiment overview

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Purpose

The goal of these experiments is to investigate the biofouling of bare and polymer brush functionalized polystyrene colloidal particles, as well as investigate their stability over a pre-determined time period. We hypothesize that the bare polystyrene colloidal particles show significant biofouling as well as being unstable over time. Whilst the polymer brush functionalized polystyrene colloidal particles show decreased biofouling and will be stable over time.

Keywords

Extracellular vesicles, flow cytometry, human blood plasma, Polystyrene colloidal particles, biofouling, polymer brushes.

Experiment variables

Experimental variables were the different types of bare or functionalized polystyrene colloidal particles. With bare polystyrene colloidal particles not being functionalized. Polystyrene colloidal particles functionalized with *N*-(2-hydroxypropyl)methacrylamide (HPMA), 2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate (MPC), or 3-[[2-(methacryloyloxy)ethyl]dimethylammonio] propionate (CBMA) polymer brushes.

All types of colloidal particles were tested on their biofouling in 10% HiLyte fluor 488™ labelled plasma (Plasma-HF488), 5 mg/mL Fluorescein isothiocyanate labelled bovine serum albumin (BSA-FITC), and Dulbecco's phosphate-buffered saline (DPBS).

Experiment design and quality controls

All samples were measured using an autosampler, which facilitates subsequent measurements of samples in a 96-well plate. Flow rate, and light scattering calibrations were performed daily.

Dates

Flow cytometry experiments were performed between August and September 2024.

Conclusions

As hypothesized the bare polystyrene colloidal particles showed significant biofouling and were unstable over time. Whilst the HPMA and MPC functionalized colloidal particles showed no biofouling and were stable over time. Surprisingly the CBMA functionalized colloidal particles showed significant biofouling and were unstable over time.

Sample details

Sample storage

Samples were stored at 4 °C prior to analysis.

Sample characteristics

Four different types of polystyrene colloidal particles were measured. The bare polystyrene colloidal particles and Polystyrene colloidal particles functionalized with *N*-(2-hydroxypropyl)methacrylamide (HPMA), 2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate (MPC), or 3-[[2-(methacryloyloxy)ethyl]dimethylammonio] propionate (CBMA) polymer brushes.

Sample dilution

As the concentration of particles in plasma differs $>10^2$-fold between donors, samples require different dilutions to (1) avoid swarm detection and (2) detect a statistically significant number of events within a measurement time of a few minutes. The optimal dilution factor is the minimum dilution factor that is required to prevent swarm detection. For the flow cytometer and settings used, the optimal dilution factor for plasma is $\geq 1.1 \cdot 10^2$ -fold and should result in a count rate $<1.1 \cdot 10^4$ events$\cdot s^{-1}$ [2].

Concentrations of the colloidal particles were kept as low as possible, count rates of 100-300 counts per second were used for the colloidal particles.

Flow cytometer

Model and manufacturer

A60-Micro, Apogee Flow Systems, Hemel Hempstead, UK. The flow cytometer has not been altered. All components are original and came with the flow cytometer.

Configuration and settings

Flow rate and acquisition time

The flow cytometer is equipped with a syringe pump with volumetric control. Samples were analysed for 120 s at a flow rate of 3.01 µL/min.

Light sources

The flow cytometer has three lasers that illuminate a fixed-alignment cuvette flow cell. The laser powers were 100 mW, 150 mW and 150 mW for the 405-nm, 488-nm, and 638-nm laser, respectively.

Detectors

Table 1 shows an overview of the detectors used in this study.

Detector name	Detected property	Voltage (V)	Spectral filter bandwidth (nm)
405-SALS	Forward scattered light	470	
405-LALS	Side scattered light	367	
488-Green	FITC fluorescence and HiLyte Fluor488 fluorescence	560	575/30

Table 1. Detector name, detected property, voltage and spectral filter bandwidth of the detectors used in this study. FITC: fluorescein isothiocyanate; SALS: small angle light scattering; LALS: large angle light scattering.

Trigger detector and threshold

Based on the buffer-only control, a trigger threshold of 14 arbitrary units was applied to the side scattering detector. As (1) the A60-Micro applies the trigger threshold analogically, thus before digitalization of the signal, and (2) the arbitrary unit channel numbers differ between the data acquisition software and the flow cytometry datafiles, the trigger threshold was expressed in standard units by taking the mode of the effective side scattering cross section distribution and optical diameter distribution of a plasma sample. Plasma samples have a size distribution with a peak far below the detection limit of the A60-Micro. The modes of the effective side scattering cross section distribution and optical diameter distribution therefore represent the trigger threshold. The threshold of 14 arbitrary units, corresponds to a side scattering cross section of 3 nm^2

Data analyses

FlowJo software (Version 10.10.0) was used for the analysis of the nanosphere peaks. Singlets were gated out and the resulting counts were calculated back to the original sample volume for an undiluted concentration.

For fluorescence positivity the threshold was defined as the median plus 4 times the standard deviation of the fluorescence intensity histogram of the DPBS control measurement.

Calibrations

Flow rate

At the start of each measurement day, we applied the automated quality control system ApoCal (#0152, Apogee Flow Systems), which checks whether the flow rate is within 20% of the adjusted flow rate of 3.01 $\mu L \cdot min^{-1}$. For all days, the flow cytometer passed this quality control check.

Fluorescence calibration

Fluorescence calibration is not applicable to this study.

Light scattering calibration

Rosetta Calibration

Rosetta Calibration (v2.06, Exometry, The Netherlands) was used to relate the forward and side scattering intensities measured at a wavelength of 405 nm to the effective scattering cross sections[1] and optical diameter[2] of the colloidal particles. The colloidal particles are modelled as core particles with a core refractive index of 1.635, Figure 11 shows print screens of the light scatter calibrations.

[1] The effective scattering cross section is a hypothetical area of a particle that incoming light must impinge in order to be scattered towards the lens. The calibrated effective scattering cross section axis is independent of refractive index assumptions, but depends on the illumination wavelength and collection angles of the flow cytometer[5].

[2] The optical diameter equals the physical diameter of a particle when (1) the particle is spherical and (2) the particle has the same refractive index distribution as assumed in the model.

A B

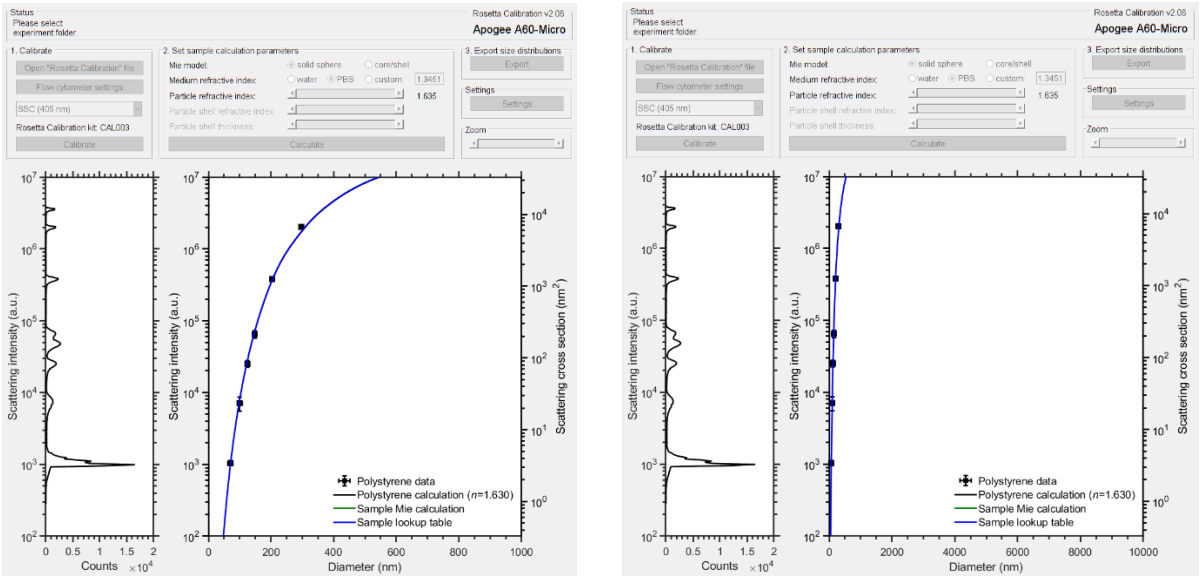

Figure 1. Forward scattering and side scattering calibration of the A60-Micro by Rosetta Calibration. To relate scatter to the diameter of EVs, EVs are modelled as core-shell particles with a core refractive index of 1.38, a shell refractive index of 1.48, and a shell thickness of 6 nm.

Gate description and boundaries

For fluorescence positivity the threshold was defined as the median plus 4 times the standard deviation of the fluorescence intensity histogram of the DPBS control measurement.

In the side scattering cross section histogram gates were set around the singlet peaks of the colloidal particles. The counts of these gates were used for the calculations of the undiluted concentration.

References

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