Supplementary Information

Interfacial Interactions between Natural RBC Membranes and Synthetic Polymeric Nanoparticles

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1. Theoretical Calculations

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

Density of PLGA polymer: $\rho = 1.25 \text{ g mL}^{-1}$ [1]

For 65 nm cores:

Radius of the PLGA core: $r = 32.5 \text{ nm}$

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

Mass per PLGA core: $M_{\text{core}} = \rho \times \frac{4}{3} \pi r^3 = 1.80 \times 10^{-16} \text{ g per particle}$

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

Surface area of each RBC-NP: $S_{\text{np}} = 4 \pi r_{\text{np}}^2 = 0.020 \mu\text{m}^2 \text{ per particle}$
Total surface area of RBC-NPs made of 1 mg of PLGA polymer:

\[ S_{\text{total}} = N_{np} \times S_{np} \approx 1.12 \times 10^{11} \, \mu\text{m}^2 \]

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

Blood volume = \( S_{\text{total}} / \) average surface area of mouse RBC (75 \( \mu\text{m}^2 \)) \[2\] / concentration of RBCs in mouse blood (10^{10}/mL) \[3\]

\[ \approx 150 \, \mu\text{L} \]

For 100 nm cores:

Radius of the PLGA core: \( r = 50 \, \text{nm} \)

Radius of the RBC-NP: \( r_{np} = 57.5 \, \text{nm} \)

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

Number of nanoparticles made of 1 mg of PLGA polymer: \( N_{np} = \frac{1 \, \text{mg}}{M_{\text{core}}} = 1.53 \times 10^{12} \)

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

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

\[ S_{\text{total}} = N_{np} \times S_{np} \approx 6.35 \times 10^{10} \, \mu\text{m}^2 \]

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

Blood volume = \( S_{\text{total}} / \) average surface area of mouse RBC (75 \( \mu\text{m}^2 \)) \[2\] / concentration of RBCs in mouse blood (10^{10}/mL) \[3\]

\[ \approx 85 \, \mu\text{L} \]

For 120 nm cores:

Radius of the PLGA core: \( r = 60 \, \text{nm} \)

Radius of the RBC-NP: \( r_{np} = 67.5 \, \text{nm} \)

Mass per PLGA core: \( M_{\text{core}} = \rho \times \frac{4}{3} \pi r^3 = 9.05 \times 10^{-16} \, \text{g per particle} \)
Number of nanoparticles made of 1 mg of PLGA polymer: \( N_{np} = \frac{1 \text{ mg}}{M_{core}} = 8.84 \times 10^{11} \)

Surface area of each RBC-NP: \( S_{np} = 4 \pi r_{np}^2 = 0.057 \mu \text{m}^2 \) per particle

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

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

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

Blood volume = \( S_{total} / \text{average surface area of mouse RBC (75 \mu \text{m}^2)} \) [2] / concentration of RBCs in mouse blood (10^{10}/mL) [3]

\[
\approx 68 \mu \text{L}
\]

**For 200 nm cores:**

Radius of the PLGA core: \( r = 100 \text{ nm} \)

Radius of the RBC-NP: \( r_{np} = 107.5 \text{ nm} \)

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

Number of nanoparticles made of 1 mg of PLGA polymer: \( N_{np} = \frac{1 \text{ mg}}{M_{core}} = 1.9 \times 10^{11} \)

Surface area of each RBC-NP: \( S_{np} = 4 \pi r_{np}^2 = 0.145 \mu \text{m}^2 \) per particle

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

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

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

Blood volume = \( S_{total} / \text{average surface area of mouse RBC (75 \mu \text{m}^2)} \) [2] / concentration of RBCs in mouse blood (10^{10}/mL) [3]

\[
\approx 37 \mu \text{L}
\]

**For 340 nm cores:**
Radius of the PLGA core: \( r = 170 \text{ nm} \)

Radius of the RBC-NP: \( r_{np} = 177.5 \text{ nm} \)

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

Number of nanoparticles made of 1 mg of PLGA polymer: \( N_{np} = \frac{1 \text{ mg}}{M_{core}} = 3.9 \times 10^{10} \)

Surface area of each RBC-NP: \( S_{np} = 4 \pi r_{np}^2 = 0.396 \mu m^2 \text{ per particle} \)

Total surface area of RBC-NPs made of 1 mg of PLGA polymer:
\[ S_{total} = N_{np} \times S_{np} \approx 1.54 \times 10^{10} \mu m^2 \]

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

Blood volume = \( S_{total} / \text{average surface area of mouse RBC (75 } \mu m^2) [2] / \text{concentration of RBCs in mouse blood (10}^{10}/mL) [3] \)
\[ \approx 21 \mu L \]

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

Sialic acid content per \( 10^9 \) RBCs = 0.013 \( \mu mol \) [4]

Amount of blood for complete coating of 1 mg of 100 nm PLGA nanoparticles = 85 \( \mu L \)

Total sialic acid content on fully coated RBC-NPs with 100 nm cores = concentration of RBCs in mouse blood \( (10^{10}/mL) \times 85 \mu L \times 0.013 \mu mol/10^9 \text{ RBCs} = 0.01105 \mu mol \)

Number of 100 nm nanoparticles made of 1 mg of PLGA polymer: \( N_{np} = \frac{1 \text{ mg}}{M_{core}} = 1.53 \times 10^{12} \)

Average sialic acid content per RBC-NP = 0.01105 \( \mu mol \times 6.022 \times 10^{23} / M_{core} = 43,500 \)

sialic acid per RBC-NP
2. **Supporting Figures**

![Diagram showing zeta potential comparison between PLGA NP and biotinylated PLGA NP.](image)

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

![Diagram showing particle size comparison between non-biotinylated and biotinylated PLGA cores.](image)

**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.
**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).

**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.
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).

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

3. Supporting References


