Supplementary Information

Paracrine signalling of inflammatory cytokines from an in vitro blood brain barrier model upon exposure to polymeric nanoparticles

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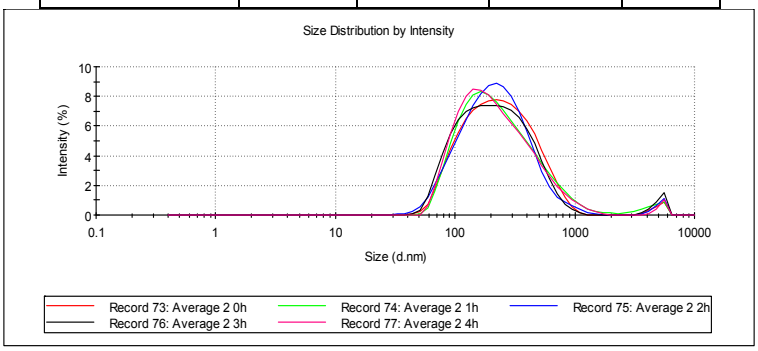
Exposure time (h)	z average diameter (nm)[a]	PDI [b]	Diameter (nm)[c]	Width (nm)
0	190	0,36	264	165
1	186	0,36	247	166
2	181	0,38	301	249
3	185	0,37	240	151
4	183	0,41	148	64

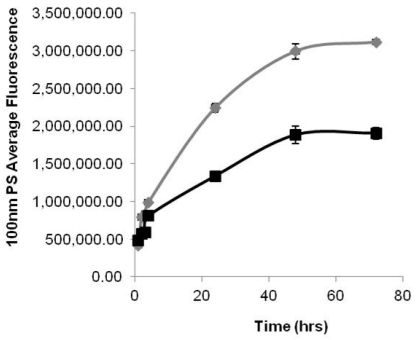


[a] z-average hydrodynamic diameter extracted by cumulant analysis of the data. [b] Polydispersity index from cumulant fitting. [c] Average hydrodynamic diameter determined from CONTIN size distribution.

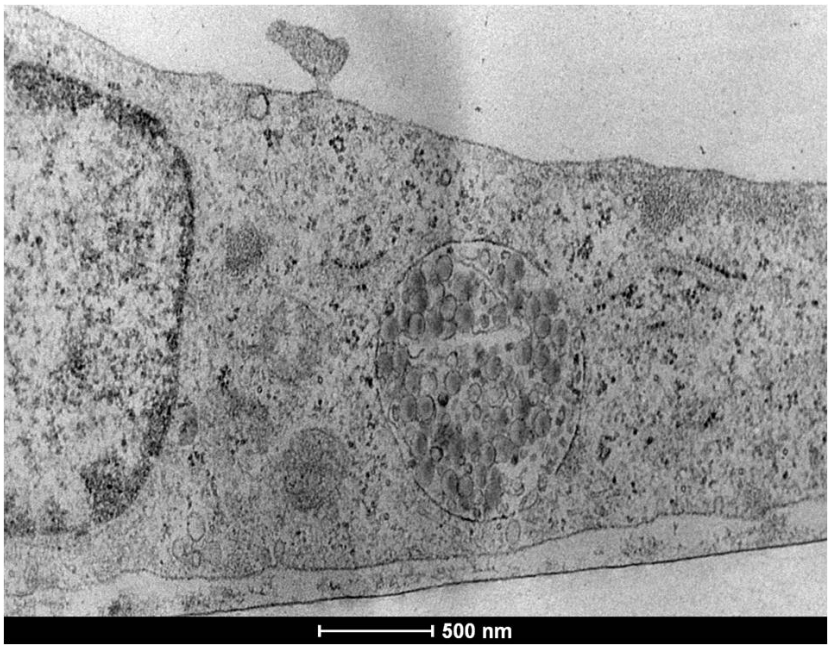
Supplementary Figure 1. DLS data of 100 nm PS COOH NPs dispersed in *in vitro* BBB cell culture medium over time. The average diameter of 100 µg/ml 100 nm PS COOH NPs in cell culture medium was measured over 4 h at 37°C. The table shows the average diameter and polydispersity index obtained by DLS. Below, the size distributions obtained after dispersion (0 h) and 1-4 h incubation at 37°C are also shown. The size distribution of 100 nm PS COOH remained relatively stable over 4 h of incubation in cell culture medium containing 2% serum proteins, confirming that the available dose of NPs remained constant thoughout the exposure period.

Solute	Temperature (°C)	Zeta-Potential (mV)
Water	37	-30
PBS	37	-35
Cell Culture Medium	37	-13

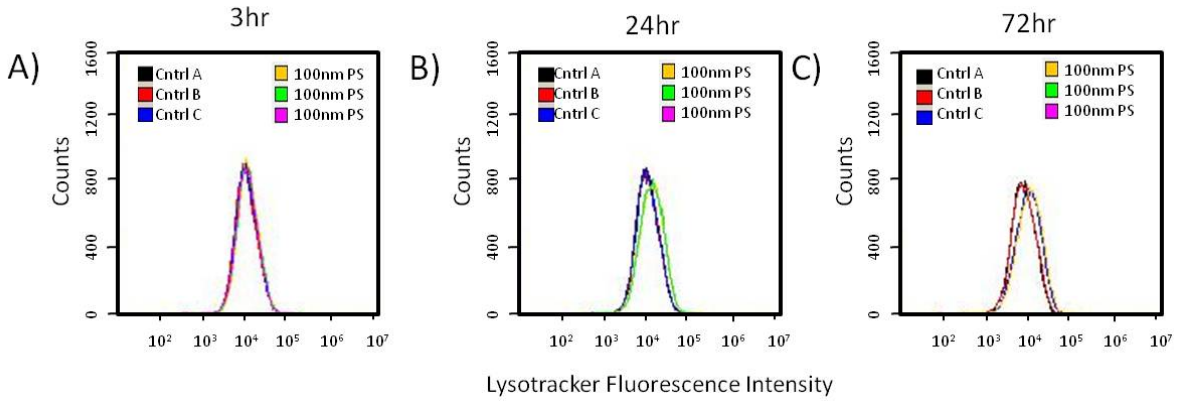
Supplementary Figure 2. Zeta-potential measurement of 100 nm PS COOH NPs. 100 nm PS COOH NPs were incubated in the different solutions over 4 h at 100 µg/ml. A decrease in (absolute) zeta potential was observed when NPs were dispersed in cell culture medium supplemented with 2% serum proteins compared to the value in water or PBS, as a consequence of protein adsorption on the NPs and corona formation.



Supplementary Figure 3. Low level uptake of 100 nm PS COOH NPs within the *in vitro* BBB monolayer over time. Flow cytometry showed lower NP uptake post acute exposure (0-4 h) of 100 nm PS COOH in confluent polarised hCMEC/D3 barriers (black line), in comparison to what is observed in hCMEC/D3 cells (gray line). Prolonged exposure (24-72 h) gave an increase in 100 nm PS COOH NP uptake compared to earlier time points for both barrier and hCMEC/D3 cells. (n=3, nanoparticle concentration 100 µg/ml).



Supplementary Figure 4. TEM image showing accumulation of 100 nm PS COOH NPs in a lysosome of *in vitro* BBB hCMEC/D3 cells 28 h post exposure. The barrier was exposed to 100 µg/ml PS COOH NPs.



Supplementary Figure 5. No considerable alteration in lysosomal compartment size upon accumulation of 100 nm PS COOH NPs in the *in vitro* BBB model over time. Flow cytometry analysis of *in vitro* BBB monolayer upon uptake of non-fluorescent 100 nm PS COOH NPs (100 µg/ml) post LysoTracker staining (n=3). (A) Acute exposure of BBB monolayer to 100 nm PS COOH NPs showed no difference in LysoTracker staining compared to control. (B & C) A small shift in LysoTracker staining was found upon a more prolonged (24-72 h) hCMEC/D3 barrier exposure to 100 nm PS COOH NPs compared to control, possibly indicating a minor lysosomal alteration at longer exposure time. Note from Supp. Figure 3 that mean particle uptake (fluorescence intensity) is about constant from 24-72 h.