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COMMUNICATION

Introducing Heterojunction Barriers into Single Kinked Nanowire for Probe-Free Detection of Protein and Intracellular Recording

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Nanoprobe based on single nanowire (NW) offers substantial potential for biological and in vivo determination. In regard to intracellular detection, minimal invasion and adjustable detection depth have become the crucial challenges. The nanoprobes with small, sharp tip, and long arm are thus desired. Here, we demonstrate a general strategy to prepare single kinked NW heterojunction with continuously adjustable angle, length and sharp line-type tip. It is found that the introduction of heterojunction barriers in kinked NW can act with biomolecules and cell. The prepared kinked NW nanoprobe is successfully used for highly-sensitive probe-free detection of hemoglobin and real-time intracellular recording with minimal invasion. The sensing performance is regularly dependent on the amount of the heterojunction barriers. The integrated nanoprobes with multi-shaped structure are further designed for multi-functional application. Introducing heterojunction barriers in kinked NW provides a substantial opportunity for fabricating functional and integrated nanoprobes in application of life science.

Introduction

One-dimensional nanostructures have been demonstrated as outstanding materials for fabricating ultrasensitive nanosensors. The single nanowire (NW) based field-effect transistor (FET) is the most typical configuration for biosensors.[1-6] And these nanodevices usually use a bioprobe to recognize biomolecules, such as antibody to antigen and virus, enzyme to substrate.[7,8] However, active transistor requires two contacts for operation, and until recently this constraint has made intracellular recording devices difficult to achieve. Recent advances have demonstrated that the rational design and synthesis of nanomaterials could open-up unique opportunities at the interface between nanoelectronics and biological systems.[9-19] For example, the kinked Si NW with an angle of 120° can be synthesized by variation of reactant pressure during growth and used for intracellular recording.[9] The special band structure and electronic state in interface are the chief functional factors. As we know that the heterojunction has wide selectivity of distinct energy band structure and conduction type and results in unique nanoelectronic characteristic, such as, high injection ratio and electron mobilities. It has attracted wide attention in designing nanoelectronics and nanodevices. Therefore, heterojunction is desired to be introduced into single kinked NW to develop as novel bioprobe for further exploiting and expanding the applications between nanoelectronics and biological systems. However, it is hard to synthesize many other kinked NWs using the method of synthesizing kinked Si NW, due to the specialty of Si NW. It is known that many metal oxide semiconductors (MOS) NWs, such as ZnO, NiO and CuO, are promising materials for fabricating nanoscale biosensors with excellent performance. Among these, ZnO is a wide band gap n-type semiconductor with a wide range of nanostructures, and unique optical, electrical, mechanical and chemical properties.[20] For example, Wang group have reported that the piezoelectric of single ZnO NW played an important role in biosensing.[18] The additional benefit of ZnO NWs is an oxide semiconductor with good stability in an air environment, as compared to that of nonoxide semiconductor, such as Si NWs. It may be desirable to remove native oxide on the Si NWs by a harsh wet etching process, in order to achieve more effective surface for optimal sensing performance. Therefore, ZnO NW has substantial potential for designing novel biosensor. NiO is a wide band gap p-type semiconductor. Because both side has wide band gap and distinct band position, the formed heterojunction has largely adjustable band structure scope, which is the key factor in the probe-free biosensing according to the previous reports.[17-19] According to previous reports, the heterojunctions formed by NiO and ZnO have exhibited excellent performance in photoelectric, catalytic and energy field.[21-23] So it is necessary to develop single kinked ZnO-NiO NW heterojunction based nanoprobe for biosensing applications and in vivo determination.

In regard to inserting into a cell, it is desirable to penetrate the cell membrane with the minimum damage and enough depth. So the probe with small, sharp tip, long arm and adjustable kinked angles is desired. However, it is still a challenge to fabricate single NW based three-dimensional (3D) nanoprobe with above features for sensitively detecting biomolecules and intracellular recording. Based on the fact that the fabrication of nanoprobe with more complex NW geometries and multiple nanoFETs within single 1D NWs can open up distinct sensing applications,[24-34] how to achieve a facile and flexible design and integration of multiple nanoprobes thus becomes an important topic. Electrospinning has proven to be an efficient method for the formation of long polymer fibers with diameters varying from nanometers to micrometers.[35-37] Various nano/micro inorganic/organic fibers can be facilely fabricated by this technique. Due to the flexility and cohesiveness of the precursor fiber, it is facile to assemble and integrate. So electrospinning provides a powerful tool to design nano/micro-electronic device.[38]

In this work, we first report a successfully synthetic integration of the kinked ZnO-NiO NW heterojunction with the continuously adjustable angles ranged from 10° to 150°. The small angle nanoprobe has sharp line-type tip and long arms. The facile fabrication of multifunctional and integrated devices is further demonstrated. The nanoprobe fabricated by single kinked ZnO-NiO NW exhibits sensitive response to hemoglobin (Hb) and pH value. The operation of the nanosensor is largely determined by the behavior of the heterojunction region, the response comes from the change of barrier as a result of biomolecules adsorption. On this basis, local dopant and structure modulation are introduced to design devices. New type of functional nanoprobe fabricated by single kinked ZnO NW-NiO nanosphere (NS) is further developed. It is found that the sensitivity is largely dependent on the amount of the introducing NiO NSs and shows regularly adjustable performance. The performance results from the local contact barriers introduced by embedding NiO NSs in the electronic transport channels of ZnO NW. A prepared 3D bioprobe based on single ZnO-NiO NW with small angle (~ 30°) and sharp line-type tip is successfully used for intracellular recording within single live cell.

Experiment

Synthesis and Characterization: Kinked ZnO-NiO NWs were synthesized by electrospinning and subsequent calcinations. In a typical procedure, the single composite polymer fiber with polyvinyl alcohol (PVA 10 wt%) was first prepared with controlled orientation by a paralleled metal wires electrode. The charged fiber is prone to be vertically distributed on the metal wires due to the uniformly distributed electric field between them. Under short electrospinning time, a single fiber that vertical to metal wires can be collected. Then two fibers were assembled to form a connected point with adjustable angle under microscopy on the micro-manipulation platform. The kinked angle can be controlled by coordinate marker. The length of the arm and the tip can be tailored by a metal probe on the micro-manipulation platform. Finally, the sample was calcinated at 600 ^{o}C in air, as is schematically shown in Fig. S1 (Supporting Information 1). The annealed samples with sufficient quantities were characterized by X-ray powder diffraction (XRD) and energy dispersive X-ray detector (EDX) (Supporting Information 2). Single kinked ZnO NW embedded with NiO NSs was prepared by electrospinning the precursor sol solution with dispersed NSs and then calcinations. The NiO NSs with the diameter about 600 nm were synthesized according to previous report.[39] The single kinked NW based 3D probe can be fabricated using electrospinning with special designed substrate to yield single kinked NW with the tip part isolated from substrate or oriented at an angle with respect to the substrate (Fig. S3).

Measurements: Carbon paste was used to contact the NW and metal electrode, and ohmic contact formed. Polymethyl methacrylate (PMMA) was employed as the passivation layer to isolate the metal electrodes from the aqueous medium (Fig. 2a). The sensing experiments were carried out in phosphate buffer saline (PBS). A home-built microfluidic cell was placed over all biosensor devices to control the liquid environment. To use the 3D probe for intracellular recording, it was first functionalized with phospholipid bilayer [1, 2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)], which can form on a variety of nanostructured inorganic materials and also fuse with cell membranes according previous reports.[40-42]

Results and discussion

Kinked ZnO-NiO NWs were synthesized by controlled electrospinning and subsequent calcinations, as is schematically shown in Fig. S1 (Supporting Information 1). The X-ray powder diffraction (XRD) and energy dispersive X-ray detector (EDX) reveal the formation of pure ZnO and NiO NW. (Fig. S4-8). The as-prepared kinked ZnO-NiO NWs assembled with various angles are representatively shown in Fig. 1a. It is observed that the probe can be facilely prepared as single bottom-up V-shaped NW with adjustable angles at the range of 10-150° and long arms up to millimeters. The adjustable angle and length makes it powerful in biomedical applications. Based on the preparation of single NW probe, we have extended the topological complexity by designing and synthesizing multi-shaped kinked NWs probes making up of three "Vs" (Fig. 1b left) or else. In this manner, independent, parallel multiplexing with a single probe can be implemented,[24] which has the potential to be further designed and used as diverse functional nanoprobes. Another branched nanoprobe was also fabricated (Fig. 1b right), which has two heterojunctions in different depth and two respective transport channels. It is promising in synchronous recording of the intracellular/extracellular signals or that from different depths within the same cell. These designs would allow for series and/or parallel multiplexing of intracellular/extracellular recording using a single nanoprobe. Last, the integrated network of heterojunction NWs was fabricated and diverse functional application need to be further developed (Fig. S2). The exposed interface of junctions plays a key role in the sensing since the device element is naturally localized at the depletion region of the junction.[43] Hence large exposed interface is desired by well-design. In a planar junction device, such as formed by films, the junction interface is mostly buried beneath the surface and thus only mini part is exposed. In contrast, the axial design can fully expose the junction interface for use. In our design, the tip of the probe can not only form as a point but also a line. It is prone to form a point when the angle is larger than 90°, but a line at the angle less than 60°. The exposed linear interfaces are typically divided into two types: parallel and voluble (Fig. 1c). The former is usually formed at the angle less than 30°, while the latter is usually at the angle between 30° and 60°. Both have large axial exposed interface of heterojunction for effective sensing. More importantly, the line-type tip enables the probe sharp enough to penetrate the cell membrane with minimum invasion.

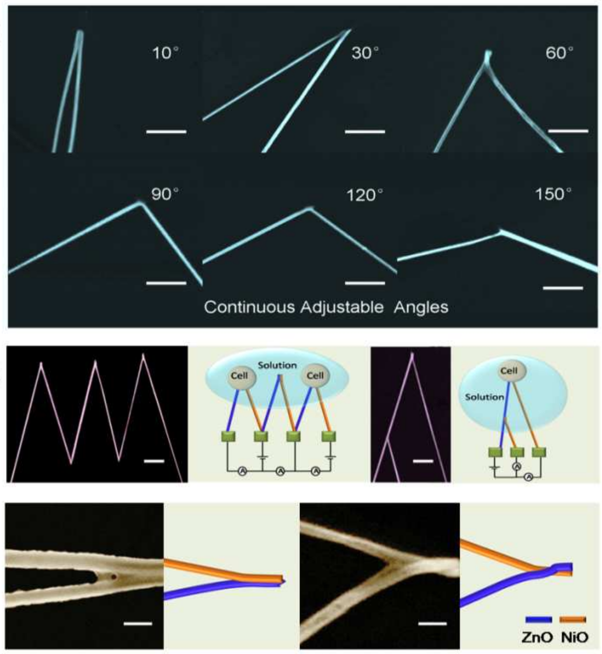

Fig. 1. SEM image and schematic diagram of the prepared kinked ZnO-NiO NWs. (a) The prepared kinked ZnO-NiO NWs with continuously adjustable angles, the tips formed as point type at the angles greater than 90°and line type lower than 60°. Scale bar, 5 μm. (b) The multi-shaped kinked ZnO-NiO NWs making up of three bottom-up "Vs" with independent, parallel multiplexed functions, which has the potential to be further designed and used as diverse functional nanoprobes. The branched nanoprobe making up of two bottom-up "Vs", which is promising in synchronous recording of the intracellular/extracellular singles or that from different depths within the same cell. Scale bar, 5 μm. (c) The kinked ZnO-NiO NWs with line type tips that are typically divided into two types: parallel and voluble, both the exposed heterojunction interfaces are larger than that of points and films. Scale bar, 500 nm.

A scanning electron microscopy (SEM) image of kinked ZnO-NiO NW heterojunction is shown in the inset of Fig. 2b. Typical current versus voltage (I−V) data (Fig. 2b) show clear rectification with no measurable current in reverse bias and an onset for current flow in forward bias. The as-prepared ZnO-NiO NW nanoprobe was first explored for localized detection of protein molecules. We used Hb as our sensing molecules; the PH was adjusted to control the electrical properties of the Hb molecules. The real-time detection of Hb with different pH value under reverse bias is shown in Fig. 2c. It is observed that the presence of Hb molecules caused a significant increase of steady current at pH = 5.7. For Hb with an isoelectric point (pI) of 7.4, the molecules are positively charged at pH = 5.7 (pI > pH). However, when the pH value increased to 7.0 (pI ~ pH), the response largely decreased. It demonstrates the response is heavily depended on the charge quantity of the protein molecules that is directly related to the pH value.[17] The increased current resulted from the charges on the adsorbed biomolecules changed the potential barriers height and conduction (Supporting Information 1 and Fig. 2d). The electrical response of the nanoprobe is characterized for various concentrations of charged

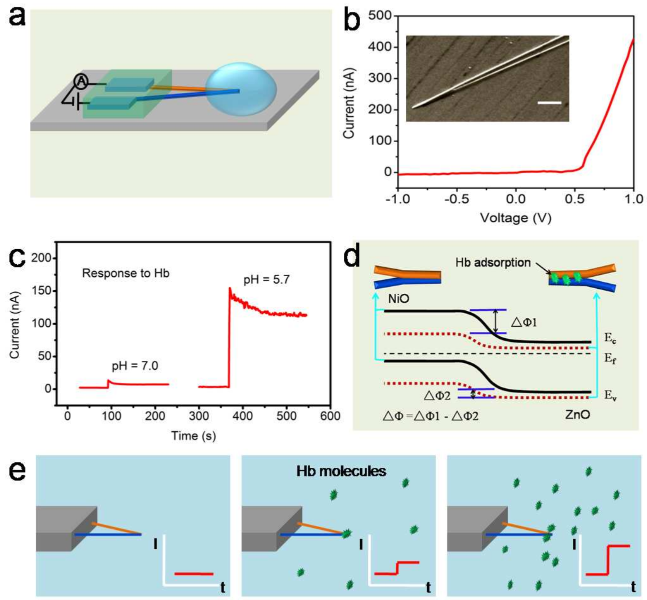

Fig. 2. Detection of hemoglobin. (a) Schematic diagram of the nanosensor for biosensing. (b) Current versus voltage (I−V) data recorded from a representative kinked ZnO-NiO NW device. Inset: SEM image of the kinked ZnO-NiO NW structure with an angle about 10°. Scale bar, 5 μm. (c) A fast response and distinct current variations at different PH values is found, when the sensor is exposed to a same concentration (500 μg/ml) of positively charged Hb molecules. (d) Schematic illustration of the adsorbed positively charged Hb molecules caused the decrease of potential barrier height. (e) Schematic demonstration of the increased current caused by the adsorbed positively charged Hb molecules, once the molecules adsorbed on the heterojunction probe the current increased, more adsorption caused enhanced increase of current.

Hb molecules at the same pH value. It is observed that the steady current increased as the increase of molecules concentration (Fig. S8). The lower detection limit is 10 μg mL^{-1}, and the upper detection limit is 800 μg mL^{-1}. The response process is schematically shown in Fig. 2e. The conductance increases as soon as the protein molecules approach and/or attach to the heterojunction NW due to the positive charges on the molecules changes the energy band structure and depresses the potential barrier height, as a result, the steady current achieves to a higher level. Furthermore, when the higher concentration of charged protein molecules is introduced, the steady current is further enhanced as the further depressed potential barrier height. However, it will tend to reach a saturated steady current without further enhancement as the barrier height has been depressed to the lowest.

Single kinked ZnO NW (diameter ~ 500 nm) embedded with NiO NSs (diameter ~ 600 nm) (Fig. 3b) was prepared by electrospinning the precursor sol solution with dispersed NSs and then calcinations. The performance of this type of probe is shown in Fig. 3a. It is observed that the pure single kinked ZnO NW based probe showed negligible response to the charged Hb molecules. However, the probe constructed by single kinked ZnO NW embedded with NiO NSs showed significant response comparatively. This phenomena is attributed to the formation of local heterojunction potential barrier (Φ) in the electron transport channels after embedding NSs. As the ZnO NW and NiO NS have opposite conductivity types, the p-type NiO NS will block the electrons transport in the n-type ZnO NW channel by forming a local potential barrier. The schematic energy band diagram is shown in Fig. 3c. Once positively charged molecules begin to adsorb on the probe, the local barrier height decreases owing to the local build up of positively charged molecules (Fig. 3d). In such a case, the resistance of the device drops as more charged molecules are adsorbed, and thus the magnitude of the transmission current increases. As is shown experimentally in Figure 3a, the steady current increases sharply to a high level once positively charged molecules are added into the solution. Further experiment showed that the sensitivity increased as the

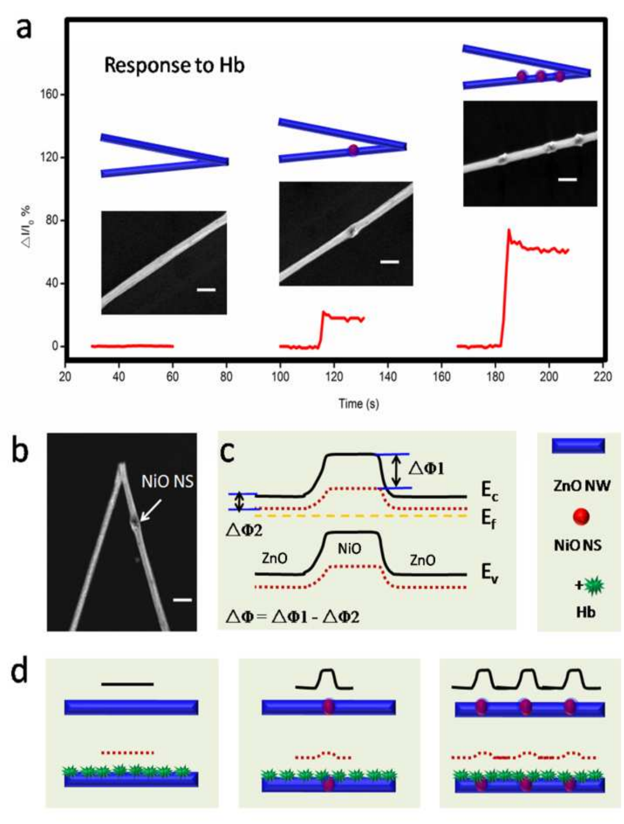

Fig. 3. Adjustable performance by structure modulation. (a) The compared response of the nanosensor with various amounts of embedded NiO NSs in ZnO arm to the same concentration of Hb, which increases as the increase of NSs. Scale bar, 1 μm. (b) SEM image of the kinked ZnO NW embedded with one NiO NS in the right arm. Scale bar, 1 μm. (c) Schematic band diagram (red curves) and band diagram change (dotted line) of kinked ZnO NW embedded with NiO NSs after adsorbing positively charged Hb molecules. The adsorbed charges cause the decrease of potential barrier height. (d) Schematic illustration of the relation between the embedded NiO NSs and the response, more NiO NSs introduce more potential barriers and result in enhanced sensitivity.

increased amount of the embedded NSs (Fig. 3a, supporting information 4). These data indicate that the sensing performance can be achieved and adjustable through introducing local barriers in the electrons transport channel. In other words, the probe-free nanosensor can be prepared and adjusted by local dopant and structure modulation, which is a general strategy to design diverse biosensing devices and can extend to other semiconductor materials by further experiments.

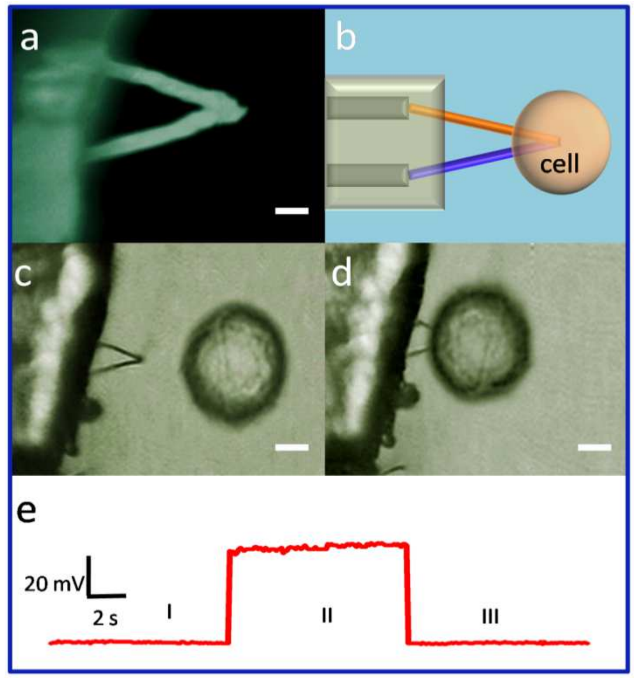

Fig. 4. Intracellular recording. (a) SEM image of the kinked ZnO-NiO NW based 3D nanoprobe with a kinked angle about 30°. Scale bar, 1 μm. (b) Schematic diagram of the nanoprobe entrance into a cell. Microscopy images of the procedure that the kinked NW probe approaches (c) and internalizes (d) the cell. Scale bar, 5 μm. (e) Electrical recording of an HL-1 cell and kinked NW probe as the probe approaches (I), internalizes (II), and is retracted from (III) the cell.

A kinked ZnO-NiO NW was configured as 3D probe with the tip part separated from substrate for localized interaction with living cells (Fig. 4a). The single kinked NW based 3D probe was fabricated using electrospinning with special designed substrate (Fig. S3). The SEM image of the 3D kinked NW with an angle of 30° was shown in Fig. 4a. The microscopy images of the procedure that the single kinked ZnO-NiO NW probe inserted into cell is shown in Fig. 4c, d. The real-time potential change was monitored while the HL-1 cell was moved into contact and then away from the nanoprobe using a glass micropipette under microscopy visualization. It is observed that the recorded potential showed a sharp change as soon as the probe was inserted into the cell and then maintained a relatively constant value (Fig. 4e). Finally, the potential returned to baseline when the cell was detached from the NW probe end, which is consistent with intracellular action potentials recorded using Si NW probe.[9] The intracellular recording was done in five cells and the key features of the intracellular action potential was not lost. The results demonstrate that the single kinked ZnO-NiO NW can be used for intracellular recording. The sharp tip and small kinked angle minimize the damage of the cell. Further work needs to be done to develop its specific and diverse applications in intracellular detection.

Conclusion

In conclusion, we demonstrate that the single kinked ZnO-NiO NW heterojunction based nanoprobes with continuously adjustable angles and sharp line-type tip can be conveniently fabricated and integrated by electrospinning technique. It is a general strategy to fabricate single kinked metal oxide NW. The heterojunction barriers introduced into kinked NW can be used as a functional factor to act with biomolecules and cell. The charged Hb molecules are probe-free detected by introducing the heterojunction barriers in the nanoprobe. The probe-free response results from the change of the potential barrier by adsorbing charged Hb molecules and largely depends on the amount of introduced heterohunction barriers. It is an adjustable strategy to design single NW based nanoprobe with adjustable performance. The real-time intracellular recording using a 3D kinked NW nanoprobe is finally achieved. The facile design of kinked NW heterojunctions with adjustable angles and multi-shaped structures provide powerful tools for fabricating highly compact and multiplexed nanoprobes for promising applications in life sciences, including (1) minimally invasive, deeply intracellular and deep tissue recordings using the nanoprobe with small kinked angle, long arms and sharp tip, (2) improving the probe-free biosensing performance by local dopant and structure modulation or integration, (3) independent recording of more than two intracellular or extracellular signal from different spatially defined positions. Introducing heterojunction barriers in kinked NW offers unique opportunities at the interface between nanoelectronics and biological systems.

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Notes and references

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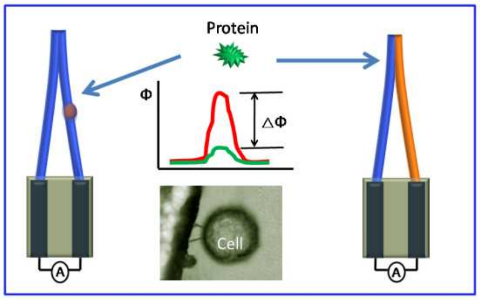

Kinked ZnO-NiO nanowires with adjustable angles were controllably fabricated by tailoring polymer fibers and used for probe-free detection of protein and intracellular recording.