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Vertical SiNWAs for Biomedical and Biotechnology Applications

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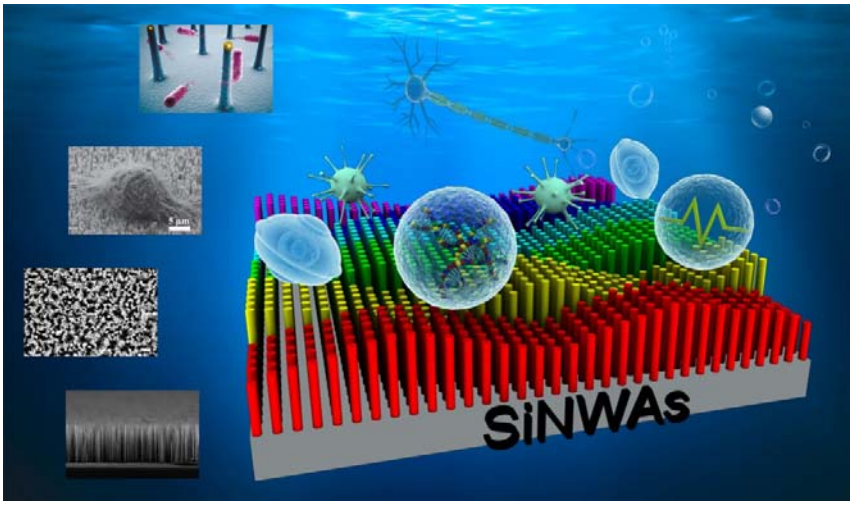
Abstract:

Vertical silicon nanowire arrays (SiNWAs) are considered as one of the most promising nanomaterials. Notably, silicon-based nanomaterials exhibit excellent biocompatibility, and the diameters of silicon nanowires are comparable to the dimensions of many biological molecules, providing SiNWAs with great potential for life science applications. In this review, we first briefly introduce the synthesis, patterning and surface functionalization of SiNWAs and then focus on the recent progress in the application of SiNWAs for biosensors, studies on mammalian cells or bacteria with nanomaterials, controlled capture/adsorption and release of cells or proteins, drug delivery, DNA transformation, antifouling surfaces, and nanozyme. We conclude with a brief perspective on future research directions and on the major challenges in this promising field.

Keywords: silicon nanowire arrays (SiNWAs); biomedical; biotechnology nanomaterials;

TOC graphic



1 Introduction

The past decades have witnessed rapid progress in the field of nanotechnology. Several novel nanomaterials with well-defined structures at the nanoscale have been developed that have great potential for various applications, including, but not limited to, energy, catalysis, electronics, photonics, biotechnology and biomedical engineering.[1-7] Among the well-investigated nanomaterials, vertical silicon nanowire arrays (SiNWAs), in which numerous silicon nanowires are oriented perpendicular to the silicon substrate with uniform lengths and densities, have attracted increasing research attention due to their unique structural features and excellent electronic, optical, thermoelectric, and mechanical properties.[8-14] Notably, silicon-based nanomaterials exhibit excellent biocompatibility, and the diameters of silicon nanowires are comparable to the dimensions of many biological molecules, providing SiNWAs with great potential for various biomedical and biotechnology applications.[15-17] For example, the high surface-to-volume ratio of SiNWAs greatly enhances the sensitivity for biomolecule detection, making SiNWAs a good substrate for biosensors. SiNWAs also provide the possibility for fundamental studies on the interfacial interactions between synthetic nanomaterials and living cells and tissues. After proper modification with a polymer or immobilization of biological ligands, SiNWAs with large surface areas can be used as a platform for the controlled capture and release of target cells and proteins. In addition, due to their good biocompatibility, SiNWAs have been applied in drug delivery, DNA transformation, and in antifouling surfaces. SiNWAs also exhibit photocatalytic activity and enzyme-like activity, making them useful for degrading organic dye molecules and toxic pollutants. However, to the best of our knowledge, few reviews have systematically summarized these recent promising achievements and their substantial opportunities.

In this review, we summarize recent (particularly over the past 5 years) representative works on the development of SiNWAs as a versatile and powerful platform for biomedical and biotechnology applications. First, we briefly introduce the synthesis, patterning and surface functionalization of SiNWAs. Next, we highlight the recent progress on the application of SiNWAs for biosensors, studies on mammalian cells or bacteria with nanomaterials, controlled capture/adsorption and release of cells or proteins, drug delivery and DNA transformation, antifouling surfaces, and nanozyme. Finally, we provide a brief perspective on future research directions and on the major challenges in this promising field.

2 Synthesis and modification of SiNWAs

2.1 Synthesis of SiNWAs

Generally, there are two main approaches to synthesize vertical SiNWAs including top-down strategy (such as reactive ion etching (RIE) and metal-assisted chemical etching (MACE)) and bottom-up strategy (such as chemical vapor deposition via vapor-liquid-solid (VLS) mechanism and laser abrasion).[8-9, 18] Among all the approaches, MACE is a simple, fast and low-cost method to produce large-area, highly oriented, porous or non-porous SiNWAs.[14, 19] In this method, the silicon wafers coated with a thin layer of noble metal particles are incubated in etching solution containing hydrofluoric acid (HF) and oxidant (e.g. H_2O_2). The noble metal atoms deposited from HF solution on the silicon wafer surface could form nuclei that behave as a nanoscale cathode, and the silicon surrounding these nuclei behaves as an anode, and can subsequently be etched and dissolved into the solution by the galvanic cell reaction (the detailed process is illustrated in **Figure 1**)[20]. The density and length of resulted SiNWs can

be easily controlled by changing the reaction time, temperature and the concentration of HF and oxidants.[14, 19] Compared with other synthesis methods, MACE does not require high temperature, high vacuum, complex equipment or hazardous silicon precursors, making it a facile wafer-scale production of SiNWAs.[12-13] In this review, we mainly focus on the applications of SiNWAs prepared by this method.

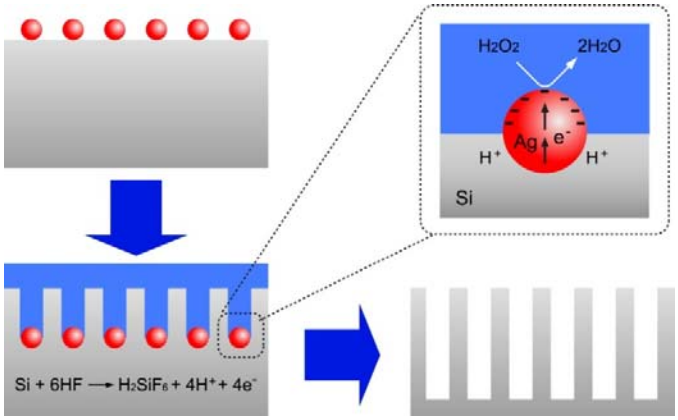


Figure 1. Schematic illustration of growth mechanism of SiNWAs. [Reprinted with permission of REF[20], Copyright 2008, Wiley-VCH.]

2.2 Patterning of SiNWAs

Normally, the SiNWAs prepared by one-step MACE method are very dense but lack spatial ordering, and have a broad diameter distribution, restricting their applications where the well-defined morphology and architectures are needed. The combination of modern lithography techniques and MACE provides a solution to fabricate ordered and patterned SiNWAs with controlled diameter, length and density. This two-step strategy includes (i) pre-patterning silicon substrate by laser interference lithography,[21-24] nanosphere lithography,[25-27] electron beam lithography,[28] block copolymer lithography[29] and photolithography,[30-31] and (ii) etching selective areas of silicon to achieve well-defined SiNWAs. Several typical fabrication processes of patterned SiNWAs are illustrated in **Figure 2**.

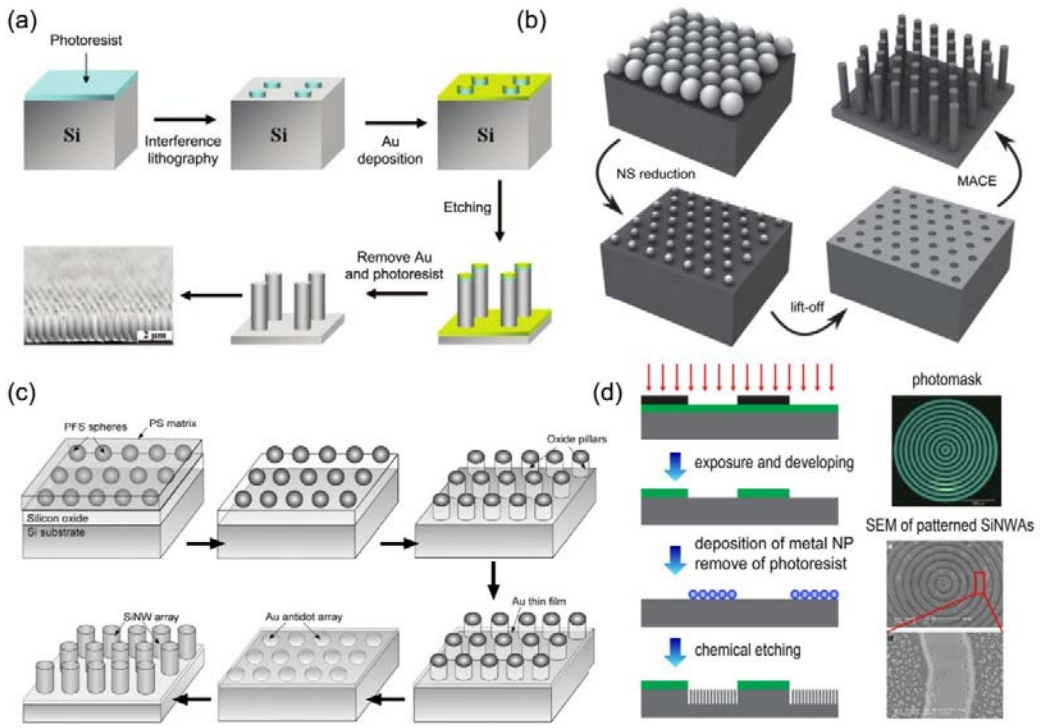


Figure 2. Fabrication of patterned SiNWAs by the combination of lithography techniques ((a) laser interference lithography; (b) nanosphere lithography; (c) block copolymer lithography (BCL); and (d) photolithography) and MACE method. [Reprinted with permission of REF[21], Copyright 2008, American Chemical Society; REF[26] Copyright 2014, Wiley-VCH.; REF[29] Copyright 2009, Wiley-VCH.; REF[31] Copyright 2010, Springer]

2.3 Modification of SiNWAs

The as-prepared SiNWAs usually have a thin silicon oxide layer on the topmost surface, providing the possibility of modification by the common silanization reaction with various silane molecules containing different functional end groups such as fluoroalkylsilane (FAS),[32] perfluorooctyltrichlorosilane (PFTS),[33] dodecyltrichlorosilane (DTS),[34-35] octadecyltrichlorosilane (OTS),[36-37] and (3-aminopropyl) triethoxysilane (APTES)[38] (**Figure 3, step1**). Especially, if the end

groups have initiate activity, the modified SiNWAs can be further functionalized by grafting polymers via surface-initiated polymerization[39-43] (**Figure 3, step2**). On the other hand, the silicon oxide layer can be easily removed by HF treatment to give hydrogen atom-terminate SiNWAs (H-SiNWAs, **Figure 3, step3**), which can be either functionalized by immobilization of organic molecules containing aldehyde groups (**Figure 3, step 4**) or by hydrosilylation with an electron-deficient alkyne 1,2-ethanediol dipropiolate (EDDP) (**Figure 3, step 5**) for further click coupling of organic molecules containing azide groups. (**Figure 3, step 6**) Apart from covalent functionalization, SiNWAs can also modified by directly physisorption of polyelectrolytes such as polyethylene imine,[44] plasma-enhanced chemical vapor deposition of fluorine carbon polymer,[45] or a simple contact printing of siloxane oligomers[46]. H-SiNWAs are also capable of *in situ* reducing metal ions into metal nanoparticles (NPs), leading to uniform deposition of metal NPs on the surface of SiNWAs. This method provides a simple and facile way to fabricate metal NPs coated SiNWAs hybrids, which have been widely used as SERS substrates or photocatalysits as discussed later.

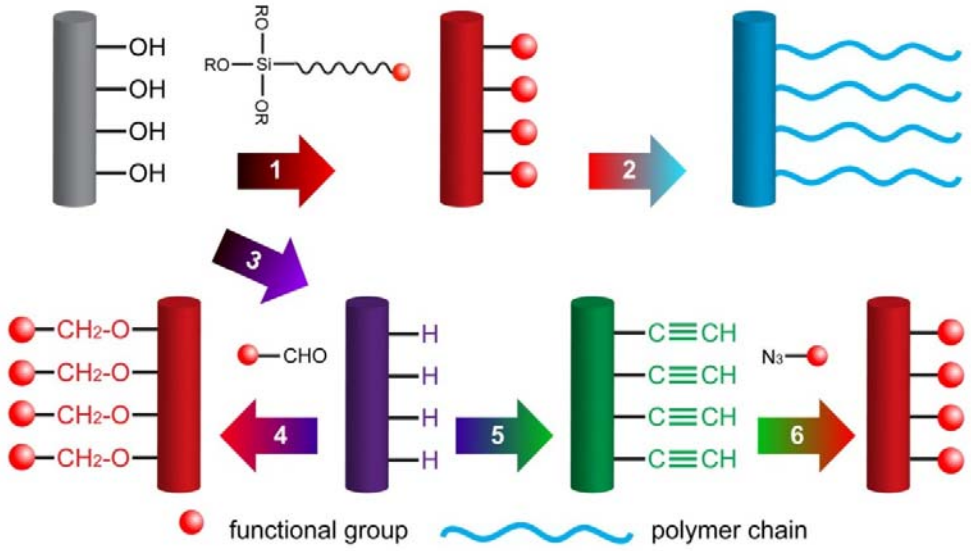


Figure 3. Different ways to surface functionalization of SiNWAs. Step 1: silanization; Step 2:

surface-initiated polymerization; Step 3: HF treatment; Step 4: reaction between Si-H bonds and CHO groups; Step 5: hydrosilylation; Step 6: alkyne-azide click chemistry.

3 Bioapplications of SiNWAs

3.1 Biosensors

Nanostructured materials have provided new opportunities as biosensing platforms due to their exceptional physiochemical and electrical properties.[47-50] Silicon nanowires (SiNWs) have attracted considerable attention because of their high surface areas and surface reactivity, which enhance the limit of detection (LOD) to fM concentrations of targets and thus provides ultrahigh sensitivity. In addition, the diameter of a SiNW is comparable and compatible with the dimensions of most biological molecules, thereby facilitating interactions between the targets and the recognition molecules that are pre-immobilized on the SiNWs and leading to a rapid detection response. To date, considerable efforts have focused on developing field-effect transistors (FETs) based on SiNWs for the detection of a wide range of biological and chemical species. In this type of device, the SiNWs are usually prepared by bottom-up strategy (such as chemical vapor deposition and laser abrasion) and then integrated into an electrode surface, followed by immobilization of bioactive ligands. The biological events happen on the SiNWs such as protein binding, DNA hybridization, and enzymatic reaction can be direct conversion into signals that can be electrically detected.[51-53] On the other hand, vertical SiNWAs biosensors prepared by MACE method are usually based on optical/fluorescent sensing or SERS sensing. These biosensors mainly utilize the special 3D nanostructure and high surface area of SiNWAs to enhance the signals. These two types of nanowire biosensors are fabricated by different methods and have different

sensing mechanisms. The advantages of FETs include high sensitivity, direct and real-time electrical signal transductions, and multiplexed detection for different biological species, while the high processing costs/complexity and relatively low yields are still problems. In contrast, vertical SiNWAs biosensors are more easily prepared, however, compared with FETs, the current detection limitation of this kind of biosensors are still not low enough for the applications where high sensitivity is needed. In the following section, we focus on the recent progress in biosensors based on vertical SiNWAs.

3.1.1 Surface-enhanced Raman scattering (SERS)

The most widely investigated area for the use of vertical SiNWAs as a biosensing platform is surface-enhanced Raman scattering (SERS) spectroscopy. SERS is a surface-sensitive technique that enhances the normally weak Raman scattering by molecules adsorbed on rough metal surfaces or by nanostructures such as metal nanoparticles (metal NPs), providing a detection of biomolecules with up to single molecule sensitivity.[54-55] Direct sedimentation of aggregated metal NPs on the substrates is a facile method for the fabrication of SERS substrates; however, this method suffers from problems of poor reproducibility and stability. Alternatively, SiNWAs with a 3D nanostructure can offer an increased surface area and promote the formation of hot spots and the capture of target analytes, making them suitable for use as SERS substrates.[56-57]

SiNWAs incorporated with silver nanoparticles (SiNWAs@AgNPs) were used as a SERS substrate for a label-free immunoassay that was capable of detecting trace amounts of mouse immunoglobulin G (mIgG) and goat-anti-mouse immunoglobulin G (gamIgG) down to 50 ng.[58] Notably, the complex formed from 4 ng of mIgG and 4 ng of gamIgG still presented distinct Raman peaks, suggesting that the SiNWAs@AgNPs were more sensitive to the conformational changes of the immune reagents after the

immune reaction. The SiNWAs@AgNPs-based SERS substrate can also be used for the rapid detection of trace of molecules related to human health and food safety, such as antibiotics (amoxicillin),[59] insecticides (Carbaryl),[60] and artificial dyes (Sudan I-IV and G)[61]. The unique 3D configuration of SiNWAs@AgNPs enables the dipole vibration on every AgNP to transfer, couple, and resonate throughout the entire wire surface, and the field effect would significantly increase the electromagnetic effect, thereby providing more hot spots for Raman scattering. The observed enhancement factor of the substrate was 8-10 orders of magnitude, which is several orders of magnitude greater than that of common substrates. Moreover, these substrates possess good storage stability and reproducibility, providing the potential for practical applications in the fields of biology, medicine, environment, and safety control.

Most SERS substrates are based on a signal-on strategy, that is, the signal intensities are amplified in the presence of targets. However, a SERS substrate based on a signal-off strategy was developed to provide a selective for the detection of DNA with high sensitivity.[62] As shown in **Figure 4**, the stem-loop DNA tagged with organic dyes was first immobilized on SiNWAs@AuNPs as capture and reporter probes, and distinct SERS signals were observed due to the close distance between the dye molecules and AuNPs. However, when the stem-loop DNA hybridized with target DNA, the stem-loop configuration was disrupted, and thus, dye molecules separated from the AuNPs, resulting in a remarkable decrease in the SERS signal. These signal-off SERS substrates can be used to detect specific DNAs with a low detection limit of 10 fM. In particular, these substrates are effective for simultaneous and multiple DNA analysis through observing the weakening or disappearance of Raman peaks of the Raman labels.

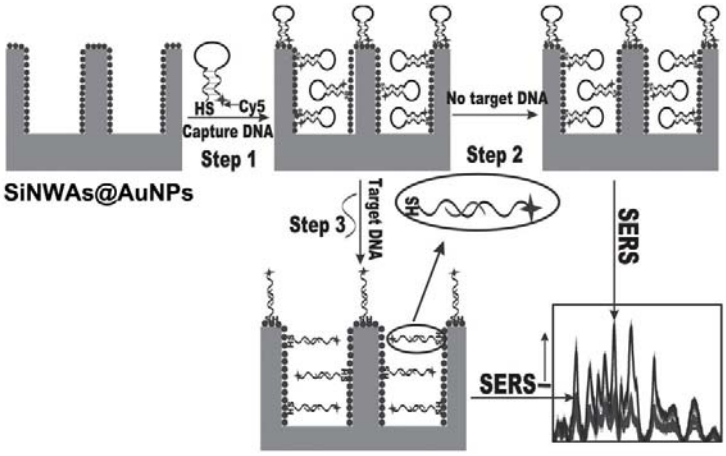


Figure 4. Schematic illustration of the SiNWAs@AuNPs-based SERS substrate with the signal-off strategy. [Reprinted with permission of REF[62], Copyright 2013,Wiley-VCH.]

3.1.2 Other biosensors

SiNWAs with incorporated metal NPs can be used not only as a SERS substrate but also directly used as the working electrode for electrochemical biosensors.[63] For example, a biosensor based on SiNWAs@AuNPs was used to detect bovine serum albumin (BSA) in phosphate-buffered saline (PBS) solution via linear sweep voltammetry.[64] Compared with conventional gold electrodes, this sensor exhibited higher sensitivity and selectivity due to its larger surface area and higher conductivity.

In another report, a multiscale protein immunosensor was developed by overlaying SiNWAs with micropatterned polyethylene glycol (PEG) hydrogels.[65] Due to the non-adhesiveness of PEG hydrogels toward proteins, proteins were selectively immobilized on the pre-modified SiNWAs regions to create protein micropatterns. The increase in surface area increased the protein-loading capacity of the SiNWAs by more than 10 times the capacity of a smooth silicon substrate. Immunobinding assays between IgG and anti-IgG and between IgM and anti-IgM that were performed on the micropatterned SiNWAs

emitted stronger fluorescent signals and exhibited higher sensitivities compared with those from assays performed on smooth silicon substrates. Finally, microfluidic channels were successfully integrated into the micropatterned SiNWAs to enable the simultaneous performance of multiple immunoassays on a single microarray platform.

In addition to the detection of proteins, SiNWAs can also be used for other sensing applications. A SiNWAs-based pH-sensitive chip was developed for the real-time and *in situ* monitoring of pH changes for live HeLa cells.[66] A pH-sensitive molecule, 5-amino fluorescein, was immobilized on the surface of the SiNWAs. The observed fluorescence intensity of the modified surface increased gradually with pH with a good linear relationship (pH values from 3 to 9). SiNWAs were also employed for low laser energy desorption/ionization mass spectrometry (LDI-MS).[67-68] Compared with other conventional LDI-MS substrates, the SiNWAs exhibit several advantages, such as excellent light trapping ability over a broad wavelength range, high efficiency of light-to-heat conversion to desorb/ionize analytes (such as proteins and peptides), large surface area to allow for the retention of analytes, and an open interwire space to allow the ionized analytes to leave, making them good substrates for the LDI-MS analysis of small biomolecules.

Despite the considerable efforts have been made to develop SiNWAs based biosensors, it should be noted that so far most of these biosensors are still on the laboratory stage but are not widely popularized for industry applications. The major challenges for this situation include (1) how to optimize the fabrication to get SiNWAs with controllable and reproducible parameters (such as length, diameter, and surface chemistry); (2) how to enhance the stability and reusability of SiNWAs to increase their working life. It is thus required the collaboration of scientists and engineers from the materials science,

fabrication, chemistry and nanotechnology areas to commercialize the SiNWAs biosensors for real applications in the future.

3.2 Studies on cells or bacteria with SiNWAs

3.2.1 Effects of nanostructure on cellular behaviors

Understanding how living cells interact with nanostructures is crucial for obtaining a deeper understanding of the fundamental principles of biology and for developing next-generation biomedical devices.[69-71] In addition, the biocompatibility of nanomaterials is critically important for meeting the increasing demands of their bioapplications. Before SiNWAs can be incorporated into new and existing biomedical devices, their cytotoxicities and potential adverse effects on biological systems should be thoroughly investigated. Here, we highlight several examples using SiNWAs as model nanostructural materials to investigate how nano-topography affects cell adhesion and proliferation, alters cell morphology, and initiates intracellular signaling.

Yang and co-workers first investigated the direct interfacial phenomenon between vertical SiNWAs and mammalian cells (mouse embryonic stem cells) without any external force.[44] The results indicated that the cells were penetrated by SiNWs due to the small diameter and high aspect ratio of the nanowires (**Figure 5a**). The introduction of SiNWs inside the cells did not meaningfully affect the cell viability, as the cells survived for up to 1 week. Furthermore, the same group explored bacteria-nanostructure interactions using *Shewanella oneidensis* MR-1 as a model microorganism and well-defined SiNWAs as a platform.[72] Compared with mammalian cells, bacteria cells have a smaller size, which is comparable with the nanoscale dimensions of a single SiNW (**Figure 5b**). Their results indicated that nanoscale topographies on surfaces play an important role during the early stage of biofilm formation. MR-1 cells

can recognize nanoscale structures, and their initial attachment showed preference to the nanowires. Analyses of bacterial trajectories suggested that the presence or absence of nanowires resulted in different cell diffusion modes.

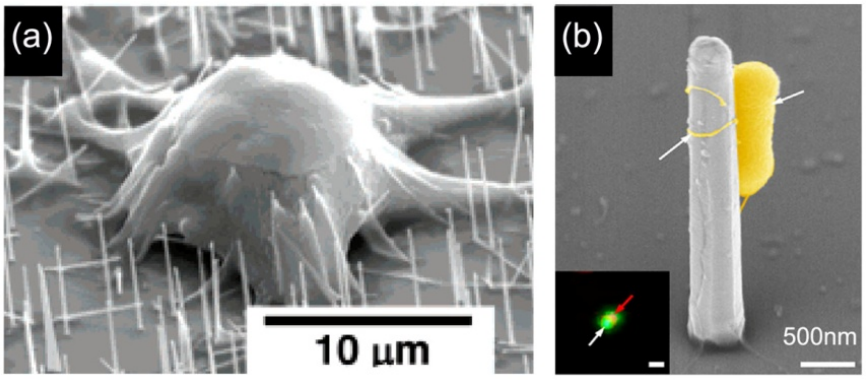


Figure 5. SEM images of (a) an individual mouse embryonic stem cell penetrated with SiNWs and (b) a single MR-1 cell that was preferentially attached to the individual SiNW with alignments along the length direction of the nanowires. [Reprinted with permission of REF[44, 72], Copyright 2007 and 2013, American Chemical Society]

SiNWAs can not only enhance the cell-substrate adhesion force but also can restrict cell spreading.[73] From a molecular perspective, Yang and co-workers analyzed the expression levels of several genes known to be related to the cell adhesion and spreading process. Based on the results, a related mechanism on how SiNWAs might guide cell adhesion and spreading was proposed. When cells first come into contact with the SiNWAs, the needle-like nanostructures stimulate the cells to extend more filopodia, form focal adhesion points with the substrate, and reach out to as many SiNWs around and underneath as possible. At the same time, the relatively large interval space between every cluster of SiNWs makes it impossible for the cells to reach out for the SiNWs at a distance from their first contact

point. Consequently, further spreading of the cell skeleton is restricted. The guiding effect of SiNWAs might make them beneficial for the development of cell microarrays, tissue-engineering scaffolds, and molecule delivery vehicles where strong cell-substrate adhesion forces and weak cell-cell interactions are valuable.

The collective cellular behaviors, including the surface area of cells, membrane trafficking, focal adhesion distribution and dynamics, and cytoskeletal protein distribution on amine-coated SiNWAs with different physical properties were systematically investigated.[74] The length of the SiNW is unlikely to be a significant factor that influences cell spreading behavior, whereas denser SiNWAs promote cell spreading, which suggests that cells could sense the presence of SiNWs on a two-dimensional surface and actively adjust their spreading behaviors in response to the physical properties of these obstacles. The reduced number of focal complexes per focal complex area and their diffusive behavior suggested that the cells were weakly adhered on the SiNWs. The significant decrease in the number of actin filaments due to the SiNWs appeared to affect the focal complex dynamics, which presumably accounts for the observed cell shape and behavior on the SiNWs. These results are in contrast to those of other reports, which showed that cells on SiNWAs exhibited enhanced adhesion. The difference might be attributed to the different physical properties of the SiNWAs and cell lines used in these reports. Thus, a detailed understanding of the interactions between SiNWs and cells and the induced cellular responses is required for adequate exploitation of their distinct characteristics.

SiNWAs can also be used as unique probes for quantifying biological processes at a high spatial resolution. The elasticity of SiNWs enables measurements of the traction force from a single cell. For example, Wang and co-workers cultured both cancer cells and normal cells on the top of SiNWAs and

measured the maximum cell traction forces (CTFs) by quantitatively analyzing the bending of the nanowires through the use of atomic force microscopy (AFM) and finite element calculations. They found that the cancer cells exhibited significantly larger CTFs compared with the normal cells. The study of the mechanical properties of single cells and their migration characteristics potentially provides a new cell-level diagnostic technique.

It has been suggested that the special nanotopography of SiNWAs affects the differentiation of mesenchymal stem cells (MSCs).[75] The interactions between MSCs and SiNWAs caused the stem cells to preferentially differentiate toward osteocytes and chondrocytes but not adipocytes in the absence of supplementary growth factors. The Ca^{2+} ion channels were transiently activated in MSCs upon mechanical stimulation, which eventually led to the activation of the Ras/Raf/MEK/ERK signaling cascades to regulate the adhesion, proliferation, and differentiation of MSCs. The stretch-mediated transient Ca^{2+} ion channel activation and cytoskeleton reorganization during stem cell-nanowire interactions may be early events of the lineage-specific potentiation of MSCs in determining the fates of mesenchymal stem cells cultured on microenvironments with specific mechanical properties.

3.2.2 Effects of surface wettability on cellular activities

Wettability is a very important property of solid surfaces, and it has numerous practical applications in daily life and industry.[76-78] In particular, it is well known that the adhesion of cells on a solid substrate is highly affected by surface wettability.[79] As-prepared SiNWAs with a thin silicon oxide layer exhibited superhydrophilicity. In contrast, after coating with a hydrophobic material, the surface became superhydrophobic with a water contact angle greater than 150°. Using standard optical lithography techniques, Boukherroub and co-workers fabricated a micropatterned

superhydrophilic/superhydrophobic SiNWAs surface for investigating cellular responses to different wettabilities.[80-81]

The results from Chinese Hamster Ovary K1 (CHO) cells cultured on patterned SiNWAs indicated that cell adhesion was selective to the superhydrophilic region rather than the superhydrophobic region (**Figure 6a**).[81] The non-fouling properties of superhydrophobic SiNWAs might originate from the absence of initial adhered cells or from the removal of adhered cells during the rinsing step. Further analyses using transmission electron microscopy revealed that the cell cytoplasmic projections penetrate the hydrophilic layer of silicon nanowires and coat the nanowires, leading to an intimate surface contact and thus strong adhesion (**Figure 6b**). On the superhydrophobic surface, the cell cytoplasmic projections remained on the top of the wires. Interestingly, the adhesion of *Bacillus cereus* spores on the same patterned surface exhibited the opposite trend, *i. e.*, there is a high propensity for spores to move toward the superhydrophobic regions of patterned SiNWAs surfaces.[80] However, the population of spores on superhydrophobic SiNWAs was less compared with that on a smooth hydrophobic silicon surface with the same chemistry, which resulted from the discontinuous contact between the bacterium and SiNWs. The different responses of bacteria and mammalian cells to superhydrophilic/superhydrophobic SiNWAs suggested different adhesion mechanisms.

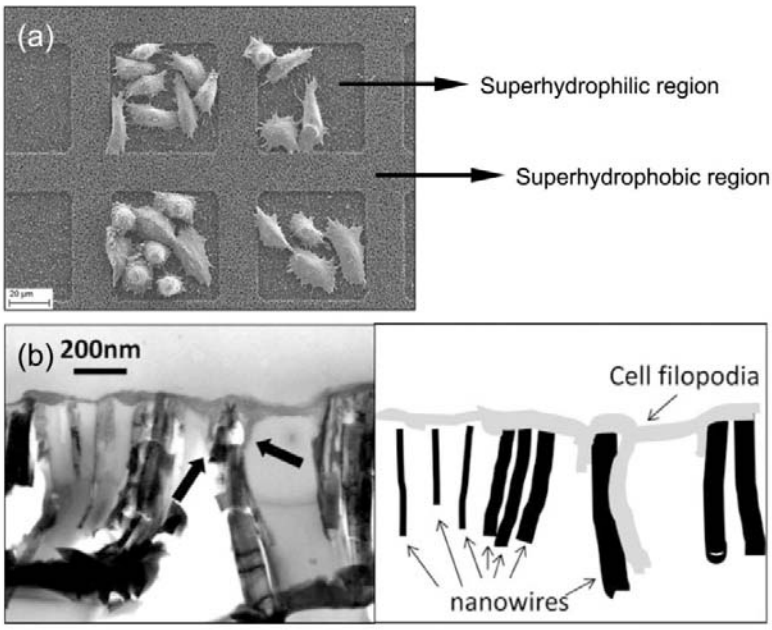


Figure 6. (a) SEM images of CHO cells trapped within the superhydrophilic SiNWAs patterns. (b) TEM image of CHO cells adhering to superhydrophilic SiNWAs surface. The arrows indicate the SiNWs in contact with the cells and their filopodia. [Reprinted with permission of REF[81], Copyright 2011, The Royal Society of Chemistry.]

The effect of surface wettability on biological interactions provides a way to control cell adhesion on surfaces. For example, SiNWAs modified with the ionic strength-responsive polymer poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) exhibited responsive surface wettability between highly hydrophobic and hydrophilic values with increasing or decreasing NaCl concentrations.[41] The ionic strength-responsive wettability can be further used to control bacterial attachment. Because type I pilus for bacterial attachment interacts strongly with hydrophobic surfaces but is resisted by hydrophilic surfaces, increasing NaCl concentrations resulted in decreased attachment of *Escherichia coli* bacteria on the surface.

3.3 Controlled capture/adsorption and release of protein and cells

3.3.1 Capture and release of cells

The capture and release of target cells from patient samples with high efficiency is critical for cell-based cancer diagnostics of early diseases.[82] Compared with unstructured substrates (i.e., smooth silicon surface), SiNWAs with a specific 3D nanostructure not only provide a large surface area but also enhance local topographic interactions between the surface of the SiNWAs and the nanoscale components of the cellular surface (e.g., microvilli and filopodia), leading to an effectively improved cell-capture affinity when SiNWAs are integrated with cell-affinity ligands (such as the antibody of epithelial-cell adhesion-molecule (anti-EpCAM); EpCAM is a common marker of cancer cells[83]). The application of SiNWAs as a cell-capture platform was first demonstrated in 2009 by Wang and Tseng (**Figure 7a**).[84] The SiNWAs coated with anti-EpCAM exhibited specificity for capturing circulating tumor cells (CTCs) and vastly improved the capture yields (>40%), suggesting that this platform can provide a convenient and cost-efficient alternative for sorting CTCs in clinics. Furthermore, they integrated SiNWAs into a fluidic handling system (so called "NanoVelcro chip") to increase the cell–substrate contact frequency.[85] As shown in **Figure 7b**, this system contained two parts: patterned SiNWAs pre-coated with anti-EpCAM for high-affinity cell enrichment and a polydimethylsiloxane (PDMS) chip with a serpentine chaotic mixing channel capable of improving the CTC/substrate contact frequency. The resulting synergistic effects led to high CTC-capture performance being observed for both spiked and clinical blood samples.

To conduct molecular and functional analyses of cells, it is required that the platform not only capture cells with high efficiency but also release cells with minimal contamination of the surrounding

white blood cells (WBCs) and negligible disruption to the viability and functions of the CTCs. To date, several methods have been developed to achieve this target based on different surface-cell interactions. The first method is to use DNA aptamers rather than antibodies as the capture and release mediator molecules because they can be easily synthesized to bind with specific molecular or cell targets and can be decomposed by exonuclease without cell damage (**Figure 7d**).[86] This SiNWAs-DNA surface was demonstrated to be capable of effectively capturing up to two-fold more T lymphocytes than planar surfaces. Additionally, 97± 2% of captured cells can be released by a simple treatment with exonuclease. Similarly, a new generation of NanoVelcro chip was developed, in which DNA aptamers were used rather than an antibody-based capture agent (anti-EpCAM) to capture non-small cell lung cancer (NSCLC) CTCs (**Figure 7c**).[87] A genetically engineered endonuclease was then introduced to specifically degrade the SiNWAs-grafted DNA aptamer, thereby enabling the recovery of viable NSCLC cells. The capture and release features enable the isolation of CTCs with minimal contamination of the surrounding WBCs and negligible disruption to the viability and functions of CTCs, thus paving the way toward molecular and functional analyses of CTCs.

Although SiNWAs-DNA is effective for the one-time capture and release of cells, it is more desirable to achieve reversible specific capture and release of target cells for multiple assays, which are required for cell-based cancer diagnostics. Surfaces modified by the thermo-responsive polymer poly(*N*-isopropylacrylamide) (PNIPAAm) exhibited reversible cellular attachment and detachment in response to temperature due to the transition of the chain conformation between the hydrophobic collapsed state and the hydrophilic extended state.[88] Using this unique property, two groups independently developed PNIPAAm-based SiNWAs platforms for the temperature-induced capture and

release of CTCs. In one report, a PNIPAAm-based copolymer was first grafted onto SiNWAs, which was followed by the covalent incorporation of biotin molecules (**Figure 7e**).[89] At 37°C, biotins are present on the surfaces, leading to the binding of biotinlyted anti-EpCAM through a streptavidin as a bridge and thus facilitating the capture of cancer cells with high efficiency. As the temperature decreased to 4°C, the PNIPAAm chains became hydrophilic and extended to encapsulate the anti-EpCAM, which stimulated the release of captured cells. In another report, the capture and release of cells was achieved by controlling the hydrophobic interactions between the protein ligand and the PNIPAAm grafted on SiNWAs (**Figure 7f**).[90] Here, a biotinlyted bovine serum albumin (biotin-BSA) was used as a hydrophobic anchor, which can be reversibly adsorbed/desorbed on SiNWAs-PNIPAAm in response to temperature. Similarly, using streptavidin as a bridge, biotin-BSA was connected with the cell-capture agent biotinlyted-anti-EpCAM. The capture and release of MCF-7 cells were achieved by simply switching the temperature between 37°C and 20°C. Notably, compared with the use of an aptamer-degraded enzyme as the release agent, the use of temperature is mild, easy to conduct, and keeps the cells undamaged during the capture/release process, thereby facilitating subsequent cell culturing and single-cell analyses.

Recently, a pH and glucose dual-responsive SiNWAs-based surface was fabricated through grafting poly(acrylamidophenyl boronic acid) (PAAPBA) to obtain a platform for the capture and release of breast cancer cells (**Figure 7g**).[91] The working mechanism is based on the reversible molecular recognition between the PAAPBA brushes and sialic acid moieties (the molecules that are over-expressed in the cancer cell membrane). Specific cells captured on PAAPBA can be released by changing the environmental pH and/or glucose concentration to break the interaction between sialic acid

and PAAPBA. The 3D nanostructure of SiNWAs provided an enhanced local interaction to amplify the responsiveness.

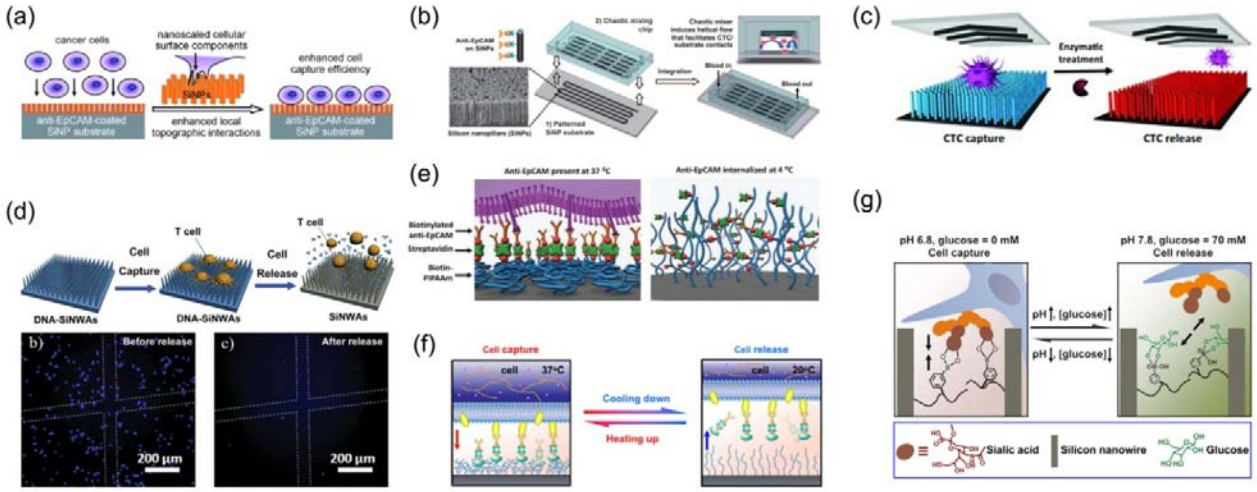


Figure 7. (a) Schematic illustration of anti-EpCAM-coated SiNWA substrate for the enhanced capture of CTCs; (b) Schematic illustration of a NanoVelcro chip with SiNWAs integrated in a PDMS fluidic device for capturing CTCs; (c) Schematic illustration of an aptamer-coated NanoVelcro Chip for capturing and releasing CTCs upon enzymatic treatment; (d) Schematic illustration of DNA-coated SiNWAs for capturing and releasing T cells (top) and fluorescence microscopy images of captured cells before and after treatment with exonuclease (bottom); (e) (f) Schematic illustration of PNIPAAm-based polymer-coated SiNWAs for capturing and releasing CTCs in response to temperature; (g) Schematic illustration of PAAPBA-coated SiNWAs for capturing and releasing CTCs in response to pH and glucose[91]. [Reprinted with permission of REF[84], Copyright 2009, Wiley-VCH; REF[85], Copyright 2011, Wiley-VCH; REF[87], Copyright 2013, Wiley-VCH; REF[86], Copyright 2011, Wiley-VCH; REF[89], Copyright 2013, Wiley-VCH; REF[90], Copyright 2013, Wiley-VCH; REF[91], Copyright 2013, American Chemical Society]

3.3.2 Protein separation

In addition to capturing and releasing cells, SiNWAs can also be used as good platforms for immobilizing and separating proteins. Chen and co-workers employed SiNWAs modified with various stimuli-responsive polymers and systematically investigated the synergistic effect of the surface chemistry and nanostructures on protein adsorption under external stimuli. Inspired by the pH-responsive protein adsorption on smooth silicon surface modified with poly(methacrylic acid) (PMAA)[92], a pH-switchable system based on PMAA-modified SiNWAs (SiNWAs-PMAA) was developed for protein adsorption and release.[40] Due to the high surface area, 3D structure and enhanced local topographic interactions of SiNWAs, SiNWAs-PMAA possesses several unique advantages, including (i) an extremely high capacity for binding protein at low pH, (ii) high efficiency (greater than 90%) for releasing adsorbed proteins by increasing the environmental pH without a loss of protein activity, and (iii) reversible and repeatable binding and release of protein. In another report, SiNWAs were modified with thermo-responsive PNIPAAm (SiNWAs-PNIPAAm), and this system exhibited very low levels of native-protein adsorption; however, this system favored the adsorption of more hydrophobic denatured proteins.[93] In particular, the adsorption of denatured protein on SiNWAs-PNIPAAm is thermo-responsive because the amount of denatured lysozyme adsorbed at 40°C was approximately twofold greater than that at 10°C. Moreover, the SiNWAs-PNIPAAm surface could selectively adsorb denatured proteins from native/denatured protein mixtures of either the same or different proteins. The above results suggest that SiNWAs modified with stimuli-responsive polymers provide an effective platform for bioseparation and protein purification.

3.4 Drug delivery and DNA transformation

As previously mentioned, vertical SiNWAs can support cell cultures, and needle-like SiNWs can penetrate cellular membranes, providing potential to deliver biomolecules into living cells.[44] Park and co-workers explored SiNWAs as a robust, monolithic platform for introducing various bioactive molecules (such as DNAs, RNAs, peptides, proteins, and other small molecules) into living cells with high throughput.[94] The method relies on the ability of the SiNWs to penetrate cellular membranes and subsequently release surface-bound molecules directly into the cytosol, thus enabling the highly efficient delivery of biomolecules without the chemical modification or viral packaging protocols required by other methods. On the other hand, SiNWAs possess several structural features that make this system suitable as a host for the controlled release of drugs, such as a high porosity and surface reactivity and a relatively large reservoir volume.[95]

Using the thermo-responsive properties of PNIPAAm, a SiNWAs-PNIPAAm-based substrate was developed for transforming DNA in *E. coli* cells with high efficiency, and the amount of transformation achieved using this substrate was 400 times greater than that using the chemical method.[96] Several features of the nano-catalyst contribute to the observed high DNA transformation efficiency, as illustrated in **Figure 8**. Initially, when DNA and cells are incubated with SiNWAs-PNIPAAm at 4°C, the superhydrophilic surface induces the precipitation of DNA on the *E. coli* cell surfaces. During heating to 42°C, the PNIPAAm-SiNWAs become highly hydrophobic and allow the cells to adhere with a high density, thereby increasing the number of cells to be transformed. Finally, when the temperature is decreased to 4°C following the transformation, the PNIPAAm layer becomes hydrophilic to release virtually all of the cells, facilitating molecular screening of the transformants and recycling of

SiNWAs-PNIPAAm.

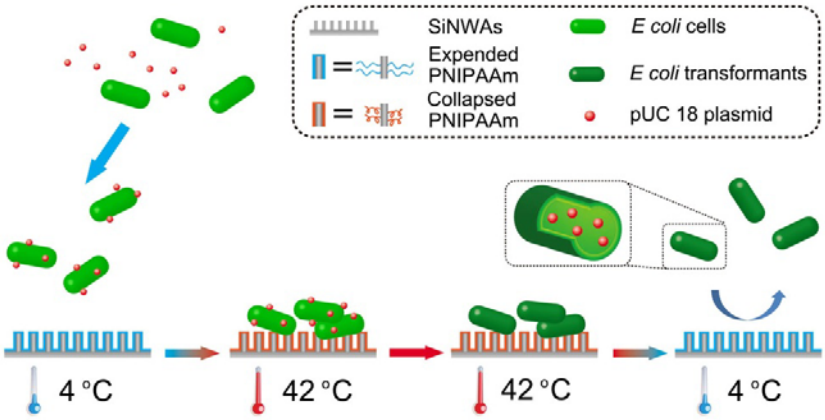


Figure 8. Schematic illustration of DNA transformation of *E. coli* cells on SiNWAs. [Reprinted with permission of REF[96], Copyright 2011, The Royal Society of Chemistry.]

3.5 Antifouling

Developing methods to prevent biofouling (such as non-specific protein adsorption, platelet adhesion and microbial contamination) on synthetic surfaces is of great interest for biomedical applications.[97-99] With proper functionalization, SiNWAs can serve as good antifouling surfaces for either preventing protein adsorption[39] and adhesion of platelets[43] or for killing attached bacteria[38, 42]. In particular, compared with their corresponding modified smooth silicon surfaces, the introduction of nanoscale structures significantly enhances the antifouling properties.

Previous studies have indicated that PNIPAAm-modified smooth surfaces exhibit temperature-dependent bioadhesion behaviors. Above the LCST, the relatively hydrophobic surfaces facilitate the adsorption of proteins and the subsequent adhesion of platelets, bacteria and mammalian cells. In contrast, below the LCST, the surfaces become hydrophilic and exhibit antifouling properties.[100-107] Notably, however, PNIPAAm-modified SiNWAs exhibited good resistance to proteins[39]

and platelets[43] regardless of the temperature. As shown in **Figures 9a** and **9b**, SiNWAs-PNIPAAm significantly reduced more than 99.9% of fibrinogen adsorption and the density of adhered platelets at temperatures both above and below the LCST. The elimination of thermo-responsive behavior might be due to the water molecules trapped in the interstices of the nanowire arrays, which formed a strong hydration layer to support intimate molecular contact between proteins/platelets and the surface regardless of temperature (as illustrated in **Figure 9c**). This novel surface is a promising candidate for biomaterial and biomedical applications when non-fouling properties are required.

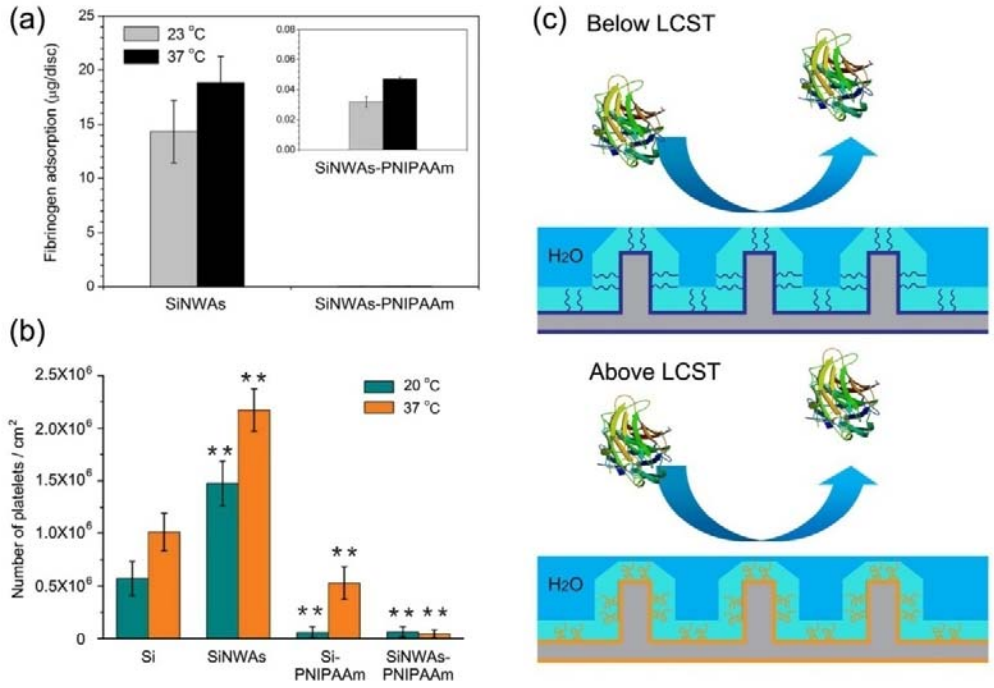


Figure 9. (a) Adsorption of 1 mg/mL fibrinogen from PBS solution over a 3-h period on pristine SiNWAs and SiNWAs-PNIPAAm surfaces at 23°C and 37°C (the ‘‘apparent’’ surface area of one disc is 0.5 cm^2). (b) The number of adhered platelets on different surfaces at 20°C and 37°C. ** indicates significant differences ($p < 0.01$) in comparison with smooth silicon. (c) Schematic illustration of protein resistance of SiNWAs-PNIPAAm surfaces below and above the LCST. (Reprinted with permission of

REF[39], Copyright 2011, The Royal Society of Chemistry; REF[43], Copyright 2009, American Chemical Society)

On the other hand, after the integration of biocides, the unique nanostructure of SiNWAs can enhance biocidal activity because the high surface-to-volume ratio facilitates the immobilization of biocides and increases the biocide density. For example, SiNWAs modified with quaternized PDMAEMA is a highly effective antibacterial material due to its high density of biocidal quaternary ammonium moieties. More than 95% of *E. coli* bacteria were killed after incubation on this surface for 18 h.[42] In another report, SiNWAs@AgNPs exhibited strong antibacterial activity toward *E. coli* bacteria but low cytotoxicity to HeLa cells.[108] Recently, a bacteriolytic enzyme lysozyme was covalently immobilized on SiNWAs, resulting in a platform with efficient broad-spectrum bacterial capture and killing ability against several bacterial strains.[38] Notably, the SiNWAs-lysozyme complex could be transferred to a flexible substrate, which might be used as an antibacterial dressing for the treatment of skin or bacterial wound infections.

3.6 Nanozyme

Chen and co-workers found that H-SiNWAs possess catalytic activities similar to those of biological enzymes. The H-SiNWAs exhibited catalase-like activity to catalyze the decomposition of H_2O_2 to water and oxygen and peroxidase-like activity to catalyze the oxidation of o-phenylenediamine (OPD) to 2,3-diaminophena-zine (DAP) by H_2O_2.[110] Based on the results of Raman spectroscopy, it is suggested that the formation of reactive intermediates $(Si{-}H)_2$…(O species) is responsible for these enzyme-like catalytic activities when H-SiNWAs are reacted with H_2O_2. Furthermore, they found that H-SiNWAs

behaved like reductase to reduce the dye MTT (3-(4,5-di methyl-2-thiazol)-2,5 -diphenyl-2 H-tetrazolium bromide) and nitrobenzene derivatives (such as *p*-nitrophenol (PNP)) and to degrade various organic azo dyes *via* their ability to break N=N bonds.[111-112] These findings suggest the potential use of SiNWAs as nanozyme in biotechnology and environmental chemistry.

4 Summary and perspectives

Over the past 5 years, there have been a considerable amount of achievements regarding vertical SiNWAs for various biomedical and biotechnology applications, ranging from fundamental studies on cell-nanomaterial interfacing to practical applications of biosensing, biocatalysis, and controlled separation and delivery. These developments depend on the significant interdisciplinary research between materials science, surface chemistry, biology and engineering. Despite the considerable progress that has been made, many difficult challenges remain and need to be addressed. Further research efforts may be guided by the following possibilities.

1. ***Optimization of the fabrication process and surface functionalization.*** It is suggested that the physical parameters of SiNWAs, such as their diameter, density, and length, are important for the performance of the resulting SiNWAs and are crucial for affecting cellular behavior when mammalian cells interface with SiNWAs. In addition, as discussed above, proper modification of SiNWAs provides unique surface properties (such as superhydrophobicity, high protein loading capability, and enhanced photocatalytic activity) compared with the corresponding smooth silicon wafers. It would be interesting to extensively explore whether the synergistic effects of surface chemistry and surface topography result in new biointerfacial phenomena and novel biological functionalities.
2. ***Deeply understand the influence of SiNWAs on cell function.*** It has been demonstrated that cells

can sense the nanoscale topographical cues of the substrates and that these structural features can inversely affect cellular behaviors such as attachment, alignment, proliferation, and differentiation. Although some pioneering works have investigated this topic, several questions remain to be answered. For example, whether the silicon nanowires penetrate the cell membrane is still under debate. Additionally, whether the SiNWAs after loading with information molecules can guide the differentiation of stem cells is unclear. Furthermore, although several reports have demonstrated the good biocompatibility of SiNWAs, thoroughly evaluating the cytotoxicity and biodegradation ability of SiNWAs is still required for practical *in vivo* applications.

3. ***Enhancement of stability and reusability of SiNWAs.*** To date, the majority of reports on the biomedical and biotechnology applications of SiNWAs have been proof-of-concept experiments conducted in laboratories. However, the requirements of high stability and reusability should be considered for practical applications. For example, for biosensing applications, the platform not only requires high sensitivity to target molecules but must also be capable of being regenerated to increase its working life; for antifouling surfaces, it is ideal to endow the modified SiNWAs with the capability to release contaminants to keep the surface clean.

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