

Platelet-like Nanoparticles (PLNs): Mimicking Shape, Flexibility and Surface Biology of Platelets to Target Vascular Injuries

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SI Text

SI Figure 1: FTIR of PLNs and PLN composition materials. Spectra of PAH, BSA, PS, PLNs prior to core dissolution and PLNs following core dissolution.

SI Figure 2: Device schematic. (a) Schematic of microfluidic device used for *in vitro* adhesion of spheres, discs and PLNs. (b) Brightfield and fluorescent image of microfluidic channel coated with fluorescent BSA.

SI Figure 3: Attachment of peptides to PLNs. (a) Peptide coupling to dendrimers, (b) amine modification of PLNs. (c) (i) Method of attachment of peptides to PLNs and (ii) final PLN schematic. (d) Quantification of peptides attached per PLN. (e) Qualitative confirmation of peptide attachment via confocal microscopy.

SI Figure 4: Schematic of parallel plate flow chamber. Experimental setup for PPFC *in vitro* adhesion and aggregation studies.

SI Figure 5: PLN Aggregation. Aggregation of PLNs before, during, and after flow through a glass capillary. Scale bars = 20 μm unless otherwise noted.

SI Figure 6: Histology. Representative histology sections of liver, brain and lung for saline injected (n=3) and PLN injected (n=3) BALB/c mice at 24 hours. Scale bars = 100 μm .

Methods

Device synthesis

Single layer, microfluidic channels were fabricated out of a glass slide and PDMS (Dow Sylgard 184). Thin PDMS films with a thickness of 50 μm were made by spinning uncured PDMS on Kapton film (DuPont Electronics) at 1500 rpm for 30 seconds. The films were baked for at least 1 hour at 80°C, where upon curing, the Kapton film and PDMS form a reversible bond, as previously reported.¹ A laser cutter (Trotec) was used to cut channels into the PDMS film, which was then ozone bonded to the glass slide and baked for at least 4 hours at 80°C. The Kapton film

was then carefully removed from the PDMS film. A thick PDMS slab, with pre-drilled inlet holes, was ozone bonded to the top of the PDMS film and baked for at least 1 hour at 80°C.

Scanning electron microscopy (SEM)

An FEI XL40 SEM at 5 kV with a 5 mm working distance was used for imaging particles. Samples were coated with palladium (at 10 kV) via a Hummer sputtering system.

Dynamic light scattering (DLS)

DLS measurements for size and zeta potential were performed in water using a Malvern Zetasizer Nano ZS.

Capillary flow experiments

Glass capillaries of 0.8 mm diameter and 100 mm length were connected to a syringe containing particles of identical concentrations as above. Particles were then flown through the capillary at a flow rate of 5.7 mL/h, to match the average velocity in the microfluidic device channel, and the effluent was collected and immediately imaged along with particles remaining in the syringe.

Histology and histopathology

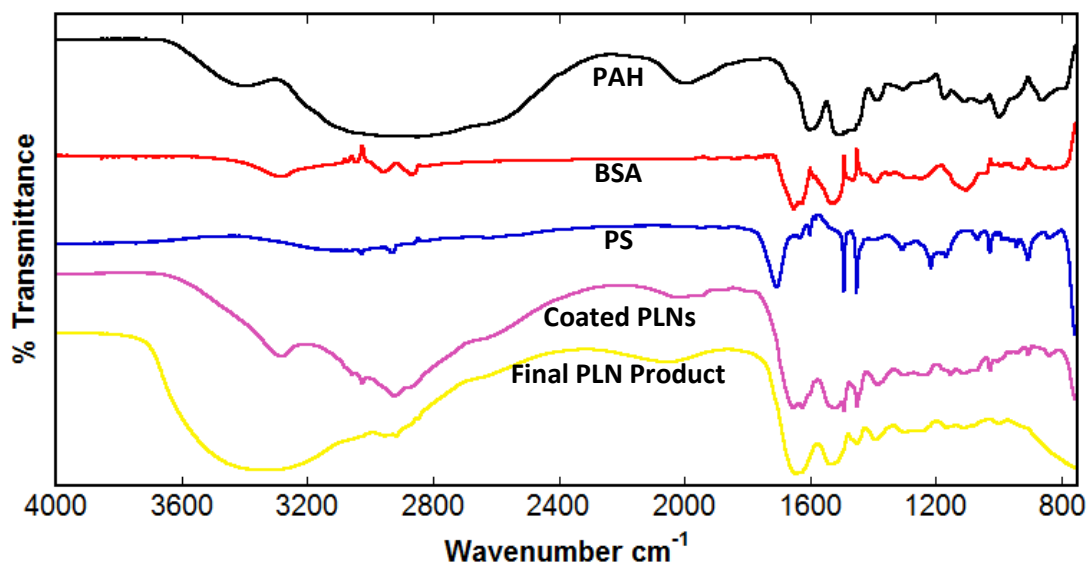
100 µl saline or 15 mg/kg PLNs in 100 µl saline were injected into healthy female BALB/c mice (18-20g, n=3). Animals were sacrificed via CO₂ overdose and whole organs (liver, lung and brain) were collected and fixed in 10% formalin. Tissues were sectioned and stained with hematoxylin and eosin. The sections were imaged using an inverted light microscope and assessed by a veterinary pathologist at Mass Histology services for further analysis.

FTIR

All FTIR samples were suspended in identical concentrations and volumes of a water/acetone mixture. Samples were pipetted onto a zinc selenide ATR crystal and the water/acetone mixture was evaporated completely, leaving a film of the sample. Samples were then placed into an FTIR spectrometer (NICOLET 4700, Thermo Electron Corporation) and the chamber was purged with nitrogen for 30 minutes. Dry crystal backgrounds were subtracted from each sample's spectrum.

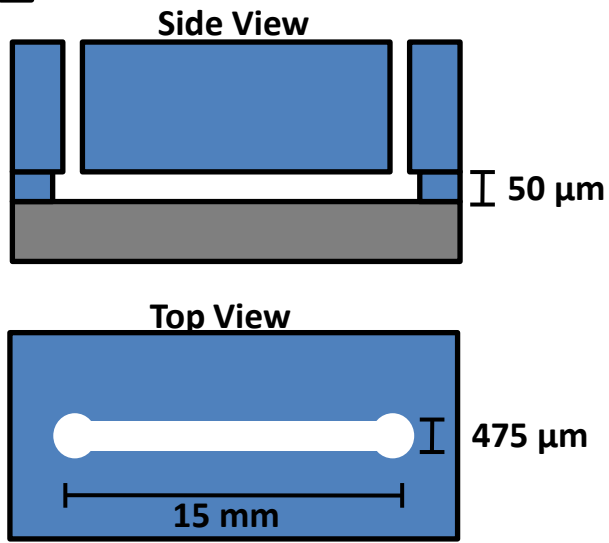
References

1. Epshteyn, A. A.; Maher, S.; Taylor, A. J.; Holton, A. B.; Borenstein, J. T.; Cuiffi, J. D. Membrane-integrated microfluidic device for high-resolution live cell imaging. *Biomicrofluidics* 2011, 5, 046501.

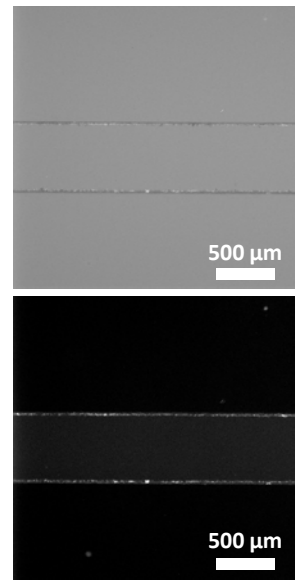


SI Figure 1

(a)

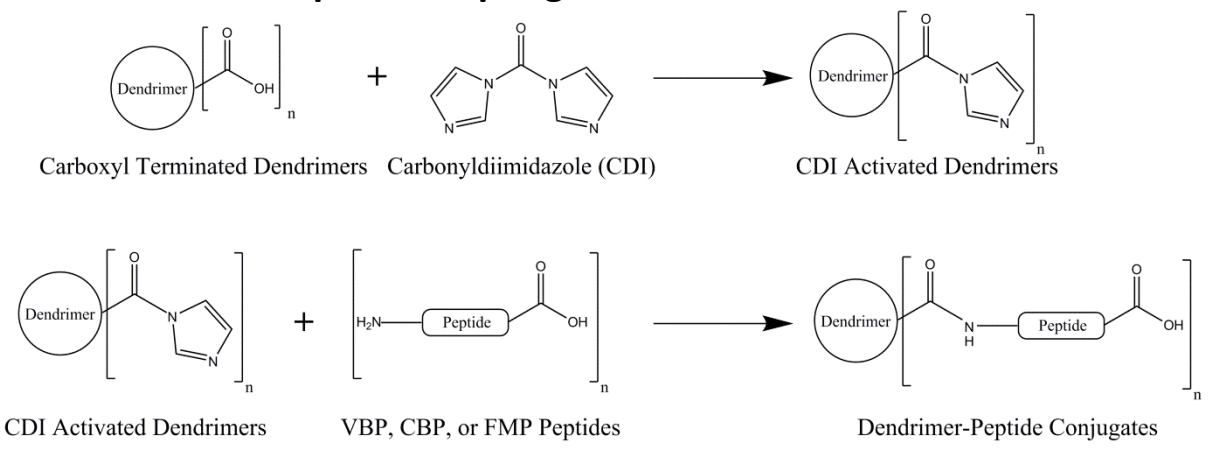


(b)

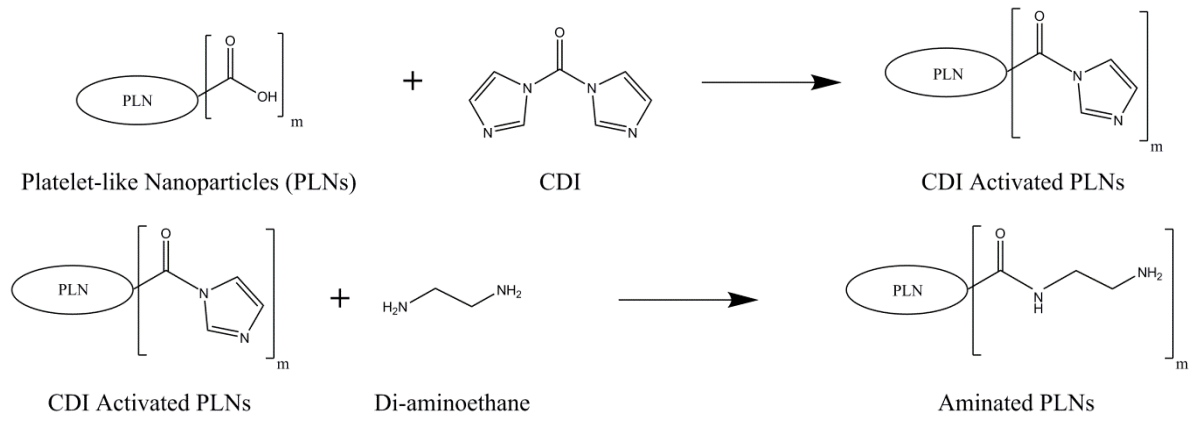


SI Figure 2

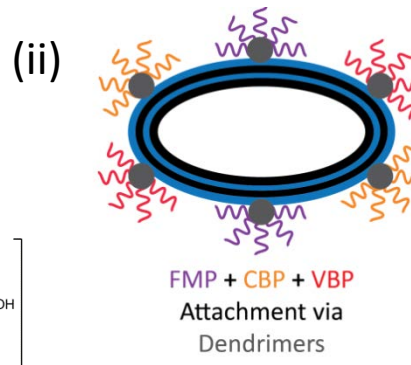
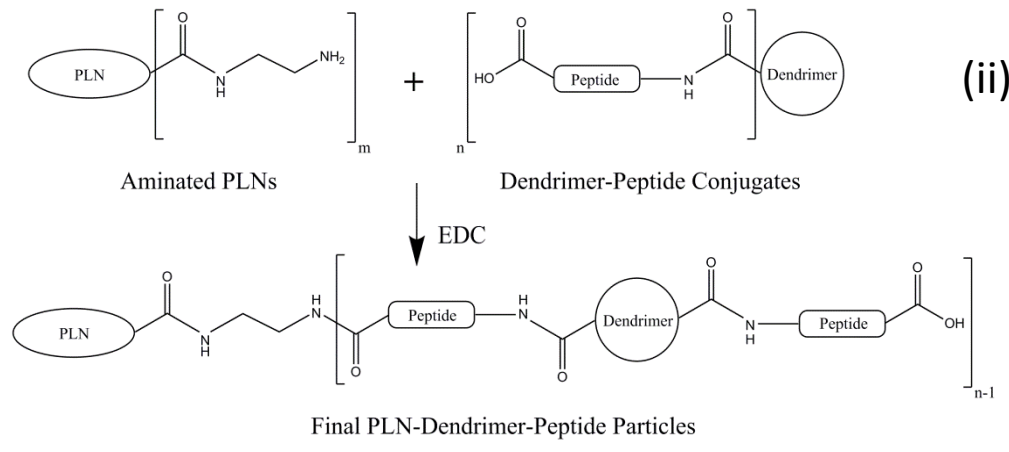
(a) Peptide coupling on dendrimers



(b) Amine modification of PLNs

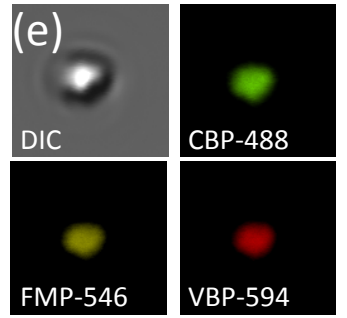


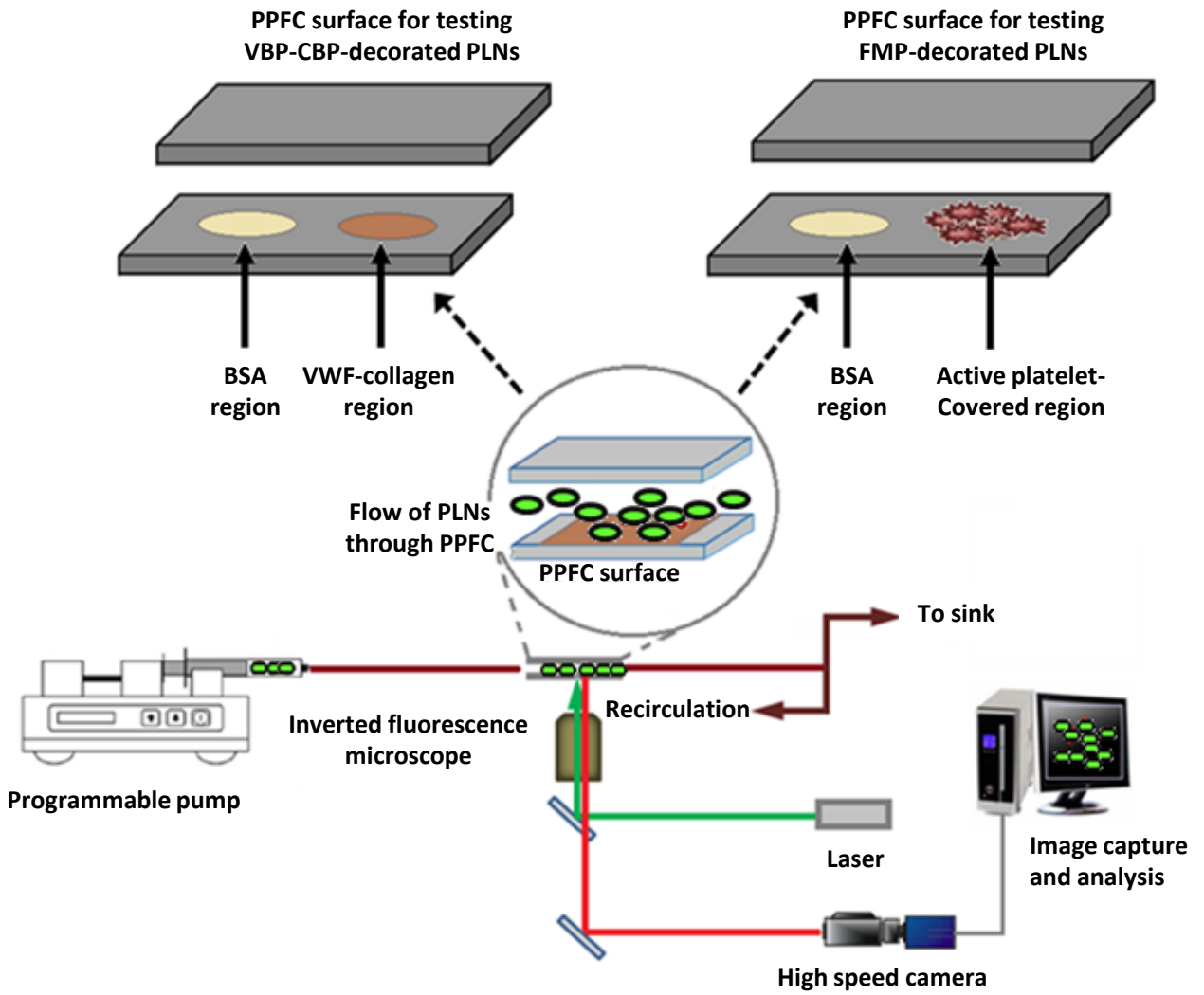
(c) Dendrimer-Peptide Conjugation to PLNs



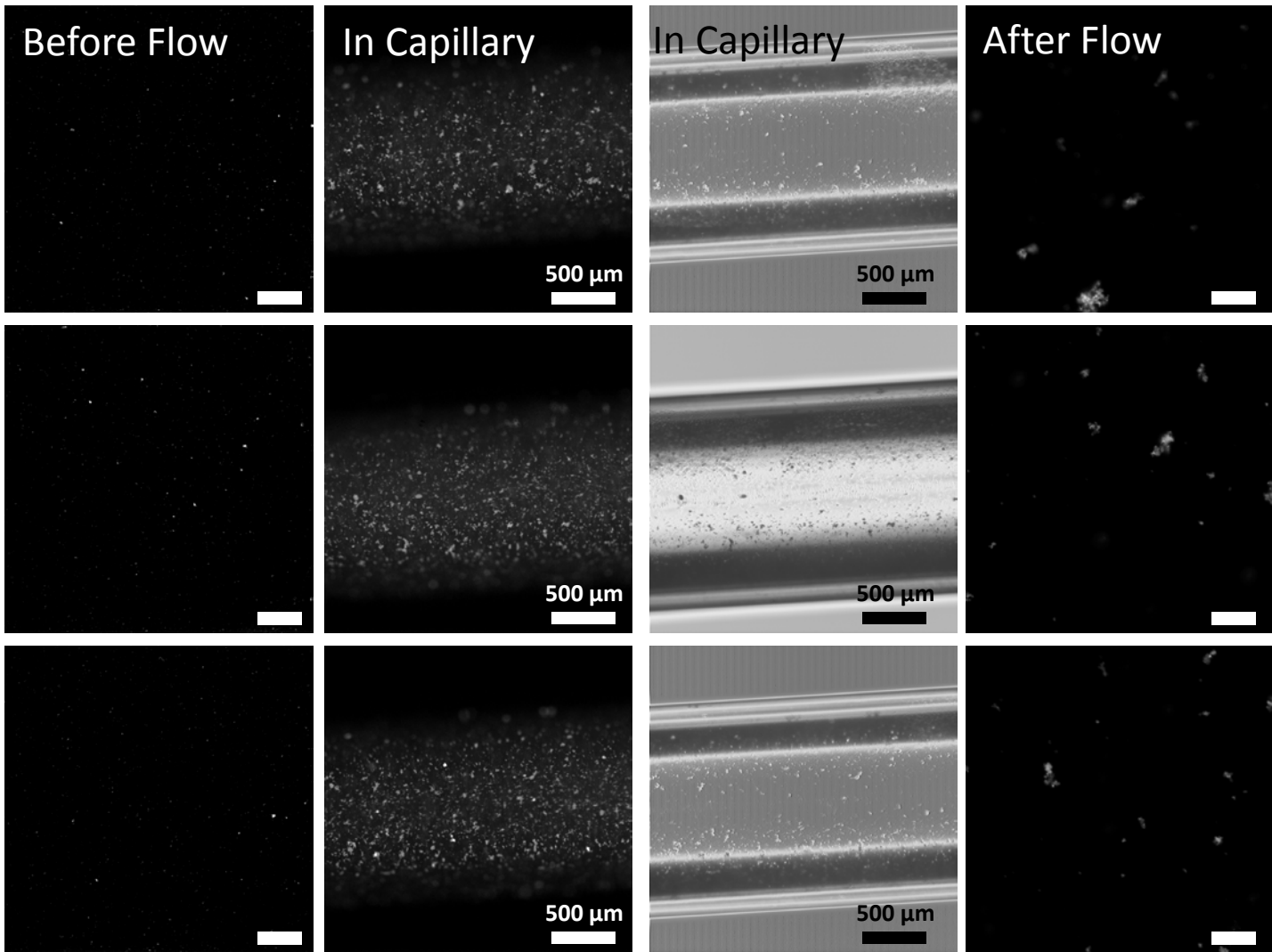
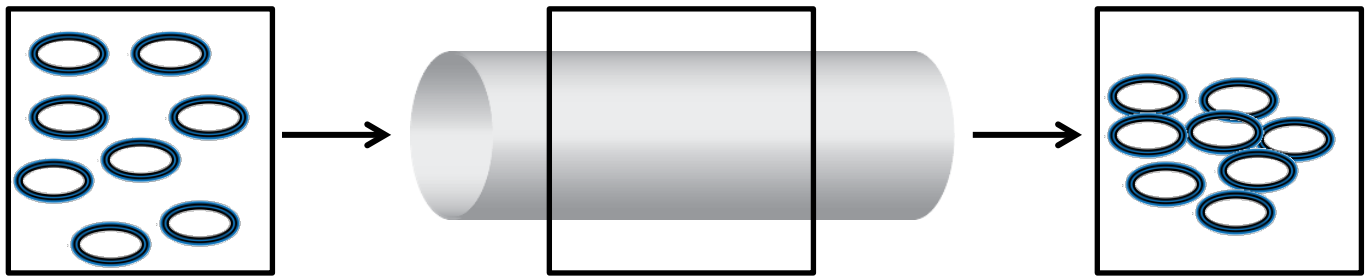
(d) Peptide per mg of PLN and Peptide Molecules per mg PLN

Peptide	Peptide per mg of PLN ($\times 10^{-3}$ mmol/mg particle)	Peptide Molecules per mg PLN ($\times 10^{17}$ peptides/mg particle)
CBP	1.01 +/- 0.020	6.44 +/- 0.12
VBP	1.11 +/- 0.027	6.65 +/- 0.16
RGD	2.31 +/- 0.032	14.0 +/- 0.19

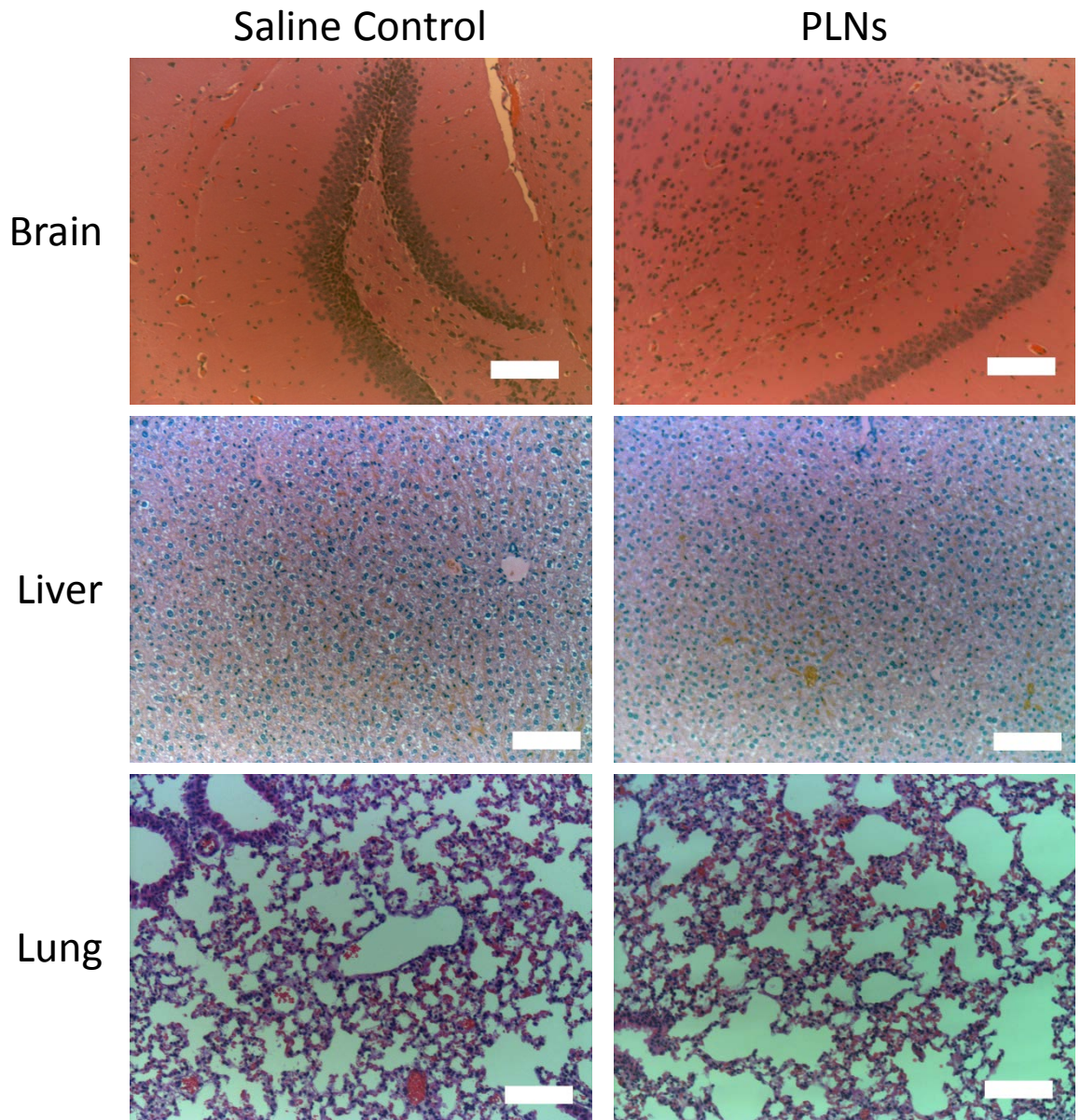




SI Figure 4



SI Figure 5



SI Figure 6