Supplementary Information

Interfacial Interactions between Natural RBC Membranes and Synthetic Polymeric Nanoparticles

Brian T. Luk[ac‡], Che-Ming Jack Hu[bc‡], Ronnie H. Fang[bc], Diana Dehaini[bc], Cody Carpenter[bc], Weiwei Gao[bc], and Liangfang Zhang[bc*]

[a] Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA
[b] Department of Nanoengineering, University of California, San Diego, La Jolla, CA 92093, USA
[c] Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA
[‡] These authors contributed equally to this work.

*Corresponding author: zhang@ucsd.edu

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1. <u>Theoretical Calculations</u>

RBC membrane materials required to fully cover differently sized PLGA cores:

Density of PLGA polymer: ρ = 1.25 g mL^{-1} [1]

For 65 nm cores:

Radius of the PLGA core: r = 32.5 nm

Radius of the RBC-NP: r_{np} = 40 nm

Mass per PLGA core: $M_{core} = \rho \times \frac{4}{3}\pi r^3 = 1.80\times10^{-16}$ g per particle

Number of nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} = 5.57×10^{12}

Surface area of each RBC-NP: S_{np} = 4 π r_{np}^2 = 0.020 μm^2 per particle

1

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

$$S_{total} = N_{np} \times S_{np} \approx 1.12 \times 10^{11} \ \mu m^2$$

Estimated amount of blood for complete coating of 1 mg of 65 nm PLGA particles

Blood volume = S_{total} / average surface area of mouse RBC (75 μm^2) [2] / concentration of

RBCs in mouse blood (10^{10}/mL) [3]

$$\approx \mathbf{150 \ \mu L}$$

For 100 nm cores:

Radius of the PLGA core: r = 50 nm

Radius of the RBC-NP: r_{np} = 57.5 nm

Mass per PLGA core: $M_{core} = \rho \times \frac{4}{3}\pi r^3 = 6.54 \times 10^{-16}$ g per particle

Number of nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} = 1.53×10^{12}

Surface area of each RBC-NP: $S_{np} = 4 \ \pi \ r_{np}{}^2 = 0.042 \ \mu m^2$ per particle

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

$$S_{total} = N_{np} \times S_{np} \approx 6.35 \times 10^{10} \ \mu m^2$$

Estimated amount of blood for complete coating of 1 mg of 100 nm PLGA particles

Blood volume = S_{total} / average surface area of mouse RBC (75 μm^2) [2] / concentration of

RBCs in mouse blood (10^{10}/mL) [3]

$$\approx \mathbf{85 \ \mu L}$$

For 120 nm cores:

Radius of the PLGA core: r = 60 nm

Radius of the RBC-NP: r_{np} = 67.5 nm

Mass per PLGA core: $M_{core} = \rho \times \frac{4}{3}\pi r^3 = 9.05 \times 10^{-16}$ g per particle

Number of nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} = 8.84×10^{11}

Surface area of each RBC-NP: $S_{np} = 4 \pi r_{np}^2 = 0.057$ μm^2 per particle

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

$$S_{total} = N_{np} \times S_{np} \approx 5.06 \times 10^{10} \ \mu m^2$$

Estimated amount of blood for complete coating of 1 mg of 120 nm PLGA particles

Blood volume = S_{total} / average surface area of mouse RBC (75 μm^2) [2]/ concentration of

RBCs in mouse blood (10^{10}/mL) [3]

$$\approx \textbf{68 } \boldsymbol{\mu}\textbf{L}$$

For 200 nm cores:

Radius of the PLGA core: r = 100 nm

Radius of the RBC-NP: r_{np} = 107.5 nm

Mass per PLGA core: $M_{core} = \rho \times \frac{4}{3}\pi r^3 = 5.23 \times 10^{-15}$ g per particle

Number of nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} = 1.9×10^{11}

Surface area of each RBC-NP: $S_{np} = 4 \pi r_{np}^2 = 0.145$ μm^2 per particle

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

$$S_{total} = N_{np} \times S_{np} \approx 2.77 \times 10^{10} \ \mu m^2$$

Estimated amount of blood for complete coating of 1 mg of 200 nm PLGA particles

Blood volume = S_{total} / average surface area of mouse RBC (75 μm^2) [2]/ concentration of

RBCs in mouse blood (10^{10}/mL) [3]

$$\approx \textbf{37 } \boldsymbol{\mu}\textbf{L}$$

For 340 nm cores:

Radius of the PLGA core: r = 170 nm

Radius of the RBC-NP: r_{np} = 177.5 nm

Mass per PLGA core: M_{core} = $\rho \times \frac{4}{3}\pi r^3$ = 2.57×10^{-14} g per particle

Number of nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} = 3.9×10^{10}

Surface area of each RBC-NP: S_{np} = 4 π r_{np}^{2} = 0.396 μm^2 per particle

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

$$S_{total} = N_{np} \times S_{np} \approx 1.54 \times 10^{10} \text{ μm}^2$$

Estiamted amount of blood for complete coating of 1 mg of 340 nm PLGA particles

Blood volume = S_{total} / average surface area of mouse RBC (75 μm^2) [2]/ concentration of

RBCs in mouse blood (10^{10}/mL) [3]

≈ 21 μL

Estimation of sialic acid density on fully coated RBC-NPs with 100 nm PLGA cores:

Sialic acid content per 10^9 RBCs = 0.013 μmol [4]

Amount of blood for complete coating of 1 mg of 100 nm PLGA nanoparticles = 85 μL

Total sialic acid content on fully coated RBC-NPs with 100 nm cores = concentration of

RBCs in mouse blood (10^{10}/mL) × 85 μL × 0.013 μmol/10^9 RBCs =

0.01105 μmol

Number of 100 nm nanoparticles made of 1 mg of PLGA polymer: N_{np} =1 mg / M_{core} =

1.53×10^{12}

Average sialic acid content per RBC-NP = 0.01105 μmol × 6.022 × 10^{23} / M_{core} = **43,500**

sialic acid per RBC-NP
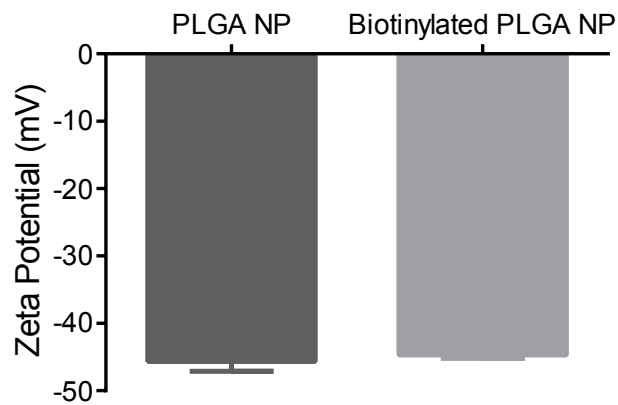
2. <u>**Supporting Figures**</u>

Figure S1. Bare PLGA cores and biotinylated PLGA cores have similar surface zeta potential. Error bars represent standard deviation (n=3).
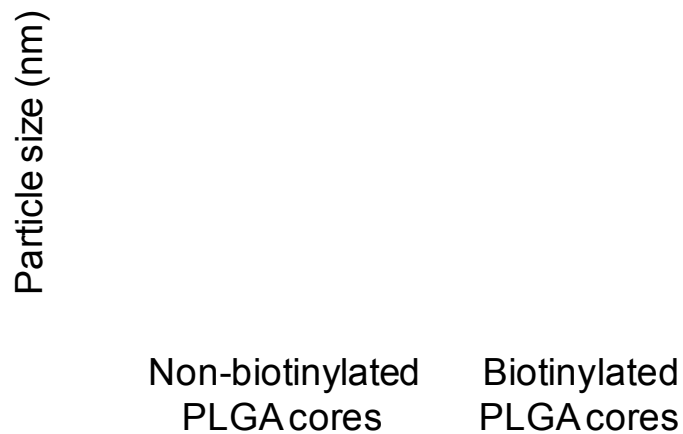

Figure S2. Effect of the presence of streptavidin on the size of non-biotinylated and biotinylated PLGA cores. Non-biotinylated cores did not show significant change in size upon addition of streptavidin, but the size of the biotinylated cores increased dramatically in the presence of streptavidin due to cross-linking of the biotinylated cores.

A

Particle Size (nm)

B

Zeta Potential (mV)

Figure S3. Effect of sialidase treatment on the size and surface zeta potential of bare PLGA NPs and RBC-NPs. (A) Sialidase treatment did not cause any significant change in size for bare NPs and RBC-NPs. (B) Sialidase treatment of RBC-NPs shifted the zeta potential from -23 mV to -0.6 mV, but had negligible effect on the zeta potential of bare PLGA NPs. Error bars represent standard deviation (n=3).
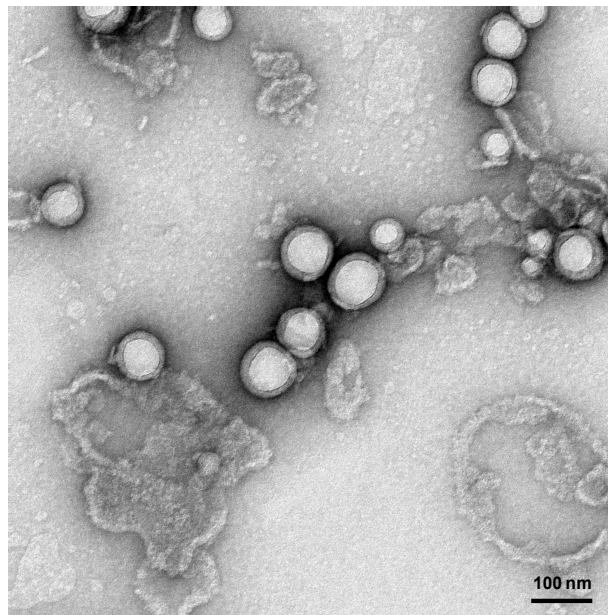

100 nm

Figure S4. RBC-NPs prepared with membrane-to-particle ratio of 200 μL of blood per 1 mg of polymer. RBC-NPs show unilamellar membrane coating despite there being excess membrane materials, which remained in vesicular form.

A

Size (nm)

B

Zeta Potential (mV)

Figure S5. The sizes (A) and surface zeta potential (B) of negatively (PLGA) and positively (PLGA-PEI) charged cores, measured by dynamic light scattering (DLS). Error bars represent standard deviation (n=3).
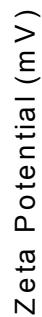
Zeta Potential (mV)

Figure S6. Surface zeta potential of RBC membrane vesicles measured by DLS. Error bars represent standard deviation (n=3).

3. <u>Supporting References</u>

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[2]   R. Waugh, I. Sarelius, *Am. J. Physiol.-Cell Ph.*, 1996, **271**, C1847.

[3]   E. Russell, E. Neufeld, C. Higgins, *P. Soc. Exp. Biol. Med.*, 1951, **78**,761.

[4]   A. Miller, J. Katz, J. Sullivan. *Cancer Res.,* 1963, **23**, 485.