Electronic Supplementary Information

Background free *in vivo* ^{29}Si MR imaging with hyperpolarized PEGylated silicon nanoparticles

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Materials

The 50 nm PEGylated silicon particles and identically sized regular silicon particles were sourced from US Research Nanomaterials, Inc. (TX, USA). Phosphate-buffered saline PBS (0 mM, pH 7.4) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). 4-Amino-TEMPO and dimethyl sulfoide-d_6 (DMSO-d_6) were purchased from Sigma-Aldrich (St. Louis, MO, USA). TEMPOL was purchased from Alfa Aesar (Waltham, MA, USA). Deuterium oxide (D_2O) was purchased from Deutero GmbH (Kastellaun, Germany). ^{29}Si powders with 99.34% purity were purchased from Cambridge Isotope Laboratories (MA, USA). All the other chemicals and reagents used were of analytical grade.

Preparation for DNP Experiment

For the hyperpolarization experiments, specific quantities of Si nanoparticles were prepared in a short NMR glass tube. An equivalent mass of TEMPO solution, twice that of the Si NPs, was integrated into the same container. After ensuring complete penetration of the TEMPO solution into the silicon particles, the tube was sealed with Teflon tape and stored in a dark environment. All the TEMPOs were dissolved in a 1:1 mixture of DMSO-d_6 and D_2O.

DNP System and Hyperpolarization

A laboratory-constructed DNP system was used in this study. (Supplementary Figure 1A). The sample tube was pushed and fitted onto the end of a 5-mm NMR glass tube and inserted into the laboratory-built solid-state DNP device, which consisted of a superconducting magnet (5.0 T) and a helium flow cryostat (3.6 K). The microwave source (500 mW) was set at 140.00 GHz to accommodate a relatively wide silicon ESR spectrum. The microwaves were directed to the sample tube using a waveguide and launched into an open-type antenna. Inside the cryostat, the sample resides within an *in situ* NMR coil, allowing quality assurance using an NMR spectrometer, and is then transported to a resistance thermocouple (for temperature monitoring). The ^{29}Si signal of the Si nanoparticles was monitored using a 90˚ pulse after hyperpolarization for 30 min at a temperature of 3.4 K. The buildup of the ^{29}Si signal was monitored by applying a single pulse of 10° every 15 min for 6 h at a temperature of 3.4 K. In the decay test conducted at room temperature, after at least 6 h of hyperpolarization at 3.4 K, the sample tube was quickly removed from the cryostat and transferred to the MRI within 1 min using a magnetic carrier. The ^{29}Si signal was monitored 16 times by applying pulses with 20° flip angle. The T_1 time was measured using a decay experiment, not the real T_1 time measured using standard saturation/inversion recovery pulse sequence. For all ^{29}Si MR images, hyperpolarization was performed at a temperature of 3.4 K for at least 6 h and then transferred for MRI within 1 min. Hyperpolarization results were analyzed using the TNMR program (Tecmag, TX, USA).

MR imaging

The MRI experiments were performed on a 9.4 T Bruker Biospec MRI system (Ettlingen, Germany). Images were recorded using an 86-mm ^{1}H transceiver volume coil and a homemade ^{29}Si surface coil. Anatomical ^{1}H images were acquired using a rapid acquisition with relaxation enhancement (RARE) sequence. The imaging parameters were TE = 11 ms, TR = 2,000 ms, FOV = 9.31 × 5.43 mm, and matrix = 256 × 192. ^{29}Si images were acquired with RARE and short-echo time protocols using the following parameters: TE = 0.86 ms, TR = 2,000 ms, FOV = 9.31 × 4.65 mm, and matrix = 32 × 32. All ^{29}Si MR data were normalized from daily fluctuations in scanner performance and coil sensitivity in comparison with the ^{29}Si MR signal of 99.34% ^{29}Si powder, which was acquired immediately prior to the given experiment. The MR images were analyzed using ParaVision (Bruker, Ettlingen, Germany) and ImageJ software (NIH, Bethesda, MD, USA).

***In Vivo* Delivery of PEGylated Silicon Nanoparticles**

Five-week-old male athymic BALB/c nude mice (Orient Bio, Seoul, Korea) were used for MR. The mice were kept in micro-isolator cages under sterile conditions and observed for at least 1 wk before study initiation to ensure proper health. Temperature, lighting, and humidity were centrally controlled. All the experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee. The anesthesia was induced using 3% and maintained using 2% isoflurane in a mixture of 70% N_2O and 30% O_2. The respiration rate was monitored using a small animal respiration pad (Model 1025 Small Animal Monitoring and Gating System; SA Instruments, Inc., Stony Brook, NY), and body temperature was supported by a warm water tube integrated into the animal bed. Prior to the experiment, the same 50 mM TEMPOL radical solution used in the hyperpolarization condition was mixed with 200 μL of saline and injected intraperitoneally and intravenously into the mice, confirming the lack of toxicity. All PEGylated Si NPs used in the *in vivo* experiments were hyperpolarized for more than 6 h at temperatures below 3.4 K. For subcutaneous and intraperitoneal delivery, 20 and 15 mg of PEGylated Si NPs were used, respectively. Sufficiently hyperpolarized PEGylated Si NPs were carefully dispersed in 200 μL of saline and then injected using a 24 G needle. For oral delivery, 20 mg of PEGylated Si NPs were dispersed in 250 μL of saline and injected using an 18 G gavage tube. All ^{29}Si MR data were normalized from daily fluctuations in scanner performance and coil sensitivity in comparison with the ^{29}Si MR signal of 99.34 % ^{29}Si powder, which was acquired immediately prior to the given experiment. The MR images were analyzed using ParaVision and ImageJ software. The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for animal experimental investigations. All animal experimental procedures were approved by the Yonsei University College of Medicine Institutional Animal Care and Use Committee and carried out in accordance with the committee's guidelines.

Supplementary Figures

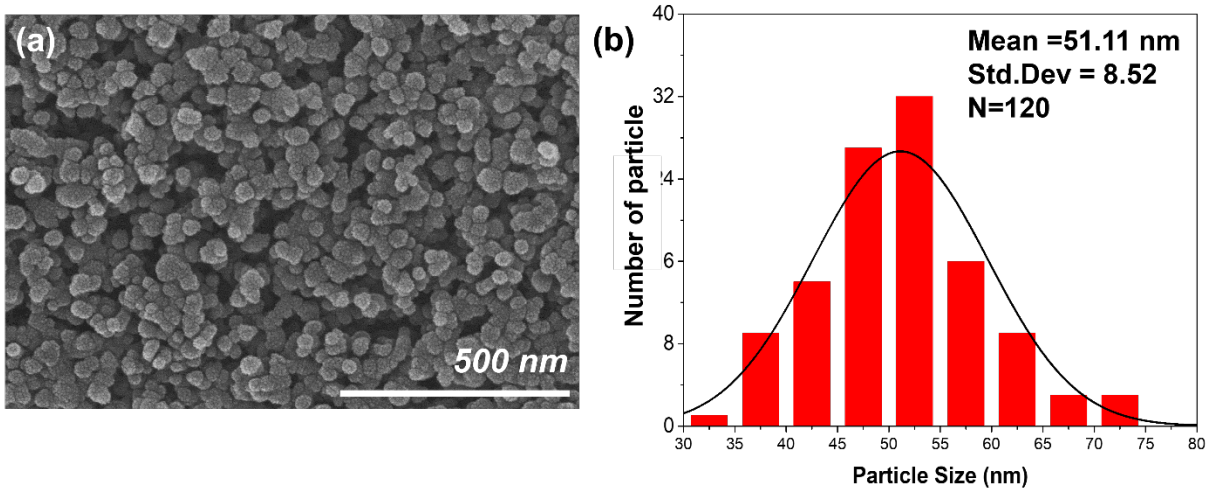

Fig. S1 a) SEM image and b) size distribution histogram of normal Si NPs. Based on the experimental results, it was conclusively determined that silica nanoparticles of approximately 50 nm in size were synthesized uniformly.

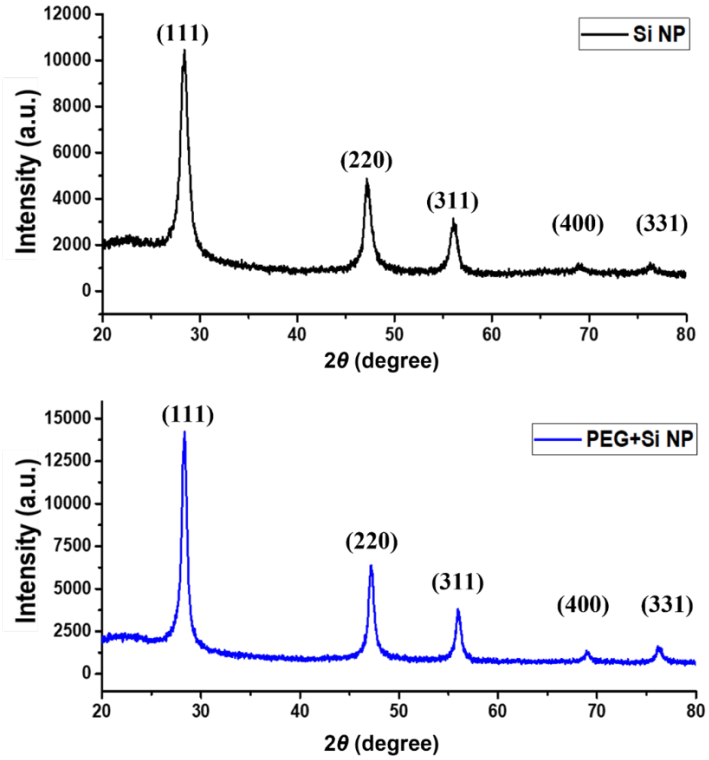

Fig. S2 The XRD pattern of normal (black) and PEGylated (blue) Si NPs. It was confirmed that both samples exhibit the same representative silicon phase.

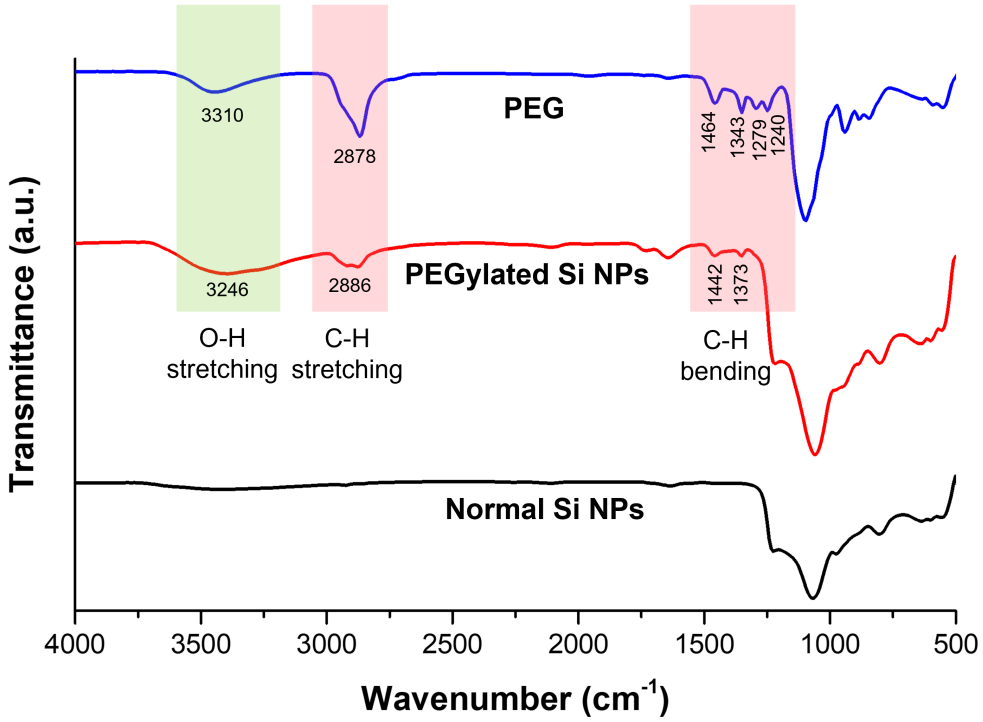

Fig. S3 FT-IR spectra of pure PEG (blue), PEGylated Si NPs (red) and normal Si NPs (black). PEGylated Si NPs demonstrated PEGylation by showing the same representative functional group peaks as pure PEG.

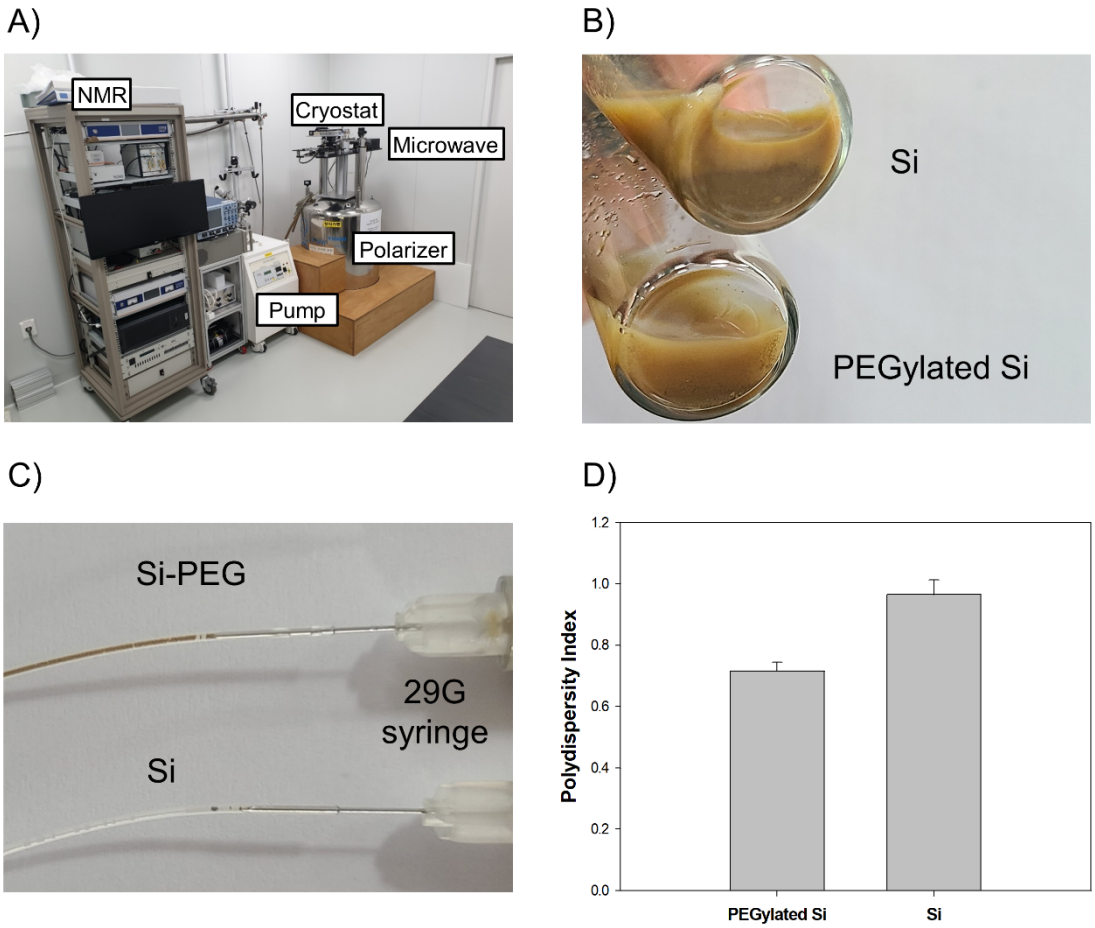

Fig. S4 Photo of a) DNP system and b) silicon and PEGylated Si NPs in water. C) Images of PEGylated Si NPs passing through the 29-G needle and catheter. D) Polydispersity index graph of silicon and PEGylated Si NPs

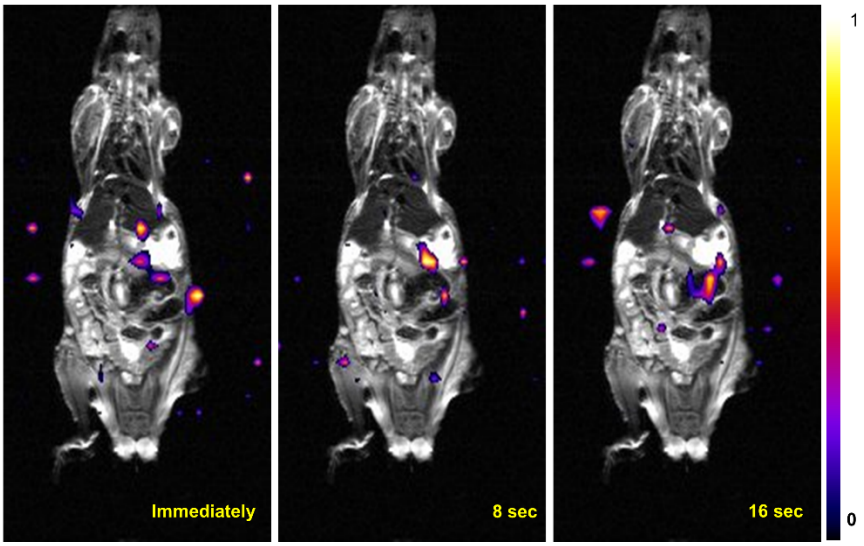

Fig. S5 *In vivo* ^{29}Si MR images of a mouse with orally delivered hyperpolarized PEGylated Si NPs. Using the surface coil made stomach alignment difficult, and particle spatial delocalization led to a reduced S/N level compared to other methods.

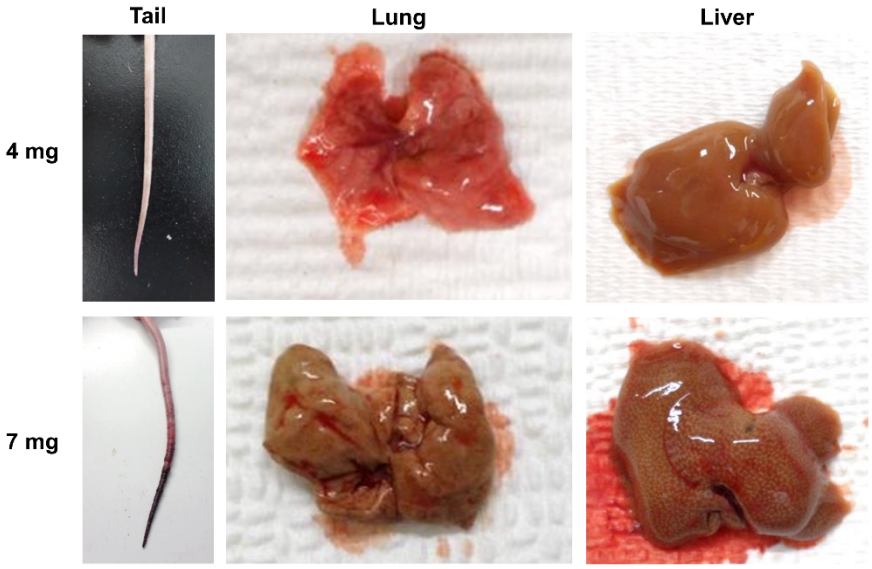

Fig. S6 Photos of mouse tail, lung, and liver after injections of 4 and 7 mg of PEGylated Si NPs. When 4 mg of PEGylated Si NPs were injected, the mice were alive and showed no abnormal behavior, and no organ changes were observed. However, when 7 mg of PEGylated Si NPs were injected, some mice died, and Si NPs were confirmed in the lungs and liver. Even if the mice did not die, the tail vein was blocked, and necrosis ensued 2 d after injection.