Specific rare cell capture using micro-patterned silicon nanowire platform

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We report on the rapid and direct quantification of specific cell captures using a micro-patterned streptavidin (STR)-functionalized silicon nanowire (SiNW) platform, which was prepared by Ag-assisted wet chemical etching and a photo-lithography process. This platform operates by high-affinity cell capture rendered by the combination of antibody-epithelial cell surface-binding, biotin-streptavidin binding, and the topologically enhanced cell-substrate interaction on a 3-dimensional SiNWs array. In this work, we developed a micro-patterned nanowire platform, with which we were able to directly evaluate the performance enhancement due to nanotopography. An excellent capture efficiency of \( 96.6 \pm 0.7\% \), which is the highest value achieved thus far for the targeting specific A549 cells on a selective area of patterned SiNWs, is demonstrated. Direct comparison between the nanowire region and the planar region on the same substrate indicates dramatically elevated cell-capture efficiency on nanotopological surface identical surface chemistry (\(< 2\% \) cell-capture efficiency). An excellent linear response was seen for quantifying captured A549 cells with respect to loaded cells. This study suggests that the micro-patterned STR-functionalized SiNWs platform provides additional advantage for detecting rare cells populations in a more quantitative and specific manner.

1. Introduction

Specific cell separation, further enumeration and further characterization (e.g. molecule analyses) of immune and tumor cells are necessary in a variety of immunology, neuroscience, stem cell, and cancer research including cell transplantation (Michalek et al., 2006; Ugelstad et al., 1987) and anti-tumor therapy (Schriebil et al., 2010; Wang et al., 2011a). Previous reports have suggested that the clinical value of separation and enumeration of special subsets of cells such as circulating tumor cells (CTCs) since these cells are shed into blood and served as a liquid biopsy, which permits frequent sampling and longitudinal monitoring of patients for early stage detection of metastasis (Chung et al., 2011; Smerage and Hayes, 2006). The enumeration of intact CTCs can be used to accurately predict the survival of patients with cancer. To date, various technologies have been developed to enrich CTCs among the many millions of normal blood cells including microfluidics approaches (Augustsson et al., 2012; Dong et al., 2013), immunomagnetic bead-based separation (Beyor et al., 2008; Chen et al., 2007), and flow cytometry (Bianchi et al., 1996; Leary et al., 1991). However, many of these technologies are still limited for separating and enumerating specific target CTCs, which are present as only \(< 0.004\%\) of all mononucleated cells in the blood (e.g. 1–100 CTCs/mL) (Dong et al., 2013). Current technology requires between 1 and 10 million cells for optimal performance. In this respect, a novel platform for separating T-lymphocytes from splenocytes was recently demonstrated using streptavidin (STR)-functionalized and vapor–liquid–solid (VLS)-grown silicon nanowire (SiNW) (Kim S.T. et al., 2010) and transparent quartz nanopillar (QNP) arrays (Kim D.J., et al., 2012c) with a high separation efficiency of more than \( \sim 93\% \) compared to other reports (Nagrath et al., 2007; Wang et al., 2011b). Our studies have proven that these improvements in cell-capture efficiency are due to high-affinity binding (e.g., anti-EpCAM and biotin) and the 3D nanotopographic feature of SiNW substrates (e.g., VLS-grown SiNW and QNP arrays). For example, due to the low quantities of CTCs in the metastatic cancer patient, \(< 3–10 \) cells/7.5 mL (Bulowski et al., 2010) in the CTC analyses required in clinical medicine, it is necessary to develop a rapid, direct, and specific evaluation method for targeting low-quantity cells, with reasonable capture efficiency (\( \sim 93\% \)) and in a low-cost manner.

Here we report on the direct quantification of a human lung carcinoma cells (A549) captured by micro-patterned STR-functionalized SiNW substrate, which was prepared by Ag-assisted
chemical etching method and a photo-lithography process. Coupled with laser scanning cytometry, this approach is rapid, low-cost, simple, and quantitative compared to other techniques (Kim D.J. et al., 2012b, 2012c; Lee et al., 2012; Wang et al., 2011b). This platform operates based on high-affinity binding and the nanotopography surface features of STR-functionalized SiNWs, and with an integrated STR-SiNW platform that contains polydimethylsiloxane (PDMS) nine-well cell-capture reservoirs. The results exhibit an excellent capture efficiency of $\sim 96.6 \pm 6.7\%$ for target tumor cells on selectively micro-patterned SiNWs on an STR-SiNW substrate. The platform is capable of capturing small quantities of specifically targeted cells (less than $\sim 6$ cells/cell-capture reservoir) and qualifying the captured cells within an hour.

2. Materials and methods

2.1. Fabrication process of silicon nanowire (SiNW) arrays through Ag-assisted chemical etching

Silicon nanowire arrays used for tumor cell-capture experiments were fabricated by the Ag-assisted chemical etching of p-type Si (100) wafers, which have a resistivity of $1–10 \Omega \text{cm}$. This method is a relatively simple and useful technique to produce well-aligned SiNW arrays (Fig. 1a) (Lee et al., 2012; Peng et al., 2008). First, a large-scale Si wafer (4 in. in diameter) was cut into pieces of $2.5 \text{ cm} \times 2.5 \text{ cm}$ in size, which were cleaned by ultrasonication in acetone, isopropyl alcohol (IPA), and deionized (DI) water for 15 min. The cleaned samples were immersed in 10 wt% hydrofluoric (HF) acid for 5 min to remove the native oxide layer, and treated in boiling RCA cleaning solution ($\text{H}_2\text{O}_2$:NH$_4$OH:H$_2$O=1:1:5) for 1 h to convert the surface into a hydrophilic surface. An Ag film (~30 nm) was coated onto the cleaned Si substrates by electroless deposition in an aqueous solution containing $10^{-3}$ M AgNO$_3$ solution at room temperature for 5 min. The Ag-coated Si samples were then immersed in an aqueous solution containing 10% HF and 0.3% H$_2$O$_2$ at room temperature for 30 min. Finally, the Ag metal remaining on the Si substrates was completely removed by aqua regia (HCl:HNO$_3$=3:1) for 1 h, followed by amorphous Si etching for 30 s in buffered oxide etchant (BOE, NH$_4$F:HF=6:1). Fig. 1b shows a schematic and scanning electron microscopy (SEM) images (tilt, top, and cross-section views) of the as-prepared SiNW arrays on Si substrates obtained using the Ag-assisted chemical etching method. The lengths of the SiNWs (Fig. 1b) were typically 60–100 nm in diameter and 5–10 $\mu$m in length. These dimensions were strongly dependent on the size of Ag nanoparticles and the etching time, respectively (Peng et al., 2006).

2.2. Functionalization of SiNW surface

Prior to the surface functionalization (Fig. 1c), as-prepared SiNW arrays were carefully cleaned with $\text{H}_2\text{O}_2$:H$_2$SO$_4$ (1:1) for 10 min to remove all of the organic materials and impurities on

Fig. 1. Fabrication and surface-functionalization of silicon nanowire (SiNW) substrate. (a) Schematic views of silicon nanowire substrate preparations including electroless deposition of Ag nanoparticle, Ag nanoparticles, and Ag removal process via (Ag)-assisted chemical etching method of p-type Si (100) wafer ($2.5 \times 2.5 \text{ cm}^2$). (b) Schematic image and scanning electron microscopy images of as-prepared SiNW (tilt and top-view). The diameters and lengths of as-prepared SiNWs were determined to be 60–100 nm and 5–10 $\mu$m, respectively after a process time of less than 20 min. (c) Schematic images of the functionalization of SiNW surface with APTES, GA, and STR for capturing of tumor cells loaded into cell-capture chambers.
the surface. We then washed the substrates using a three-step cleaning process (acetone, isopropyl alcohol, and distilled water) and dried them with air. For sterilization of nanowire platform, we first immersed the nanowire platform into 100% ethanol for 2–3 h and then kept it under UV light for at least overnight prior to usage. The surface was treated with O₂ plasma for 20 s to confer the hydroxyl groups on the SiNW surface after a piranha cleaning process (96% H₂SO₄:30% H₂O₂ = 1:1) for 10 min. The surface was subsequently subjected to a three-step surface functionalization process using 1% (v/v) (3-aminopropyl)-triethoxysilane (APTES, Sigma-Aldrich, USA) in ethanol for 30 min at room temperature, 12.5% (v/v) glutaraldehyde (GA, Sigma-Aldrich, USA) in distilled water for 4 h on a 3D-rocker, and 50 μg/mL streptavidin (STR, Sigma-Aldrich, USA) solution in phosphate buffered saline (PBS) overnight in an incubator at 37 °C in 5% CO₂ as shown in Fig. 1c (Kim S.T. et al., 2010).

2.3. Cell-capture reservoir and cell preparation

Cell-capture reservoirs with nine circular wells (5 mm in diameter) were fabricated using PDMS elastomer (Fig. 2a). A mixture of GE RTV 615 PDMS prepolymer parts A and B (10:1) (GE Silicones, USA) was prepared, homogenized, and applied to a blank Petri dish. After degassing for 1 h, the PDMS mold was cured at about 80 °C for 120 min. The solidified PDMS mold was cut at size of 25 mm × 25 mm, and cell-counting reservoir holes were fabricated with a stainless-steel hole punch (5 mm in diameter). The nine-holed PDMS mold was then bonded thermally to the GA-coated SiNW substrates after cleaning with 75% alcohol in an ultrasonic bath. Finally, the surface was covered with STR (~50 μg/mL) in PBS solution overnight. The STR-functionalized SiNW substrates were placed overnight in an incubator (37 °C containing 5% CO₂). Prior to loading the integrated STR-SiNW arrays in the PDMS reaction chamber, the surfaces were rinsed 2–3 times with PBS to flush away-unreacted molecules, including STR. For cell separation, A549 (human lung carcinoma cells, CCL-185), MDA-MB-231 (human breast carcinoma cells), and U937 (monocytes) were purchased from the American Type Culture Collection (ATCC) in the USA. These cell lines were first pre-stained and tagged prior to loading 10 μL/mL of the cells three different Vybrant cell-labeling solution in the STR-SiNW arrays (DiI, and DiD, for emission of 565 nm, and 665 nm, respectively, Invitrogen, USA). Then, the stained-SiNW arrays were rinsed with PBS at least three times to wash off non-specific cells. Prior to the introduction the cells into the PDMS wells on the SiNW substrates (i.e. the cell-capture assembly, Fig. 2a), the cells were pretreated with biotinylated anti-EpCAM (eBioscience Inc., USA) and then loaded into the wells (10, 20, and 30 cells per each PDMS well).

Fig. 2. Cell-capture chamber reservoir preparation, laser-scanned cytometry preparation, and cell-capture efficiency in a small number of cells. (a) Optical images of cell-capture assemble (nano-patterned SiNW) with cell-capture PDMS reservoir having nice circular wells (5 mm in diameter). (b) Schematic images of cell-counting and quantification steps using microarray scanner (Axon Genepix 4000B, Molecular Devices, USA). (c) 10–200 Cells per cell-capture chamber. (d) Correlation of total capture cells to loaded cell population ranging from 10 to 200 cells on STR-functionalized SiNW substrates from cell suspension, indicating excellent linear response (R² = 0.979 and n = 3–6). Inset shows the distribution of cell-capture efficiency of captured cells on STR-SiNW platform. (d) Cell-capture efficiency of captured cells (A549) on STR-SiNW substrates as a function of loaded A549 cells. The solid-line denotes a linear fitting. Inset shows the microarray scanned images of the captured A549 cells on STR-SiNW substrates for the loaded cells (10, 20, and 30 cells per each cell-capture chamber). Green-colored numbers (right-top) indicate the number of captured cells, while red-colored numbers (right-bottom) denote the total number of loaded cells into the cell-capture chambers.
as a universal biomarker for most of the epithelial tumor cells and then stored at 4 °C for 20 min. A solution of the cells conjugated with biotinylated anti-EpCAM was pipetted into each of the nine wells. The cells loaded into the PDMS reaction chambers were manually counted using a conventional hemocytometer (Hauser Scientific Co., USA) within 10% error prior to the reaction with biotinylated anti-EpCAM. A series of counted and differently diluted cells in culture medium (F12K, 500 mL, Invitrogen, USA) with a final volume of about 60 μL for each well was introduced into nine wells (Fig. 2a) with cell populations in the range of 10–3000 cells/well. After incubation at 37 °C and 5% CO2 for 40 min, the PDMS wells were washed out using 1 × PBS with Tween-20 (PBST, KPL Inc., USA) at least five times to remove unbound tumor cells. The captured cells (A549, MDA-MB-231, and U937) on the STR-functionalized SiNW substrate were then fixed with 4% paraformaldehyde (PFA, Santa Cruz Biotechnology Inc., USA) in PBS for 15 min, followed by washing out with PBS. After peeling off the PDMS wells, the stained STR-SiNW arrays were finally subjected to a three-step cleaning process, using PBS, PBS in deionized (DI) water (1:1), and DI water, before a microarray scanner (laser scanning cytometry) was used to image the captured cells and carry out further analysis.

2.4. Counting the captured tumor cells on STR-SiNW arrays

An Axon Genepix microarray scanner 4000B (Molecular Devices, USA, Fig. 2b) was used to image various Vybrant-stained cells (at emission wavelengths of 484, 565, and 635 nm) on STR-SiNW substrates. The microarray scanner contained green (YAG laser, 532-nm wavelength) and red (He–Ne laser, 635-nm wavelength) channels, and was used to scan and visualize the square-shaped STR-SiNW substrates with 5-μm resolution (Fig. 2b).

Consequently, the scanned images showing the captured tumor cells on the substrates were visualized with Genepix 6.0 software (Molecular Devices, USA). The visualized cell images exported from the Genepix 6.0 were then transferred into CellProfiler™ cell image analysis software for further analysis, and for quantitation of the population of the captured cells.

3. Results and discussion

3.1. Capture of ultra-low quantities of cancer cells on STR-SiNW arrays

Single crystalline SiNWs prepared by Ag-assisted wet chemical etching method were utilized (Hochbaum et al., 2008; Peng et al., 2002). This technique did not require any high-cost chemical vapor deposition (CVD) growth equipment, such as growth furnaces, gas-control units and other vacuum equipment. This method is able to synthesize vertical nanowire arrays more easily and quickly (less than ~20 min for ~10 μm-long nanowire) than previous methods for VLS-grown SiNWs (>3–10 h depending on the process condition) as shown in Fig. 1b. To assess the potential of the STR-functionalized SiNW platform for capturing with an extremely low abundance population of cells (e.g., CTCs), a quantitative experiment was conducted to evaluate the capture of tumor cells spiked at numbers as low as ~10 cells/cell-capture well. For this experiment, the cell capture cells (A549 cell-line as an artificial CTC) were pre-stained and then introduced to STR-functionalized cell-capture chambers to test cell-capture wells with nominal cell numbers ranging from 10 to 200 per cell-capture chamber. Fluorescence imaging was used to quantify the exact number of tumor cells (Dil, green-532 nm) loaded in each well.

Fig. 3. Cell-capture efficiencies on three different cell-lines and on whole sheep blood. (a) Comparison of cell-capture efficiency for three different cell lines (A549, MDA-MB-231 for EpCAM-positive cells, U937 for EpCAM-negative cells). The loaded cells were in the range of 200, 400, 600 cells per cell-capture chambers. The cells were pre-stained by Dil (green-532 nm) or Dd (red-635 nm). (b) Microarray scanned images of the capture cells. A549, MDA-MB-231, and U937. Yellow (first two rows)- and red (third row) numbers (right-bottom) indicate the number of captured cells for each loaded cells (200, 400, and 600 cells). (c) Microarray scanned images of the captured cells with only A549 (green-532 nm) and overlapped images, showing A549 cells and other blood cells from whole sheep blood (stained by DID-red-635 nm). (d) The cell-capture efficiency of the A549 cells in RBC-lysed sheep blood.
chamber, as shown in the inset of Fig. 2d. Next, capture and rinsing steps identical to those described in Section 2.3 were performed and the imaging cytometry approach was used to enumerate all single cells captured on STR-SiNW substrates. Fig. 2c shows the correlation of the total captured cells on STR-SiNW substrates with the initially loaded cells from cell suspension, indicating excellent linear correlation between the number of captured cells vs loaded cells for down to \( < 8 \) cells/cell-capture chamber (\( n = 3–6 \) and \( R^2 = 0.979 \)). As shown in the insets of Fig. 2c and d, the results demonstrate that the capture yield (\( \sim 82.3 \pm 11.4\% \)) remains constant in the loaded-cell range of 10–200 cells/cell-capture reservoir. It is clearly revealed that the STR-functionalized SiNW platform with the imaging cytometry system can easily capture and quickly enumerate the captured target cells (EpCAM-positive A549 cell-lines) even when the cell number is extremely small. It is anticipated that this technology can be a promising cell capture platform for detecting disseminating tumor cells in various metastatic cancer patients (1–100 cells/mL) in clinical analysis.

3.2. Selectivity characteristics using STR-SiNWs in single cell and sheep-blood suspension

To assess the selectivity performance of the STR-functionalized SiNW platform with anti-EpCAM, two more cancer cell-lines were utilized: MDA-MB-231 (human breast carcinoma cell line, EpCAM-positive) and U937 (monocytes, EpCAM-negative) purchased from the American Type Culture Collection (ATCC, USA). The processes for staining and capturing characteristics were described in the experimental Sections 2.3 and 2.4. Fig. 3a shows the capture efficiency distribution with three different cancer cell-lines (A549, MDA-MB-231, and U937) with three different cell populations ranging from 200 to 600 cells/well. Fig. 3b shows the fluorescence images of three different immobilized cancer cell-lines on STR-SiNW substrates via laser scanned cytometry (microarray scanner). In Fig. 3a and b, we are easily able to confirm EpCAM-positive cancer cell-lines among the three cell-lines, indicating relatively higher capture efficiencies of \( \sim 92.8 \pm 5.9\% \) and \( \sim 79.9 \pm 11.2\% \) for

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**Fig. 4.** Cell-capture performance on nano-patterned SiNW substrates. (a, b) Microarray scanned images of the captured cells on six-different SiNW substrates. The cell-capture chambers were pre-patterned with six different ratios of SiNW portions to the total substrates (1/6, 2/6, 3/6, 4/6, 5/6, and 6/6) using the conventional photolithography method. For this experiment, \( \sim 3500 \) pre-stained (DiD-red-646 nm) A549 were introduced into each cell-capture chamber. Yellow-colored numbers (right-top) indicate the number of captured cells, while red-colored numbers (right-bottom) denote the total number of loaded cells into the cell-capture chambers. (c) Correlation of the captured cells (A549 cells) for two portions of the substrates in a single cell-capture chamber, SiNW and Si part, as a function of portion of SiNW in a single cell-capture chamber, indicating excellent linear response with \( R^2 = 0.996 \). (d) Summary of cell-capture efficiencies for SiNW and Si (planar) substrates on a single cell-capture chamber.
A549 and MDA-MB-231 cell-lines, respectively. On the other hand, it was observed that ~8.8 times lower number (capture yield ~10.5 ± 5.1%) of EpCAM-negative U937 monocytes were seen on the same nanowire region as shown in Fig. 3a and b.

The optimized cell-capture conditions were then applied to validate the performance in a more complex cell population by spiking similar quantities of tumor cells (A549 cells) in whole sheep blood. This experiment was conducted by first spiking a series of Dil-stained A549 cells at cell densities of 200, 400, and 600 cells per cell-capture chamber (n=3). Prior to performing tumor cell capture, the red blood cells (RBCs) were removed by a selective RBC lysis process. RBC-lysis solution was added to the whole blood sample (10:1 v/v ratio) and incubated for 10 min at room temperature. Then, the supernatant of the solution was completely aspirated after centrifugation at 300 g for 10 min, and the cell pellet was re-suspended in a DMEM:F12K medium. Prior to loading the cell-suspension into the cell-capture chamber, all of the cells in the whole sheep blood were pre-stained by DiD (red-635 nm) to differentiate between spiked A549 cells (green-Dil+) and blood cells (red-DiD+), including white blood cells (WBC), remnants of RBC, etc. from whole sheep blood. Finally, RBC-lysed cell suspensions containing artificial tumor cells (~200–600 cells) were introduced into the 9 PDMS cell-capture chambers (wells), where each PDMS well retains ~60 μL of cell suspension. After fixing the captured cells on the capturing substrates and a few more washing processes (see details in Section 2.3), the captured cells on STR-SiNW substrates were quantified by laser scanning cytometry (see Section 2.4). Fig. 3c and d shows the images with a 2-color microarray scanner including captured cell images (green-532 nm, first row), and overlapped images (green-532 nm and red-635 nm, second row of the figure), of the captured A549 cells (green-Dil+, red-DiD-) in whole sheep blood (blood cells, green-Dil-/red-DiD+) with an average capture efficiency (n=3). Fig. 3c and d shows that the STR-SiNW substrates consistently exhibited...

**Fig. 5.** Cell-capture performance on nano-patterned SiNW substrates (Yale logo and emblem) and SEM images of the captured cells. (a) Microarray scanned images of the captured cells on nano-patterned SiNW substrates where Yale logo and emblem were patterned. (b) Tilted SEM images of the captured cell on STR-functionalized SiNW substrates with low-magnification (first column image). Top and tilt-view images of region B in first-column image. The cells were highlighted in red for easy differentiation. For the FE-SEM measurements, the surface-bound cells were first fixed with 4% GA in the refrigerator for 2 h, followed by a post-fixing in 1% osmium tetroxide for 2 h. The captured A549 cells on STR-conjugated substrates were then dehydrated by successive immersion in 25%, 50%, 75%, 95% and 100% ethanol for 5 min. Subsequently, the cells with substrates were slowly dried under vacuum for 24 h.
excellent cell-capture performance (~92.6 ± 6.0%). It has clearly been demonstrated that the STR-SiNW platform can capture tumor cells with reasonable cell-capture efficiency.

3.3. Micro-patterned SiNWs to evaluate tumor cell capture performance

We compared our optimized tumor cell-capture conditions on micro-patterned SiNW platform with planar (unstructured) substrate, where two surfaces (SiNW array and planar smooth Si surface) are created in close vicinity on the cell-capture chip (Fig. 4a and b). For this experiment, we performed photo-lithography and subsequent wet chemical etching processes to fabricate patterned SiNW substrates (2.5 × 2.5 cm²) that contain nine cell-capture chambers on substrate where six different area ratios of micro-patterned SiNW arrays to planar Si surfaces (1/6, 2/6, 3/6, 4/6, 5/6, and 6/6) were patterned in individual cell-capture chambers by a photo-lithography process as shown in Fig. 4a and b (first column images). More details of the process are described in Section 2.1. The A549 cells (~3500 cells in F12K culture medium) were first pre-stained by DiD (red-635 nm) prior to the loading into the cell capture chamber on the STR-functionalized SiNW platform. After the fixing and washing processes, fluorescence images (red-635 nm) of the captured cells were taken using a red diode laser (red-635 nm wavelength) built in a microarray scanner, and the results were then transferred to a computer to enumerate and quantify the captured cells on STR-SiNW substrates (shown in Fig. 4a and b). Fig. 4a and b shows images of all capture chambers (9 × 2 = 18 cell-capture chambers, n = 3) to analyze the cell capture performance on six different portions of micro-patterned SiNW substrates. Fig. 4c and d summarizes the cell-capture efficiencies for micro-patterned SiNW and planar Si substrates. The cell-capture efficiencies on the nano-patterned SiNW platform were determined to be ~96.6 ± 6.7% (Fig. 4c), indicating the highest performance in cell-capture efficiency compared to previous nanostructure-based capture platforms (Kim D.J. et al., 2012c; Kim S.T. et al. 2010; Wang et al., 2011b). This is also consistent with cell-capture performance results achieved, as described in Section 3.1. We further evaluated the cell-capture performance on more complicatedly patterned SiNW substrates (2.5 × 2.5 cm²). Fig. 5a shows the microarray scanned images of the captured cells on micro-patterned SiNW substrates, with an enlarged image of an emblem and logo of Yale University. These results clearly confirm the enhanced effect of high-affinity binding between anti-EpCAM and biotin and the 3D nanotopography-feature of SiNW substrates regarding cell-capture efficiency.

To quantify the morphological properties of the captured A549 cells bound to STR-conjugated and micro-patterned SiNW substrates, scanning electron microscopy (SEM) analysis was performed using a cell freezing technique (Kim D.J. et al., 2012c; Kim S.T. et al. 2010). For this measurement, the immobilized A549 cells on STR-functionalized SiNW substrates were first fixed with 4% GA, followed by post-fixing in 1% osmium tetroxide, dehydrated by successive immersion in 25%, 50%, 75%, 95% and 100% ethanol, and slowly dried under vacuum for 24 h. Quantitative characterization of the cellular morphologies for the surface-bound A549 cells on STR-functionalized SiNW substrate was performed on the SEM images. Fig. 5a and b shows representative FE-SEM images of A549 cells bound on the surfaces of STR-functionalized SiNW substrate. It was observed that the captured A549 possessed several cellular components (i.e. microvilli or filopodia) protruding from the surface-bound A549 cells, even after a very short incubation period (~40 min), as shown in Fig. 5a and b. Previous results showed that the surface-bound A549 cells on STR-nanostructures possess higher adhesion force due to 3D surface accessibility (e.g. nanoholes or nanopillars), where the nanoscale structures of SiNW and surface filopodia are closely and locally interacting with each other when the cells adhered to the surface. In contrast, the cells on planar substrates exhibit lower adhesion force at the same stage, corresponding to 2-D movement on the surface (Kim D.J. et al., 2012a). This mechanism results in elevated cell-capture efficiency on the STR-nanostructures with enhanced cell adhesion force occurring at similar stages.

4. Conclusion

We have demonstrated a rapid and more direct method for the quantification of specific cells captured by STR-functionalized and wet-chemically prepared SiNWs via laser scanning cytometry. This platform operates based on high-affinity binding between anti-EpCAM and biotin, and on 3D surface-features of SiNW substrates. An excellent capture efficiency of ~96.6 ± 6.7% was achieved, which is the highest value compared to other published reports for targeting specific A549 cells on selective areas of micro-patterned SiNWs. The results also suggest that the STR-functionalized SiNWs platform holds great potential for qualifying ultralow numbers (< 10) of rare cells (e.g. circulating tumor cells) in an hour.

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