

Supporting Information

of

Nanoscale Bupivacaine Formulations to Enhance the Duration and Safety of Intravenous Regional Anesthesia

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Supporting Tables and Figures

Table S1. Anesthesia duration resulting from different tourniquet times.

Time (min)	Successful Anesthesia (%)	Anesthesia Duration (h)
5	0	0
10	25	0.3 ± 0.5
15	100	2.0 ± 0.6
20	100	1.9 ± 0.5
30	100	2.0 ± 0.5

0.5% bupivacaine (5 mg/mL (w/v), 0.4 mL) was administered by IVRA, and the tourniquet removed at predetermined intervals. Data are means ± S. D.; n = 4. There was no statistically significance difference between 15 min and 20 min treatment.

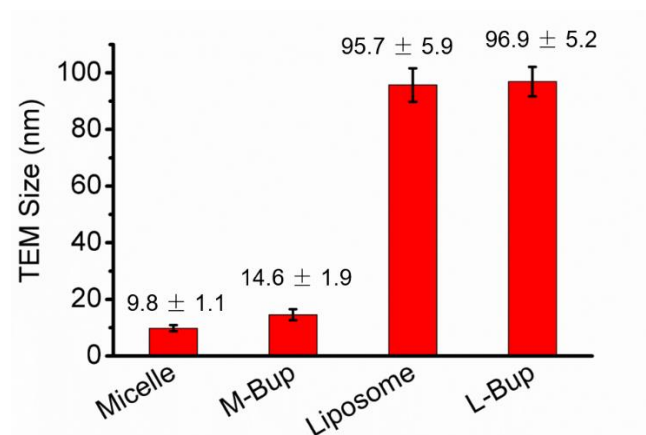


Figure S1. The particle size distribution from transmission electron microscope (TEM) images. Data are presented as mean \pm S. D. (n = 3).

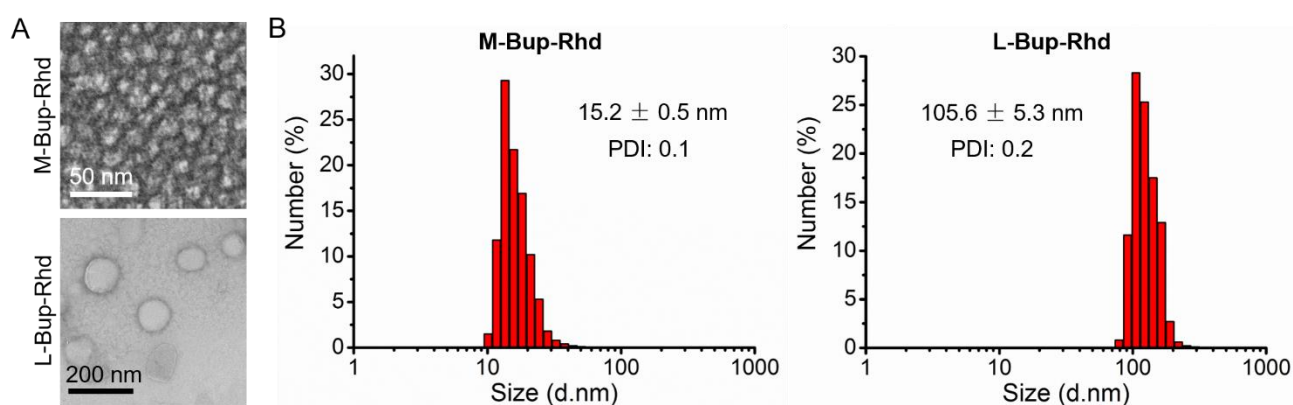


Figure S2. Characterization of M-Bup-Rhd and L-Bup-Rhd. (A) Transmission electron microscopy (TEM) images and (B) Dynamic light scattering (DLS) of M-Bup-Rhd and L-Bup-Rhd. Their sizes are similar to the formulations without Rhd labeling. Data are means \pm S. D.; n = 3.

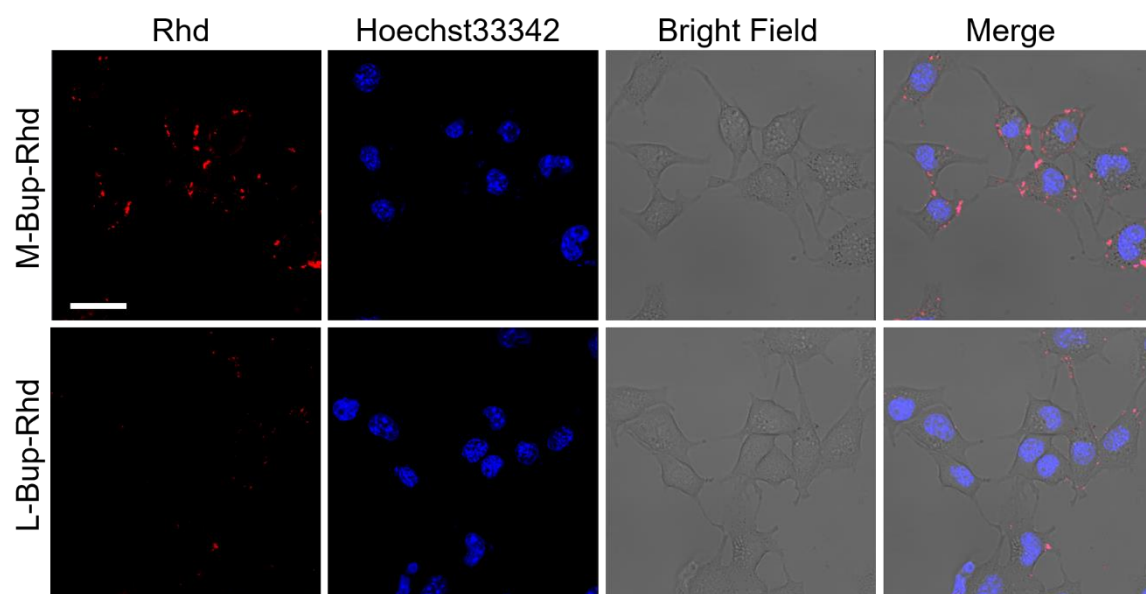


Figure S3. Whole images and separate panels of Figure 3A. Red: rhodamine (Rhd); blue: nuclei (Hoechst33342). Scale bar = 50 μm .

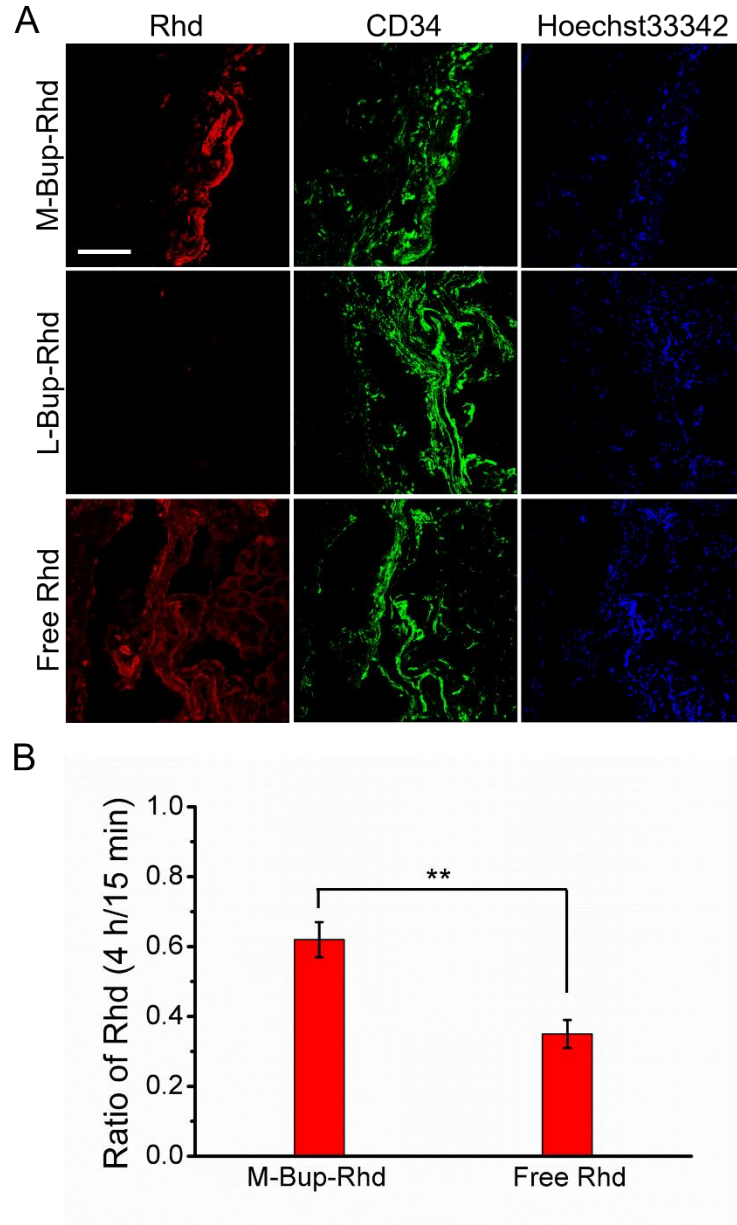


Figure S4. Representative (of 4 animals) images of the distribution of Rhd-labeled (red) formulations 4 h after releasing the tourniquet. (A) The blood vessels were IHC labeled with anti-CD34 mAb (green), and the nucleus were stain with Hoechst33342 (blue). The scale bar is 200 μ m. (B) Ratio of total Rhd signal at 4 h to that at 15 min (see images in Figure 5). The ratios were 0.62 ± 0.05 (M-Bup-Rhd) and 0.35 ± 0.04 (free Rhd), respectively. Data are means \pm S. D.; n = 4.

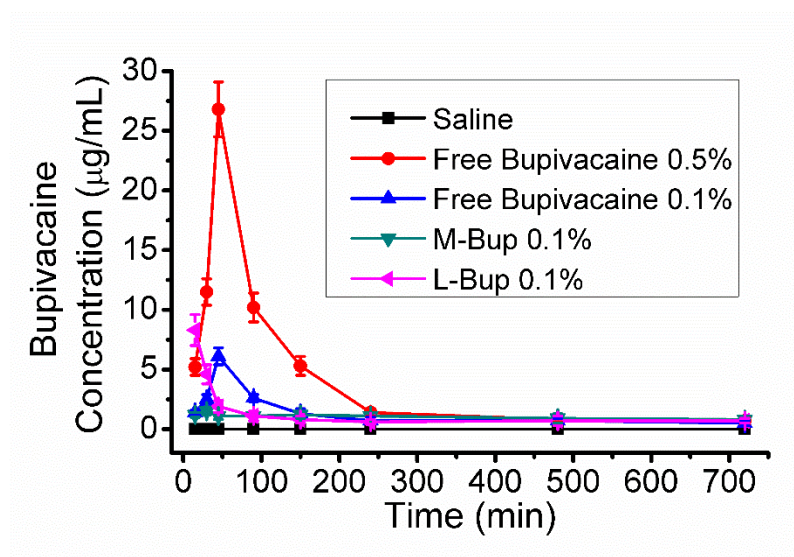


Figure S5. Bupivacaine concentration in blood after releasing the tourniquet (at time zero). All the injections were 0.4 mL. No bupivacaine was detected in saline treated animals. Data are means \pm S.D.; n = 4.

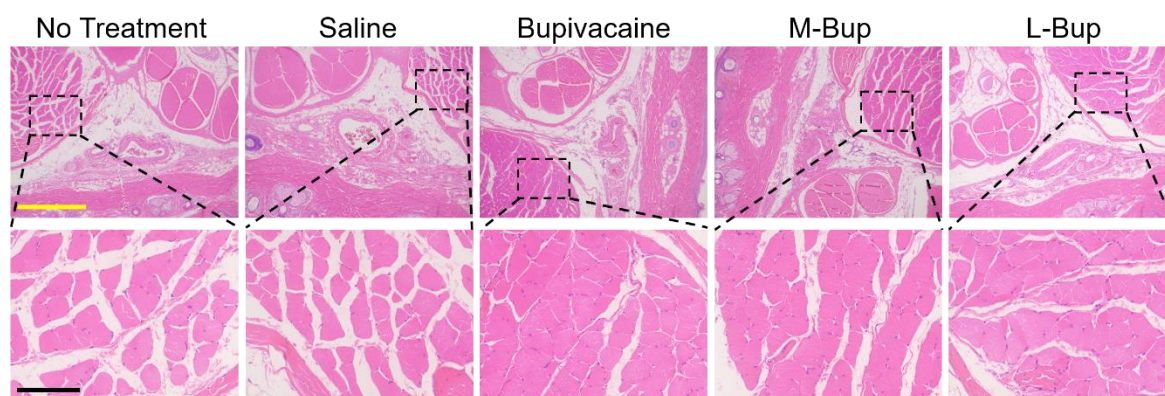


Figure S6. Histological evaluation of H&E-stained sections 24 h after treatment. The dotted lines in the top panels are shown in greater detail in the corresponding bottom panels. There were no pathological findings. The yellow scale bar is 200 μ m, and the black scale bar is 50 μ m.

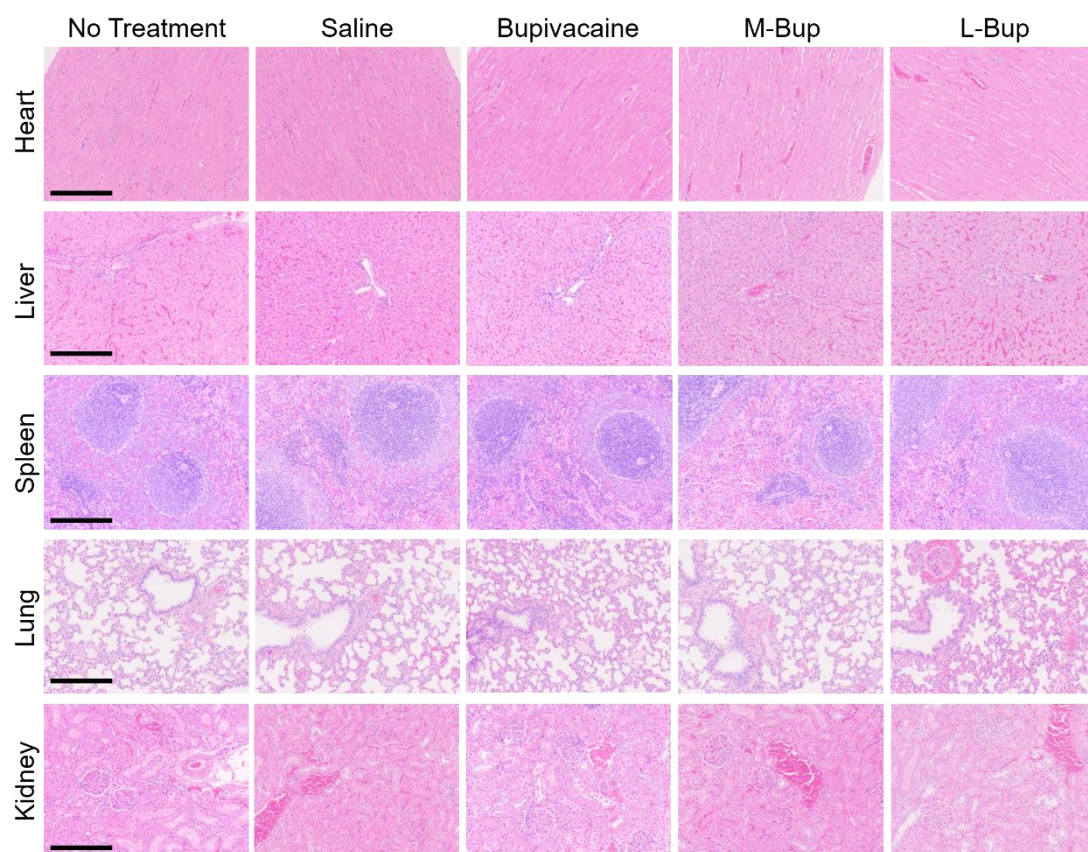


Figure S7. H&E-stained sections of heart, liver, spleen, lung and kidney 24 h after IVRA treatment with each formulation. There were no pathological findings. The scale bars are 200 μm .