Experimental Section

Materials

Fluorescent yellow-green polystyrene 3μm carboxyl functionalized particles (PS) were purchased from Polysciences Inc. (Warrington, PA). Poly(2-(methacryloyloxyethyl)-2-(trimethyl-ammoniumethyl)phosphate,inner salt / 3-(2-amminoethylsulfanyl)-2-hydroxypropyl methacrylate hydrochloride) was a generous gift of NOF Co. Ltd. (Tokyo, Japan). AlexaFluor 647 ovalbumin (OVA) was obtained from Life Technologies (Grand Island, NY). Ovalbumin was obtained from InvivoGen (San Diego, CA). O-(2-Aminoethyl) polyethylene glycol (PEG, MW 5000) was obtained from Sigma Aldrich (St. Louis, MO). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was obtained from Thermo Scientific (Rockford, IL). N-hydroxysulfosuccinimide (Sulfo-NHS) was obtained from G-Biosciences (St. Louis, MO). 2-(N-Morpholino) ethanesulfonic acid hydrate (MES) was obtained from Acros Organics (City, NJ).

Cells

J774A.1 macrophages (American Type Culture Collection (ATCC), Manassas, VA) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (ATCC) at 37°C in a humidified atmosphere containing 5% CO_2. All media was supplemented with 10% fetal bovine serum (Seradigm, Radnor, PA) and 1% penicillin/streptomycin (Amresco, Solon. OH). Cells used in experiments were between passages 4 and 10.

Methods

MPCAM Coating

18 mg of MPCAM polymer (5 wt% in water) was added to 1 ml of an 80% ethanol-water solution. 1.64 mg of carboxyl functionalized particles were added to the solution and sonicated for 15 min. The particles were centrifuged at 5000g for 3min, washed twice in PBS, and stored at 4°C.

PEG Conjugation

1.64 mg of carboxyl-functionalized particles was washed twice in 0.1 M MES buffer (pH 5.5). The activated particles were then suspended in 0.1 M MES buffer containing 8 mM Sulfo-NHS and 15 mM EDC and reacted for 15 min. Then, 0.625 mM PEG was added to the solution and the reaction was continued overnight at 4°C. The particle suspension was centrifuged at 7500g for 5 min and washed twice with phosphate buffered saline (PBS) and stored at 4°C .

Characterization of Particles

Zeta potential was determined by measuring the electrophoretic mobility of particles in 10 mM NaCl using Zetasizer Nano ZS90 (Malvern Instruments Ltd., Westborough, MA). The existence of MPC groups on the particle surface was confirmed by X-ray photo- electron spectroscopy

(XPS; Thermo K-Alpha XPS, Waltham, MA). A small drop of MPCAM coated particles was placed on a glass slide and allowed to dry for 24 hours prior to XPS characterization.

Ovalbumin Adsorption on Particles

MPCAM coated, PEG conjugated, and carboxyl uncoated particles (14.9 μg each) were incubated with 0.01 mg/ml fluorescent OVA for one hour. The particles were spun down at 7500g for 5min and washed twice with PBS. The particles were interrogated by a flow cytometer (Accuri C6, Beckton Dickenson Biosciences, Ann Arbor, MI) to monitor any increase in particle fluorescence due to OVA adsorption.

Ovalbumin Adsorption on Particles at High Concentration, Long Time Condition

MPCAM coated, PEG conjugated, and carboxyl uncoated particles (0.149 μg each, after 6 months of storage in PBS at 4°C) were incubated with 10.01 mg/ml OVA protein solution for twenty four hours at room temperature. The OVA protein solution was a combination of fluorescent and non- fluorescent OVA. The fluorescent OVA constituted 0.1% of the total OVA protein by mass. The particles were spun down at 17900g for 10 min and washed twice with PBS. The particles were interrogated by a flow cytometer (Accuri C6, Beckton Dickenson Biosciences, Ann Arbor, MI) to monitor any increase in particle fluorescence due to OVA adsorption.

Stability of MPCAM Coating on Particles

The stability of the MPCAM coating on particles was tested in PBS buffer. The particles were left in PBS for four weeks at 4°C and their effectiveness against OVA adsorption was tested as described above and compared to PEG conjugated particles.

Adsorbed OVA Secondary Structure

Circular dichroism (CD) spectra were acquired on a Jasco J-810 spectrometer (Easton, MD) with the sample chamber maintained at 25°C. Measurements were made using a 0.2 mm path length quartz cell. The average spectra of five measurements were obtained from a wavelength range of 200-250 nm with 1 nm increments. PBS without protein was used as a blank. Spectra for ovalbumin (OVA) (0.01 mg/ml) were measured in solution and in the presence of MPCAM coated, PEG conjugated, and carboxyl uncoated particles (0.07μg each). The particle concentration was optimized to reduce scatter and absorbance. OVA and particles were incubated for one hour prior to acquisition. Spectra were acquired in millidegrees and converted to mean residue ellipticity using the following equation.

$$[\theta] = \frac{[\theta]_{obs}\, MW}{10lCn}$$

The mean residue ellipticity in units of degrees cm^2/dmol ($[\theta]$) is a function of the observed signal in millidegrees, $[\theta]_{obs}$, the average molecular weight of the protein (MW), path length in cm (l), protein concentration in g/L (C), and the total number of amino acids (n). Estimation of

secondary structure content was performed with the CDSSTR program provided in the CDPro software package.

Phagocytosis Assay

Cells were seeded in 24 well plates at a concentration of 10^5 cells per well and were allowed to adhere overnight. Particles (MPCAM coated, PEG conjugated, carboxyl uncoated) were added to cells in supplemented media at 10 particles/cell and incubated for two hours at 4°C. After cold incubation, samples were moved to 37°C, 5% CO2, humidified for one hour. Cells were then scraped from the wells, washed three times with cold PBS to remove unattached particles and analyzed by flow cytometry. For serum independent experiments, particles were incubated with OVA for one hour and subsequently added to cells in serum free media with the same incubation procedure described above. 10,000 cells were counted in each condition and was repeated with freshly coated particles.

Statistical Analysis

All quantitative experiments were performed in triplicate and are presented as arithmetic mean ± SD. Phagocytosis assay was performed in duplicate. One-way ANOVA was used to determine significance among groups. p values <0.05 among groups were considered statistically significant.

Supplementary Figures

Structure of MPCAM Polymer

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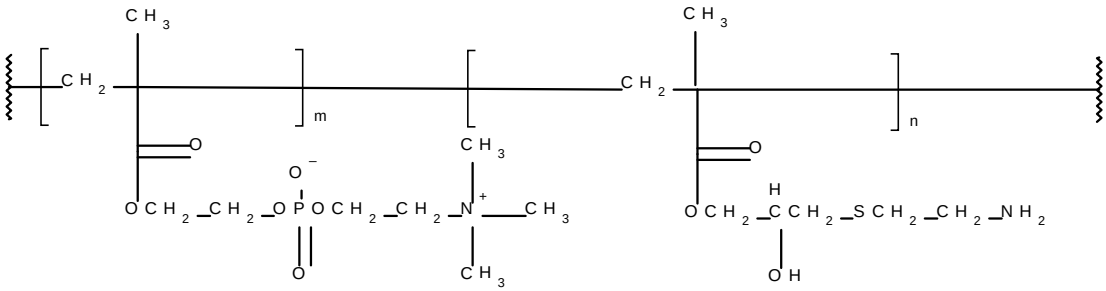

Fig. S 1 Poly(2-methacryloyloxyethyl)-2-(trimethyl-ammoniumethyl)phosphate, inner salt/ 3-(2-aminoethylsulfanyl)-2-hydroxypropyl methacrylate) polymer. m:n 85:15 %

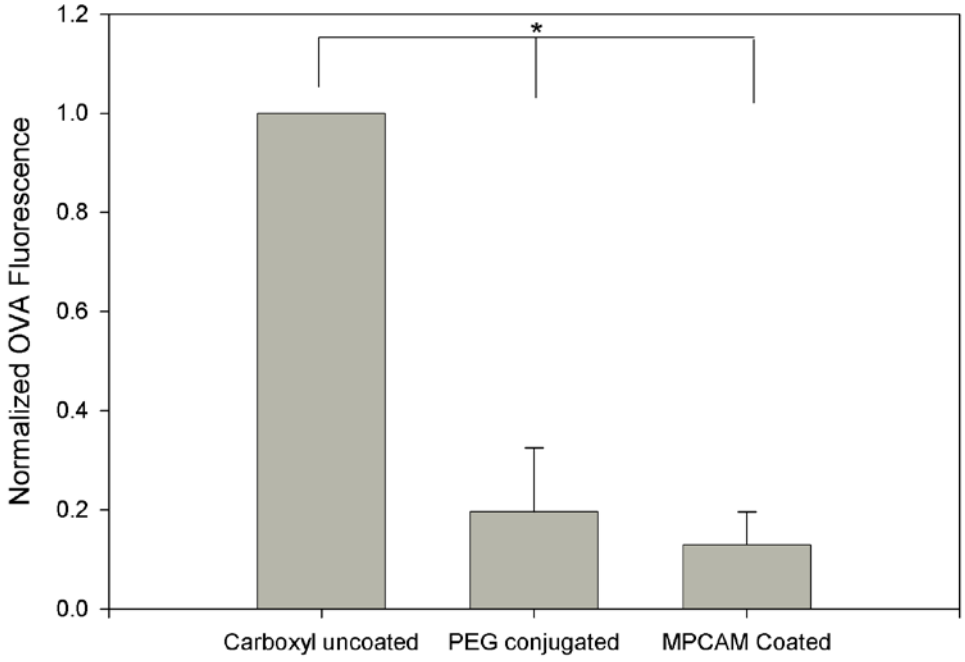

Fig. S 2 Normalized OVA fluorescence of particles was obtained with respect to adsorption on carboxyl particles. These fluorescence values were obtained after OVA adsorption for one hour at room temperature. The median fluorescence value of particles was normalized with respect to median fluorescence value of carboxyl particles upon OVA adsorption.

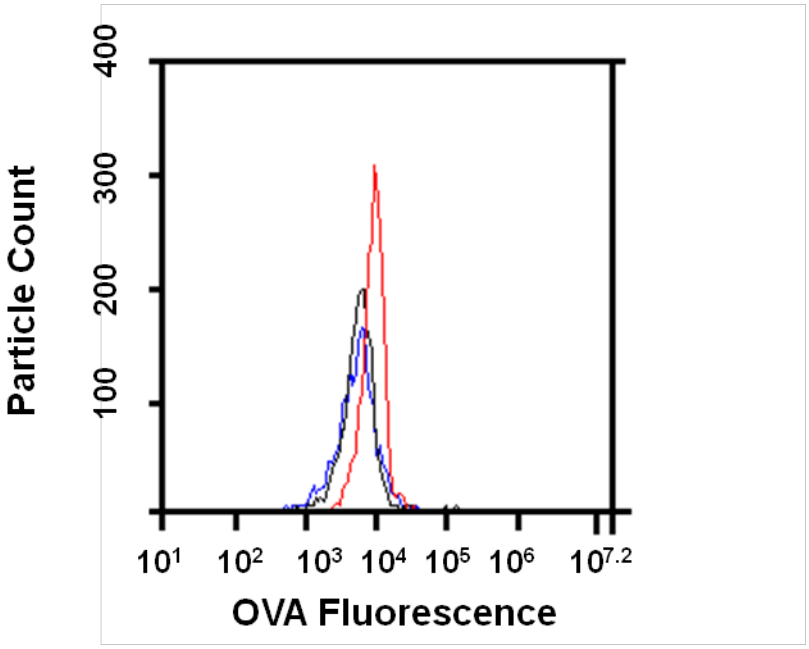

Fig.S 3 Fluorescent OVA adsorption measured by particle fluorescence after twenty four hours of incubation with a high concentration mixture of fluorescent and non-fluorescent OVA at room temperature. MPCAM coated (blue), PEG conjugated (black), carboxyl (red) particles upon OVA adsorption after storing in PBS for 6 months at 4°C.

CD Spectroscopy of Particles

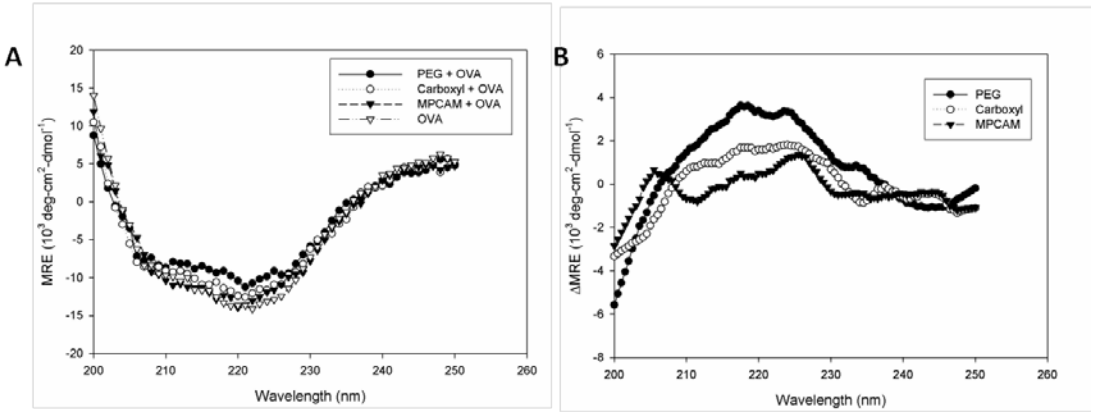

Fig. S 4 CD Spectra of OVA in the presence of PEG conjugated (●), Carboxyl uncoated (○), MPCAM coated Particles (▼) and in the absence of particles (▽). Spectra reported in units of mean residue ellipticity (MRE). Spectra was recorded after one hour of incubation with OVA at room temperature.(A) Raw CD Spectra. (B) CD difference spectra calculated by subtracting and smoothing the spectra of OVA from the spectra of OVA in the presence of PEG conjugated particles (●) or Carboxyl uncoated particles (○) or MPCAM coated particles (▼).