Hyperpolarized Long-$T_1$ Silicon Nanoparticles for Magnetic Resonance Imaging


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Silicon nanoparticles are experimentally investigated as a potential hyperpolarized, targetable MRI imaging agent. Nuclear $T_1$ times at room temperature for a variety of Si nanoparticles are found to be remarkably long ($10^2$ to $10^4$ s)—roughly consistent with predictions of a core-shell diffusion model—allowing them to be transported, administered and imaged on practical time scales without significant loss of polarization. We also report surface functionalization of Si nanoparticles, comparable to approaches used in other biologically targeted nanoparticle systems.

The use of nanoparticles for biomedical applications has benefited from rapid progress in nanoscale synthesis of materials with specific optical and magnetic properties, as well as biofunctionalization of surfaces, allowing targeting in-vivo, and therapeutic action. For magnetic resonance imaging (MRI), superparamagnetic nanoparticles have extended susceptibility-based contrast agents toward targeted imaging, though achieving high spatial resolution with high contrast remains challenging. An alternative approach is direct MRI of hyperpolarized materials with little or no background signal. Hyperpolarized noble gases and $^{13}$C-enhanced biomolecules have demonstrated impressive image contrast, but are limited by short in-vivo enhancement times ($\sim 10$ s for noble gases, $\sim 30$ s for $^{13}$C biomolecules). It is known that bulk silicon can exhibit multi-hour nuclear spin relaxation ($T_1$) times at room temperature, and that silicon nanoparticles can be hyperpolarized via dynamic nuclear polarization (DNP).

Nuclear magnetic resonance (NMR) in silicon has been widely investigated for half a century and with renewed interest in the context of quantum computation. The low natural abundance of spin-$1/2$ $^{29}$Si nuclei (4.7%) embedded in a lattice of zero-spin $^{28}$Si nuclei isolates the active nuclear spins from one another and from the environment, leading to multi-hour spin relaxation ($T_1$) times, and decoherence ($T_2$) times of tens of seconds. Moreover, the weak dipole-dipole coupling of the sparse $^{29}$Si atoms, together with the isotropic crystal structure and the absence of nuclear electric quadrupole moment conspire to keep any induced nuclear polarization aligned with an external magnetic field, even as the nanoparticle tumbles in space. This is critical for tracking hyperpolarized nanoparticles in a fluid suspension using MRI, as the nuclear polarization direction can be fixed using a small (mT-scale) applied field.

Particle size determines regimes of application to biomedicine as well as predicted NMR properties. Here, we report NMR properties of Si particles spanning four orders of magnitude in mean diameter, from $\sim 40$ nm to millimeter-scale granules. We investigate particles made by ball milling high-resistivity ($30$–$100$ k$\Omega$-cm, residual p-type $\langle 111 \rangle$, Silicon Quest International), and low-resistivity ($0.01$–$0.02$ $\Omega$-cm, boron-doped (p-type), $\langle 100 \rangle$ oriented, Virginia Semiconductor) commercial silicon wafers, followed by centrifugal segregation by size. We also investigate chemically synthesized Si nanoparticles with mean diameters of 40 nm (Meliorum), 60 nm (Meliorum), 140 nm (MTI) and 600 nm (NanoAmor), obtained commercially. Figure 1 shows representative scanning electron microscope (SEM) images of all measured particles, along with volume-weighted size distributions obtained by SEM image analysis. Dilute suspensions of silicon nanoparticles in ethanol were sonicated for ten minutes before being pipetted onto a vitreous carbon planchett which was mounted on a standard specimen holder with conducting carbon tape. For each sample, $> 1000$ particles were analyzed, sourced from $\sim 50$ images, with particles in contact excluded from the analysis. Particle agglomeration seen in dry Meliorum and MTI samples has been reported in similarly sized silica nanoparticles, but is significantly reduced after pegylation. In these cases (Meliorum, MTI), individual measurement of the particle diameter from SEM images was used instead of software analysis.

Nuclear $T_1$ times of the Si nanoparticles, segregated by size and packed dry in teflon NMR tubes, were measured at room temperature at a magnetic field of 2.9 T using a spin-echo Fourier transform method with a saturation recovery sequence. Following a train of sixteen hard $\pi/2$ pulses to null any initial polarization, the sample was left at field to polarize for a time $\tau_{pol}$, followed by a CPMG sequence $(\pi/2)_X - [\tau - (\pi)_Y - \tau - \text{echo}]^m$ with $\tau = 1$ ms and $m = 200$. In Si and other nuclear-dipole-coupled materials echo sequences can yield anomalously.
FIG. 1: Electron micrographs of Si nanoparticles. (a)-(e) ball milling high-resistivity silicon wafer, (f)-(g) wet synthesis (Meliorum), (h) plasma synthesis (MTI), (h) electrical explosion (NanoAmor), (j) ball milling low-resistivity wafer. Insets: Volume-weighted histograms of diameters following size segregation along with averages $d_0$ and standard deviations $\sigma$ based on gaussian fits to distributions.

long decay tail$^{[20]}$. However, the Fourier amplitude of the echo train still provides a signal proportional to initial polarization$^{[20]}$. Values for $T_1$ are extracted from exponential fits, $A \propto 1 - e^{-\tau_{\text{pol}}/T_1}$, to the Fourier amplitude, $A$, of the $n = 200$ echoes as a function of polarization time (see Fig. 2a, inset for an example).

Figure 2a shows $T_1$ as a function of (volume-weighted) average particle diameter for the various samples. The high-resistivity ball-milled samples follow a roughly linear dependence on size, $T_1 \propto d_0$, for $d_0 < \sim 10\mu m$, saturating at $T_1 \sim 5$ h for larger particles. The trend of increasing $T_1$ in larger particles is qualitatively consistent with a simple shell-core spin diffusion model,$^{[21]}$ which predicts $T_1 \propto d_0^2$ for particles with no internal defects or dopants. The low-resistivity ball-milled particles have $T_1 \sim 200$ s, independent of size. Smaller commercial particles formed by wet synthesis (Meliorum) and plasma synthesis (MTI) have $T_1$ times as long as 700 s. This is significantly longer than the predicted values, as this model assumes instantaneous spin relaxation at the surface of the particle. Larger commercial particles formed by electrical explosion (NanoAmor) have shorter $T_1$ than the comparably sized high-resistivity ball-milled particles. Powder x-ray diffraction measurements (not shown) indicate that ball milling induces partial polycrystallinity, consistent with previous studies.$^{[21]}$ We speculate that $T_1$ is reduced for the smallest ball-milled particles, compared to the synthesized particles, by coupling of nuclear spins to paramagnetic defects at the interface between crystallites. Electron Spin Resonance (ESR) measurements (see Supplementary Material S1) on ball-milled and synthesized particles show a single peak corresponding to a g-factor of $g=2.006$, characteristic of $P_h$-
enhanced the $^{29}$Si nuclear spin polarization by approximately a factor of 15 over room temperature. We then removed the sample from the polarizing cryostat and transferred it to the 4.7 T Bruker DMX-200 imager with a micro-imaging gradient set, requiring $\sim$1 minute for the transfer, and imaged the phantom using a small tip angle gradient echo sequence\textsuperscript{23}. Imaging parameters were: tip angle $\theta = 9^\circ$, echo time $\tau = 1.2$ ms, field of view = 15 mm, sample thickness = 2.5 cm, single pass (no averaging), acquisition time = 11 s. The resulting image is shown in Fig. 3. We note that much higher image resolution will be possible with DNP polarization\textsuperscript{9,10,11,12,13}.

To examine the applicability of Si nanoparticles to targeted MRI, we prepared the Si nanoparticle surface for attachment to biological-targeting ligands. Ball-milled high resistivity nanoparticles ($d_0 = 200$ nm) were aminated using either (3-Aminopropyl)triethoxysilane (APTES, Sigma, 99%) or a 1:2 mixture by volume of APTES with bis-(triethoxysilyl)ethane (BTEOSE, Aldrich, 96%) or (3-trihydroxysilyl)propyl methylphosphonate (THPMP, Aldrich, 42 wt% in H$_2$O) (see Fig. 4 a\textsuperscript{23}). The surface oxide was first etched with a dilute solution of hydrofluoric acid (8% in ethanol) followed by resuspension of the particles in ethanol. Approximately 100 mg of silicon nanoparticles were added to 45 mL of acidified 70% ethanol (0.04% v/v, adjusted to pH 3.5 with HCl) and the solution was placed in an ultrasonic bath for five minutes. Saline (0.15 M) was then added and the solution was shaken for 18-24 hours. Silanes were removed from the nanoparticle solution by washing and resuspending three times in methanol buffer, with the final resuspension performed with 10 mL of methanol buffer. Successful amination was assessed using fluorescence spectroscopy (Fig. 4b) using an excitation at 390 nm and emission at 465 nm (SpectraMax Plus, Molecular Devices). The high level of fluorescence observed for aminated particles results from the covalent bonding of surface amino groups with fluorescamine, showing these functional groups were accessible for further reaction.

In addition to chemical assays, the accumulation of amines was indirectly monitored by measuring the particles’ surface charge in solution, known as the zeta potential\textsuperscript{26} (Fig. 4c). The surface of the unmodified silicon nanoparticles is composed of hydroxy groups from the silicon dioxide and thus shows a negative zeta potential. Particles treated with APTES have surfaces coated with propylamines, which become protonated and positively charged in acidic solutions and show a positive zeta potential\textsuperscript{26}.

Aminated particles were coated with polyethylene glycol (PEG) polymers to confer stability and biocompatibility. PEG coating of silica and iron-oxide nanoparticles has been shown to be non-toxic and to reduce the rate of clearance by organs such as the liver or kidneys, thus increasing the particle’s circulation time \textit{in-vivo}\textsuperscript{26}. Pegylation was performed with either $\alpha$-methyl-PEG-succinimidyl $\alpha$-methylbutanoate (mPEG-SMB) (Nektar)
or maleimide-PEG-N-hydroxysuccinimide (MAL-PEG-NHS) (Nektar). Both SMB and NHS are reactive with amine-reactive groups when compared to the negative control. A change in the sign of the surface charge, or zeta potential fluorescence was evident with the negative control (N/C). (c) Fluorescence spectroscopy confirmed the success of the amination reaction. No methylphosphonate (THPMP in HSO4) or (3-trihydroxysilyl)propyl methyloxysilane (BTEOSE) or (3-trihydroxysilyl)propyl methyloxysilane (BTEOSE) was added to this solution and it was placed in an ultrasonic bath for 13 h. To remove the unreacted PEG, samples were centrifuged and resuspended twice in methanol and finally in a phosphate-buffered saline solution (PBS, 0.1 M Na2HPO4, 0.015 M NaCl buffer).

The stability of nanoparticles in solution was assessed using both dynamic light scattering (DLS) (Nano ZS90, Malvern) as a measure of the particles’ hydrodynamic radius, and visual determination of flocculation and sedimentation. The particles treated with mPEG-SMB and NHS-PEG-MAL were both stable in phosphate-buffered saline (PBS) for a period of two days, with no significant change in the particles’ hydrodynamic radius (see Supplementary Information S2). As a control, mPEG-Amine polymer, which does not contain amine-reactive groups, was used. The aminated particles treated with mPEG-Amine aggregated after centrifugation and resuspension in PBS. These results are consistent with other reports of the successful pegylation of SiO2 nanoparticles.

In conclusion, we demonstrate that Si nanoparticles show promise as biologically targeted MRI imaging agents based on their exceptional NMR properties, including their receptivity to hyperpolarization and long nuclear relaxation ($T_1$) times, in the range of minutes to hours. We investigated Si $T_1$ times as a function of nanoparticle size, dopant concentration and synthesis method. Furthermore, we have demonstrated techniques for fabricating, size-separating and coating Si nanoparticles to satisfy a broad spectrum of design criteria. Future developments in the chemical synthesis of larger, monodisperse single-crystal silicon nanoparticles may provide even longer $T_1$ times. We note that Si nanoparticles may be combined with other material components to provide MRI tracking of the delivery of drugs or as a therapeutic agent that allows simultaneous MRI tracking.

The addition of APTES and PEG to the surface of these nanoparticles is a critical step for further surface functionalization and, ultimately, biological targeting.

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