

Supplementary Materials for

Neutrophils preferentially phagocytose elongated particles—An opportunity for selective targeting in acute inflammatory diseases

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This PDF file includes:

Tables S1 to S3
Figs. S1 to S7

Supplementary Materials

Table S1. The dimensions of polystyrene particles of different aspect ratios and sizes measured from SEM images.

Aspect ratio	Equivalent Spherical Diameter (ESD)					
	500 nm		1 μm		2 μm	
	Major axis	Minor axis	Major axis	Minor axis	Major axis	Minor axis
2	820 \pm 110 nm	450 \pm 51 nm	1.9 \pm 0.7 μm	830 \pm 85 nm	5.0 \pm 0.5 μm	1.4 \pm 0.1 μm
4	1.15 \pm 0.2 μm	340 \pm 50 nm	3.0 \pm 0.35 μm	740 \pm 100 nm	6.1 \pm 0.7 μm	1.3 \pm 0.1 μm
6	2.0 \pm 0.5 μm	300 \pm 48 nm	3.9 \pm 0.6 μm	650 \pm 90 nm	9.2 \pm 3 μm	1.5 \pm 0.5 μm

Table S2. The theoretical dimensions of polystyrene particles of different aspect ratios if they were perfect spheroids.

Aspect ratio	Equivalent Spherical Diameter (ESD)					
	500 nm		1 μm		2 μm	
	Major axis	Minor axis	Major axis	Minor axis	Major axis	Minor axis
2	800 nm	400 nm	1.6 μm	800 nm	3.2 μm	1.6 μm
4	1.25 μm	300 nm	2.5 μm	630 nm	5 μm	1.25 μm
6	1.65 μm	280 nm	3.3 μm	550 nm	6.6 μm	1.1 μm

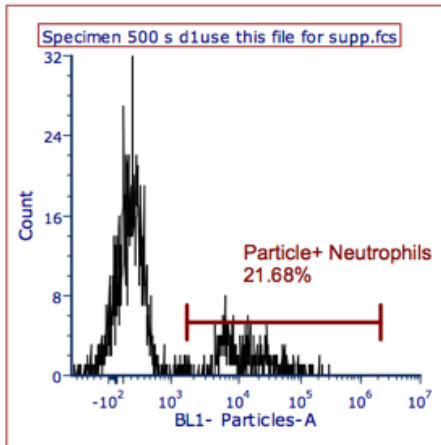
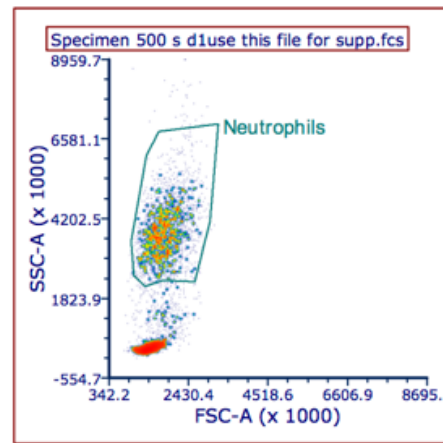
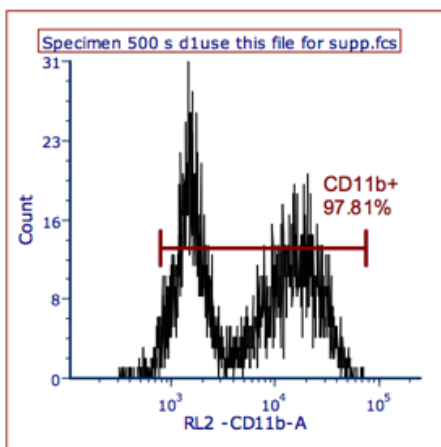
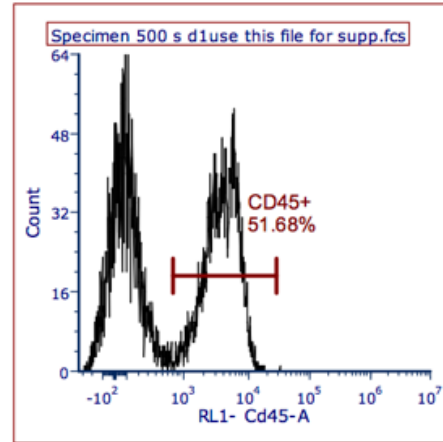
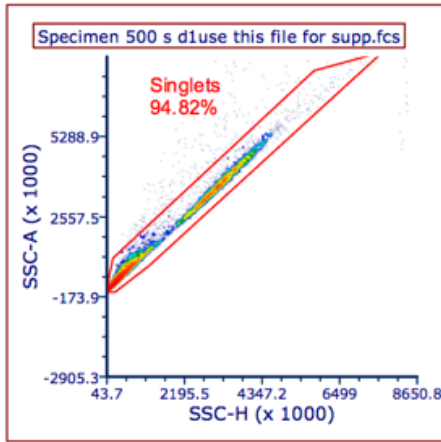


Fig. S1. Representative gating of particle-positive human neutrophils via flow cytometry using FCS express software - 500 nm particles shown here.

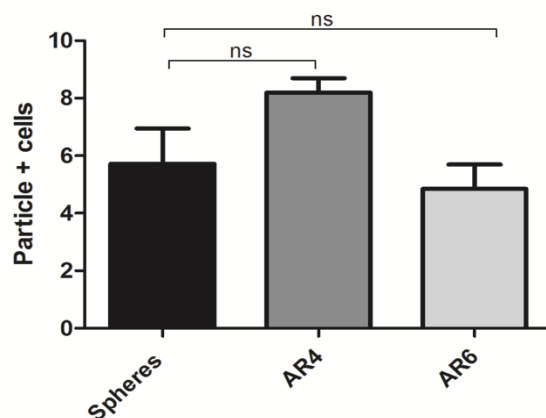


Fig. S2. Representation of the minimal uptake of the particles with equivalent spherical diameter of 500 nm by primary human neutrophils in whole blood with particle concentration set at 10^7 particle/ml of blood.

Table S3. Zeta potential measurements of polystyrene AR6 rods and spheres of different sizes fabricated via heat stretching technique.

Equivalent Spherical Diameter (ESD)	Spheres	AR6 Rods
500 nm	-45.0 ± 5.2 mV	-26.4 ± 5.2 mV
1 μ m	-44.3 ± 5.0 mV	-21.8 ± 3.9 mV
2 μ m	-41.4 ± 5.1 mV	-29.7 ± 6.0 mV

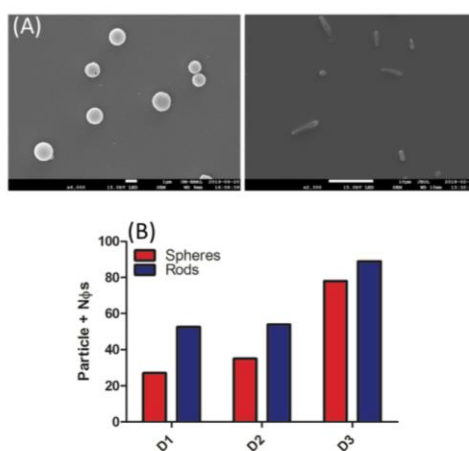


Fig. S3. *Ex vivo* uptake of PLGA rods and spheres by primary human neutrophils. (A) SEM image of the Cy5.5-loaded PLGA spheres and AR6 rods with the equivalent spherical diameter of 1.5- μ m fabricated via the two-step emulsion solvent evaporation technique, and (B) uptake of the PLGA rods and spheres by primary human neutrophils separated for individual donors. The particle concentration in blood was set at 5×10^6 particles/ml, and the uptake study was performed for 30 minutes.

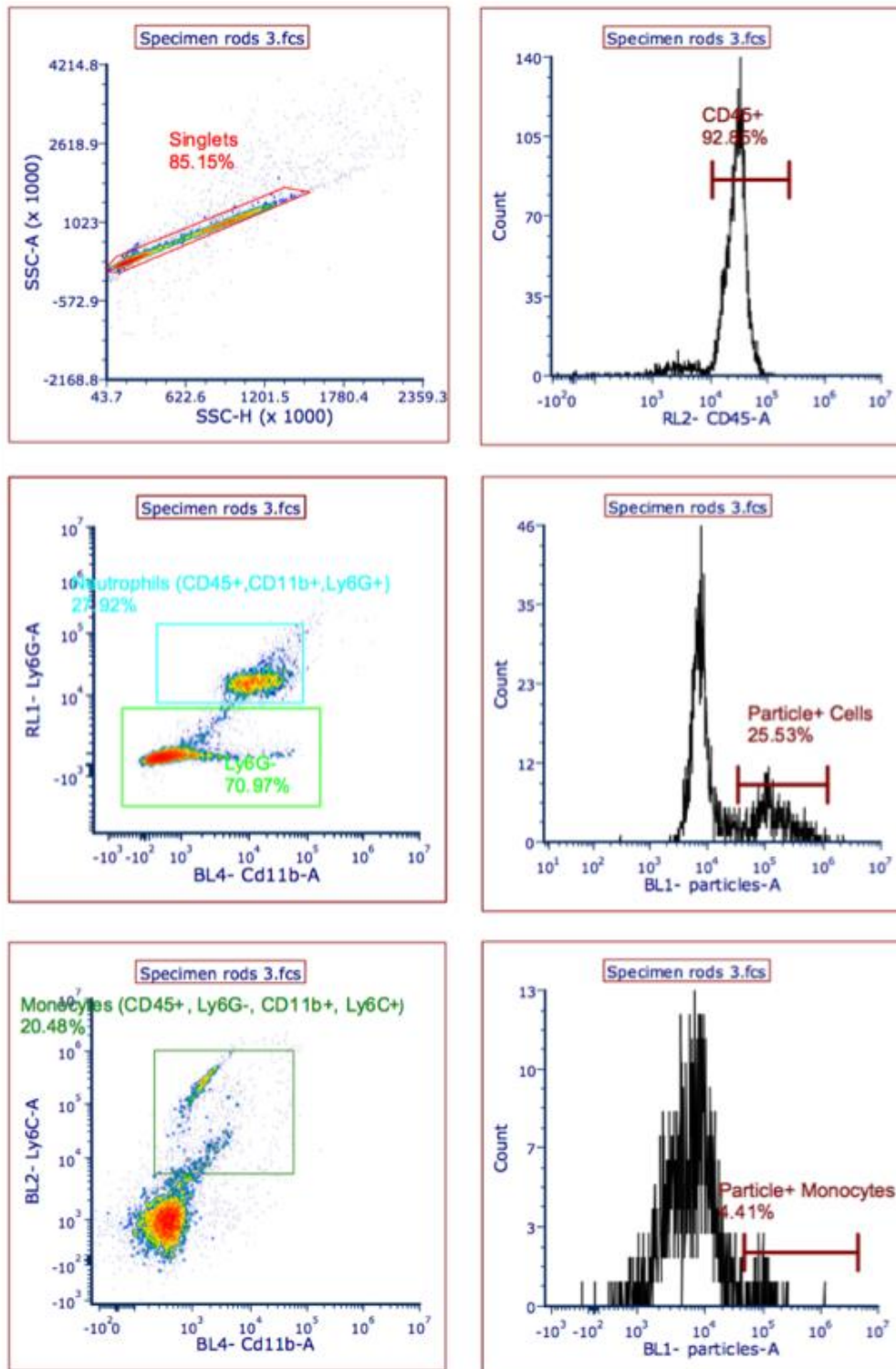


Fig. S4. Representative flow-cytometry panels for gating mouse neutrophils and monocytes in uptake assays – 500 nm particles shown here. After gating the singlets, neutrophils can be identified as CD45+, CD11b+, and Ly6G+ cells, and monocytes can be identified as CD45+, Ly6G-, CD11b+, and Ly6C+ cells. Particle+ cells are identified as the population of the cells, which are positive for the FITC signal.

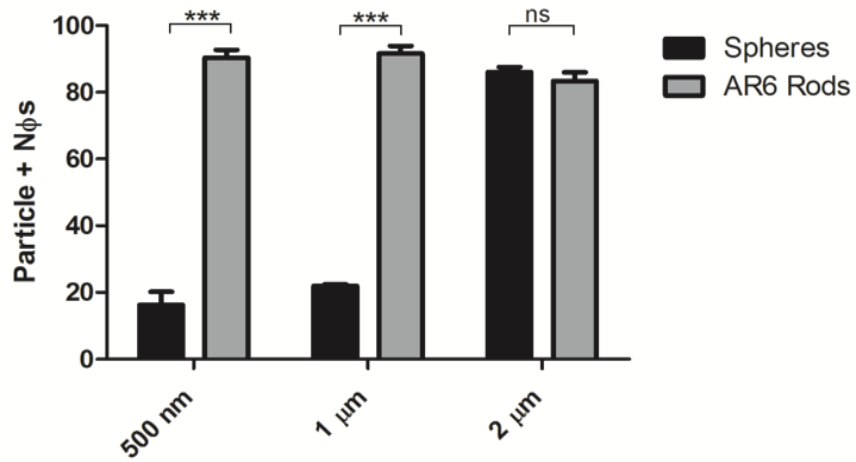


Fig. S5. Uptake of the polystyrene AR6 rods and spheres of different sizes via SJL/J mouse strain after 2-hr incubation of the particles in whole blood. The concentration of the particles in blood was set at 10^7 cells/ml.

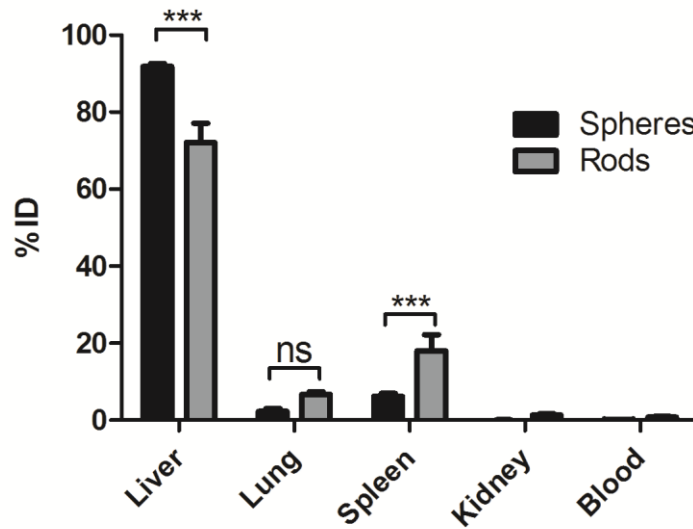


Fig. S6. Biodistribution of 500 nm spheres and AR6 rods in 6-8 weeks old male BALB/c mice following 30 min circulation. 5×10^8 particles were injected per animal.

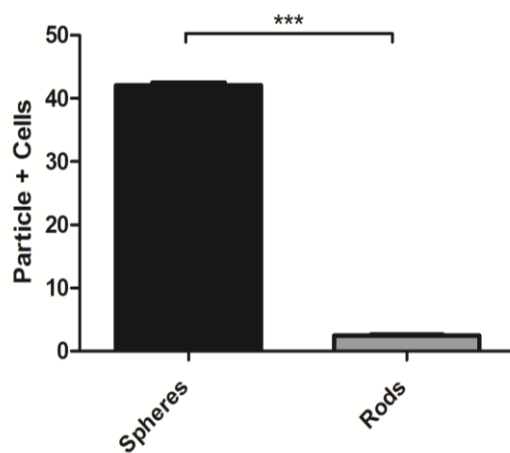


Fig. S7. Uptake of the 500 nm spheres and AR6 rods of the same volume by rat alveolar macrophages. The concentration of the cells and particles were respectively set at 10^6 cells/ml and 10^8 particles/ml.