Differential Response of Macrophages to Core–Shell Fe_3O_4@Au Nanoparticles and Nanostars: Electronic Supplementary Information

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Synthesis of SCS-NPs and NSTs

Core–shell Fe_3O_4@Au NPs were prepared using freshly synthesized Fe_3O_4 NPs (12.8 ± 0.9 nm), which was in turn prepared according the procedure described in Ref. 8 (Fig. S1). Fe_3O_4 NPs (30 mg, 0.13 mmol), $AuCl_3$ (280 mg, 0.923 mmol), oleic acid (1.5 g, 5.8 mmol), oleylamine (1.5 mL), and octyl ether (15 mL) were combined in a 125-mL round-bottomed flask. The reaction mixture was heated to 190 ºC and stirred for 1.5 hours under an argon atmosphere, then cooled to room temperature. 25 mL of hexane was added, followed by 100 mL of ethanol to precipitate the NPs. Centrifugation (9500 *g*) followed by redispersion in water was performed twice prior to statistical analysis by TEM, which indicated a mean size of 15.4 ± 1.3 nm (polydispersity 8.4%).

SCS-NPs and NSTs were prepared by seeded growth. For SCS-NPs, 15.4-nm core–shell Fe_3O_4@Au particles (1 mg) were dispersed in 0.2 M CTAB (250 μL) by immersion in an ultrasonic cleaning bath for 3 minutes. The growth solution was prepared by combining 0.2 M CTAB (5 mL) with 7.8 mM $HAuCl_4$ (5 mL) and 0.08 M ascorbic acid (0.3 mL), using a vortex mixer in between each addition. The seed and growth solutions were then combined and allowed to sit at room temperature for 15 min. The reaction mixture was centrifuged at 7300 *g* for 15 min to yield CTAB-stabilized SCS-NPs with an average diameter of 34 nm (Figure S1). The CTAB-stabilized SCS-NPs were twice resuspended in 1% sodium polystyrenesulfonate (PSS, 70 kDa) followed by centrifugation to remove excess CTAB, then resuspended in 1% bovine serum albumin (BSA) to produce SCS-NPs in their final, biocompatible form (Fig. S2a).

The NST growth solution was prepared by the sequential mixing of 0.2 M CTAB (5 mL), 4 mM $AgNO_3$ (0.9 mL), 7.8 mM $HAuCl_4$ (5 mL), and 0.08 M ascorbic acid (0.3 mL), using a vortex mixer in between each addition. The freshly prepared mixture was then treated with a dispersion of 15.4-nm core–shell Fe_3O_4@Au NPs (1 mg) in 2.5 mM sodium citrate (250 μL). NST growth was complete within 15 minutes after seed addition, and separated from the reaction mixture by centrifugation at 7300 *g* for 15 min. The CTAB-stabilized NSTs were twice resuspended in 1% 70-kDa PSS followed by centrifugation to remove excess CTAB, then resuspended in 1% BSA to produce NSTs in their final, biocompatible form (Fig. S2b).

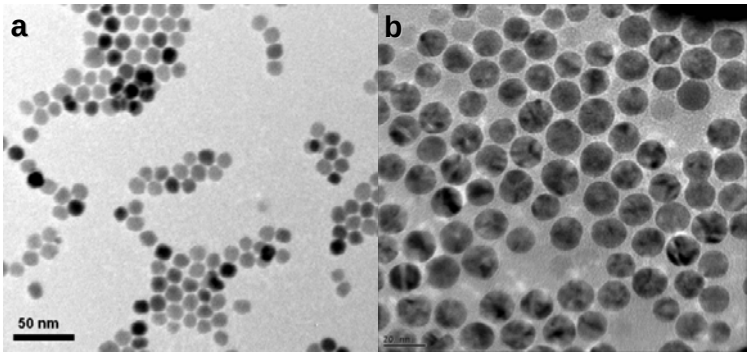

Figure S1. TEM images of (a) Fe_3O_4 NPs with an average diameter of 12.8 ± 0.9 nm, and (b) Fe_3O_4@Au core–shell NPs with an average diameter of 15.4 ± 1.3 nm.

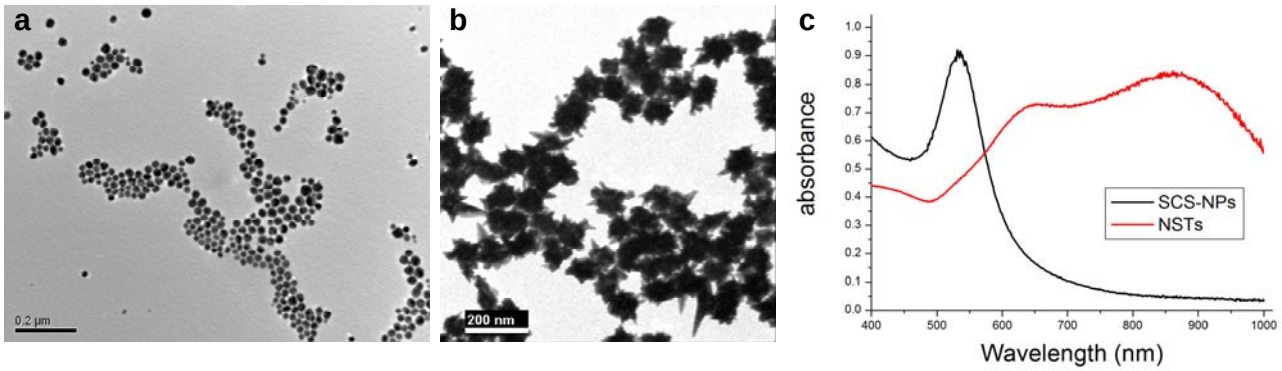

Figure S2. (a,b) TEM images of Fe_3O_4@Au spherical core–shell nanoparticles (SCS-NPs) and nanostars (NSTs). (c) Optical extinction spectra of SCS-NPs and NSTs.

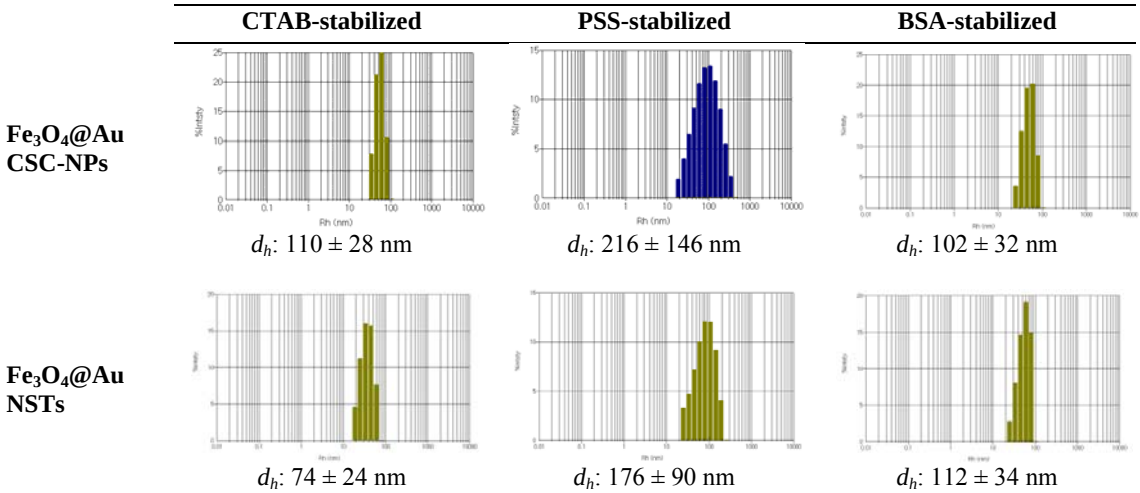

Figure S3. DLS analysis of Fe_3O_4@Au SCS-NPs and nanostars (NSTs), dispersed in PBS (pH 7.4).

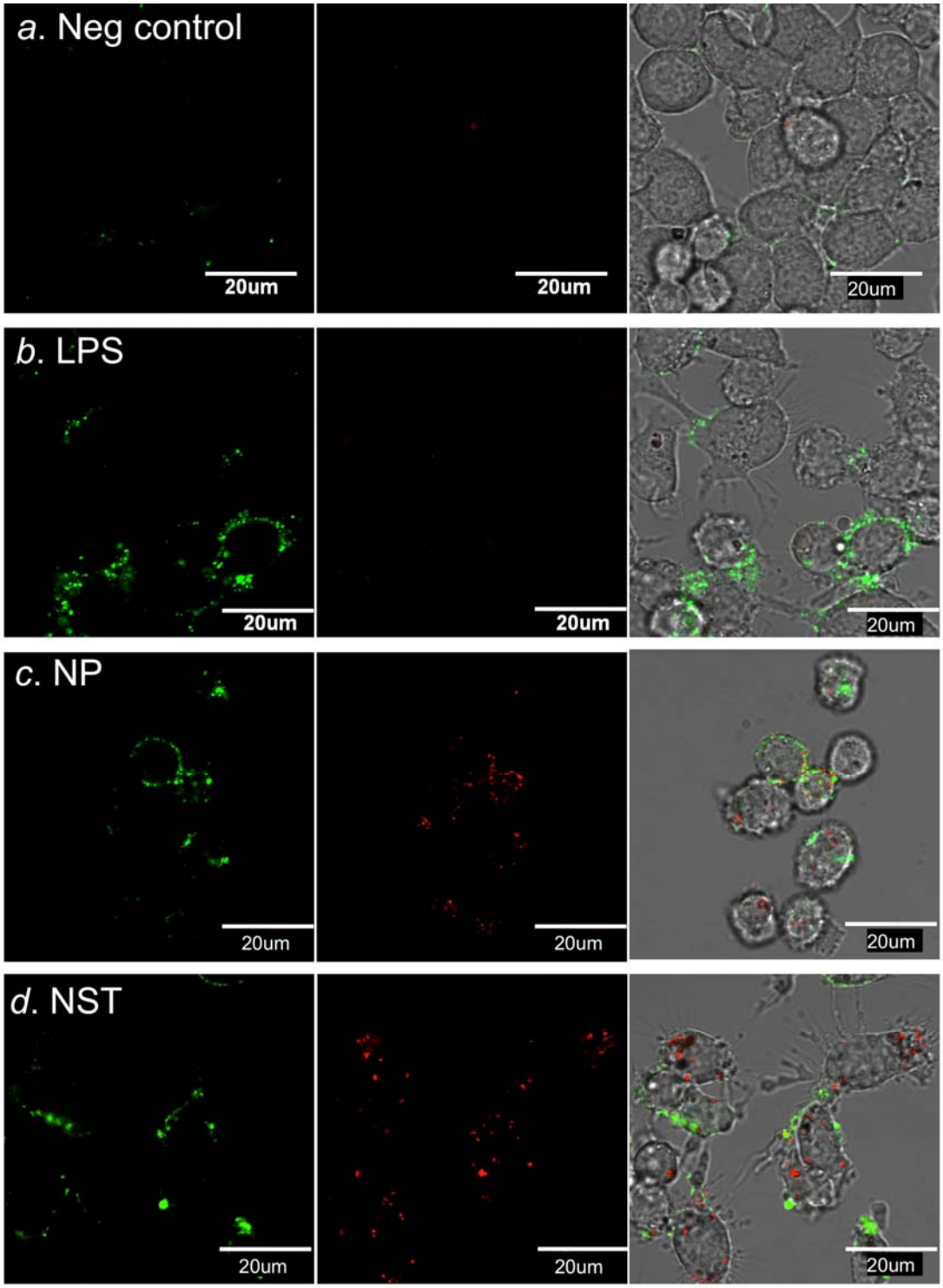

Figure S4. Multi-channel confocal imaging of ROS production by RAW 264.7 cells exposed to: (a) sterile PBS, (b) LPS, (c) CSC-NPs, and (d) NSTs, using DCF fluorescence (green, λ_{ex} = 488 nm; 55-nm bandpass filter). Nanoparticle signals were visualized by confocal reflectance (red, λ_{ex} = 635 nm; 100-nm bandpass filter).

Macrophage response to rotating magnetic field gradients, following phagocytosis of SCS-NPs or NSTs

Petri dishes containing RAW cells were incubated at 37 °C with SCS-NPs or NSTs for a period of 4, 12, or 24 hours. During the last two hours of incubation, cells were exposed for 2 hours to a rotating magnetic field gradient ($|B| \sim 100$ *G*), with plates exchanged every 15 minutes to ensure uniform field exposure, prior to being evaluated for ROS production. After 4 hours, cells with exposure to magnetic field gradients produced slightly less ROS than cells incubated without exposure to magnetic fields, or cells treated with LPS (Fig. S3). This trend was observed again for RAW cells after 12 hours, but cells treated with NSTs exhibited higher levels of ROS production than those treated with SCS-NPs and continued to do so over a 24-hour period, in accord with our earlier observations (cf. Fig. 7b). However, LPS treatment produced much higher ROS levels at 12 and 24 hours than either SCS-NPs or NSTs. This experiment was only conducted once, so the results do not necessarily represent a statistically valid sample.

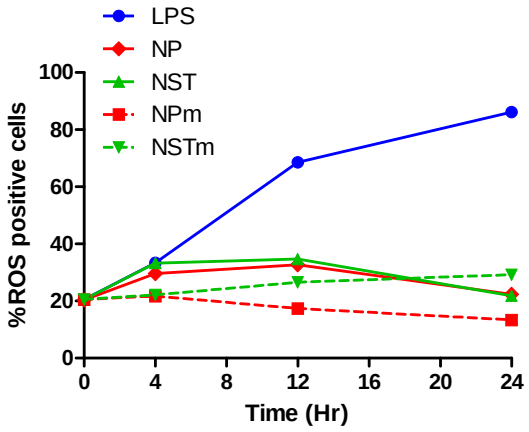

Figure S5. ROS levels of macrophages incubated with LPS (blue circle), SCS-NPs (red diamond), NSTs (green triangle), and SCS-NPs and NSTs with 2-h exposure to a rotating magnetic field gradient (red square and inverted green triangle, respectively).