Particle
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Supporting Information

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Macrophage-Targeting Poly(lactide-*co*-glycolic acid) Nanoparticles Decorated with Multifunctional Brush Polymers

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Synthesis of CBMA and PCBMA brushes

CBMA was synthesized from DMAEMA (2-dimethylamino (ethyl methacrylate)) and b-propiolactone according to a procedure adapted from literature [1] (Figure S1). A solution of b-propiolactone (0.87 g, 12 mmol) in 10 mL anhydrous acetone was added dropwise to a solution of DMAEMA (1.57 g, 10 mmol) in 50 mL anhydrous acetone. The mixture was stirred under argon at room temperature for 5 h. Then, the resulting precipitate was washed with dried acetone, and the solvent was evaporated using a rotary evaporator. The resulting white product was further dried under reduced pressure to obtain the final product CBMA. CBMA was kept in the refrigerator until further use and characterized via 1H-NMR (D_2O) [1]: 6.06 (s,1H, dCH), 5.68 (s, 1H, dCH), 4.55 (t, 2H, OCH2), 3.70 (t, 2H, CH2N), 3.59 (t, 2H, NCH2), 3.10 (s, 6H, NCH3), 2.64 (t, 2H, CH2COO),1.84 (s, 3H, dCCH3).

[1] Ref.: Z. Zhang, S. Chen, S. Jiang, *Biomacromolecules*, **2006**, *7*, 3311-3315.

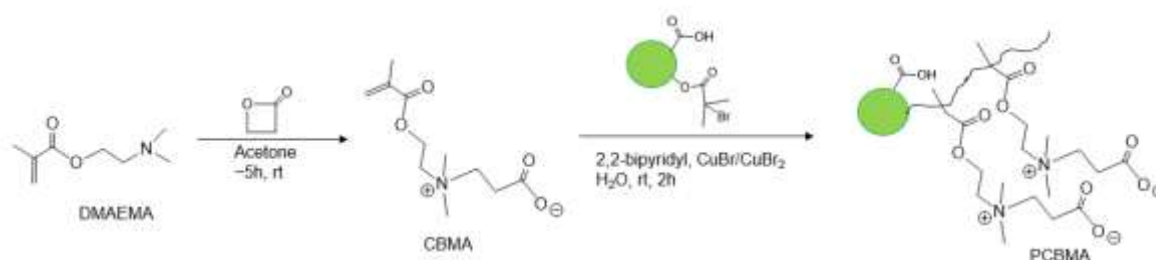

Figure S1. Synthesis of PCBMA-coated PLGA nanoparticles: CBMA monomer is synthesized from DMAEMA through ring-opening reaction of b-propiolactone [1]; ATRP of CBMA on BiBB-functionalized PLGA particles yields PCBMA brushes.

Statistical differences of nanoparticle parameters

Unpaired t test with Welch's correction was used for statistical analysis of nanoparticle circularity, roundness, diameter, and anisotropy (the raw data are shown in Figure S2).

Circularity:

Formulation: PLGA/PLA(BiBB) ratio	Statistical difference
9:1	****
7.5:2.5	No
5:5	****

Roundness:

Formulation: PLGA/PLA(BiBB) ratio	Statistical difference
9:1	****
7.5:2.5	No
5:5	****

Diameter:

Formulation: PLGA/PLA(BiBB) ratio	Statistical difference
9:1	***
7.5:2.5	****
5:5	****

Anisotropy:

Formulation: PLGA/PLA(BiBB) ratio	Statistical difference
9:1	****
7.5:2.5	No
5:5	**

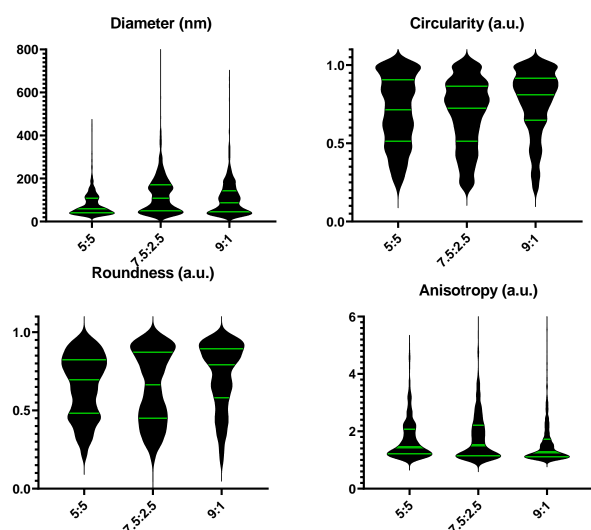

Figure S2. FIJI (a distribution of ImageJ v1.53c) was used for the analysis of diameter, roundness, circularity, and anisotropy of the PLGA/PLA(BiBB) nanoparticles prepared with PLGA/PLA(BiBB) ratios of 5:5, 7.5:2.5, and 9:1. For each formulation, multiple SEM images were analyzed for a total of 500 particles/formulation.

Pooled variance calculation

Calculation of the error for the number of particles per aggregate was made by pooled variance. The pooled variance is used to obtain the variance from different subgroups of the data. In this case, the pooled variance for each of the surface modifications was calculated combining the different subgroups (time points). If the number of observations in each subgroup is the same, the pooled variance is an average; however, if the number of observations is different, the pooled variance becomes a weighted average. By obtaining the pooled variance of the group, deviation and standard error can be calculated.

$$s_p^2 = \frac{\sum_{i=1}^{k}(n_i-1)s_i^2}{\sum_{i=1}^{k}(n_i-1)} \quad \text{(S1)}$$

$$s_p^2 = \frac{(s_1^2+s_2^2)}{2} \quad \text{(S2)}$$

Equation S1 represents the pooled variance estimation and Equation S2 is the simplification for the same number of observations per group, where s_p is the pooled standard deviation and k is the number of samples.

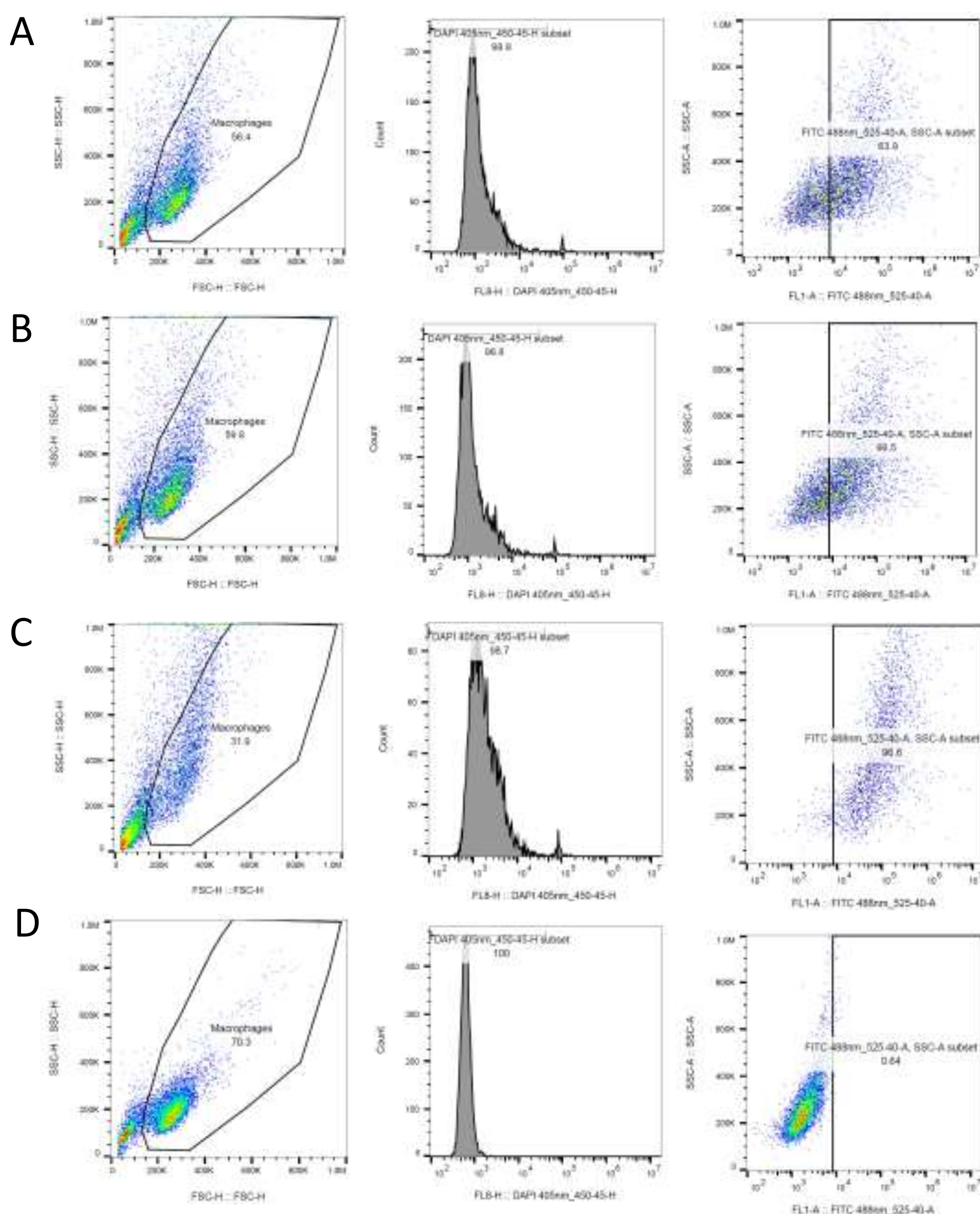

Figure S3. Flow cytometry data used to evaluate the uptake of the fluorescently labeled nanoparticles by RAW 264.7 macrophages after a 30 min incubation period. The uptake of PLGA nanoparticles with (A) PEG monolayer coating, (B) mannose monolayer coating, and (C) PCB-mannose brush coating was evaluated; (D) is the control group (RAW 264.7 macrophages without nanoparticles). Uptake experiments were conducted in triplicate. From left to right: Ungated; FSC-width, SSC-H subset; DAPI 405nm_450 subset. Figure 4 (left) in the main text compares the MFI values of the FITC 488nm_525-A, SSC-A subset for the different nanoparticle groups after 30 min incubation time.

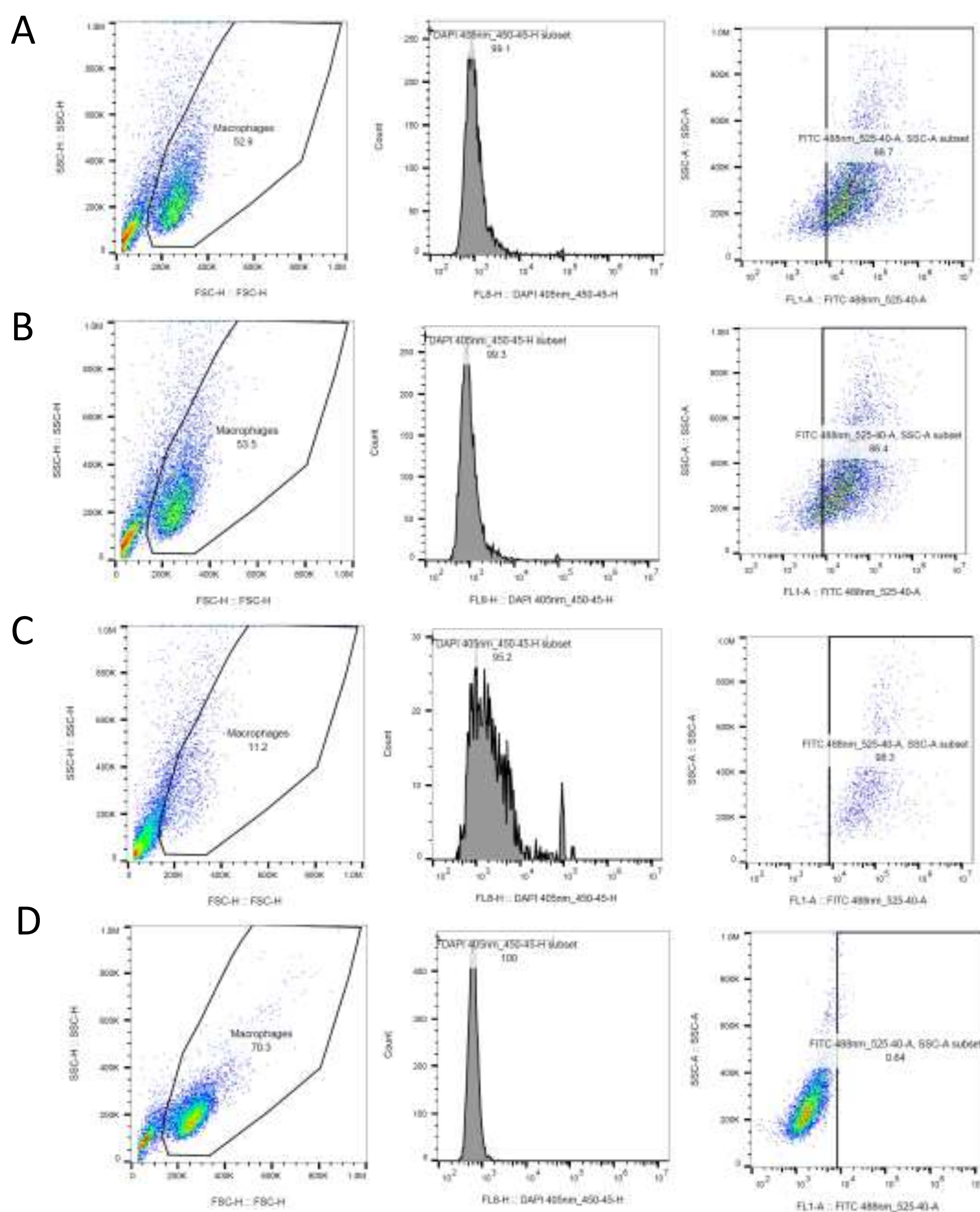

Figure S4. Flow cytometry data used to evaluate the uptake of the fluorescently labeled nanoparticles by RAW 264.7 macrophages after a 5 h incubation period. The uptake of PLGA nanoparticles with (A) PEG monolayer coating, (B) mannose monolayer coating, and (C) PCB-mannose brush coating was evaluated; (D) is the control group (RAW 264.7 macrophages without nanoparticles). Uptake experiments were conducted in triplicate. From left to right: Ungated; FSC-width, SSC-H subset; DAPI 405nm_450 subset. Figure 4 (right) in the main text compares the MFI values of the FITC 488nm_525-A, SSC-A subset for the different nanoparticle groups after 5 h incubation time.