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Supplementary Materials for

Deciphering neutrophil dynamics: Enhanced phagocytosis of elastic particles and impact on vascular-targeted carrier performance

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

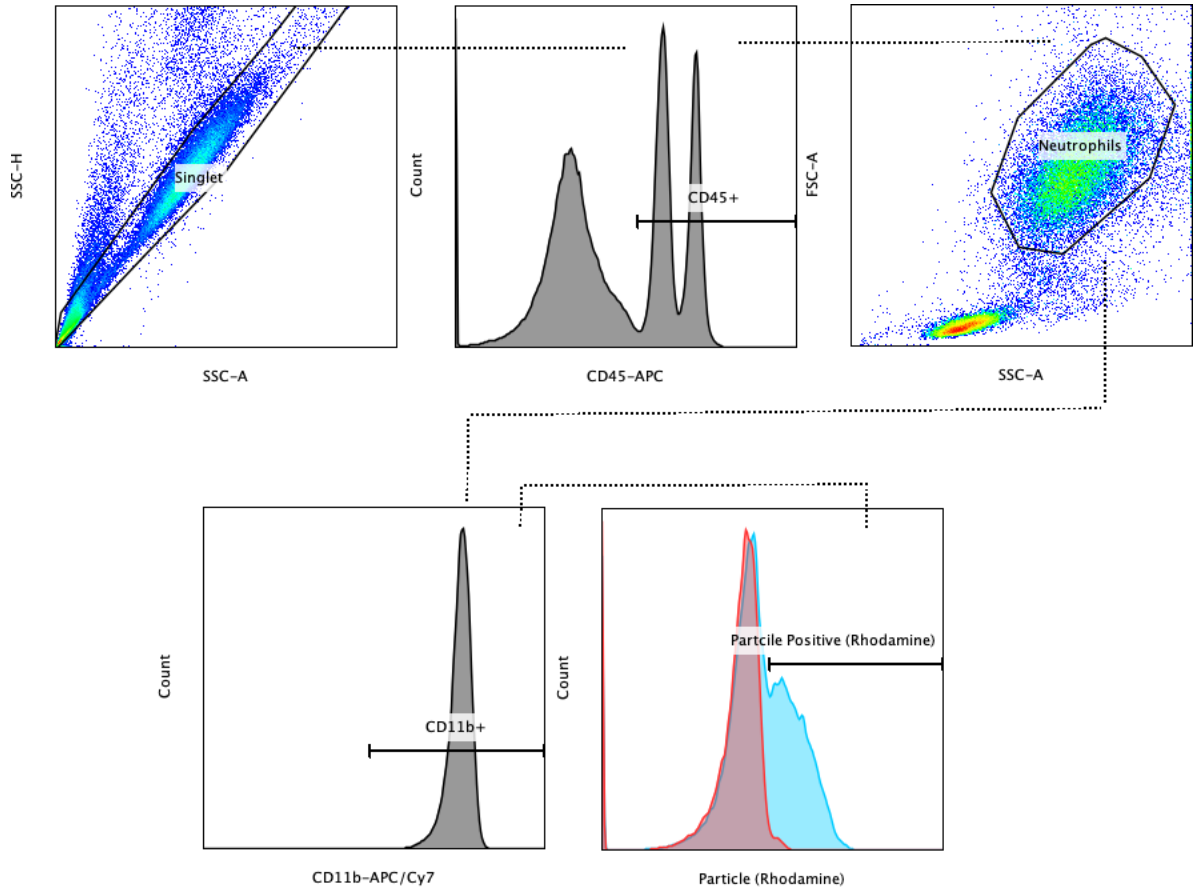


Fig. S1. Flow cytometry scheme to determine particle-positive immune cells. Immune cells in whole human blood are identified with $CD45^+$ and $CD11b^+$ markers, and neutrophils were isolated using forward and side scatter panels. Particle-positive cells are determined by the positive signal of the particle fluorescence (FITC for PS particles or rhodamine for PEG-based hydrogels). Gates for particle-negative cells are established by the negative signal of the particle from untreated samples (As seen in Red).

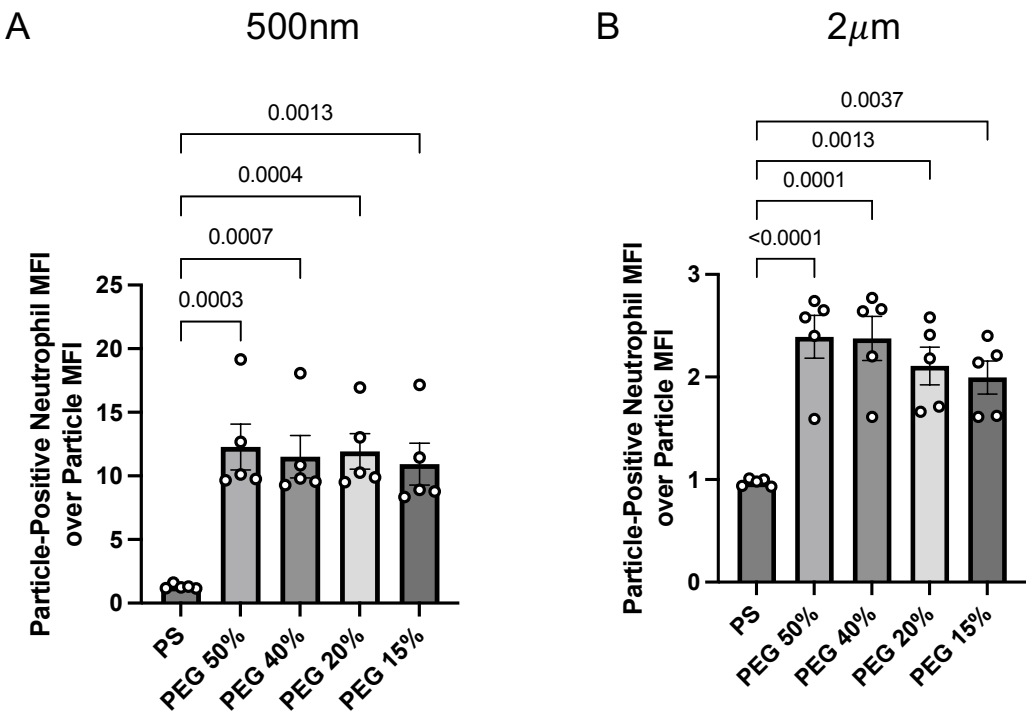


Fig. S2. Particle per cell analysis of particle-positive neutrophils for PS and PEG hydrogels. Particle per cell quantification after particle uptake by human neutrophils in whole blood is shown in (A) for 500nm PS and PEG particles and in (B) for 2μm PS and PEG hydrogels. We quantified the median fluorescence intensity (MFI) of the particle-positive neutrophil population and divided it by the MFI of each particle type to determine the approximate number of particles per neutrophil. The normalization by each particle MFI was done due to PS particles and PEG hydrogels having different fluorescent intensities.

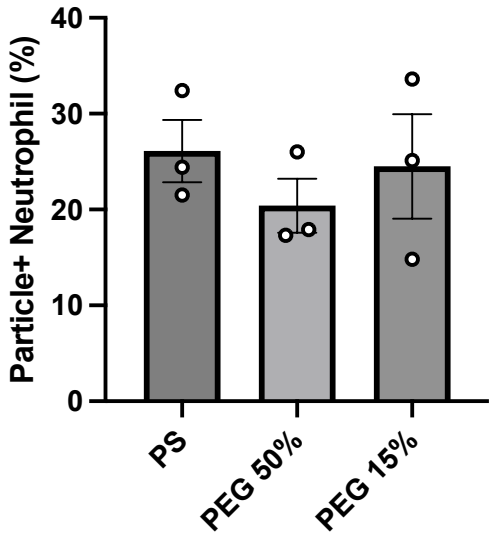


Fig. S3. Ex vivo uptake of 2μm PS and PEG particles by BALB/c mouse neutrophils in whole mouse blood. 2μm PS and 50% and 15% PEG hydrogels were phagocytosed by mouse neutrophils to a similar extent despite the large range of particle elasticities. No statistical differences between groups were found. Statistics were performed using one-way ANOVA with Tukey's multiple comparisons test. Error bars represent SEM with *N* = 3 replicates from pooled mouse blood.

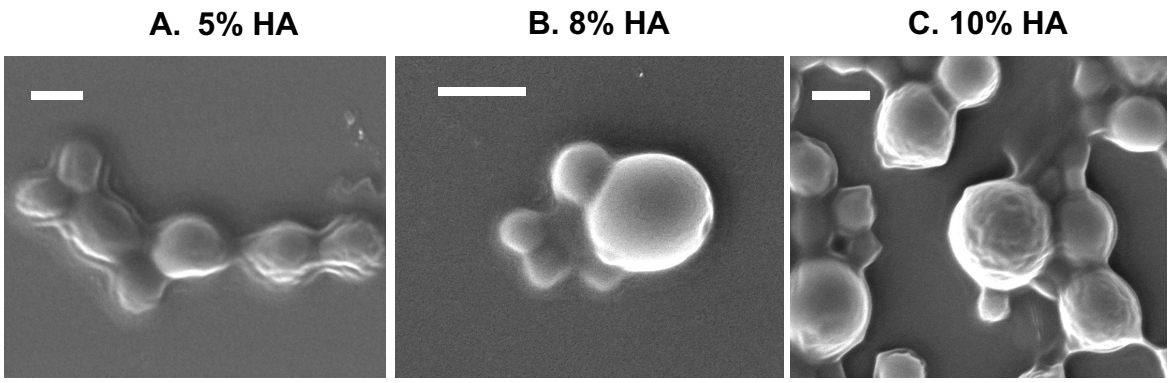


Fig. S4. Scanning electron microscopy (SEM) images of HA-based hydrogels. 2μm HA-based spherical hydrogels were fabricated with (A) 5% HA, (B) 8% HA, and (C) 10% HA weight percentages to obtain particles of different elasticities. The particle diameter of at least 50 hydrogels was determined using ImageJ. The scale bar is 2μm.

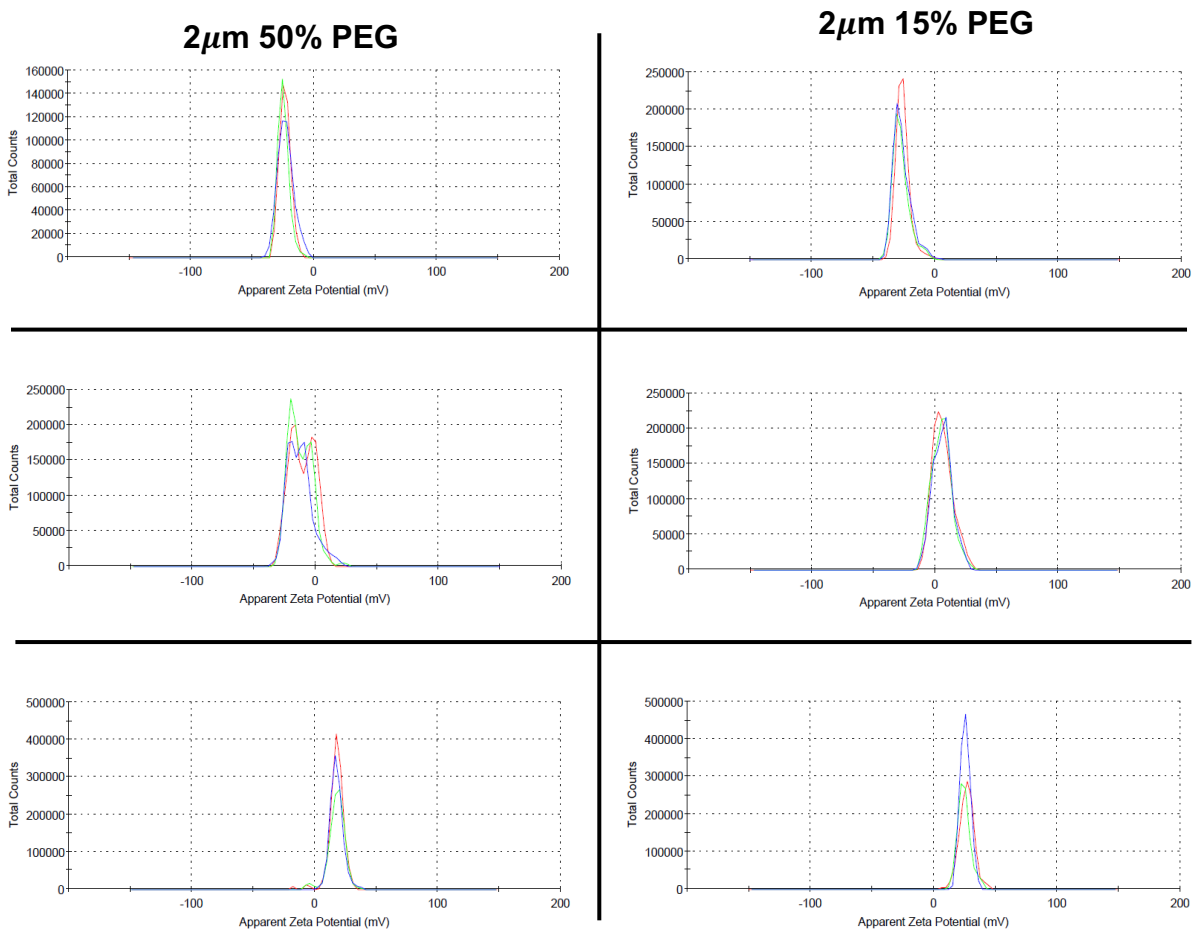


Fig. S5. Zeta potential measurements for both 2μm 50% and 15% PEG particles with varying ratios of – COOH and –NH_2 surface functional groups. Particle formulations were designed to either have a negative (top), neutral (middle), or positive charge (bottom).

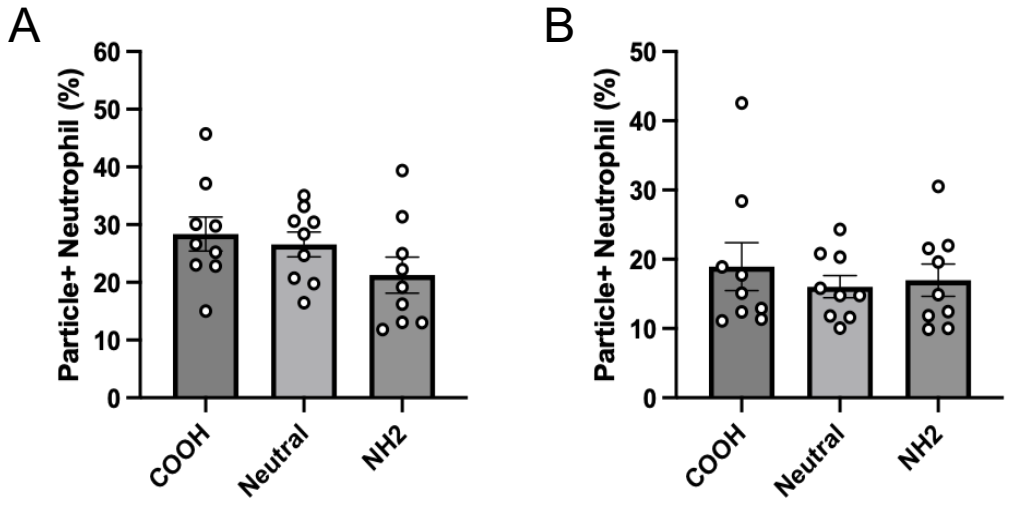


Fig. S6. Uptake of particles of varying surface charges by isolated human neutrophils in RPMI media. (A) 2μm 50% PEG hydrogels and (B) 2μm 15% PEG hydrogels incubated with isolated neutrophils in RPMI for 2 hours to allow for phagocytosis. No significant differences in particle uptake were seen for either particle type. Statistics were performed using one-way ANOVA with Tukey's multiple comparisons test. Error bars represent SEM with N = 9 independent blood donors.

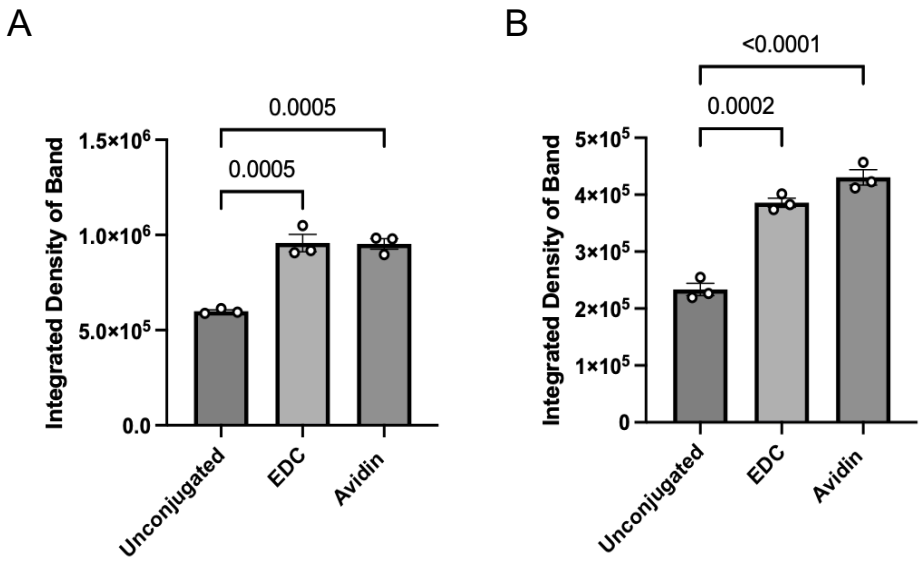


Fig. S7. Protein corona characterization of EDC- and avidin-coated PEG Hydrogels. Band intensity of 2μm (A) 50% and (B) 15% PEG hydrogels after incubation in human plasma from 3 independent donors. No differences were seen in the protein corona content between EDC- and avidin-modified PEG hydrogels. Statistics were performed using one-way ANOVA with Tukey's posttest.

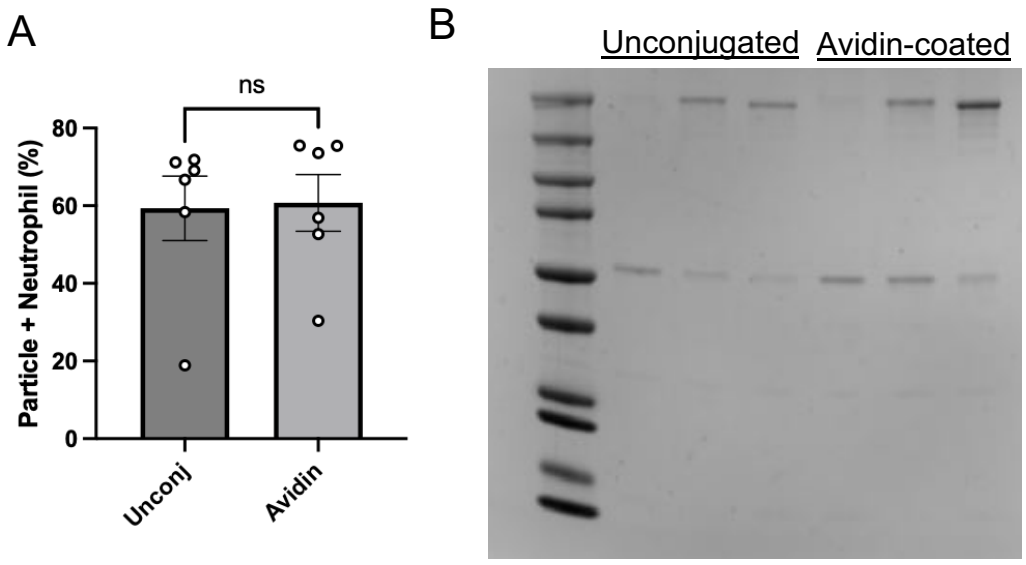


Fig. S8. Uptake of unconjugated and avidin-coated PEG particles by neutrophils and corresponding protein coronas using SDS-PAGE. Particles were conjugated with avidin using thiol-ene click chemistry, and there were no significant differences in neutrophil uptake between unconjugated particles and avidin-coated particles. (A) Particle-positive neutrophils treated with 2μm 50% PEG hydrogels. (B) SDS-PAGE of PEG particles to confirm the lack of protein absorption around the 66kDa band (albumin). No notable differences were seen for the protein corona of these particle types. Statistics were performed using an unpaired *t*-test and n=6 for blood donors.

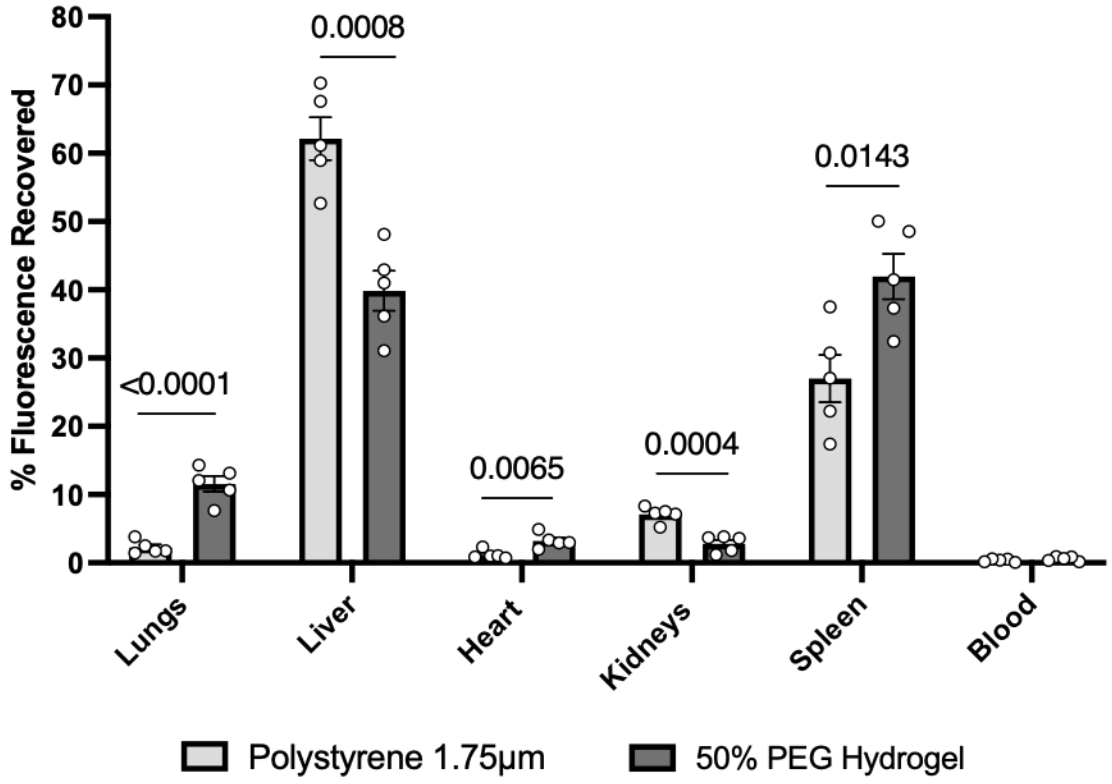
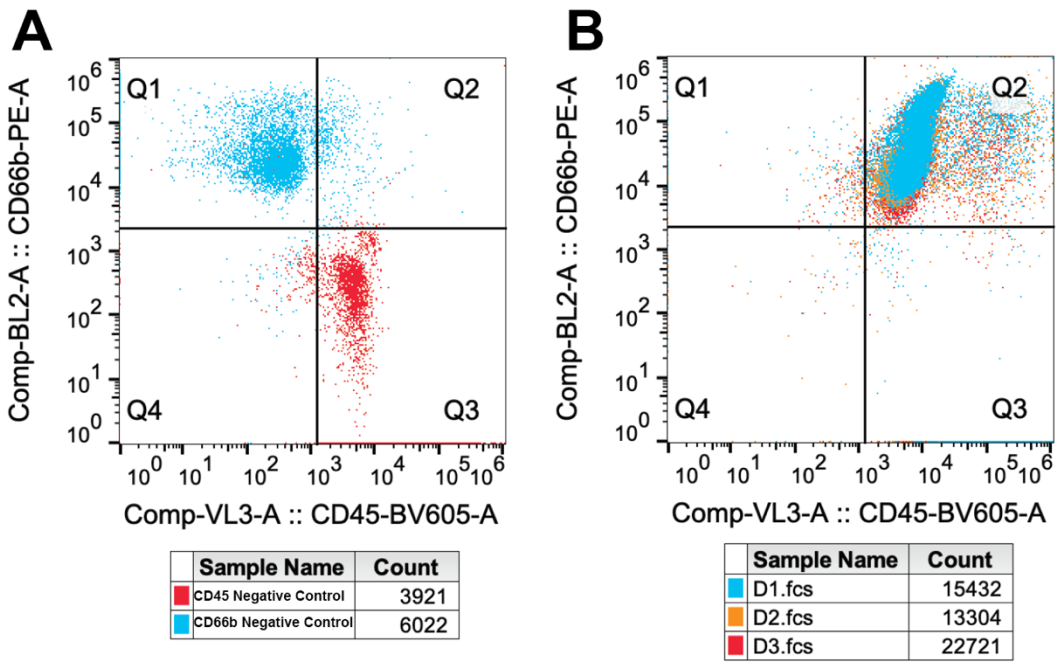


Fig. S9. Biodistribution of PS and 50% PEG Hydrogels in female BALB/c mice. 1.75μm PS and 2μm 50% PEG hydrogels were injected intravenously, and organs were collected 2 hours post particle injection. Statistics were performed using an unpaired *t*-test and n=5 female mice.



C

Sample Name	Neutrophil Purity (%)
Donor 1	95.3%
Donor 2	93.0%
Donor 3	95.8%

Fig. S10: Isolated Neutrophil Purity. Isolated neutrophils were stained with CD45-BV605 and CD66b-PE and an Attune NxT was used for data acquisition. (A) Dot plot gates were drawn using single stain control samples. (B) Isolation purity was determined across three different donors. Table (C) displays quantified purity from each donor.

Table S1: Particle Characterization of PS and PEG hydrogels of varying size and elasticities

Particle Type	Size (μm)	Zeta Potential (mV)
2μm PS	2.08*	- 47.7 ± 5
2μm 50% PEG	1.91 ± 0.7	- 37.3 ± 18
2μm 40% PEG	2.02 ± 0.7	- 46.8 ± 6
2μm 20% PEG	1.61 ± 0.3	- 36.7 ± 13
2μm 15% PEG	1.85 ± 0.5	- 41.1 ± 17
2μm 10% HA	1.95 ± 0.8	- 33.0 ± 8
2μm 8% HA	1.84 ± 0.4	- 31.6 ± 10
2μm 5% HA	2.17 ± 0.8	- 29.6 ± 11
500nm PS	0.52*	- 53.9 ± 7
500nm 50% PEG	0.60 ± 0.1	- 41.7 ± 7
500nm 40% PEG	0.64 ± 0.1	- 44.9 ± 7
500nm 20% PEG	0.64 ± 0.2	- 51.5 ± 8
500nm 15% PEG	0.81 ± 0.2	- 49.9 ± 5.0

*Reported particle diameter from PolySciences

Table S2: Particle Characterization of 2μm PEG hydrogels of varying particle charge and elasticities.

Particle Type	Monomer	Zeta potential (mV)	Particle size (μm)
2μm 15% PEG	CEA/AEM	- 1.1 ± 4	1.96 ± 0.7
2μm 15% PEG	AEM	22.1 ± 4	1.59 ± 0.7
2μm 50% PEG	CEA/AEM	12.9 ± 4	1.67 ± 0.5
2μm 50% PEG	AEM	21.0 ± 10	1.99 ± 0.7

Movie S1.
A three-dimensional visualization of neutrophils that phagocytosed hydrogel particles constructed from sections obtained from confocal imaging.

Movie S2.
A sample visualization of a neutrophil engulfing a hydrogel particle