Facile fabrication of networked patterns and their superior application to realize the virus immobilized networked pattern circuit†

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A facile route to fabricate a protein-immobilized network pattern circuit for rapid and highly sensitive diagnosis was developed *via* the evaporation directed impromptu patterning method and selective avian influenza virus (AIV) immobilization. The response to the 10 fg mL^{-1} anti-AI antibody demonstrates that this easy and simple circuit has about 1000 times higher sensitivity compared to those of conventional approaches.

Avian Influenza Virus (AIV) is the cause of one of the highly contagious diseases attracting global attention,[1,2] thus the early recognition and identification of AIV are very important in the prevention of an avian influenza outbreak. Recently, several methods including an enzyme-linked immunosorbent assay[3] and a polymerase chain reaction[4] have been developed for the detection of AIV. However, the current important issue is how to realize a new method for the rapid and highly sensitive diagnosis of the target virus.

Recently, carbon nanotubes (CNTs) have attracted great attention on account of their unique properties such as their high surface areas and fast electron transfer abilities.[5–7] Furthermore, it has been reported that patterned CNTs can be used to immobilize many kinds of viruses and to transfer electrons to deliver signals.[8,9] However, these patterning methods are still suffering from complex and expensive processes to pattern CNTs with a high precision and to obtain highly sensitive electrical signals.[10,11] Moreover, a low biocompatibility of CNTs with viruses resulting in the reduction of the biological activity is now a crucial bottleneck to realizing a rapid diagnosis circuit for the target virus.

In this work, we report a new network patterning method directed by evaporation of a hybrid solution. Hereafter we refer to this method as the evaporation directed impromptu patterning (eDIP) process. By using this method, first, the gold–CNT hybrid patterns can be prepared promptly and easily without any surface pretreatment or functionalization *via* a simple two-step process: (1) application and evaporation of a liquid solution that incorporates polyvinylpyrrolidone (PVP) and a source material over a heated substrate; as the evaporation goes on, materials form a networked pattern in a targeted shape, and (2) removal of residual PVP to leave only gold–CNT hybrid patterns on the substrate. Then, the protein immobilization was enabled on the surface of a patterned gold–CNT hybrid by the use of a gold binding polypeptide (GBP)[11,12] fused with an AIV surface antigen (AIa). The genetically engineered fusion proteins allowed for a direct and easy immobilization requiring merely one step to immobilize the recognition element and to allow successful binding of the targets onto the gold surface. Finally, with the above three steps of pattern and immobilization, we fabricated an AIV-immobilized electric circuit on the designed platinum electrode and evaluated its electrical properties.

To prepare for the eDIP process, first, the gold–CNT hybrid solution was prepared by solubilizing gold–CNT hybrids in ethanol after modifying the entire surface of a gold–CNT hybrid with PVP in 1,5-pentanediol at a high temperature to eliminate the hydrophobic interface between the tube and the hydrophilic medium.[14] In this solution, the ethanol molecules control the movement of gold–CNT hybrids in the solution. After making this stock solution, the eDIP process can be started by transferring a drop of the gold–CNT hybrid solution to the evaporation guide on a heated silicon substrate at 60 °C.

Fig. 1a illustrates the eDIP process using a line-based evaporation guide and a gold–CNT hybrid stock solution with a concentration of 0.08 g L^{-1}. In this process, the evaporation guide acts as a shape-template in the patterning process by transfixing the droplet in its shape. An evaporation process generates an outward flow of the solution and then precipitates a line of gold–CNT hybrids along the air and liquid solution contact line. As time goes by, more gold–CNT hybrids can be stacked while the volume of the solution droplet is decreased by evaporation. When the surface tension is increased to the critical value larger than the pinning force of the precipitated hybrids for the solution, the droplet can be reduced in size and then the evaporation can start again. The repeated precipitation and contraction of the droplet in the self-pinning process result in repeated patterns. The scanning electron microscope (SEM) images of gold–CNT hybrid patterns in the eDIP process are shown in Fig. 1b–f, showing various types of patterns such as line, circle and grid type patterns, which are made by using stick and dot type evaporation guides. The detailed transmission electron microscope (TEM) image of a gold–CNT hybrid is also shown in Fig. 1g.

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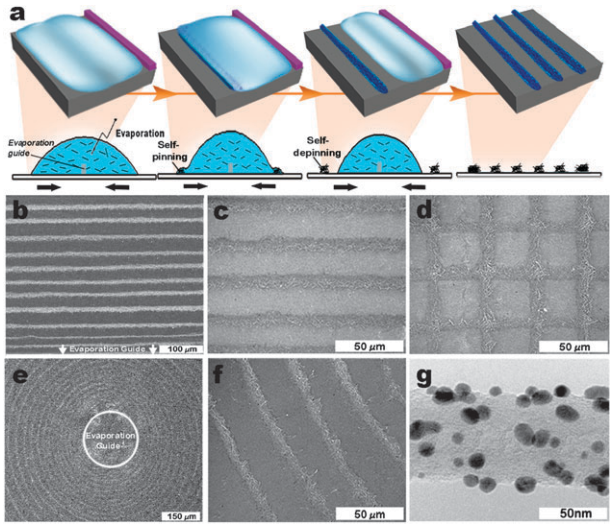

Fig. 1 Illustration and results of the eDIP process. (a) The schematic diagram to show the eDIP process, (b) SEM image for line-type patterns of gold–CNT hybrid networks, (c) magnified SEM image for line-type patterns, (d) SEM image for grid-type patterns, (e) SEM image for circle-type patterns, (f) magnified SEM image for circle-type patterns, and (g) TEM image for a gold–CNT hybrid.

In this eDIP patterning method, the width of a patterned network was linearly proportional to the concentration of a stock solution (Fig. 2a–d). The best pattern quality, however, was found to be in the concentration range of 0.04 g L^{-1} to 0.12 g L^{-1}. In a higher concentration, all of the hybrids were not controlled making a random distribution in the intra-space of patterns. In a lower concentration, the lack of gold–CNT hybrids made a pinning force too weak to form the network pattern. The dependence of the evaporation rate in the eDIP method was also investigated by changing the substrate temperature. The optimized temperature was 60 °C to obtain a high-quality pattern (Fig. 2e–g) in the ethanol-hybrid system (0.08 g L^{-1}). At a lower temperature, the lack of the outward flow in the droplet was found not to make the dense gold–CNT hybrid network. Meanwhile, at a higher temperature, the rapid evaporation was determined to disperse gold–CNT hybrids on the whole area of the droplet. It should be also noted that those optimal ranges for concentration and temperature could be changed on using different solvents.

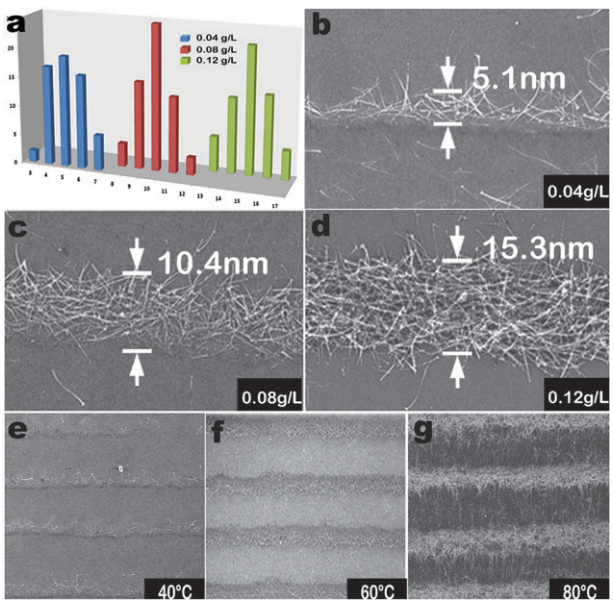

Fig. 2 The effects of the stock solution concentration and evaporation rate. (a) The width distribution of gold–CNT hybrid patterns in different concentrations of the solution, (b)–(d) the SEM figures for patterned networks to show the width variation on the different concentrations of the stock solution, and (e)–(f) the SEM figures for network patterns to show the effect of the evaporation rate.

Also, to illustrate the potential of this approach in realizing protein immobilized nanopatterns and for the future extension to fabricate rapid biosensors, we fabricated first a protein-immobilized network on gold–CNT hybrid patterns (Fig. 3). The protein immobilization was enabled by the use of a GBP-AIa fusion polypeptide. About 10–15% of a non-specific binding could occur and the biomolecular affinity by the specific binding using GBP was above 95% because the binding of the GBP sequence onto the gold surface was independent of the thiol linkage.[12] The strong affinity between the GBP and the gold surface helps expose sensing molecules outward to react with their targets.[11–13] On these findings and application of the eDIP method for gold–CNT hybrids, we realized biomolecule-immobilized patterns through the selective immobilization of a GBP-AIa fusion protein onto the gold–CNT hybrid patterns using an incubation process (Fig. 3a). We also bound the new Cy3-labeled anti-AI antibody to the GBP-AIa on the gold–CNT hybrid patterns. Fig. 3b–d show the formation of spatially resolved patterns of Cy3-labeled anti-AI antibodies bound to the immobilized GBP-AIa. In order to explore the specificity of GBP-AIa binding onto the patterned CNT hybrid line, bovine serum albumin (BSA, 100 μg mL^{-1}) was incubated with the patterned CNT hybrid lines followed by the Cy3-labeled

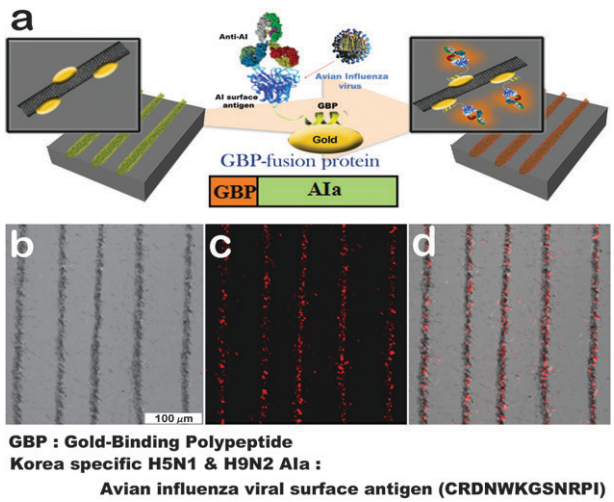

Fig. 3 Illustration and results for the immobilization process for the GBP-AIa fusion protein onto the gold–CNT hybrid patterns. (a) Illustration of selective immobilization process, (b) optical image for CNT patterns after GBP-AIa immobilization, (c) fluorescence image after the sequential binding of GBP-AIa and Cy3-labeled anti-AI onto the gold–CNT hybrid line pattern, and (d) merged image of Fig. 3b and c.

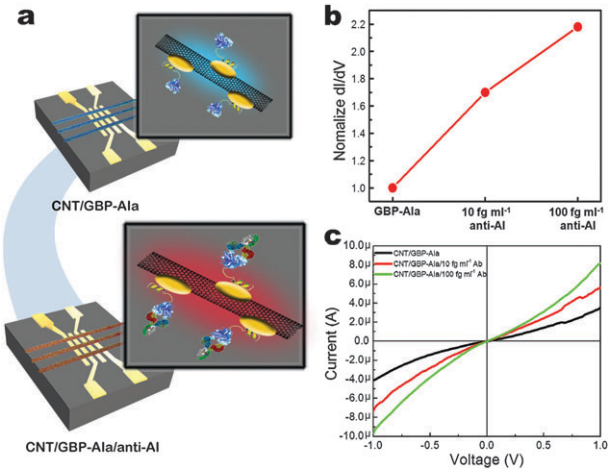

Fig. 4 Electrical properties of the gold–CNT hybrid network by the GBP-AIa fusion protein and anti-AIa antibody interaction. (a) Illustration of the circuit preparation, (b) normalized dI/dV curve by the anti-AI binding, and (c) I–V characteristic curves by the anti-AI binding.

anti-AI antibody as a negative control. In this case, there was no significant fluorescence (data not shown). These results demonstrate that biomolecules such as nucleic acids and proteins can be successfully immobilized onto the eDIP-patterned nanomaterial.

The high reproducibility and selectivity of the patterned proteins can be further exploited to develop a rapid and direct real-time electronic circuit. We fabricated a gold–CNT hybrid network pattern on the designed platinum electrode to investigate the change of electronic signal caused by specific binding between AIa and anti-AI antibodies (Fig. 4a). Fig. 4b and c show the electrical signal variations to the GBP-AIa network attributed to the anti-AI binding. It shows that a GBP-AIa network responds to the 10 fg mL^{-1} anti-AI which is about 1000 times higher compared to that by conventional approaches.[15] The current is also increased further with a large amount of the anti-AI (Fig. 4b) and this phenomenon was reproduced in repeated experiments. The current increase might be attributed to the binding event of anti-AI having the negative overall charge in the solution,[16] which is different from the activated conduction by the electron hopping mechanism in other IV results.[17]

In summary, a rapid diagnostic method for AIV was developed *via* the combination of new strategies providing both the fast and easy nanomaterial pattern and the effective immobilization of recognition elements on the surface of the patterned hybrid nanomaterials. Our eDIP method has been demonstrated to allow the formation of various patterns using hybrid nanomaterials into desired alignments *via* a simple two-step process: (1) application and evaporation of a liquid solution that incorporates polyvinylpyrrolidone (PVP) and a source material over a heated substrate; as the evaporation goes on, materials form a networked pattern in a targeted evaporation guide shape on the substrate, and (2) removal of residual PVP to leave only gold–CNT hybrid patterns on the substrate. The effects of the stock solution concentration and evaporation rate on the properties and quality of the patterned network were also investigated. The use of the GBP covalently linked to the surface antigen of an AIV particle enabled AIV immobilization on the gold–CNT hybrid patterns. A GBP-AIa fusion protein patterned network was fabricated *via* the combination of the eDIP method and the GBP-AIa incubation process. The evaluation of electrical properties of the AIV-immobilized network pattern showed that it responds to 10 fg mL^{-1} anti-AI antibody whose sensitivity is about 1000 times higher than those of conventional methods. Our results demonstrate that the integrated approach of our eDIP process and the GBP-mediated biomolecule immobilization process could allow the development of a very advanced bio-circuit due to the shortened process time, low cost, and high biocompatibility and sensitivity.

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