Magnetically Coated Bioabsorbable Stents for Renormalizaton of Arterial Vessel Walls after Stent Implantation

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Supplementary information

Synthesis of FePt NPs. FePt NPs were synthesized by simultaneous chemical reduction of $Pt(acac)_2$ and $Fe(acac)_3$ as previously reported.[1] A mixture of $Pt(acac)_2$ (0.5 mmol), $Fe(acac)_3$ (1.0 mmol), and 1,2-hexadecanediol (5.0 mmol) was added to dioctyl ether (30 mL) and stirred under Ar atmosphere for 20 min at room temperature. The mixture was then heated to 100 °C and held at 100 °C for 20 min. During this hold, oleylamine (0.05 mmol) and oleic acid (0.05 mmol) were injected into the mixture and heated to 250 °C (reflux). After 2 h, ethyl alcohol (EtOH) (20 mL) was added to terminate the reaction and centrifuged (3400 rpm for 15 min) to discard the supernatant. It was found that FePt NPs possessed the highest coercivity when mole ratio of precursors was 1:2 ($Fe(acac)_3$:$Pt(acac)_2$), yielding final particle composition of around 1:1 (Fe:Pt).

Silica coating. The FePt nanoparticles were coated with SiO_2 by base-catalyzed silica formation from tetraethylorthosilicate (TEOS).[2] FePt NPs were dispersed in Igepal CO-520 (8 mL) was mixed with cyclohexane (170 mL). NH_4OH aqueous solution (30%, 1.3 mL) was then added dropwise and stirred for 2-3 min, followed by the addition of TEOS (1.5 mL). After 72 h, methanol was added and the particles were precipitated with excess hexane.

Cell staining. Procured cells were stained with carboxyfluorscein succinmide ester (CFSE) according to manufacturer protocols for tracking. For uniform labeling, cells were labeled in as a single-cell suspension. CFSE (1 mM) was added to cells (1×10^6) in media and incubated for 15 min at room temperature. The cells were washed with culture media to remove unincorporated CFSE. CFSE labeled cells were detected at Ex=492 nm and Em=517 nm.

Labeling hHSCs with CliniMACS. CD34+ hHSCs were labeled with the clinically approved CliniMACS (a dextran-coated superparamagnetic iron oxide agent) immobilized with anti-CD34 antibodies according to protocols from manufacturer. hHSCs (1×10^6) were centrifuged at 300×g for 10 min and resuspend in 300 µL of phosphate-buffered saline (PBS, pH 7.2, 0.5% bovine serum albumin, and 2 mM EDTA). FcR Blocking Reagent (100 µL) and then CD34 MicroBeads (100 µL) was added to the cells and incubate for 30 min at 4 °C. Then, the cell suspension was loaded onto a MACS® Column, which was placed in the magnetic field of a MACS Separator. The

magnetically labeled CD34+ cells were retained within the column. The unlabeled cells ran through and were discarded. After removing the column from the magnetic field, the magnetically retained CD34+ cells were eluted as the positively selected cell fraction and suspended in the cell media.

Circulation media. Dulbecco's Modified Eagle Medium (DMEM) with Fetal Bovine Serum (FBS, 10%) and Penicillin-Streptomycin (PS, 1%) was used for macrophages. Medium 199 (M199) with an Earle's Balanced Salt Solution, (EBSS), L-glutamine, HEPES, and $NaHCO_3$ (2.2g/L) was used for HUVECs. For hHSCs, StemSpan™ SFEM serum-free medium was selected.

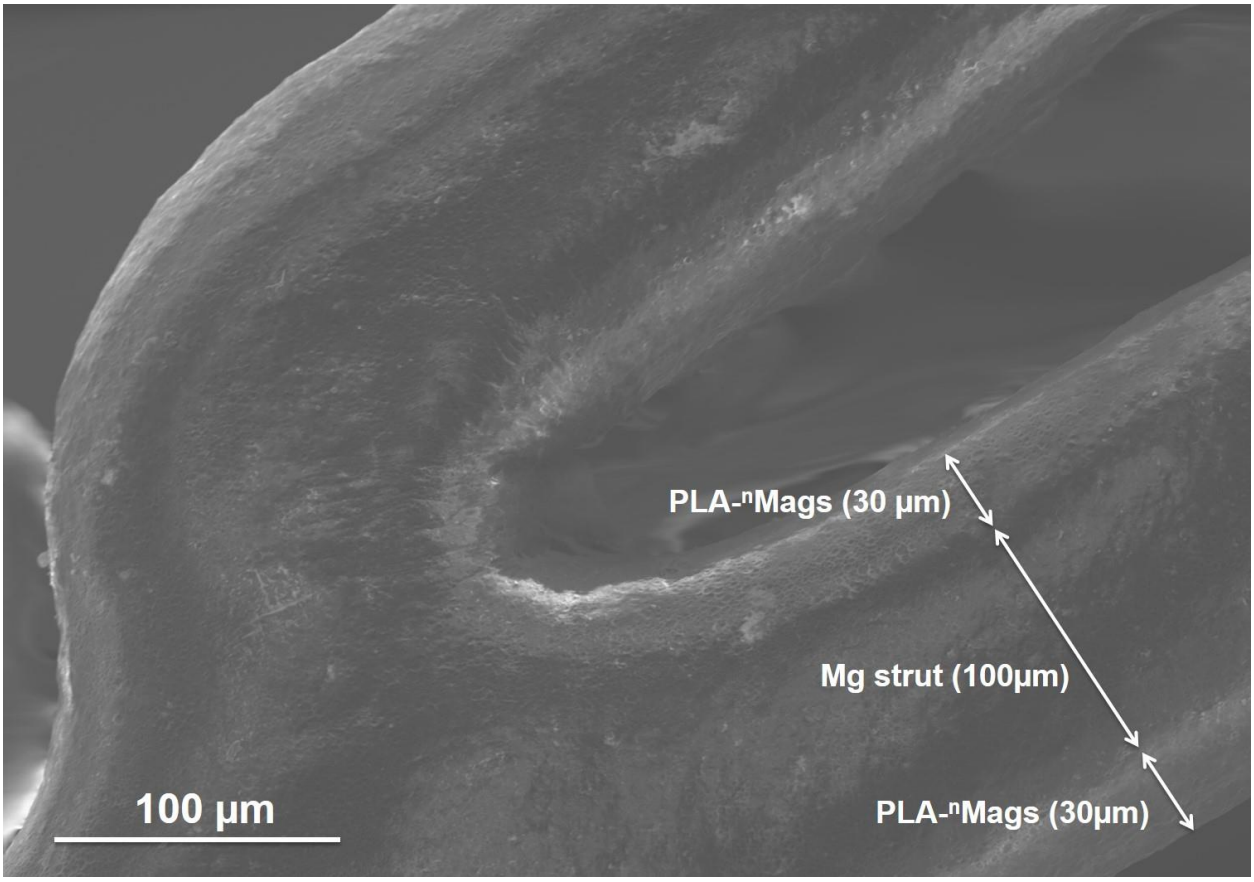

Figure S1.

Scanning electron microscopy (SEM) was performed using a Hitachi S-4800 High Resolution SEM (Hitachi High Technologies Inc.). ^{n}Mag stent was attached on the aluminum sample holder with carbon tape and sputter-coated with chromium. The samples were observed with an accelerating voltage of 10 kV.

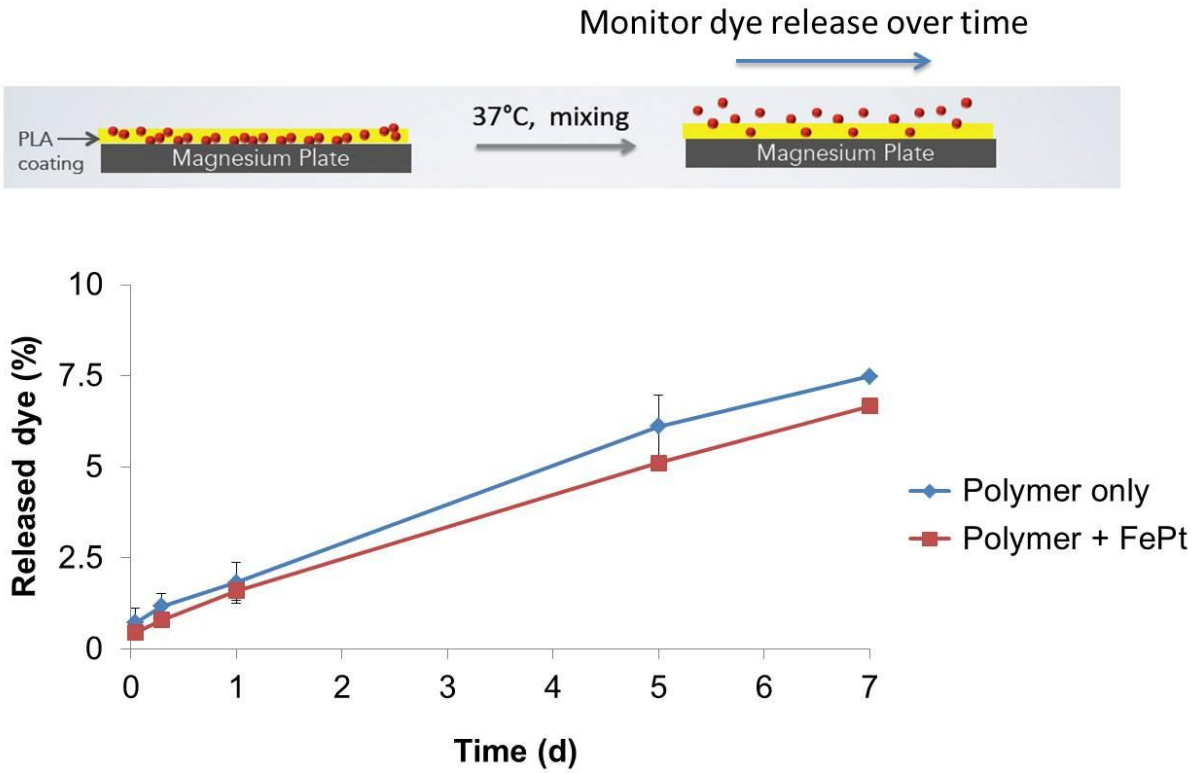

Figure S2.

Release of fluorophore from a PLA polymer coating with or without ^{n}Mags. To investigate whether ^{n}Mags mediate degradation of PLA by interfering coating integrity, Mg plates were coated with PLA (inherent viscosity 0.15-0.35 dL/g, ~25,000 g/mol) containing 30% ^{n}Mags and incubated in PBS at 37 ºC. Another Mg plates were coated with PLA alone and compared. Rhodamine B (RB, 1 wt%) was incorporated in the coatings as a reporter on polymer degradation. Dye release was monitored for 7 days by measuring fluorescence intensity in the PBS media and it is found that the presence of ^{n}Mags did not influence release of dye thus no significant changes in coating stability and degradation.

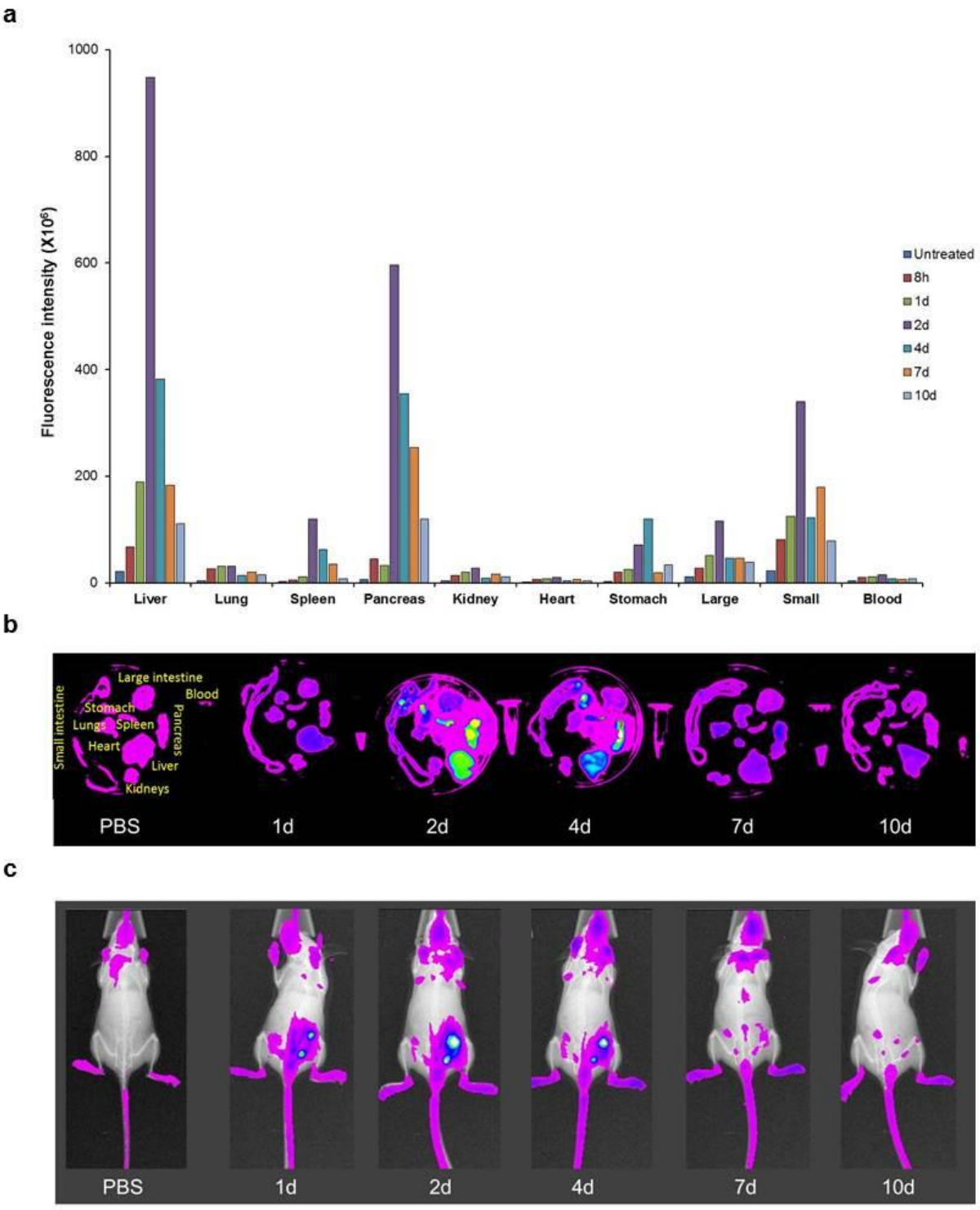

Figure S3.

Biodistribution and body clearance of ^{n}Mag-PLA. ^{n}Mag-PLA NPs were prepared and labeled using the emulsion method.[3] PLA (70 mg), ^{n}Mags (30 mg), and RB (10 mg) were dissolved in chloroform and sonicated in 5% PVA solution. The mixture was sonicated three times and then

added to 0.2% PVA solution. RB-labeled ^{n}Mag-PLA was intraperitoneally injected in C57BL/6 mice to investigate biodistribution and clearance of the stent coating (200 mg/Kg). The ^{n}Mag-PLA displayed high accumulation in the liver, pancreas, spleen, stomach, and intestines after degradation, showing the peak accumulation at day 2 and was mostly cleared from the mice after 7 d.

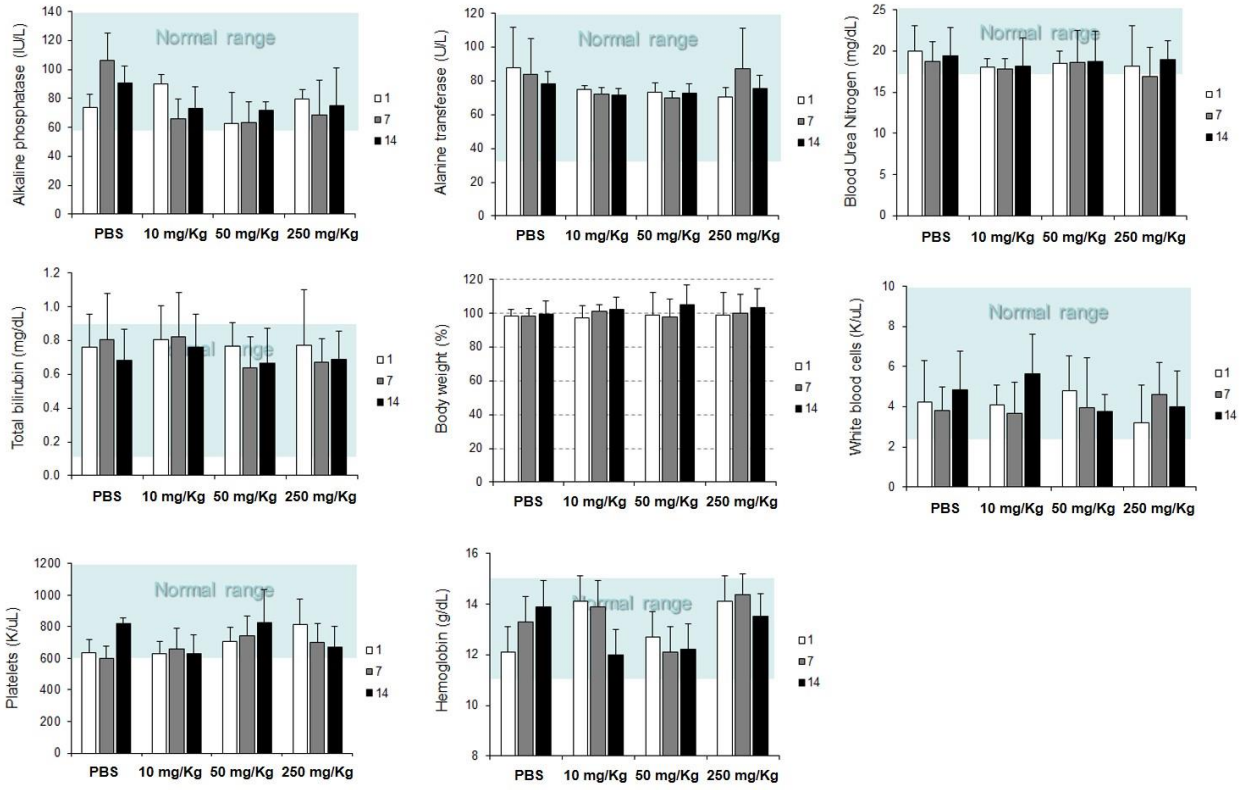

Figure S4.

Toxicology of ^{n}Mag-PLA. ^{n}Mag-PLA were prepared using the emulsion method.[3] Acute toxicity studies were performed in 10-week-old C57BL/6 female mice. Mice were dosed with indicated treatment groups on day 0. Serum concentrations of ALKP, ALT, tBIL, and BUN were measured using reagents from Teco Diagnostics at day 1, 7, and 14. C57BL/6 mice received 3 different doses of particles and compared with a PBS group. Serum clinical chemistries were within normal physiological range for alkaline phosphatase (62-209 IU/L), alanine transferase (28-152 IU/L), total bilirubin (0.1-0.9 mg/dL), and blood urea nitrogen (18-29 mg/dL). No liver or renal toxicity was observed. Mouse physiological reference ranges are from IDEXX VetTest Operator's

Reference Manual (2007). Error bars represent the standard deviation. The sample size is n = 5 mice per group. Body weight was normal. EDTA anti- coagulated blood was analyzed for hematoxicity. All CBC measurements were within the normal reference range for white blood cells (1.8 – 10.7 K/μL), platelets (592 – 2971 K/μL), and hemoglobin (11.0 – 15.1 g/dL). Mouse CBC reference ranges are from Drew Scientific Hemavet 950 Reference Ranges (2010). Error bars represent the standard deviation. The sample size is n = 5 mice per group.

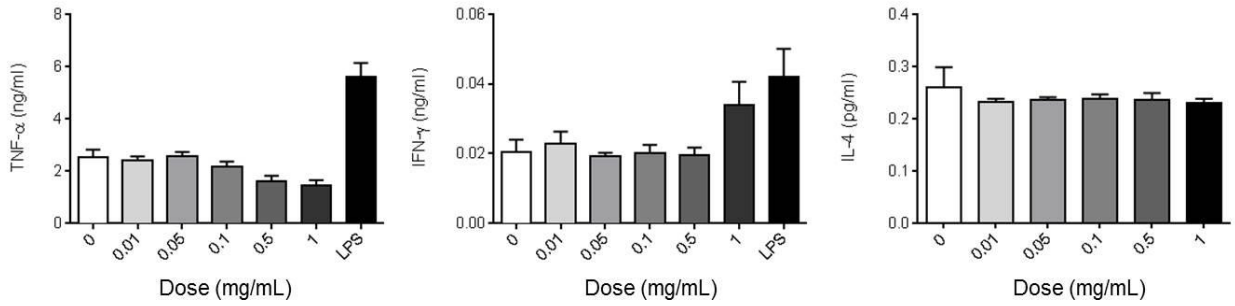

Figure S5.

Production of proinflammatory cytokines from bone marrow derived macrophages (BMM) when treated with ^{n}Mag-PLA. To ascertain levels of acute cytokines that may be induced as a result of ^{n}Mag-PLA treatment, level of TNF-α, IFN-γ, and IL-4 from BMM was measured 3 d post-treatment as a function of ^{n}Mag-PLA dose. IL-4 was measured as a proxy for potential allergic responses, and TNF-α and IFN-γ for inflammatory responses. The nanoparticle groups were compared to the PBS group (negative control) as well as lipopolysaccharides (LPS) group (positive control) in the cytokine levels. No statistically significant increases in TNF-α and IL-4 were detected, and only highest dose (1 mg/mL) induced high IFN-γ expression.

[1] K. E. Elkins, T. S. Vedantam, J. P. Liu, H. Zeng, S. H. Sun, Y. Ding, Z. L. Wang, Nano Lett 2003, 3, 1647.
[2] D. C. Lee, F. V. Mikulec, J. M. Pelaez, B. Koo, B. A. Korgel, The journal of physical chemistry. B 2006, 110, 11160.
[3] J. Park, T. Mattessich, S. M. Jay, A. Agawu, W. M. Saltzman, T. M. Fahmy, Journal of controlled release : official journal of the Controlled Release Society 2011, 156, 109.