Supporting Information

Ultrasound Monitoring of Magnet-guided Delivery of Mesenchymal Stem Cells Labeled with Magnetic Lipid–polymer Hybrid Nanobubbles

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Appendix

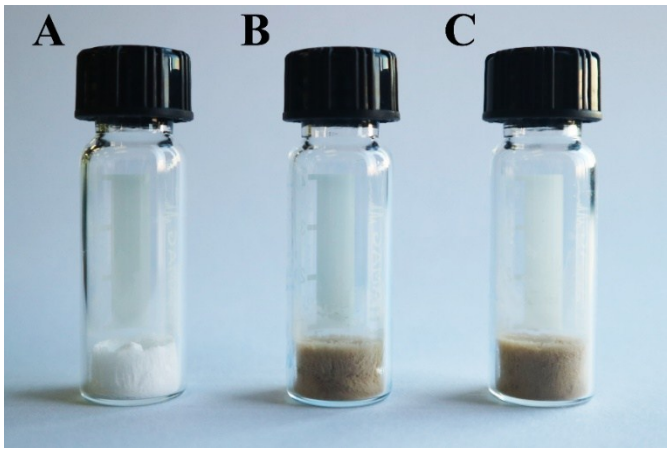

Fig. S1 Appearance of lyophilized powder of lipid-polymer hybrid ultrasound contrast agents. (A) Appearance of lyophilized powder of LPNs. (B) Appearance of lyophilized powder of Mag-LPNs. (C) Appearance of lyophilized powder of P-Mag-LPNs. The lyophilized powder of Mag-LPNs exhibit brownish color as compared to LPNs exhibiting white color. But there are no obvious differences between the appearance or color of Mag-LPNs and P-Mag-LPNs.

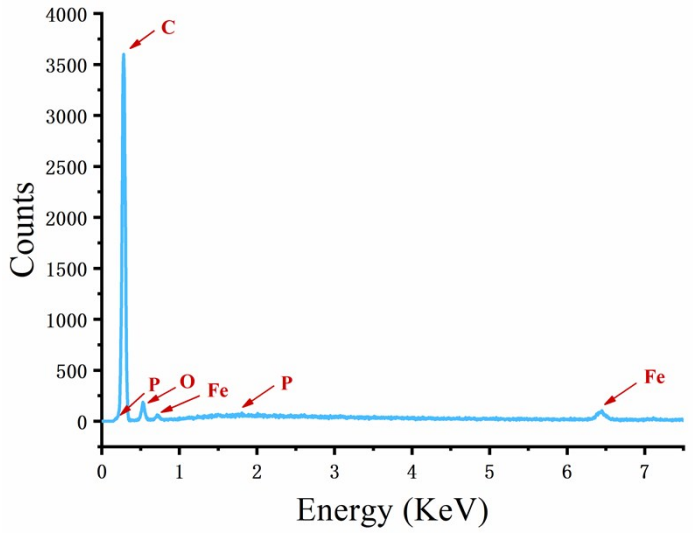

Fig. S2 The characterization of SEM-EDX spot analysis of P-Mag-LPNs. The characteristic EDX spectrum of P-Mag-LPNs validates the existence of Fe and P elements.

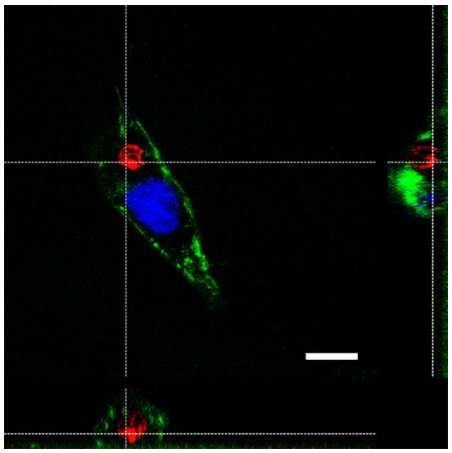

Fig. S3 Confocal laser scanning fluorescence images of MSCs labeling with P-Mag-LPNs. Orthogonal views of z-stack reconstructions, including the axial, sagittal and coronal view, exhibit that P-mag-LPN is located in the cytoplasm of MSC. The plasma membrane was stained green (WGA-FITC), the cell nucleus was stained blue (DAPI), and P-mag-LPN was stained red (DiI). Scale bar is 10μm.

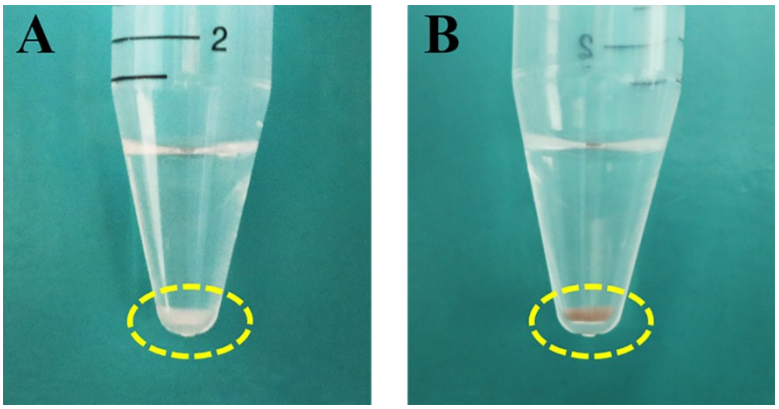

Fig. S4 Appearance of unlabeled or labeled MSCs after centrifugation. The precipitation of unlabeled MSCs (A) exhibits pure white color after centrifugation as compared to labeled MSCs (B) exhibiting brownish color.

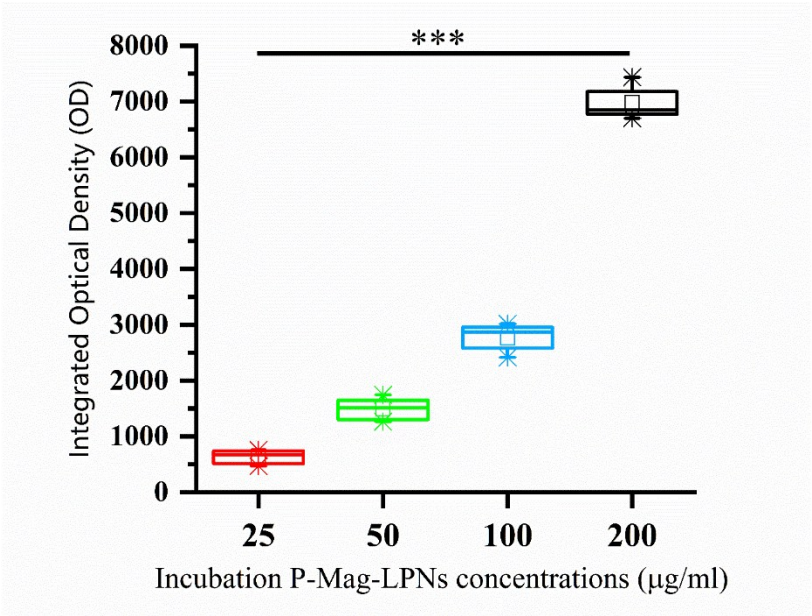

Fig. S5 Quantitative analysis of Prussian blue staining for MSCs labeled with P-Mag-LPNs at various concentrations according to integrated optical density (n = 5). *** $P < 0.001$.

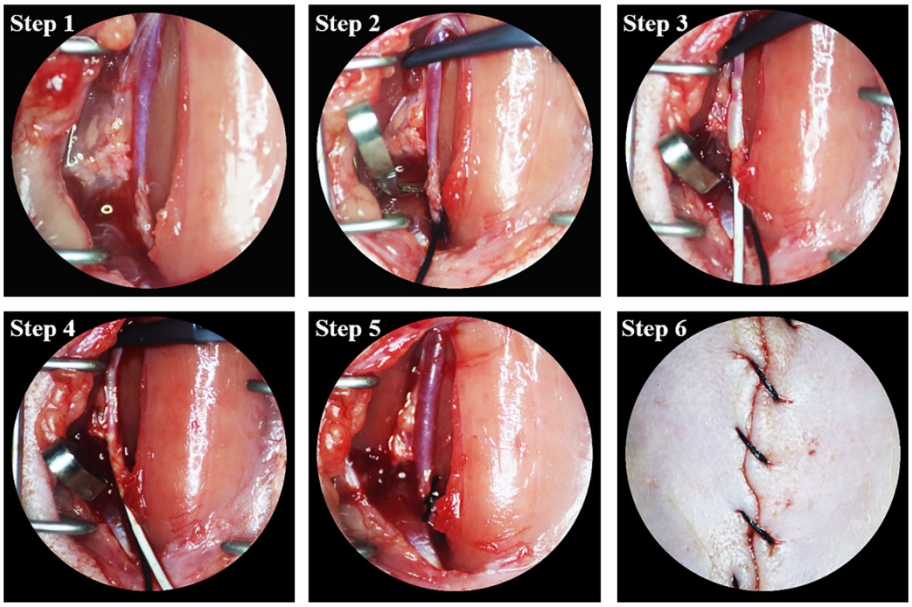

Fig. S6 Establishment of rat carotid artery balloon injury model. (Step 1) A midline neck incision was made using a scalpel. Bluntly dissect all the adjacent tissues alongside the left common carotid artery (LCCA) carefully until the LCCA was completely exposed. (Step 2) Permanently ligate the left external carotid artery (LECA) as far from the bifurcation as possible. Also permanently ligate other branches of LECA. Temporarily clip the proximal LCCA and the distal left internal carotid artery (LICA) to stop the blood flow. (Step 3) Insert 2-French uninflated balloon catheter into the LCCA through the incision of the LECA and advance the balloon catheter to the clamped site of the LCCA. (Step 4) Slowly inflate the balloon and pull the balloon rotationally back to the incision of LECA. The procedure of introduction and withdrawal was repeated another twice. (Step 5) Remove the catheter following by permanently ligating LECA, and then remove other arterial clamps on LCCA to restore the blood flow. (Step 6) To make sure there is no any leakage on the blood vessel, then close wound using skin sutures and swab the closed wound.

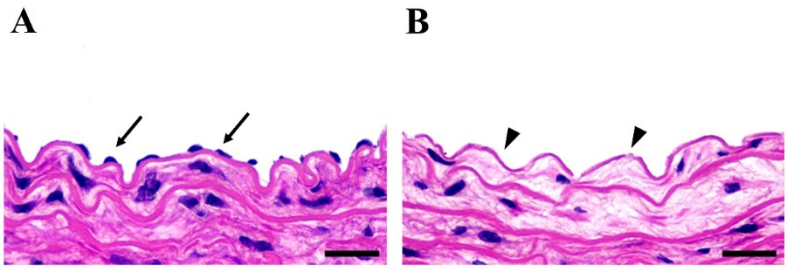

Fig. S7 The Hematoxylin-Eosin stained carotid artery cross-sections. (A) Normal right carotid artery. Note the endothelial monolayer (arrow) overlying the media. (B) Balloon-injured left carotid artery. The carotid artery cross-section exhibits endothelial denudation (arrowheads). Scale bar is 150μm.

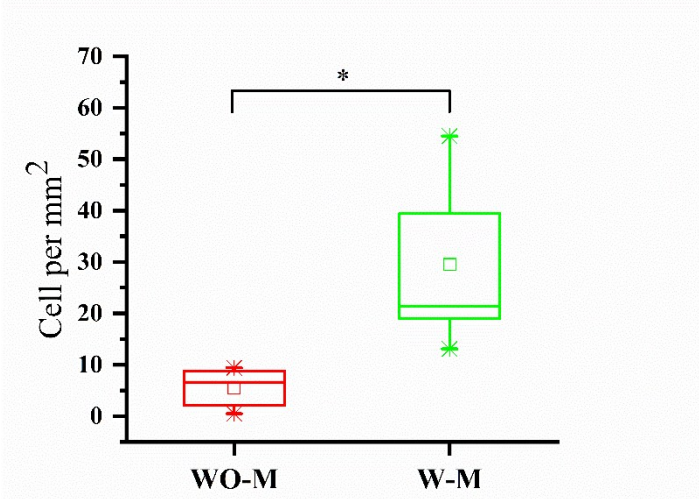

Fig. S8 Quantitative assay for number of MSCs per unit area after cell delivery with or without magnetic targeting (n = 5 animals for each group). WO-M = without a magnet, W-M = with a magnet. * $P < 0.05$.

Supplementary Video 1 (A) With the magnetic field exposure, the echo signal of the labeled MSCs were uniformly distributed in the phantom hole. Whereas, (B) without the magnetic field exposure, the echo signal of the labeled MSCs were rapidly aggregated at bottom of phantom hole.

Supplementary Video 2 (A) With a magnet field, echo signal of the labeled MSCs could be detected and retained at the arterial wall in the bloodstream. However, (B) echo signal of the labeled MSCs was not obvious at the arterial wall after cell delivery without a magnet field. MSCs = mesenchymal stem cells.