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Supplementary Materials

Influences of Surface Coating of PLGA Nanoparticles on Immune Activation of Macrophages

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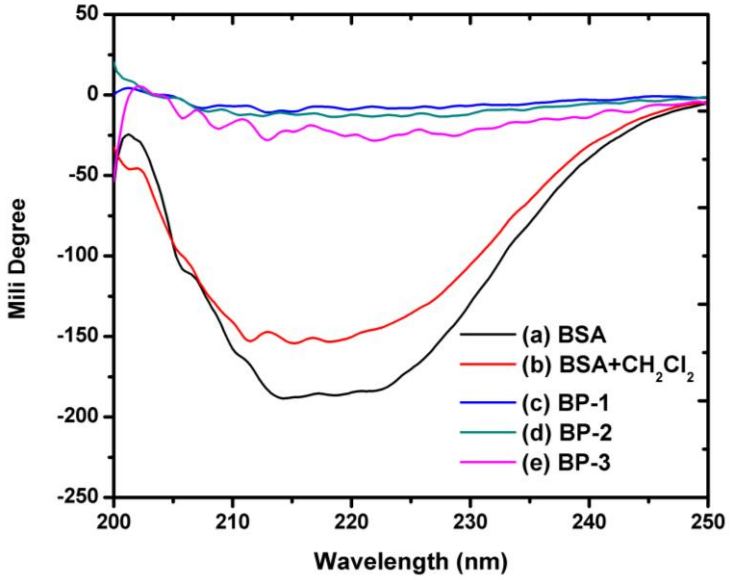

Figure S1 CD spectra of (a) BSA solution (30 mg/mL), (b) BSA solution (30 mg/mL) treated with CH_2Cl_2, and (c-e) 3 kinds of BSA/PLGA NPs (1 mg PLGA/mL).

The secondary protein structure of BSA on BSA/PLGA NPs was detected by circular dichroism spectroscopy (CD, JASCO-820, Japan). All the BSA/PLGA NPs stock solutions were diluted by PBS to form 1 mg/mL solutions for the measurement at 25 ^{o}C, respectively. As a comparison, BSA solution and BSA solution treated with CH_2Cl_2 were measured similarly.

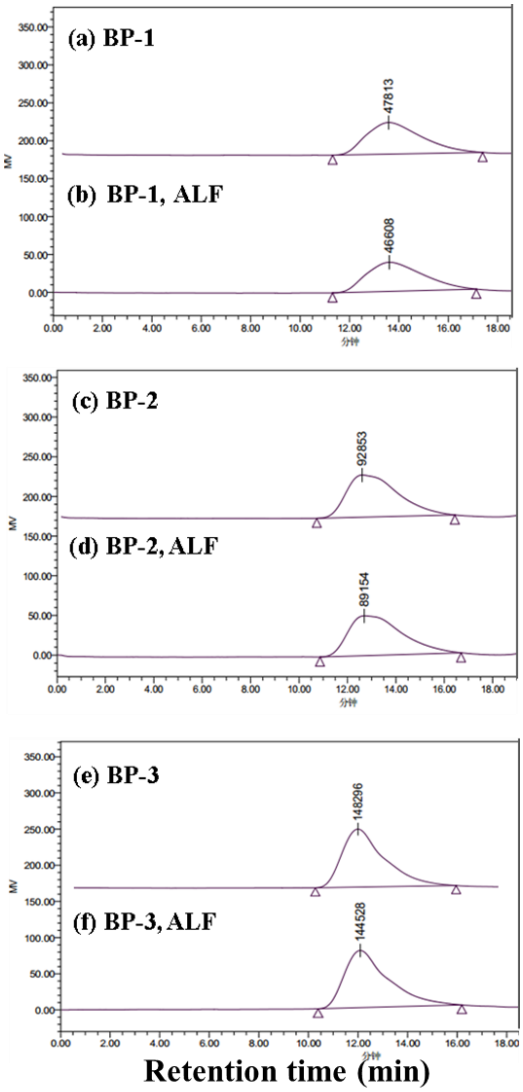

Figure S2 GPC curves of BSA/PLGA NPs before and after being incubated in ALF 4.5 for 7 days *in vitro*. The concentration of NPs was 100 μg/mL.

The degradation behavior of BSA/PLGA NPs being co-incubated with artificial lysosomal fluid (ALF) or RAW264.7 cells was evaluated by measuring the molecular weights of BSA/PLGA NPs by gel permeation chromatography (GPC, Waters 515, Water, USA). The artificial lysosomal fluid with a pH value of 4.5 (ALF 4.5) was prepared according to Midander's work [1].

The 3 kinds of BSA/PLGA NPs with different molecular weights were collected and re-suspended in the ALF solution with a concentration of 1 mg/mL. The mixtures were shaken in a thermostatic water bath at 37 ºC for 7 days. All the NPs were then collected and vacuum dried, and dissolved in

anhydrous tetrahydrofuran (THF) for GPC determination. Moreover, RAW264.7 cells were seeded into a large culture flask at a density of 5×10^5 cells/well and maintained for 12 h till adherence. Then the medium was replaced with fresh one containing 100 μL/mL BSA/PLGA NPs, respectively. After co-incubated for another 7 days, the BSA/PLGA NPs were collected and vacuum dried, and dissolved in anhydrous THF for GPC determination. The molecular weights of BSA/PLGA NPs before and after being incubated in ALF 4.5 were displayed in Figure S2, showing that they were not altered significantly. The NPs co-incubated with cells had a similar result.

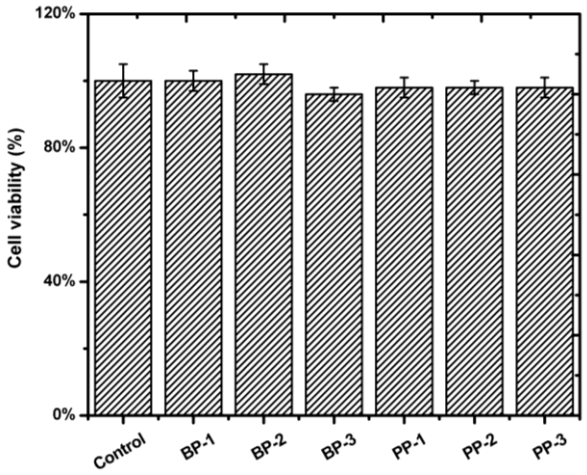

Figure S3 Cytoviability of RAW264.7 macrophages after being incubated with PLGA NPs for 24 h. The concentration of NPs was 100 μg/mL.

MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a compound that can form specific dark blue formazan crystals in the presence of metabolically active mitochondria. Thus the cytoviability was estimated by using MTT assay.

Firstly, RAW264.7 cells were plated at a density of 1×10^4 cells/well in a 96-well plate and cultured for 12 h till adherence. The medium was replaced with fresh one containing 100 μL/mL PLGA NPs, respectively. After being co-incubated for another 24 h, the medium was replaced with

100 μL fresh one containing 0.5 mg/mL MTT. After another 4 h culture at 37 °C, the dark blue formazan crystals were dissolved by dimethyl sulfoxide (DMSO), whose absorbance (i.e. the optical density (O.D.)) was measured at 562 nm by a microplate reader. Then all the O.D. values were normalized to that of the particle-free cell control (Figure S3).

Phenotypes of RAW264.7 cells were studied via immunofluorescent staining at the very beginning to ensure that the cells are not polarized and remain to be mononuclear macrophage. Generally, CD68 and CCR7 are considered to be the main specific antibody molecules for M_0 cells and M_1 cells, respectively [2-3]. After incubated with FITC-CD68/PBS or PE-CCR7/PBS antibody working solutions, the RAW264.7 cells were detached and re-suspended in PBS. The average inflorescence intensity of cells was then determined by flow cytometry (FACS Calibur, Becton Dickinson, USA) (Table S1).

Table S1 The flow cytometry results of immunofluorescence staining

	CD68 staining		CCR7 staining	
	Negative (%)	Positive (%)	Negative (%)	Positive (%)
RAW264.7 cells	94.9	5.1	97.7	2.3

The specific antibody binding experiments reveal that very small percentages of cells were stained positive, suggesting that the RAW264.7 cells used in this study remain to be mononuclear macrophages rather than transferring to M_0 cells or M_1 cells. Thus the phenotype difference of RAW264.7 cells is not considered as a factor influencing the uptake capacity of PLGA NPs.

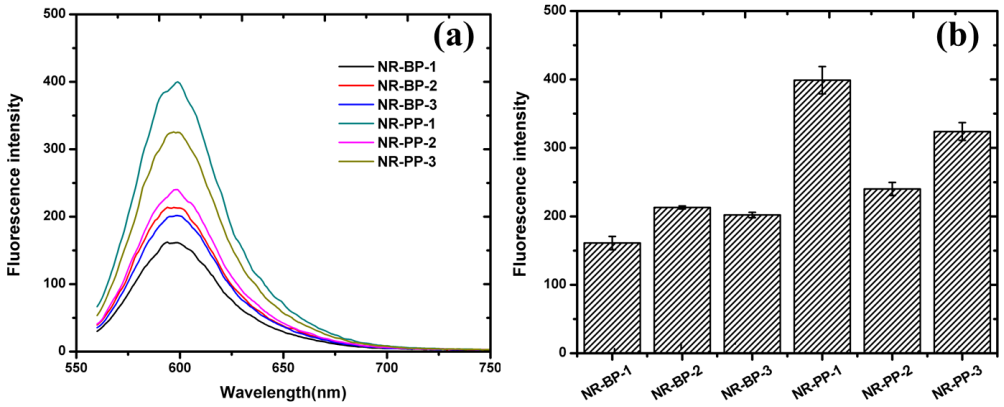

Figure S4 Fluorescence intensity of the 6 kinds of PLGA NPs. (a) The emission spectra of Nile red-labeled PLGA NPs under an excitation wavelength of 543 nm, and (b) the relative fluorescence intensity at an emission wavelength of 598 nm.

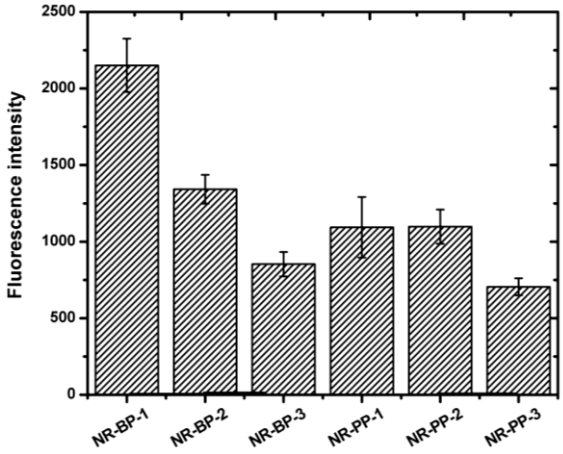

Figure S5 Fluorescence intensity of cells being incubated with 100 μg/mL PLGA NPs for 24 h. The data were obtained by flow cytometry.

To monitor the cellular uptake of the PLGA NPs by flow cytometry and to visualize the intracellular NPs distribution by confocal laser scanning microscopy (CLSM, LSM510, Zeiss, Germany), minor amount of Nile red was loaded into the BSA/PLGA and PEI/PLGA NPs.

Firstly, the florescent intensity of each kind of PLGA NPs was determined by fluorescent spectroscopy (LS55, Perkin Elmer, USA) (Figure S4a). The sample preparation procedure was as follows. A known weight of each kind of NR-PLGA NPs was dissolved in dichloromethane to form a

homogeneous solution, and then the fluorescent intensity was measured at an emission wavelength of 543 nm. The fluorescence intensity at the emission wavelength of 598 nm was quantitatively compared in Figure S4b.

Secondly, the average fluorescence intensity of RAW264.7 cells after being co-incubated with Nile red-labeled PLGA NPs for 24 h was determined by flow cytometry (Figure S5). The raw data obtained by flow cytometry were further normalized according to the unit fluorescence intensity of each Nile red-labeled PLGA NPs. In this way, the internalized amount of PLGA NPs can be quantitatively compared with each other.

Figure S6 Qualitative detection of bacterial endotoxin via Gel Clot LAL Assay. The concentration of BSA and HSA solutions was 10 mg/mL.

To acquire an endotoxin test with a detection limit of 0.25 EU endotoxin, a standard endotoxin solution with a concentration of 0.25 EU was prepared. All the standard group and test groups were preceded according to the standard procedure by incubating the test tubes at 37 ºC for 1 h without disturbance. Figure S6 shows that a solid gel was formed for both the BSA test sample and the 0.25 EU standard endotoxin solution (i.e. positive control), whereas no gel was formed for the HSA test sample and the pyrogen-free diluting water (i.e. negative control).

Reference

[1]. Midander K, De Frutos A, Hedberg Y, et al. Bioaccessibility studies of ferro-chromium alloy particles for a simulated inhalation scenario: A comparative study with the pure metals and stainless steel [J]. Integrated Environmental Assessment and Management, 2009, 6(3): 441-455.

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