Electronic Supplementary Information (ESI) for Chemical Communications

This journal is at The Royal Society of Chemistry 2013

Red blood cells decorated with functionalized core-shell magnetic nanoparticles: Elucidation of the adsorption mechanism

Thanh Duc Mai,[a,b,c] Fanny d'Orlyé,[b] Christine Ménager,[a] Anne Varenne,*[b] Jean-Michel Siaugue*[a]

[a]*UPMC University of Paris 06-CNRS-ESPCI Laboratoire Physicochimie des Electrolytes, Colloïdes et Sciences AnalytiquesPECSA UMR 7195, 4 place Jussieu, 75252 Paris, France*

[b] *Chimie ParisTech, Ecole Nationale Supérieure de Chimie de Paris ; Imagery, Chemical and Genetic Pharmacology Unit (UPCGI); UMR CNRS 8151 - U INSERM 1022; 11, rue Pierre et Marie Curie - 75231 Paris Cedex 05, France*

[c]*Centre for Environmental Technology and Sustainable Development (CETASD), Hanoi University of Science, Nguyen Trai Street 334, Hanoi, Viet Nam*

*Corresponding authors:

Jean-Michel Siaugue, email: jean-michel.siaugue@upmc.fr

Anne Varenne, e-mail: anne-varenne@chimie-paristech.fr

Materials and methods

Materials

Neuraminidase from Clostridium perfringens (C. welchii, type V), phosphate buffered saline (PBS) 10X, Sucrose, Trypton X-100, 3-(N-Morpholino)-propanesulfonic acid (MOPS), tetraethylorthosilicate (TEOS), ethylen diamine tetra-acetate (EDTA), 3-aminopropyltriethoxysilane (APTS), and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France); 2-(methoxy(polyethyleneoxy)propyl)-trimethoxysilane (PEOS) was purchased from Gelest, Inc., Morrisville, PA, USA.

Collection of erythrocytes

Red blood cells of porcine origin previously anti-coagulated with EDTA 6% w/w ($V_{blood}/V_{EDTA\ 6\%} = 25$) were washed three times with PBS 1X (10 times dilution of PBS 10X), followed by a twice rinse with a buffer of MOPS (pH 7,4 and ionic strength of 100 mM) containing sucrose (concentration of 50 mM) (M100S50) so as to maintain isotonicity of the buffer. Note that this buffer was also employed for dispersion of CSMNs as it offers a good colloidal stability of CSMNs' suspension (stable for months). After each rinse, erythrocytes were separated from the buffy solution, which subsequently was discarded, by centrifugation at 3000 rpm for 5 min. The washed RBCs were then dispersed in M100S50 to form a suspension of RBCs 10% v/v and were stored at 4 °C for maximum 1 week.

Interactions between CSMNs and RBCs

For decoration of RBCs with CSMNs, 1 mL of RBCs 10 % v/v in M100S50 was mixed with 1 mL of CSMNs dispersed in the same buffer and vortexed for 5 min. The mixture was stored at 4 °C until use. Right before zeta potential measurements or optical/ confocal/ transmission electron microscopy observations, 0.2 mL of the mixture was diluted to 1 mL with M100S50

and centrifuged at 3000 rpm for 5 min. The supernatant containing extra CSMNs was then discarded and the packed RBCs at the bottom were collected, washed twice with M100S50 and re-dispersed with this buffer to form a suspension of about 1 % v/v RBCs.

Enzymatic treatment of RBCs with neuraminidase

Incubation of erythrocytes with neuraminidase was performed by addition of an excessive amount of this enzyme in the powder form into 1 mL of the RBCs suspension (10 % v/v) in M100S50. The mixture was then gently shaken for 3h at 37°C. Controls were performed under the same conditions in the absence of neuraminidase. Every hour, a 100 µL aliquot was withdrawn, washed with M100S50, centrifuged, decanted to remove the supernatant and redispersed in 1 mL M100S50 for zeta potential measurements.

Haemolysis assay

To test the haemolytic activity, the packed RBCs decorated with CSMNs of different surface charge densities were washed twice and dispersed in M100S50 to form suspensions of 5 % v/v RBCs. These suspensions were then incubated at 37 °C for 8 hours. At different times during the incubation, 100 µL aliquots were taken out, diluted 10 times with M100S50 and centrifuged. The absorbance of the diluted supernatants ($A_{RBC\text{-}CSMN}$) was measured at 415 nm. At the same time, RBC 5% v/v in M100S50 and RBC 5% v/v in M100S50 added with 1 % v/v Trypton X-100 detergent (lysis buffer) were undergone the same incubation conditions. Their absorbance profiles were recorded and used as negative and positive controls ($A_{Negative}$ and $A_{Positive}$), respectively. The percentage of haemolysis was calculated according to the following equation:

$$Hemolysis(\%) = \frac{A_{RBC-CSMN} - A_{Negative}}{A_{Positive} - A_{Negative}} \times 100$$

Supplementary Figures

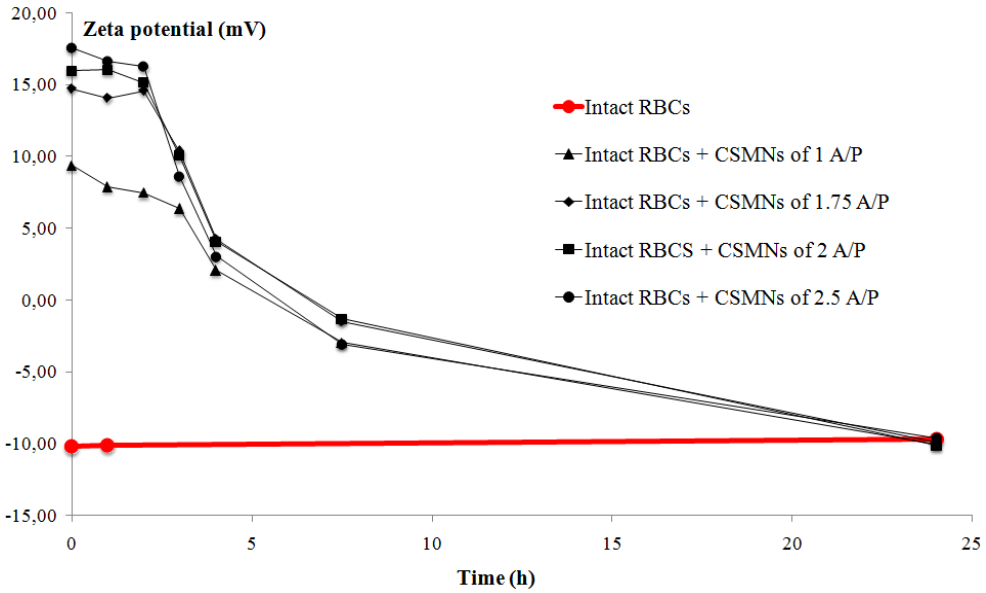

Fig. S1 Release of CSMNs from erythrocytes' surface over time. Each experimental point is the mean of 3 replicates whose relative standard deviations are less than 6 %.

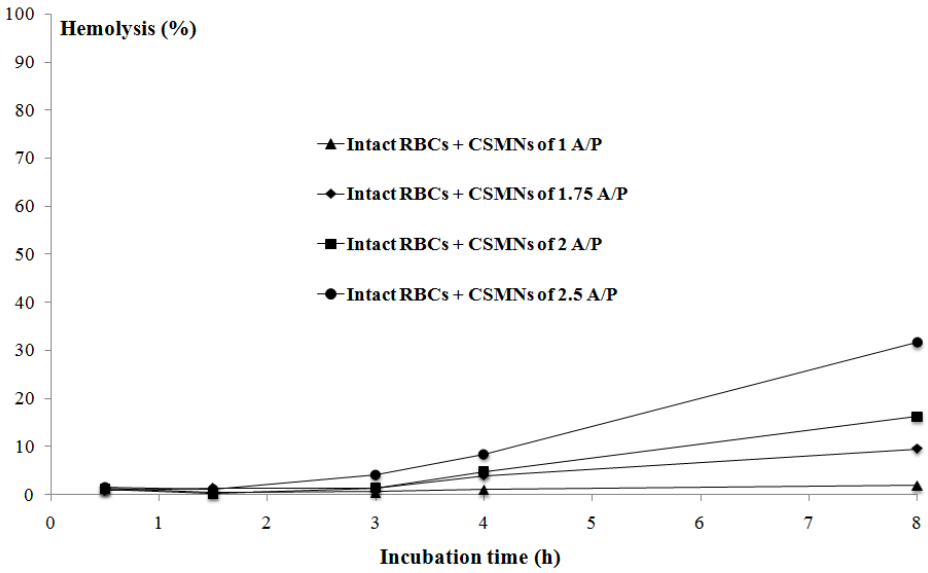

Fig. S2 Haemolysis of CSMNs-grafted RBCs over time