Supporting Information

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SI Text

Particle Characterization. Particle dimensions were determined by analysis of images from a microscope mounted camera (Zeiss AxioCam MRm) using a 100× objective (Zeiss Axio Imager D.1M). Over 50 fully hydrated particles in pH 7.4 PBS buffer were measured for each case. Mouse RBCs used for zeta potential measurements were pelleted and washed three times with cold PBS prior to cross-linking with 1% glutaraldehyde for 5 min. The mouse RBCs were washed with PBS and diluted 1,000-fold with cold deionized water for zeta potential measurements. Zeta potentials for particles and RBCs were measured on a nano ZS zetasizer (Malvern Instruments) in water (Table S2).

Assessment of Biocompatibility and Interaction with Endothelial Cells. Human umbilical vein endothelial cell (HUVEC) or HeLa cells were seeded in 200 μL of media (HuMEC containing HuMEC supplement and bovine pituitary extract or MEM containing Earle’s salts and both supplemented with 10% fetal bovine serum) at a density of 5,000 cells/cm² into a 96-well microtiter plate. Cells were allowed to adhere for 24 h and subsequently incubated with PRINT particles at concentrations ranging from 200 μg/mL to 1.56 μg/mL for 72 h at 37 °C in a humidified 5% CO₂ atmosphere. After the incubation period, all medium/particles were aspirated off cells. The ATP-lucerase assay requires 100 μL fresh medium, which was added back to cells, followed by the addition of 100 μL CellTiter-Glo® Luminescent Cell Viability Assay reagent (Promega). Plates were placed on a microplate shaker for 2 min, then incubated at room temperature for 10 min to stabilize luminescent signal. The luminescent signal was recorded on a SpectraMax M5 plate reader (Molecular Dynamics). The MTS (2-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium]-viability assay utilizes 20 μL of CellTiter 96® AQueous One Solution Reagent (Promega) into each well of the 96-well assay plate containing the samples in 100 μL of culture medium. Plates were incubated at 37 °C for 1–4 h in a humidified, 5% CO₂ atmosphere. The absorbance at 490 nm was recorded using a SpectraMax M5 plate reader (Molecular Dynamics). The viability of the cells exposed to PRINT particles was expressed as a percentage of the viability of cells grown in the absence of particles.

The HUVEC cell line was used to investigate the uptake of red blood cell mimics (RBCMs) with 1, 2, 5, or 10% cross-linker and which contained 1% fluorescein-o-acrylate as the covalently bound fluorescent dye. Cells were plated into 24-well tissue culture flat bottom plates (Corning/Costar 3526) and allowed to incubate overnight at 37 °C, 5% CO₂. The following day, particles were diluted in complete medium to obtain a final assay concentration of 15 μg/mL. Particle solution (300 μL) was then incubated with cells for a time course of 0, 1 h, 2 h, 4 h, and 24 h (37 °C, 5% CO₂). After cell/particle incubation, the cells were washed with 1× Dulbecco’s Phosphate Buffers Saline (DPBS) and detached by trypsinization (300 μL/well). Cells were resuspended in a 1:1 solution of 0.4% trypsin blue (TB) solution in 1× DPBS containing 10% FBS (500 μL/well; total sample volume 800 μL) and transferred to a 5-mL Falcon (352063) polypropylene round-bottom tube. This assay is based on the observation that, for noninternalized particles, the vital dye TB acts to quench the fluorescence emission of the fluorescein bound to the particles, shifting the emission to red, whereas an internalized particle will fluoresce green (1). Cells were analyzed by flow cytometry (CyAn ADP, Dako) for green and red fluorescence. There were 10,000 cells measured in each sample.

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sence of a significant correlation between independent model parameters (p<0.95). Secondary PK parameters \([t_{1/2\alpha}, t_{1/2\beta}, CL, CL_d, V_p,]\) and area under the curve (AUC) were calculated from the estimated pharmacokinetic parameters for the long time-point scans (Fig. S5). The two-compartment model from the WinNonlin library was found to best describe the data in all cases.

For determination of alpha and beta half-lives from the intra-vital microscopy scans, we fit the data to the best-fit model from the WinNonlin analysis. The two-compartment model assumes that particles are transported between the central and peripheral compartments with first-order kinetics and that particles are eliminated from the central compartment with first-order kinetics. The data were fit to Eq. S1, where \(C_p\) is the concentration of the particle in the plasma, and \(\alpha\) and \(\beta\) are rate constants for the distribution and elimination processes, respectively. The fit was

\[
C_p = Ae^{-\alpha t} + Be^{-\beta t}
\]

[S1] calculated by nonlinear regression, maximizing the \(R^2\) value for the fit and producing best-fit values for the coefficients and rate constants (3). Half-lives for the distribution and elimination process were calculated by Eqs. S2 and S3, respectively.

\[
\alpha_{1/2} = \frac{\ln 0.5}{\alpha}.
\]

[S2]

\[
\beta_{1/2} = \frac{\ln 0.5}{\beta}.
\]

[S3]

Although exact values for particle concentrations in the plasma could not be calculated for this study, the well-defined shape of the elimination curves (350 points over 2 h) allowed for determination of the half-life values, which are not dependant on absolute concentration.

Each elimination curve was analyzed individually for the above values, with the averages representing three to four mice per particle modulus.


**Fig. S1.** Dose-dependent cell viability of HUVEC (endothelial) cells when dosed with RBCMs with varied crosslink density (1–10%) after 72 h of incubation with the particles as determined by MTS (A) and ATP/luciferase (B) assays.
**Fig. S2.** Uptake of 1, 2, 5, and 10% cross-linked RBCMs in HUVEC cells after dosing times from 15 min to 24 h. Results are expressed in terms of percentage of cells that had taken up RBCMs (internalized) and that were unassociated with RBCMs (unassociated).

**Fig. S3.** A standard curve that correlates fluorescent efficiency (signal intensity) and concentration of 1% cross-linked RBCMs in whole blood. Deviation from linearity defines the lower limit of quantification for fluorescent signal. Data collected for the blood draw experiments fell within the linear range of this plot. Scale bars represent one standard deviation, with $n = 3$.

**Fig. S4.** A plot showing the biodistribution of 1% cross-linked RBCMs in mice over a 2- to 120-h time course in the liver, lung, spleen, and kidneys. Data are presented as fluorescent efficiency per gram of tissue weight. Error bars represent one standard deviation, with $n = 3$ for each case.
A plot of particle concentration in whole blood over time for 1% cross-linked RBCMs where $n = 4$ for each data point. These data were fit to a two-compartment pharmacokinetic model with elimination from the central compartment (red) by nonlinear regression analysis. The fit was used to calculate pharmacokinetic parameters for these particles. Scale bars represent one standard deviation, with $n = 3$.

Movie S1. RBCM particles (1% cross-linked) that are 6 μm in diameter deforming in flow to traverse a 3-μm wide restriction in a 3-μm tall poly(dimethyl siloxane) microfluidic channel. Observed restrictions are in the middle of the device, implying that the RBCMs in this movie have traversed approximately 50 such pores immediately prior to imaging without noticeable loss of structure or elasticity. The flow rate was 0.06 μL/min.

Movie S1 (AVI)

Movie S2. Intravital microscopy for 10% cross-linked RBCMs. The movie represents 2 h of scan time on a typical subject compressed to play in 10 s. Imaging scans used a 633-nm laser and proceeded for 2 h, with an image taken every 2 s at a scan rate of 0.573 s/frame.

Movie S2 (MOV)
Movie S3.  Intravital microscopy for 1% cross-linked RBCMs. The movie represents 2 h of scan time on a typical subject compressed to play in 10 s. Imaging scans used a 633-nm laser and proceeded for 2 h, with an image taken every 2 s at a scan rate of 0.573 s/frame.

Movie S3 (MOV)

Table S1. The composition of hydrogels used for RBCMs

<table>
<thead>
<tr>
<th>Monomer</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>PEG 4000 diacrylate</td>
<td>1–10</td>
</tr>
<tr>
<td>2-Carboxyethyl acrylate</td>
<td>10</td>
</tr>
<tr>
<td>1-Hydroxycyclohexyl phenyl ketone</td>
<td>1</td>
</tr>
<tr>
<td>Methacryloxyethyl thio carbonyl rhodamine B (PolyFluor 570)</td>
<td>0.1</td>
</tr>
<tr>
<td>2-Hydroxyethylacrylate</td>
<td>Remainder</td>
</tr>
<tr>
<td>Fluorescein-o-acrylate</td>
<td>0–1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Table S2. Characterization of RBCM particles and mouse RBCs

<table>
<thead>
<tr>
<th></th>
<th>1% cross-linker</th>
<th>2% cross-linker</th>
<th>5% cross-linker</th>
<th>10% cross-linker</th>
<th>Mouse red blood cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle diameter (µm)*</td>
<td>5.96 ± 0.27</td>
<td>5.68 ± 0.32</td>
<td>5.66 ± 0.34</td>
<td>5.18 ± 0.30</td>
<td>6.0 (ref. 1)</td>
</tr>
<tr>
<td>Zeta Potential (mV)*</td>
<td>–24.2 ± 5.4</td>
<td>–23.6 ± 3.4</td>
<td>–19.9 ± 6.6</td>
<td>–17.2 ± 3.4</td>
<td>–46.6 ± 4.84</td>
</tr>
</tbody>
</table>

*Error represents one standard deviation from the mean with n = 50 for particle diameters.
†Error represents standard deviations with n = 16.