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Crossbar assembly of antibody-functionalized peptide nanotubes via biomimetic molecular recognition‡

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Abstract: Previously, a large scale assembly of nanowires in a parallel array configuration has been demonstrated, and one type of nanowire could interconnect two electrodes in the high-wire density. However, to assemble nanowires into practical logic-gate configurations in integrated circuits, we need more than the parallel assembly of nanowires. For example, when the assembling nanowires are monopolar semiconductors, logic gates such as AND, OR and NOR are to be assembled necessarily from two types of semiconducting nanowires, *n*-type and *p*-type, and some of these nanowires must cross perpendicularly to form a crossbar geometry for the logical operation. In this paper, the crossbar assembly of antibody-functionalized peptide nanotubes was demonstrated by a new biomimetic bottom-up technique. Molecular recognition between antigens and antibodies enabled two types of the antibody-functionalized bionanotubes to place them onto targeted locations on substrates, where their complementary antigens were patterned. When two rectangular pads of antigens, human IgG and mouse IgG, were patterned perpendicularly on an Au substrate by nanolithography and then the antihuman IgG nanotubes and the antimouse IgG nanotubes were incubated on this substrate in solution, these bionanotubes were attached onto corresponding locations to form the crossbar configuration.

Keywords: peptide nanotube; antibody nanotube; crossbar; self-assembly; bionanotechnology; bottom-up; molecular recognition

INTRODUCTION

It is widely accepted that conventional top-down methods such as photolithography is about to hit the limit for further reduction of the patterning scale of electric components. Further miniaturization is necessary to pursue microelectronics with the increased speed and the complexity of device designs. Since various nanotubes and nanowires have been developed to possess superior and distinguished physical properties in the last decade, it is natural to seek new bottom-up technologies to assemble these superior nanoscale building blocks into the device configuration. However, addressing nanowires at precise locations for the interconnection between electrodes is a serious obstacle to overcome in the bottom-up fabrication. Recently various nanowires and nanotubes have been assembled on substrates by electric fields, microfluidics, drying effect controlled by surface topology, direct mechanical transfer and assembly on blown bubble films [1–10]. These techniques demonstrated that a large scale assembly of nanowires in a parallel array configuration is possible, and one type of nanowire could interconnect two electrodes in the high-wire density.

However, to assemble nanowires into practical logic-gate configurations in integrated circuits, we need more than the parallel assembly of nanowires. For example, when the assembling nanowires are monopolar semiconductors, logic gates such as AND, OR and NOR are necessary to be assembled from two types of semiconducting nanowires, *n*-type and *p*-type, and some of these nanowires must cross perpendicularly to form a crossbar geometry for the logical operation [5]. In general, this nonparallel assembly of nanowires is extremely difficult and there were only a few examples to demonstrate the crossbar assembly in the bottom-up approach. For example, the crossbar assembly of nanowires could be achieved by microfluidics in two steps; after the first array of nanowires was aligned in one direction, the second array of nanowires was assembled perpendicularly by changing the direction of flow [11]. Some of the nanowires can be crossed by the two-step fluidic method with no control of the crossing point of the nanowires, however, it cannot fabricate more complex geometry necessary for realistic logic gates. For example, how can we align three nanowires parallel in vertical direction and let one nanowire intercept these three nanowires at the middle point in horizontal direction when the type of these vertical nanowires is different from the type of the horizontal nanowire? One of the smart ways to achieve this complex crossbar assembly is to let nanowires recognize binding locations and directions and assemble them in a programmed manner in one step. In this manner,

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right nanowire elements can attach and interconnect desired electrodes to fabricate complex electric circuits.

Previously carbon nanotubes were aligned in a parallel array using a simple molecular hydrophobic interaction in the single step. This recognition-driven assembly method immobilized parallel carbon nanotubes in a large scale, however, this method may not be suitable to assemble multiple types of nanotubes simultaneously in different directions owing to the lack of complex-recognition function. Therefore, we need to apply molecular recognitions with more specificity to assemble nanowires in complex geometries. Recently, DNAs were applied to the building blocks with their recognition functions [12,13] and they successfully interconnected two electrodes [14]. In theory, the base pairing of DNA oligonucleotides can be used as a driving force to locate DNAs as nanowires at well-defined positions [15], however the crossbar alignment of DNAs has not been reported, which may be due to the cross-reactivity, the rigidity and the straightness of DNAs. On the other hand, in nature active recognition functions of proteins routinely address the biological nanomaterials to exact locations in cells with high specificity [16,17], and therefore, antibody is a better candidate to fulfill this task. Recently we applied the antibody–antigen recognition function to assembe antibody-functionalized peptide nanotubes at targeted locations on substrates in parallel arrays where their complementary proteins were patterned [18]. In our system, very rigid and straight peptide nanotubes were self-assembled from peptide monomers via three-dimensional hydrogen bonds, and we applied this nanotube as a template to produce the antibody nanotube by binding antibody on the template nanotube [19,20]. This antibody on the nanotube could anchor the nanotube onto the antigen-patterned areas selectively via molecular recognition to achieve their targeted placement on substrates. Due to their highly-specific molecular recognition, this fabrication method could also be applied to assemble two types of antibody nanotubes in different rows of parallel arrays where the corresponding antigens were patterned [21].

While multiple nanotubes could be placed in different positions by the antibody–antigen recognitions in parallel arrays by this biomimetic assembly method, the crossbar assembly of peptide nanotubes has not been accomplished yet. In this report, we applied the antibody–antigen recognition to assemble two types of peptide nanotubes in the crossbar geometry. Assembling one type of antibody nanotube in horizontal and the other in vertical directions is very difficult to achieve in one step, however, their molecular recognition toward antigens and patterned grooves on the substrate aligned them crossing perpendicularly. The peptide nanotubes were demonstrated to be active electric functional nanomaterials since their electric properties could be tunable by controlled metal/semiconductor coatings on mineralizing peptides of the nanotubes [22], and therefore the targeted assembly of these peptide nanotubes will enable one to produce complex electric circuits from the biomimetic bottom-up technique.

EXPERIMENTAL

Materials

α-Hydroxy ω-thiol terminated polyethylene oxide (thiol-PEG, Mw 650) was purchased from Polymer Source Inc. Human IgG, antihuman IgG, mouse IgG, antimouse IgG, bovine serum albumin (BSA), *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and 11-mercaptoundecanoic acid (MUA) were purchased from Sigma–Aldrich. All chemicals were used as received. Gold compact disk (CD) was purchased from Delkin Company. Si_3N_4 atomic force microscopy (AFM) tips (NSC15/Si3N4/Al BS) were purchased from MikroMasch.

3D Assembly of Peptide Nanotubes on Patterned Gold Substrate

Peptide nanotubes were self-assembled from bis(*N*-α-amidoglycylglycine)-1,7-heptane dicarboxylate by the previously published method [23]. In this experiment, the nanotubes with diameter of 100 nm were used after they were extracted using the size-separation column [24]. In order to fabricate antibody nanotubes, template nanotubes were coated with antihuman IgG or antimouse IgG. After the template nanotubes were centrifuged, a 1 ml solution of the nanotubes (10 mM) was incubated with a 1 ml solution of these antibodies in a pH 7.2 phosphate buffer (50 μg/ml). After 48 h, the antibody was absorbed noncovalently on the template nanotubes to form the antibody nanotubes. The resulting antibody nanotubes were washed with nanopore water and centrifuged twice to remove unbound antibodies before mixing with the antigen-patterned substrates. A groove-patterned Au substrate was obtained by removing the polycarbonate layer covered on commercial Au CD (Delkin). To remove this layer, concentrated nitric acid (12 M) was dropped on the backside of Au CD. Five minutes later, the Au substrate was rinsed thoroughly with deionized water and dried in N_2 atmosphere to complete the cleaning process. To coat the Au substrate with the protective layer, this Au substrate was immersed in thiol-PEG/ethanol solution (1 mg/ml) at room temperature for 24 h for the formation of the self-assembled monolayer (SAM) of PEG. The thiol-PEG SAM was used as the protective layer because it has the strong resistance for nonspecific protein binding [25,26]. Then the line was shaved on the PEG SAM in the groove by a Si_3N_4 tip of AFM (MFP 3D, Asylum research) with the contact force 3 μN and the sweeping speed 1 μm/s of the AFM tip. The dimension of line, 2.5 μm × 60 nm × 5 nm, was drawn by the MFP 3D nanolithography software. The resulting substrate was immersed in MUA/ethanol solution (1 mg/ml) overnight at room temperature to attach MUA molecules onto the shaved line via thiol–Au interaction. After rinsing with ethanol and drying, the resulting substrate was incubated in the aqueous solution of NHS (25 mg/ml) and EDAC (25 mg/ml) for 30 min [27]. Then the substrate was rinsed with deionized water

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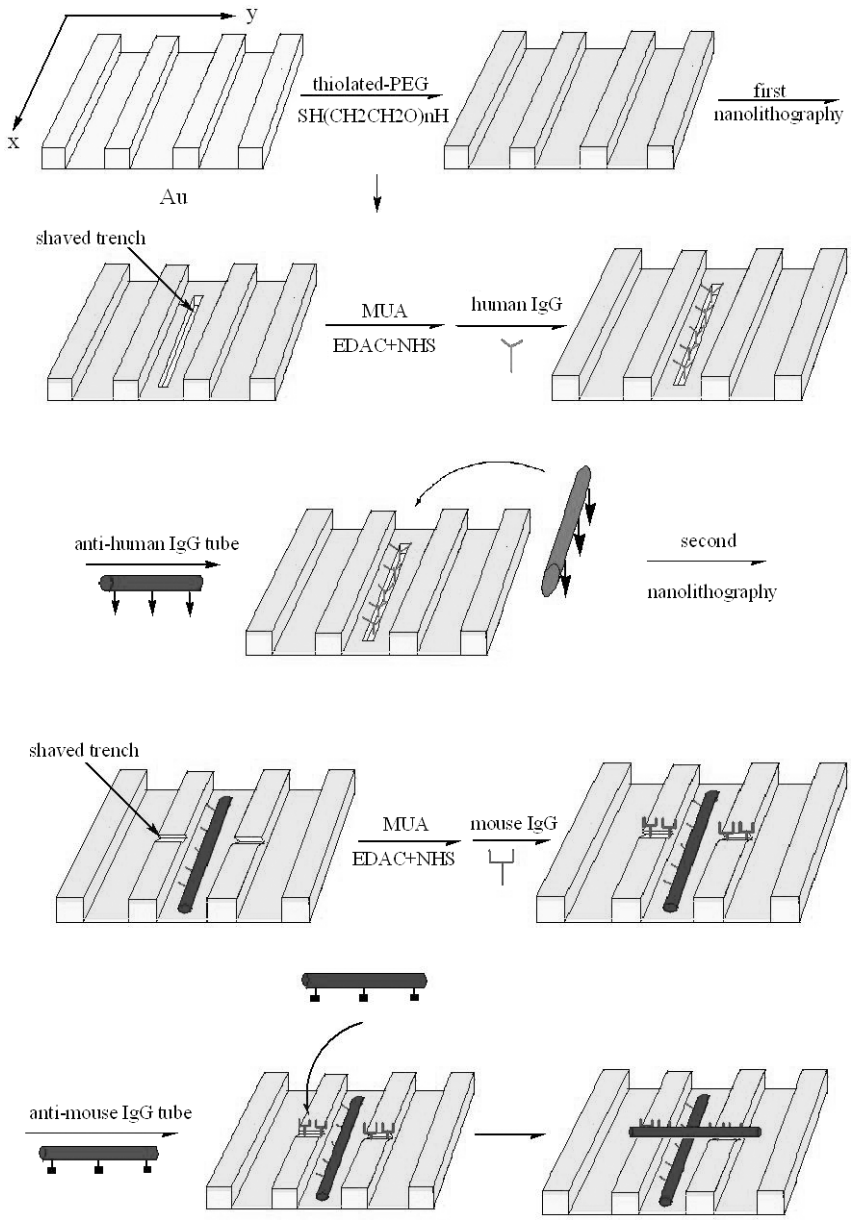
thoroughly and immersed with human IgG in pH 7.4 PBS buffer solution (1 mg/ml) for 12 h at 4 °C. Human IgG was bound on the shaved line covalently via the condensation reaction between amino group of IgG and carboxylic group of MUA on the substrate. After the IgG-patterned substrate was rinsed with nanopore water and dried in N_2 atmosphere, the antihuman IgG-coated nanotubes were incubated in the pH 8 buffer solution containing the resulting substrate for 24 h at 4 °C. Next we shaved the line in the *y*-direction on the top level of the Au substrate by the AFM tip to cross the second nanotube perpendicularly on the first antihuman IgG nanotube. Mouse IgG was deposited on the newly shaved line covalently by the same method by which we patterned the human IgG line. The resulting substrate was rinsed with nanopore water thoroughly. When the antimouse IgG-coated nanotube was incubated in the pH 8 buffer solution containing the resulting substrate for 24 h at 4 °C, the antimouse IgG nanotube attached on the mouse IgG line on the top level of Au substrate to complete the crossbar fabrication from these nanotubes. This fabrication procedure is summarized in Scheme 1.

RESULTS AND DISCUSSION

In this work, we used peptide nanotubes as templates to decorate them with selected antibodies. These peptide nanotubes were self-assembled from the peptide monomer, bis(*N*-α-amido-glycylglycine)-1,7-heptane dicarboxylate, by three-dimensional hydrogen bonds between amide and carbonyl groups, and amides on the surfaces of nanotubes that were not involved in the tube formation could bind antibodies to produce antibody nanotubes by using these



Scheme 1 The schematic representation of biological assembly of antibody-coated bionanotubes into the crossbar configuration.

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peptide nanotubes as scaffolds [19]. This antibody functionalization on the nanotube surfaces allowed the biomolecular recognition-driven nanotube alignment on the antigen-patterned substrate as shown in Scheme 1. In this experiment, we used the peptide nanotubes in the diameter of 100 nm, extracted by size-separation column [24].

To assemble these nanotubes into the crossbar geometry, we applied substrates that have grooves, as shown in Figure 1. As shown in Scheme 1, one type of nanotube was attached at the bottom of the groove in the horizontal direction while the other type of nanotube was placed on the top level of the substrate in the vertical direction via molecular recognition (Scheme 1). The gap created by the groove gave special clearance for these nanotubes to cross each other. For this crossbar assembly of nanotubes, a commercial Au CD disk was used as the groove-patterned substrate. Figure 1(A) shows AFM image of the gold substrate (Delkin), and the darker and brighter areas correspond to the grooves and the top level of the CD substrate respectively. From its sectional analysis (Figure 1(B)) the width of the groove is 600 nm and the depth of concave is 120 nm. This depth is large enough to accommodate the antihuman IgG-coated nanotube with the diameter of 100 nm inside the groove.

In order to immobilize the antihuman IgG nanotube in the groove, we patterned human IgG on the bottom of the groove by nanolithography [28]. The patterning of the antigen was achieved in two steps; shaving protective layer, thiol-PEG SAMs, on Au substrate with the Si_3N_4 tip of AFM to expose Au surfaces and then attaching the antigen on the shaved area covalently via the amine (antigen)–carboxylic acid (MUA) conjugation. Figure 2(A) shows the AFM image of the shaved substrate. The fainter line which appeared in a darker contrast in the middle of each groove is the shaved line, however these lines could not clearly be imaged in Figure 2(A) and (C) owing to the fine dimension of the line; the depth and the width of the line was about 5 and 60 nm, respectively. After MUA was attached on the shaved areas via thiol–Au interaction and then human IgG was covalently immobilized with MUA via the NHS–EDAC coupling reaction, the originally darkened lines in Figure 2(A) turned to much brighter lines in the grooves as seen in Figure 2(B). The sectional analysis of these lines before and after the IgG incubation further confirmed that the immobilization of human IgG in the groove was successful; Figure 2(C) showed that the height of the line on the groove is −5 nm, however it is increased to +10 nm after the immobilization of human IgG, as shown in Figure 2(D).

When antihuman IgG-coated nanotubes were incubated on the human IgG-patterned Au substrate in solution, these antibody nanotubes selectively attached onto the human IgG-patterned areas on the Au substrates (Figure 3(A)). This selective attachment on the antigen areas indicates that this nanotube assembly is driven by the antibody–antigen interaction. Next, in order to assemble the antimouse IgG-coated nanotube perpendicularly to the antihuman IgG nanotube on the Au substrate, we shaved a new line of mouse IgG along the *y*-direction on the top level of the substrate (black arrows in Figure 3(B)). Here, we applied the same method used for the patterning of the human IgG line; first the MUA immobilization on the shaved areas, then the covalent attachment of mouse IgG on the MUA lines via the NHS–EDAC coupling reaction. After MUA was attached on the shaved areas via thiol–Au interaction and the mouse IgG was covalently immobilized with MUA, the much brighter lines of the mouse IgG appeared on the top level of Au substrate in AFM image in Figure 3(C). The section analysis of this mouse IgG line (Figure 3(D)) shows that the height of this line is consistent with the height of the human IgG line in the groove. Finally, when the anti-mouse IgG-coated nanotube was incubated on the resulting substrate in the solution for one day, this nanotube was attached on the mouse IgG line and this attachment formed the crossbar configuration of the nanotubes, as shown in Figure 3(E). Here we fabricated the crossbar geometry of nanotubes in the two-step process since that way

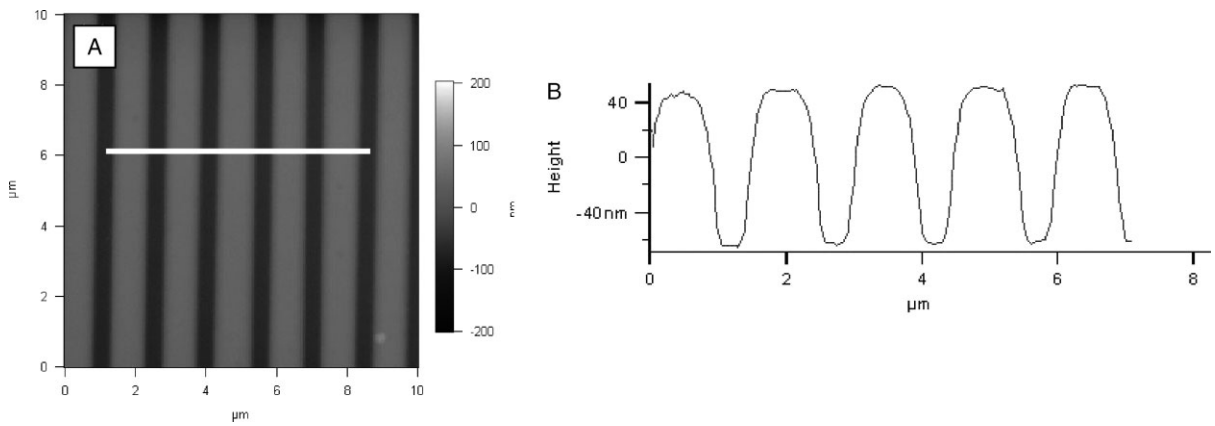

Figure 1 (A) AFM image of the bare Au substrate with grooves. (B) The sectional analysis at a white line drawn in (A).

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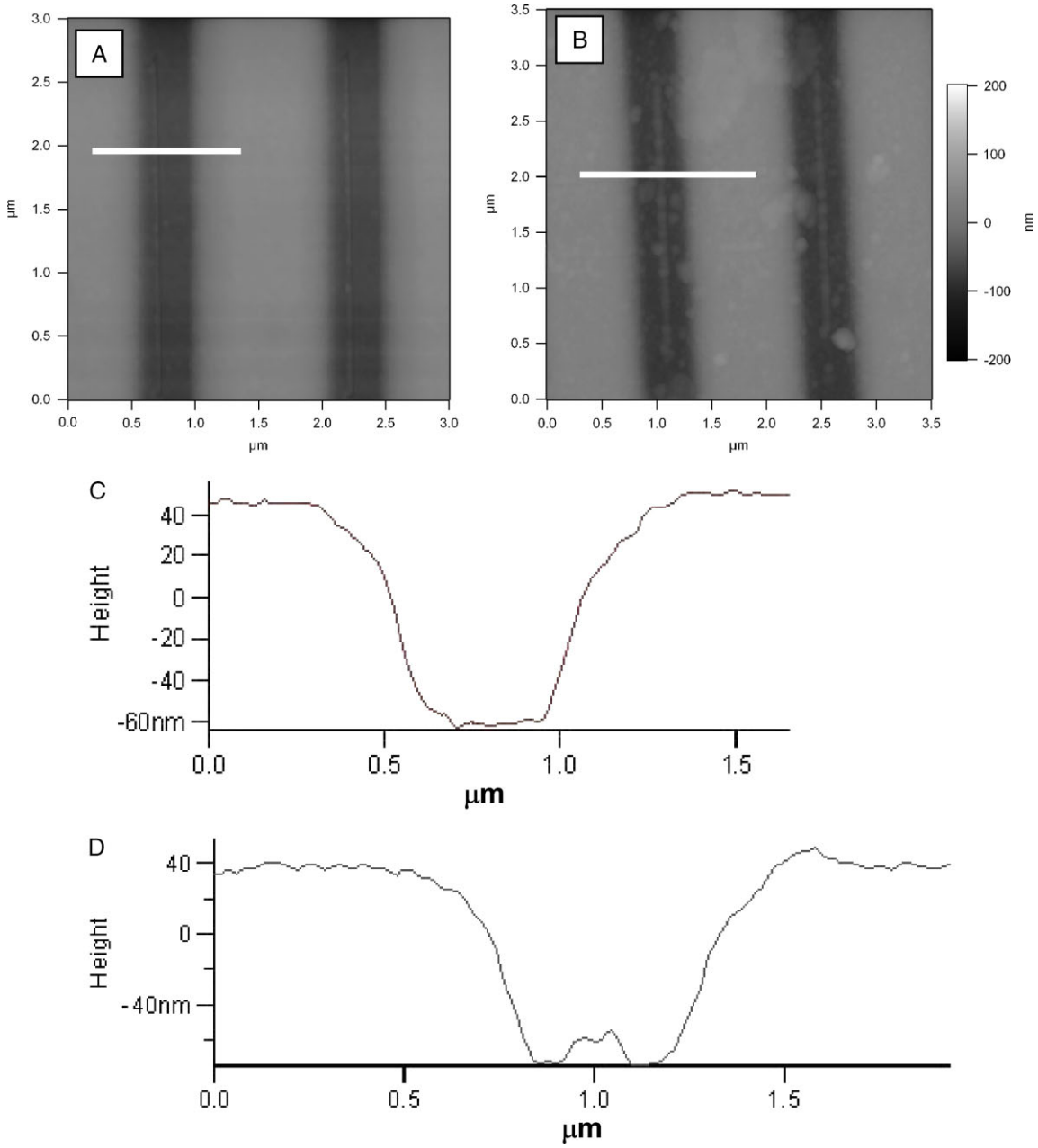

Figure 2 (A) AFM image of the Au substrate where the trench was shaved by AFM tips in the groove. (B) AFM image of the Au substrate where human-IgG was immobilized in the trench. (C) The sectional analysis at a white line drawn in (A). (D) The sectional analysis at a white line drawn in (B). This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

we could clearly show that two antibody nanotubes could be placed in different locations and directions in a controlled manner; the antihuman IgG nanotube was aligned along the groove direction and the anti-mouse IgG nanotube was immobilized perpendicular to the groove direction. However, it is totally feasible to attach both antihuman and antimouse IgG-coated nanotubes simultaneously in one step on the Au substrate where the human IgG line and the mouse IgG line are already patterned by nanolithography before the nanotube immobilization.

It should be noted that the yield of the nanotube attachment along the groove direction was almost 100% while the attachment (%) of nanotubes on the trenches perpendicular to groove direction was much lower, about 20%. Recently, there were reports showing that the capillary force could have a significant effect to drive nanowires aligning parallel to the groove direction [7,29]. In the case of our nanotube alignment, the nanotube needs to resist against this capillary force to be immobilized perpendicular to the groove. While this capillary force probably diminishes the yield of the

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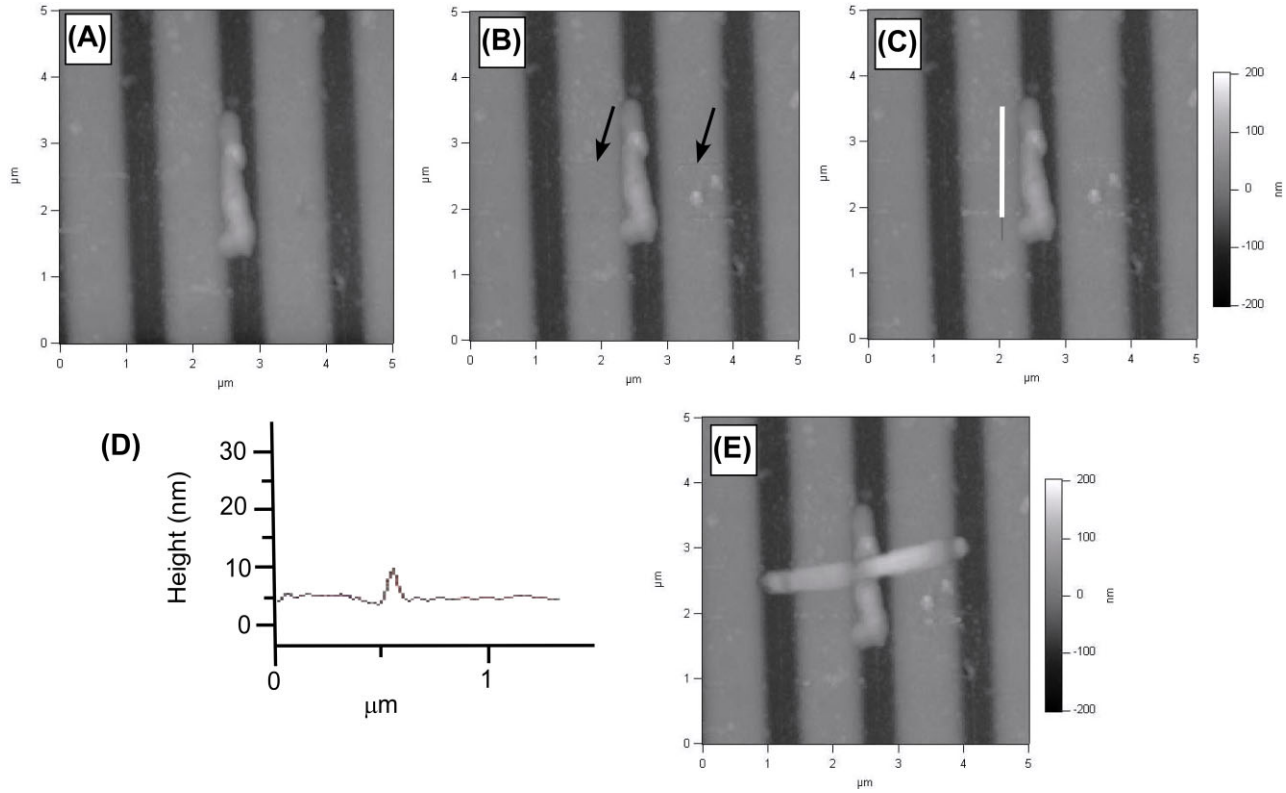

Figure 3 (A) AFM image of the antihuman IgG nanotube attached on the human IgG line on Au substrate. (B) AFM image of the Au substrate (A) where the trench was shaved by AFM tips perpendicular to the groove. Black arrows show the position of the shaved line. (C) AFM image of the Au substrate (B) where mouse IgG was immobilized in the trench. (D) The sectional analysis at a white line drawn in (C). (E) AFM image of the Au substrate (D) where the antimouse IgG nanotube attached on the mouse IgG line. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

perpendicular attachment of the nanotube, 20% of the nanotubes should not be immobilized in the direction normal to the groove without the antibody–antigen interaction. In other words, the antibody–antigen interaction could drive the nanotube to align against the capillary force. If the stronger molecular recognition such as the avidin–biotin system is applied for the perpendicular alignment of the nanotube, the yield of the perpendicular attachment could be increased drastically.

CONCLUSION

Antibody–antigen molecular recognitions enabled two types of antibody-functionalized bionanotubes to place them onto specific locations on substrates, where their complementary antigens were patterned. When two rectangular pads of antigens, human IgG and mouse IgG, were patterned perpendicularly on an Au substrate by nanolithography and then the antihuman IgG nanotube and the antimouse IgG nanotube were incubated on the Au substrate in solution, these bionanotubes were attached onto corresponding locations to form the crossbar configuration. This biomimetic bottom-up fabrication method is robust and practical, and the crossbar assembly can be expanded to more complex logic gates such as AND, NOR and OR by integrating multiple crossbar nanotubes.

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