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Electrofluidic Positioning of Biofunctionalized Nanowires

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Abstract

We functionalized nanowires with three different probe peptide nucleic acid (PNA) sequences, and assembled the three populations onto a lithographically patterned chip. Electrofluidic assembly enabled positioning each set of nanowires to span a different pair of guiding electrodes. Fluorescence imaging was used to probe whether the PNA on the individual nanowires remained able to selectively bind complementary DNA targets following assembly and integration of the positioned nanowires onto the chip surface.

INTRODUCTION

Chip-based nanowire sensing methods are promising candidates for ultraportable bioanalysis due to the small size of individual wires, and the potential for ultrasensitive electrical, electrochemical, and mass-based detection of biological target molecules. detection[1-3]. Such a device would provide critical information in emergency situations, and could aid in the early diagnosis of complex diseases such as cancers or respiratory infections. Consequently there has been intense interest in developing on-chip methods such as nanowire field effect transistors (FETs), micro/nano electromechanical systems (MEMS or NEMS), and electronic noses[4-6]. The incorporation of nanowires functionalized with probes for a large number of different targets (multiplexing) –while retaining the activity and selectivity of the probe molecules– is an obstacle currently limiting the further development of these sensors. Although some nanowire positioning [7,8] and probe molecule delivery methods [9-11] have been developed to overcome this obstacle, these methods lack the ability to place nanowires in predetermined locations needed for highly multiplexed systems, have limited types of probe molecules that can be delivered, and cannot ensure optimal attachment chemistries of the probe molecules, possibly leading to cross contamination of adjacent sensing elements. For these reasons, we have developed a versatile electrofluidic positioning method which allows us to position prefunctionalized nanowires to predetermined chip locations, such as between columns of electrodes. Scheme 1 illustrates the concept.

EXPERIMENTAL DETAILS

Nanowire Synthesis

Metallic nanowires (8 μm in length) were synthesized by galvanostatic electrodeposition into the pores of aluminum oxide templates with a nominal pore diameter of 0.2 μm [12,13]. The nanowires were rinsed by centrifugation and resuspended in 1 mL of ethanol resulting in

~10⁹ nanowires. Rh nanowires were coated with ~30 nm of SiO₂ [14] and functionalized with PNA probe molecules (P1, P2 or P3 shown in Table 1) using previously described methods [5] and suspended in ethanol for alignment. We selected sequences that were complementary to nucleic acids from pathogenic viruses hepatitis C (HCV), hepatitis B (HBV), and human immunodeficiency virus (HIV). As targets, we used synthetic DNA oligonucleotides for these sequences, with different fluorescent tags on each target strand (Table 1).

Patterning of Silicon Wafers and Electrofluidic Assembly

Electrodes (32 μ m wide, 5 mm long and separated by a 3 μ m gap) were fabricated by standard metal liftoff of 10 nm of Ti, and 90 nm of Au on a 300 nm layer of thermally grown SiO₂ on a silicon (100) substrate. Standard lithographic techniques using PMGI-SF6 and Shipley 1811 in a dual layer resist process were used to pattern them. Silicon nitride was deposited to insulate the guiding electrodes during the assembly process.

Electrofluidic Assembly of the PNA Functionalized Nanowires Between Specific Electrodes

Alignment of the three sets of PNA functionalized nanowires was accomplished by applying a non-uniform electric field with a frequency of 1 MHz and a voltage of 3 V_{rms}. Nanowires functionalized with P1, P2 or P3 were deposited on the substrate and positioned into the desired gaps, allowing the ethanol to evaporate after each set of functionalized nanowires were positioned into the gap region.

PNA Hybridization Procedure

Hybridization of T1, T2 and T3 to their complementary probe molecules was accomplished by incubating the targets at a concentration of 0.38 μ M in PBS buffer at room temperature for ~15 hours. The wafer was rinsed in excess PBS and a cover slip was added for imaging using an oil immersion 60x objective (1.4 NA). The fluorophores synthesized onto the target DNA sequences were chosen to minimize the spectral overlap of the absorbing and emitting wavelengths (listed under table 1).

DISCUSSION/RESULTS

Modeling the Positioning of Nanowires

We simulated the forces acting on the nanowires during assembly. Electric field calculations were conducted in which alternating voltages of 3V_{rms} at a frequency of 1MHz were applied between guiding electrodes (Figure 1). The area of highest electric field was at the edges of the guiding electrodes in the gap region. Dielectrophoretic forces drive the nanowires between the desired gaps in under 1 min. After a wire is positioned into a specific location, the driving force for additional nanowires to assembly nearby is greatly reduced, which results in a spacing of several microns between individual wires [5,15].

Positioning of PNA Functionalized Nanowires

Three batches of SiO₂ coated Rh nanowires were functionalized with three different probe PNA sequences (P1, P2 and P3) in suspension prior to positioning them onto the chip. They were then positioned one batch at a time into pre-determined locations by applying non-uniform electric field gradients between desired electrodes. The ethanol suspending the populations of injected nanowires was allowed to fully evaporate before subsequent injections of the other nanowire populations. This helps ensure that when the electric field is removed and applied to other sets of guiding electrodes for subsequent injections, the nanowires positioned in prior steps remain in their desired locations rather than migrating to the newly activated gaps. Following sequential alignment of the three PNA probe coated Rh nanowire populations, a mixture of all three fluorescently labeled DNA targets (T1, T2 and T3) was incubated with the

aligned nanowires to check whether probe molecules remained active and selective following the electrofluidic assembly process (electric fields, being suspended in ethanol). Figure 2 shows reflectance and fluorescence microscopy images of the three-column nanowire arrays after target hybridization. All of the nanowires can be seen in the reflectance image (far left). Wires from only one column are visible in each of the fluorescence images, which from left to right show the position of Alexa488-labeled T1, TAMRA-labeled T2, and Alexa647-labeled T3. Since a color overlay was not possible for this Proceeding, we have numbered the wires identically in each image to facilitate comparisons between them. These data show both that the fluorescently labeled target molecules hybridized selectively to their complementary PNA probe molecules on the wires (indicating retention of PNA function after assembly), and that the sequential nanowire assembly process led to the intended placement of different nanowire populations across the different electrode pairs.

Integration of Biofunctionalized Nanowires

An important challenge for the bottom-up assembly of nanoscaled building blocks is integration with the on-chip circuitry to form arrays with a high density of devices. To address this problem, biocompatible parameters were developed for use with standard photolithographic techniques to maintain the activity and selectivity of the sensitive PNA molecules functionalized to the nanowires. Following alignment as described above, photoresist was applied over top of the substrate and the aligned nanowires. The photoresist helps protect the PNA probe molecules bound to the central section of each nanowires while electrical contact is made to the nanowire ends. This is done by patterning windows over the ends of the aligned nanowires, into which Ti is evaporated, anchoring the aligned nanowires. Following evaporation, standard lift-off procedures were used, exposing the PNA probe molecules to their environment. To ensure that the PNA remains active and selective following nanowire anchoring, fluorescently labeled targets T1, T2 and T3 were again incubated in PBS buffer to hybridize to their complementary PNA probes functionalized to the aligned nanowires. Figure 3 shows the results of this experiment. As in Figure 2, where no post-assembly processing was performed, the fluorescently labeled targets T1, T2 and T3 successfully hybridized to their complementary PNA probe molecules showing that the PNA remains both active and selective following both the electrofluidic alignment and integration procedures. Some of what appear to be wires in the reflectivity image shown in panel A are in fact regions where nanowires have been lost during processing, leaving behind Ti-free “shadows”; these have not been numbered, since they cannot be expected to show up in any of the fluorescence images. Improved clamping procedures should prevent this in the future. Importantly, these data show that standard photolithographic techniques can be compatible with biorecognition probe molecules such as PNA, paving the way for biocompatible photolithographic methods that will be required to increase the (bio)functionality of integrated circuits.

CONCLUSIONS

Bottom-up assembly is a promising route to for the incorporation of different materials or molecules with lithographically defined features. The electrofluidic assembly method described here shows that it is possible to position nanowire building blocks to pre-determined locations on the chip surface. By using pre-functionalized nanowires and positioning them to predetermined locations on the chip surface, we hope to increase the multiplexing capabilities of chip-based sensors, which will have a tremendous impact in emergency situations, and increase the functionality of integrated chip devices revolutionizing the chemical and biomedical industries.

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Scheme 1.

(A) Selective positioning of pre-functionalized nanowires. Populations of peptide nucleic acid (PNA)/SiO₂ Rh nanowires were sequentially injected onto the chip surface while alternating voltages were applied to specific sets of guiding electrodes guiding the nanowires to the gap area. (B) Schematic illustrating the integration procedure of aligned nanowires. Following alignment a second photoresist was applied patterned exposing the ends of the nanowires while protecting the biomolecules functionalized to the middle segment. Ti was evaporated anchoring the exposed ends of the nanowires. Standard lift-off was used to remove excess Ti and to expose the biomolecules on the once protected middle segment of the nanowire to hybridize with their fluorescently labeled target DNA sequences.

Figure 1. Cross-sectional view of a single gap (3 μm) plotted as $\log_{10} \nabla E^2$; the arrows show the direction of the long range dielectrophoretic forces.

Figure 2.

Positioning of PNA coated Rh nanowires. (A) Bright field reflectance image showing the positions of all three populations of nanowires functionalized with P1, P2 and P3 probe PNA aligned in Gap 1, Gap 2 and Gap 3 respectively, the numbers were added to the images after acquisition to easily determine the locations of the aligned nanowires. (B, C, and D)

Fluorescence images showing that the fluorescently labeled targets T1, T2 and T3 hybridized selectively to the nanowires functionalized with P1, P2 and P3 aligned in image B (Alexa 488), image C (TAMRA) and image D (Alexa 647), respectively.

Figure 3.

(A) Bright field reflectance image showing the positions of all three populations of nanowires functionalized with P1, P2 and P3 probe PNA aligned in Gap 1, Gap 2 and Gap 3, respectively following integration with evaporated Ti (~300nm). (B, C, and D) Fluorescence images showing that the fluorescently labeled targets T1, T3 and T2 hybridized selectively to the nanowires following integration procedures with P1, P2 and P3 aligned in Gap 1 (Alexa 488), Gap 2 (TAMRA) and Gap 3 (Alexa 647) respectively.

Table 1

Name	Sequence 5'→3'	Description
P1	Thiol-TTTTTTTTTTGAGTAGTGTGGGTCGCGAA	HCV PNA Probe
P2	Thiol-TTTTTTTTTTCTCAATCTCGGGAATCTCAA	HBV PNA Probe
P3	Thiol-TTTTTTTTTTCCATCAATGAGGAAGCTGCA	HIV PNA Probe
T1	^a Alexa Fluor 488- TTCGCGACCCAACACTACTC	HCV DNA Target
T2	^b TAMRA-TTGAGATTCCCGAGATTGAG	HBV DNA Target
T3	^c Alexa Fluor 647-TGCAGCTTCCTCATTGATGG	HIV DNA Target

^a $\lambda_{ex}=482$ nm, $\lambda_{em}=517$ nm

^b $\lambda_{ex}=559$ nm, $\lambda_{em}=583$ nm

^c $\lambda_{ex}=650$ nm, $\lambda_{em}=670$ nm