

## Ferromagnetic nickel silicide nanowires for isolating primary CD4<sup>+</sup> T lymphocytes

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Direct CD4<sup>+</sup> T lymphocytes were separated from whole mouse splenocytes using 1-dimensional ferromagnetic nickel silicide nanowires (NiSi NWs). NiSi NWs were prepared by silver-assisted wet chemical etching of silicon and subsequent deposition and annealing of Ni. This method exhibits a separation efficiency of ~93.5%, which is comparable to that of the state-of-the-art superparamagnetic bead-based cell capture (~96.8%). Furthermore, this research shows potential for separation of other lymphocytes, B, natural killer and natural killer T cells, and even rare tumor cells simply by changing the biotin-conjugated antibodies. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4704924>]

Silicides are widely used as metal contact materials in modern silicon microelectronics because of their low contact resistivity and the excellent junction interface on silicon (Si).<sup>1,2</sup> With the recent improvement in nanoscale Si devices, metal silicides have received significant attention because of their unique properties and potential applications in electronic and photonic devices.<sup>2-4</sup> Recently, several research groups have studied nickel silicide nanowires (NiSi NWs).<sup>3,5-8</sup> They confirmed that NiSi NWs show lower resistivity when formed at lower processing temperature with less reaction phase at interface compared to other metallic silicide materials. As a result, NiSi NWs are considered a promising material for use as gate and source/drain electrodes in current complementary metal oxide semiconductor devices.<sup>9</sup>

Cell separation is required in various biological and biomedical applications, including cell transplantation and anti-tumor cell therapy etc.<sup>10-12</sup> Separation using nanometer-sized superparamagnetic beads has attracted interest for capture and isolation of specific cells such as T-lymphocytes and circulating tumor cells because these cells provide important information in clinical diagnosis.<sup>13-15</sup> This approach uses capture-agent-coated superparamagnetic beads to immunologically recognize specific targeted-cells in the blood. Subsequent application of a magnetic field allows captured cells to be separated. Ferromagnetic Ni NWs are better at cell separation than superparamagnetic nanoparticles (NPs) because longer structures possess a stronger magnetic moment and exert larger force and torque on the cell.<sup>16,17</sup> In addition, the length of NWs can readily be controlled. To enhance separation using ferromagnetic metal nanowires, their biocompatibility needs to be further improved.<sup>18,19</sup> A potential alternative for biomedical applications such as cell separation that has not yet been trialed is ferromagnetic NiSi NWs. Use of NiSi NWs for separation would be simple, cost-effective, and biocompatible because cells tolerate ferromagnetism.<sup>20</sup> In this Letter, the

ability streptavidin (STR)-functionalized NiSi NWs to separate and isolate CD4<sup>+</sup> T-lymphocytes from whole mouse splenocytes with a high capture efficiency of  $\sim 93.5\% \pm 6.2\%$ , which is as good as commercial superparamagnetic beads ( $\sim 96.8\% \pm 1.5\%$ ), is demonstrated. Moreover, we observed that STR-functionalized NiSi NWs exhibit a high cell viability ( $\sim 96.8\% \pm 1.4\%$ ) up to 72 h.

Ferromagnetic NiSi NWs were fabricated by a cost-effective technique involving Ag-assisted wet chemical etching of silicon and subsequent Ni deposition/annealing to form Ni silicides on a silicon substrate (Fig. 1(a)). First, an Ag film with a thickness of 5 nm was coated on a (100) Si substrate by electroless deposition in a mixture of hydrofluoric acid (HF) and silver nitrate. Silicon nanowires (SiNWs) was then formed by wet chemical etching with HF/H<sub>2</sub>O<sub>2</sub> solution.<sup>21</sup> The SiNWs were typically 60–100 nm in diameter and 5–10 μm in length, which are strongly dependent on the size of Ag NPs and etching time, respectively. To form NiSi NWs, the SiNWs were annealed at 300 to 700 °C by rapid thermal annealing (RTA) at a pressure of  $8 \times 10^{-7}$  Torr for 90 s after deposition of Ni with a thickness of 200 nm on the SiNWs. High quality polycrystalline NiSi NWs were obtained using this process (Fig. 1(b)). The NiSi NW substrate was then functionalized with a monoclonal anti-mouse CD4-antibody using an STR-biotin immobilization method in the same manner as we previously reported (Fig. 1(c)).<sup>20</sup> Briefly, the NiSi NW substrate was treated with oxygen plasma to expose a surface with a high density of silanol groups. The surface was then immersed in (3-aminopropyl)-triethoxysilane (1% v/v). The NiSi NW surfaces were further functionalized by glutaraldehyde (12.5% v/v) and STR (50 μg/ml). Finally, the STR-conjugated NiSi NWs, which have been removed from NiSi substrates in ultrasonic bath, were placed in a suspension containing biotin-conjugated CD4 anti-mouse antibodies and mouse splenocytes. For experiments involving the isolation/separation of specific cells, T-lymphocytes were selected because they are an important immunocyte in the human body and have fundamental functions in the immune response to

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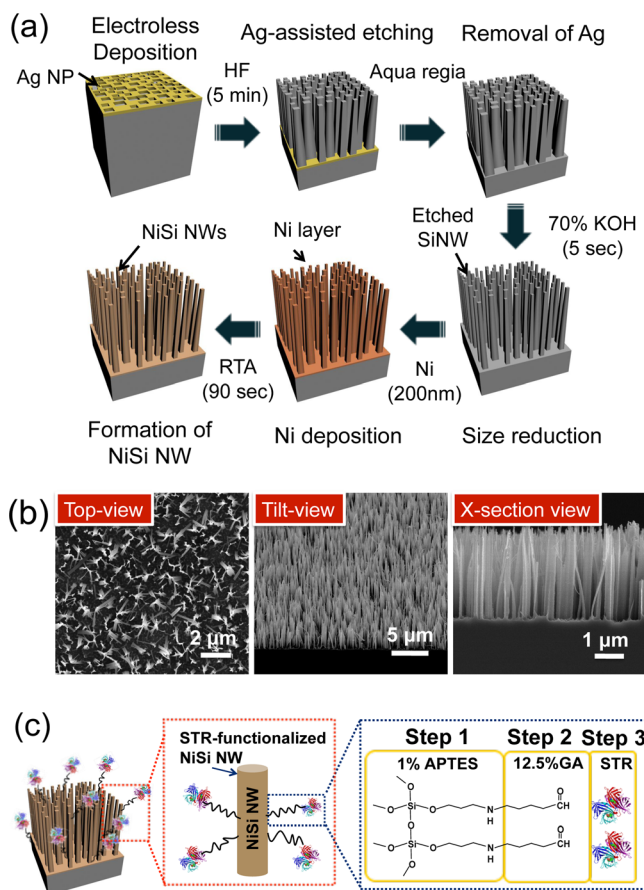


FIG. 1. (a) Schematic diagram outlining the fabrication of NiSi NWs. (b) SEM images of ferromagnetic NiSi NWs. (c) Surface functionalization of NiSi NWs on a Si substrate.

various diseases. The T-lymphocytes we used were mouse CD4<sup>+</sup> T cells from whole mouse splenocytes. Mouse splenocytes ( $\sim 10^5$  cells/sample) containing CD4<sup>+</sup> T, CD8<sup>+</sup> T, natural killer (NK), and natural killer T (NKT) cells were prepared from the spleen of C57BL/6/mice (6-8 weeks, Nara Biotech, Korea) as we described previously.<sup>20</sup> Our experiments were focused on selective isolation of targeted-CD4<sup>+</sup> T-cells from primary mouse splenocytes using ferromagnetic NiSi NWs. After incubation at  $-4^\circ\text{C}$  for 20 min, the CD4 mAb-conjugated cell suspension, i.e., mouse splenocytes, was placed under a magnetic field for 3-10 min to achieve magnetic separation using a MiniMACS<sup>TM</sup> separation unit (Miltenyi Biotec GmbH, Germany) as shown in Fig. 2(a). Finally, unbound cells were removed by rinsing with phosphate buffered saline (PBS) solution. Unbound cells were counted by flow cytometry (Becton Dickinson, USA) to quantify the proportion of captured cells. A room temperature hysteresis loop of  $M$  vs.  $\mu_0 H$  for as-prepared and annealed NiSi NWs (Fig. 2(b)) was measured using a vibrating sample magnetometer (VSM). The non-linear room temperature  $M$ - $H$  hysteresis curve with non-zero remnant magnetization and coercivity shows that the NiSi NWs possess ferromagnetic properties, consistent with the previous reports on ferromagnetic Ni nanowires.<sup>19</sup> The saturation magnetization for the as-prepared NiSi NWs ( $\sim 4.2 \times 10^{-3}$  emu) decreased as the annealing was increased up to  $700^\circ\text{C}$  ( $\sim 1.6 \times 10^{-3}$  emu). This was possibly caused by the reduction of highly ferromagnetic elemental Ni

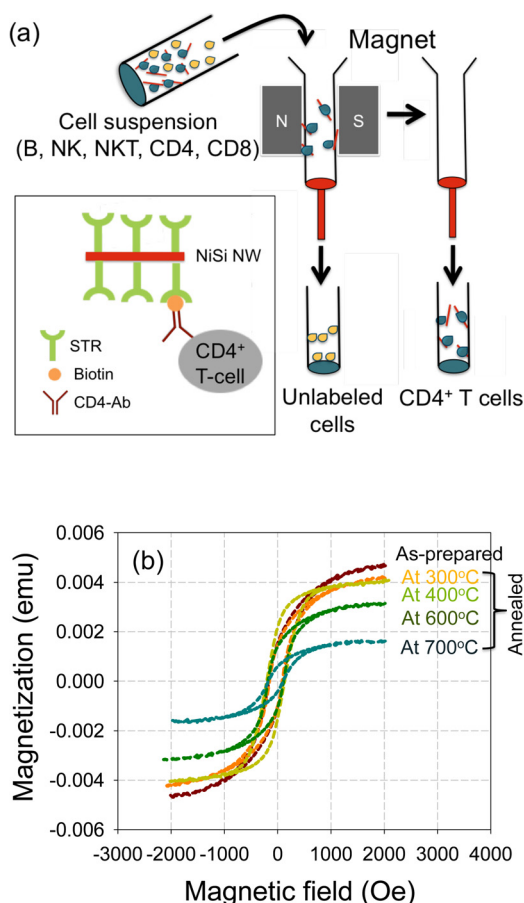


FIG. 2. (a) Schematic diagram showing magnetic separation of NiSi NWs using a MiniMACS<sup>TM</sup> separation unit. (b) Room temperature magnetization curve (hysteresis loop) for as-prepared NiSi NWs and those annealed at 300 to  $700^\circ\text{C}$ . A complete hysteresis loop was recorded using a magnetic field  $>2000$  Oe.

in the NiSi NWs during annealing.<sup>22</sup> The magnetic force on the ferromagnetic Ni nanowires ( $\sim 8.0 \times 10^{-3}$  emu in magnetization) was 20-fold greater than that on the superparamagnetic beads.<sup>22</sup> As a result, the ferromagnetic NiSi NWs can compete with the commercial superparamagnetic beads for cell separation applications.

To characterize the material and the physical properties of the NiSi NWs synthesized on a Si substrate, the transmission electron microscopy (TEM) combined with selective area electron diffraction (SAED), energy-dispersive x-ray spectroscopy (EDX), and x-ray diffraction (XRD) analyses were performed. TEM measurements provided detailed structural information about the NiSi NWs. A high-resolution TEM image (Fig. 3(c)) and SAED pattern (Fig. 3(b)) show that NiSi NWs annealed at  $700^\circ\text{C}$  were polycrystalline with a [211] preferred orientation. The presence of a thin amorphous oxide layer surrounding the NiSi NWs was confirmed by EDX, as shown in Fig. 3(d). XRD patterns of as-prepared NiSi NWs and those after annealing at  $300$ – $700^\circ\text{C}$  are shown in Fig. 3(e). The diffraction pattern of NiSi NWs annealed at  $700^\circ\text{C}$  contained signals attributed to the (200), (111), and (211) planes of NiSi. This polycrystalline nature of the NiSi NWs is consistent with TEM and SAED results and also with previous reports on NiSi NWs (Refs. 23 and 24).

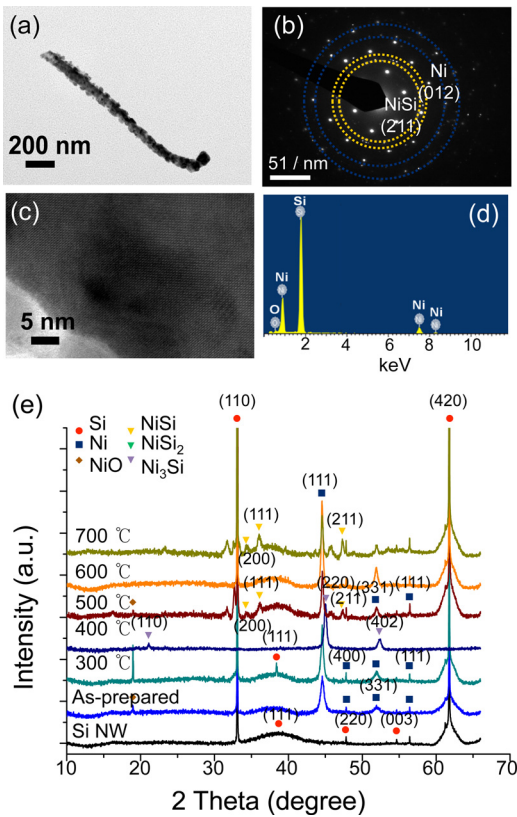
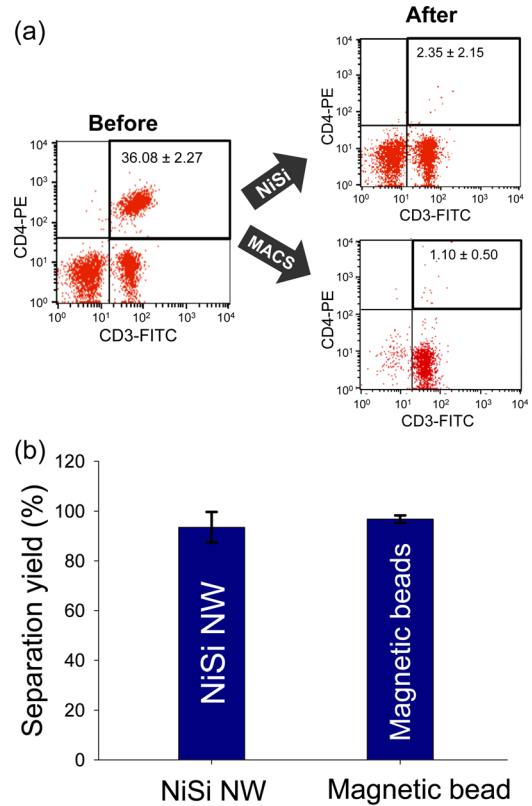


FIG. 3. (a) Low magnification and (c) high-resolution TEM images of NiSi NWs. (b) SAED patterns and (d) EDX spectra of NiSi NWs were annealed at 700 °C by RTA. (e) XRD spectra of as-prepared NiSi NWs and those annealed at 300 to 700 °C.

Fluorescence-activated cell sorting (FACS) results for CD4<sup>+</sup> T lymphocytes using STR-functionalized NiSi NWs (top right panel) before and after magnetic cell separation are presented in Fig. 4(a). Results obtained using superparamagnetic beads (bottom right panel) are shown for comparison. According to the FACS results, the mixture of whole splenocytes contained approximately ~36.08% CD4<sup>+</sup> T lymphocytes (CD3<sup>+</sup>/CD4<sup>+</sup>) and ~63.92% non-CD4<sup>+</sup> T lymphocytes (CD4<sup>-</sup>) (left panel in Fig. 4(a)). After binding with STR-functionalized NiSi NWs and magnetic separation using MiniMACS<sup>TM</sup>, the percentage of specific-CD4<sup>+</sup> T cells (double positive, CD3<sup>+</sup>/CD4<sup>+</sup>, first quadrant in Fig. 4(a)) in the cell suspension decreased to ~2.35%. This shows that most of the target-CD4<sup>+</sup> T cells were bound to STR-conjugated NiSi NWs. As shown in the bottom right panel of Fig. 4(a), the percentage of CD4<sup>+</sup> T cells in the STR-functionalized magnetic bead suspension after magnetic separation was ~1.10%. As shown in Fig. 4(b), the average capture efficiency of CD4<sup>+</sup> T cells in NiSi NWs was calculated to be ~93.5% ± 6.2% (n = 3), while that of the commercial superparamagnetic beads is ~96.8% ± 1.5% (n = 3). The comparable cell separation efficiency of the STR-functionalized NiSi NWs to the commercial superparamagnetic beads could be because of their high surface area to allow effect contact with the target cells.<sup>16,20</sup>

To evaluate the effect of NiSi NWs on cell viability, both NiSi NWs-conjugated cells and non-conjugated control cells with two specific dyes, DAPI (4'-6-diamidino-2-phenylindole, Sigma-Aldrich, USA) and PI (propidium iodide,



Samples	Separation efficiency (%)
NiSi NW	93.5 ± 6.2
Magnetic bead	96.8 ± 1.5

FIG. 4. (a) Flow cytometric analysis of CD4<sup>+</sup> T lymphocytes using cell suspensions after binding to STR-conjugated NiSi NWs (top right panel) and superparamagnetic beads (bottom right panel) before and after magnetic cell separation. In the figure, CD3-FITC in x-axis and CD4-PE denote fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated anti-mouse CD3 antibody (CD3-mAb) and anti-mouse CD4 antibody (CD4-mAb), respectively. (b) Average cell separation efficiency of CD4<sup>+</sup> T lymphocytes for STR-functionalized NiSi NWs and commercial superparamagnetic beads.

BD Science, USA), capable of detecting dead cells, where the PI (red-488 nm) passes through cell membrane of dead cells and stain DNA while the DAPI (blue-350 nm) stains all live cells, were incubated. The stained NWs-conjugated and non-conjugated cell suspensions with two specific dyes were subsequently enumerated the dead cells (PI-positive cells) out of total cells (DAPI-positive cells) bound to NiSi NWs by fluorescence microscopy analysis. As shown in Fig. 5(a), both NiSi NWs-conjugated and non-conjugated A549 cells (human lung carcinoma cell line) were found to maintain viability even up to 72 h incubation, as indicated by DAPI positive (blue) are PI negative (red). The viability, which is defined by the percentage of live cells to cells initially loaded, of NiSi NWs-conjugated A549 cells for 72 h was to be ~96.8% ± 1.4%, which are fairly comparable to that of non-conjugated A549 cells (~95.7% ± 2.0%), as shown in Fig. 5(b). As a result, we confirm the viability and safety of our ferromagnetic NiSi NWs separation platform.

In conclusion, cell separation or purification is commonly used to obtain a specific population for transplantation or gene therapy, or to isolate stem or progenitor cells for



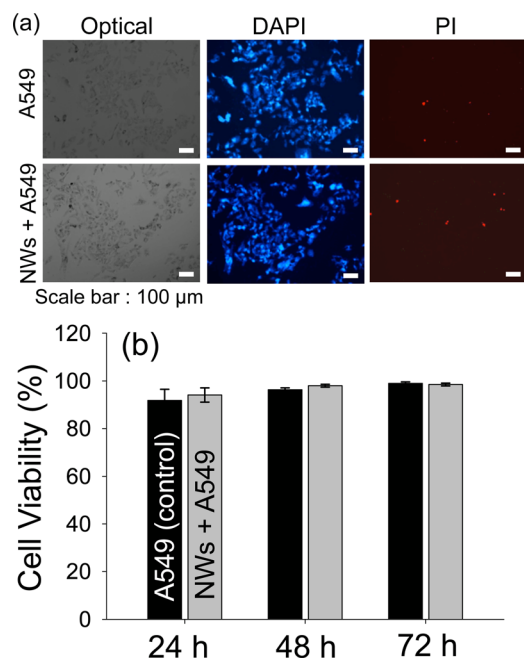


FIG. 5. (a) Optical and fluorescence images and (b) viability distribution of control cells (NW non-conjugated A549 cells) and NiSi NWs-conjugated A549 cells stained with DAPI and PI. The cells were incubated for 24 up to 72 h.

cancer treatment in clinical applications.<sup>25,26</sup> Superparamagnetic iron oxide beads (e.g.,  $\text{Fe}_3\text{O}_4$ ) conjugated with antibodies specific for the surface antigens of target cells have been widely used. Herein, we demonstrated the first example of separation of  $\text{CD4}^+$  T-lymphocytes using biocompatible NiSi nanowires. A high cell separation yield ( $\sim 93.5\%$ ) and cell viability ( $\sim 96.8\%$ ) were achieved, which is comparable of that of commercial superparamagnetic beads. Accordingly, STR-conjugated NiSi NWs are a promising tool for cell separation, which is essential for many cell-based applications in biochemistry, immunology, cell biology, and other clinical researches.

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