Biosensing using arrays of vertical semiconductor nanowires: mechanosensing and biomarker detection

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Abstract
Due to their high aspect ratio and increased surface-to-foot-print area, arrays of vertical semiconductor nanowires are used in numerous biological applications, such as cell transfection and biosensing. Here we focus on two specific valuable biosensing approaches that, so far, have received relatively limited attention in terms of their potential capabilities: cellular mechanosensing and lightguiding-induced enhanced fluorescence detection. Although proposed a decade ago, these two applications for using vertical nanowire arrays have only very recently achieved significant breakthroughs, both in terms of understanding their fundamental phenomena, and in the ease of their implementation. We review the status of the field in these areas and describe significant findings and potential future directions.

Keywords: nanowires, mechanosensing, biosensing, lightguiding, biomarkers

1. Introduction
During the last decade, semiconductor nanowires have found their way into biology and medicine, and are currently being explored in a plethora of bio-applications such as cell culture substrates, cell transfection and biosensing. Silicon nanowire field effect transistors have been used to detect biomolecules, biomarkers and viruses [1–3], as well as to record action potentials [4, 5]. Arrays of vertical nanowires, with ‘bed of nails’-like morphologies, have attracted a lot of attention since having been shown to be benign substrates for cells [6, 7]. These arrays have been the subject of multiple studies focused on investigating their interactions with living cells. For instance, culturing cells on vertical nanowire arrays can, depending on the array geometry and cell type, affect cell adhesion, motility and proliferation [8–13]. Vertical nanowire arrays have also been used to transfect cells with minimal perturbation [14, 15], to steer stem cell differentiation [16–18], or to guide cellular growth [19–22].

An increased surface-to-foot-print area is one of the interesting properties of vertical nanowire arrays. It has been exploited for the detection and capture of biomolecules, relevant for a variety of medical applications including diagnostics and drug discovery. One main obstacle to overcome in these fields is the ability to effectively identify targets, such as proteins, organic molecules, chemical species and subpopulations of cells (e.g. for capturing circulating tumor cells through membrane markers). Nanowires have been recognized as potentially invaluable tools in this respect, as they may enable researchers to achieve detection of target analytes [23–28] and cells [29–31] with higher efficiency and unprecedented specificity [32].

There are a number of review articles summarizing the interactions of vertical nanowire arrays with living cells, and the use of increased nanowire array surface area for biosensing [31, 33–36]. Two other exciting applications in biosensing with vertical nanowire arrays include (i) precise cellular force sensing with high spatial resolution, and (ii) light-guiding-induced enhancement of fluorescence-based...
biomarker detection. These two applications have recently been the subject of an increasing number of studies. The technique of using vertical nanowire arrays for cellular mechanosensing has progressed, from a method that initially used to be imprecise and cumbersome to implement, into a robust and more user-friendly tool. Concurrently, utilization of vertically aligned lightguiding nanowires has allowed researchers to substantially improve the signal-to-noise ratio when detecting the fluorescence of surface-bound biomolecules. This article reviews the use of vertical nanowire arrays in these two promising applications, which have developed rapidly as of late and thus deserve further consideration.

2. Vertical nanowire arrays for mechanosensing

Cell mechanical forces regulate many crucial biological processes, such as cell signaling and tissue morphogenesis [37, 38]. Cellular force measurements have been carried out using atomic force microscopy, optical tweezers and micro-pipettes [39–42]. With these methods, only a single point can be probed at a given time. Addressing this issue, traction force microscopy enables forces to be measured in multiple points simultaneously. This is achieved by incorporating fluorescent beads into a thin elastomer substrate onto which cells are cultured. The displacement of the fluorescent beads is then used to calculate the forces exerted on the substrate. However, since the fluorescent beads are dispersed in a three-dimensional elastomer, forces applied at a given point on the surface can cause displacement at distant locations in the elastomer matrix. Therefore, the data interpretation and force calculation requires extensive modeling, which introduces uncertainty in the measurements [42, 43]. To remove any uncertainty associated to the force application location, and to greatly simplify the data analysis, one can use substrates with elastomer micropillars with cells cultured on top of the array of pillars [42, 44, 45]. The force exerted on each pillar is calculated from its measured deflection, assuming the pillar is a cantilever [44, 45]. However, the spatial resolution of this method still remains limited to a few attachment points per cell, due to the finite size of micropillars.

Nanowires can be arranged in much denser arrays, compared to elastomer pillars, and can therefore increase the spatial resolution of force measurements. Using arrays of vertical nanowires for cellular mechanosensing was proposed in 2009 [46]. In this study, cells were cultured on top of silicon nanowires, the samples were dehydrated and scanning electron microscopy was used to quantify the bending of nanowires underneath the cell edges. Finite element simulations were performed to extract the lateral force exerted on the nanowires from the measured displacement of the nanowire tip. The measured maximum traction forces exerted by mechanocytes, L929 fibroblasts and HeLa cells, were on the order of μN. A primary drawback of this method is the necessity to perform cell fixation and dehydration prior to performing force measurements, which also precludes the possibility for longitudinal studies. Moreover, dehydrating cells can lead to cell shrinking, which affects the deflection of

Figure 1. (a) Scanning electron microscopy of a hexagonal array of GaP nanowires used to measure cellular forces, and schematic of the force exerted on a nanowire of spring constant k, calculated from the nanowire deflection, using the linear elasticity theory. Reprinted from [47], with the permission of AIP Publishing. Scale bar 2 μm. (b) Diagram of force magnitudes that can be probed using the linear elasticity theory, as a function of nanowire diameter and length (for InP nanowires). [50] John Wiley & Sons. © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
measured. A full map of the force ranges that can be probed as a function of nanowire length and diameter can be created (see figure 1(b) for indium phosphide (InP) nanowires, taken from [50]).

Using fluorescently labeled nanowires to measure the tip displacement is not a requirement for cellular mechanosensing; as recent studies have shown, detecting the reflections in confocal laser light excitation from InP nanowires also enables very precise tip position readout [50, 51]. These studies pioneered the use of vertical nanowire arrays for probing forces exerted by bacteria, in this case the phytopathogen Xylella fastidiosa (figure 2(a)). In a first study, Xylella fastidiosa biofilms (composed of a few cells surrounded by a large amount of extra cellular polymeric substances) were shown to exert higher forces than cells alone [51]. Moreover, the forces exerted by Xylella fastidiosa and their related biofilm were shown to depend on the nanowire surface functionalization. Greater forces were measured in the presence of the transmembrane trimeric autotransporter adhesin XadA1, adsorbed on the nanowires, thus shedding light on the importance of XadA1 in promoting bacterial adhesion and biofilm formation. In a second study, the effects of N-acetylcysteine on Xylella fastidiosa forces were investigated. N-acetylcysteine was tested as possible inhibitor of

Figure 2. (a) Confocal fluorescence image of a GFP-expressing Xylella fastidiosa cell adhering to vertical InP nanowires and forces measured on the nanowires highlighted. Adapted with permission from [51]. Copyright 2016 American Chemical Society. (b) Traction force heat map for MCF7 cancer cells (top) and MCF10A normal-like cells (bottom). The insets show the force direction (arrows) and magnitude (length of arrows, red scale bar: 20 nN for MCF7 cells and 10 nN for MCF10A cells). Scale bars: 10 μm (white), 1 μm (black). Reproduced from [53]. CC BY 3.0.
Xylella fastidiosa biofilm formation [52]. Exposure to N-acetylcycteine led to an abrupt loss of detectable forces exerted on the nanowires, and to the detachment of Xylella fastidiosa from the nanowire substrate [50]. The drop in force occurs too rapidly to arise from changes in gene expression. Instead, the possible dissolution of the extra cellular polymeric substances, upon N-acetylcycteine exposure, has been proposed to explain the loss of detectable forces.

In the above-mentioned studies, proper force measurement relies on the setting of the focal plane at the tip of the nanowires. Any drift in focus induces an error in the measurement, since the signal no longer arises from the nanowire tip. Using GaP nanowires with a gallium indium phosphide (GaInP) photoluminescent top segment has been proposed in order to ensure that the signal comes only from the top of the nanowires [53]. GaInP nanowires exhibit a strong and stable photoluminescence in the near infrared, which makes them ideal for imaging through cells and tissue [54–56]. This technique can accommodate for small focal drifts, as long as the GaInP photoluminescence is still detectable, and does not overlap with that of neighboring nanowires. This method has been used to show that cancer cells exert higher traction forces than normal cells, as well as to investigate the effect of the anticancer drug α-difluoromethylornithine on cellular traction forces [53]. Moreover, in that study, a robust image analysis program was developed to enable the calculation of the force exerted on all nanowires in the field of view. This image analysis program allows for the representation of results in the form of highly spatially resolved force heat maps (figure 2(b)).

When cells are cultured on top of the nanowire array, they are located in the light path and refract the light, resulting in artefacts in the observed nanowire deflection. A recent study has investigated the perceived nanowire deflection, resulting from light refraction by cells, and estimated that it is on the order of 150 nm [57]. As a possible solution, the authors suggest to culture cells on sparse nanowire array substrates, to ensure that the cells grow inside the array and not on top of the nanowires, thereby leaving the light path undisturbed [57]. Such a method removes the uncertainty in determining the forces associated with any cell-induced light refraction. However, it requires live three-dimensional imaging to precisely identify the point of contact between cells and nanowires in order to properly calculate the forces exerted on the nanowires, which can be challenging in cases where cells contact nanowires in multiple points.

An additional point to consider when using vertical nanowire arrays to measure cellular forces is the fact that the cell morphology and adhesion can be influenced by the geometry of the array, and differ from the ones of cells cultured on standard flat substrates [8–13, 58].

In summary, the use of vertical arrays of nanowires for cellular mechanosensing has developed tremendously in the past decade and has become more precise, and easier to implement. Beyond demonstrations of the technological development, biological questions have been addressed using this method, such as biofilm formation mechanisms and elucidating the mechanisms of action of anticancer drugs. In the future, this method could be further automated for a more user-friendly mode of operation. For instance, integrating a camera in an incubator where nanowire substrates could be easily mounted, would make the method accessible to cellular biology and biomedical laboratories. Cellular mechanosensing could then take place in a plethora of different research projects and one would imagine that cellular force could be used as a biomarker, on the same level as genes and proteins.

3. Enhancing biomolecule detection using lightguiding nanowires

Detecting low-abundance biomolecules in biological fluids, such as blood and urine is a challenging task. To date, only a limited number of disease biomarkers can be detected in biological fluids and often only when present in rather high concentrations, i.e. once the disease is at a rather advanced state [59]. Enhancing the signal-to-noise in fluorescence detection would be one way to achieve a lower limit of detection for fluorescently labeled biomarkers.

Although semiconductor nanowire waveguides have been reported, especially in the context of nanowire lasers [60–62], they have only recently been used to enhance the signal-to-noise ratio when detecting the fluorescence of molecules in close proximity to the nanowire surface.

Enhanced fluorescence signals on zinc oxide (ZnO) nanowire arrays have been reported in 2006. After non-specific adsorption of fluorescently labeled proteins on various substrates, an increase in fluorescence intensity was measured on ZnO nanowires compared to control substrates such as glass, silicon nanowires, and polymer substrates [63]. Enhanced fluorescence on ZnO nanowires was also measured in the case of fluorescently labeled DNA oligonucleotides hybridizing to complementary strands deposited on the substrate, as well as fluorescently labeled proteins binding specifically to proteins adsorbed on the substrate [63, 64]. ZnO nanowires and nanorods have since been used in antibody microarrays, to directly detect cancer biomarkers in diluted human serum with limit of detection comparable to that of ELISA assays [65]. In these assays, ZnO nanorods were grown on a glass slide, and micro-spots of antibodies for a cancer biomarker (carcinoembryonic antigen or α-Fetoprotein) were printed on the slide. After incubation in 10% human serum, the sample was incubated with primary antibodies to the target biomarker, followed by incubation with fluorescently labeled secondary antibodies. In similar sandwich assays, ZnO nanorods have been shown to achieve orders of magnitude lower detection limits in the duplexed detection of IL-8 and TNF-α kidney injury biomarkers in human urine, compared to ELISA [66] (figure 3). The difference in assay sensitivity between the two studies may be explained by the fact that, in the latter study, the nanorods were grown from Au catalysis particles deposited on a silicon wafer, resulting in altered nanorod dimensions and possibly differing orientation compared to the former cancer biomarker detection study.
ZnO nanorod enhanced fluorescence was reported and used in multiple studies, with nanorods arranged in vertical arrays [67, 68] and in a flower-like configuration [69, 70]. The use of ZnO nanorods can also be combined with lab-on-a-chip technologies [71–73].

The fluorescence enhancement was initially thought to arise from nanowire-induced changes in the fluorophores radiative decay rates, or from a reduced fluorescence quenching [64]. Recent investigations of the fluorescence signal on ZnO individual nanorods revealed a higher fluorescence intensity and more stable fluorescence at the nanorod extremities, compared to along the nanorod’s length [74]. This is attributed to a combination of locally enhanced electric fields at the nanorod tip, and to the waveguiding properties of ZnO nanorods. There is an in-coupling of the fluorophore signal to the nanorods, which was found to be independent of nanorod diameter, and fluorophore wavelength; it is however, highly dependent on the light polarization state [74–76].

In parallel, the guiding of light emitted by surface-bound fluorophores was also reported for III–V nanowires (figure 4(a)). This was first observed on vertically aligned GaP nanowires, coated with alumina and subsequently decorated with heavy meromyosin (HMM). The HMM propelled fluorescently labeled actin filaments over the top, and along the length of the wires [77]. In this study, the authors observed an increase in fluorescence intensity at the tip of the nanowires, when filaments were moving up or down the wires. This fluorescence intensity scaled linearly with the length of the fluorescent actin filament transported along the nanowire surface. The integration of the fluorescence signal at the nanowire tip led to a higher signal-to-noise ratio, which should enable a lower limit of detection in biosensing. Lightguiding of surface-bound fluorophore emission was also observed for InAs nanowires and later for GaAs nanowires [78–80]. In the latter case, the increase in fluorescence at the nanowire tip was used to achieve high surface-to-noise ratio and highly localized excitation volumes, which was applied to the imaging of membrane proteins from cells cultured on the nanowires [79]. Theoretical simulations were performed for InAs nanowires, which considered only the fundamental waveguide mode HE_{11} at a single nanowire diameter of 100 nm. These simulations agreed with experimental results, showing clear lightguiding of green and blue light, but not red light [78]. A subsequent, and more comprehensive description of lightguiding in nanowires as a function of nanowire diameter d (d was varied between 50–260 nm) and fluorophore emission wavelength \( \lambda \) was performed for GaP. This study showed that the normalized frequency parameter \( V = \frac{2 \pi d}{\lambda} \sqrt{n_{\text{core}}^2 - n_{\text{clad}}^2} \), frequently used in optical fiber studies [81], can be used to predict lightguiding properties in semiconductor nanowires, with lightguiding of surface-bound fluorescence occurring in cases where \( V > 2 \) [82]. Here, \( n_{\text{core}} \) is the optical refractive index of the nanowire core and \( n_{\text{clad}} \) of the coating. In the same study, finite-difference time-domain (FDTD) simulations showed that higher order modes, beyond HE_{11}, must be taken into account to understand and describe lightguiding in larger diameter nanowires (figures 4(b), (c)).

By achieving lower detection limits, compared to existing biosensing technologies, lightguiding nanowires could be used for the discovery of new antibodies in biological fluids. This would, for instance, enable early disease diagnostics from easily accessible blood and urine samples. Such a technology would dramatically improve the prognosis of patients with asymptomatic cancers, such as pancreatic and ovarian cancer.
4. Conclusions

In conclusion, we review the status of two exciting applications, currently under development, for arrays of vertical semiconductor nanowires: cellular mechanosensing and enhanced fluorescence detection by use of lightguiding in nanowires. As shown in the recent studies reviewed here, using nanowires for cellular mechanosensing can be achieved with both high spatial resolution and high force precision. The measurement of cellular traction forces can be used as a valuable tool in many research areas, such as the investigation of biofilm formation, cancer onset and spreading, as well as morphogenesis. Lightguiding in semiconductor nanowires for fluorescence detection enhancement is now well understood, and the degree of lightguiding for various nanowire materials and geometries can be predicted as a function of fluorophore emission wavelength. This knowledge will enable the design of nanowire arrays with optimal lightguiding properties, in order to achieve lower limits of detection in biosensing devices.

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