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Label-Free Pathogen Detection with Sensor Chips Assembled from Peptide Nanotubes**

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Advances in bottom-up nanofabrication have led to the use of various nanomaterials with superior physical properties as building blocks for the assembly of devices with complex configurations.[1–11] Peptide nanotubes are useful nanomaterial building blocks that have been used to construct a variety of device geometries, as their self-assembly is robust, and locations for their immobilization on substrates can be targeted by biomolecular recognition.[12,13] However, one of the unexplored areas in devices based on peptide nanotubes is the lab-on-chip sensor. Herein, we examine the feasibility of assembling peptide-nanotube sensors with a simple chip geometry (Figure 1) for the electrical detection of viruses with an extremely low detection limit.

Pathogen sensors based on peptide nanotubes have a few distinctive features. First, peptide nanotubes can incorporate any antibodies for viruses without destroying the recognition function, so that targeted viruses are trapped selectively on the peptide-nanotube surface with strong affinity. Second, the shape and dimensions of a peptide nanotube are ideal for detecting the binding event with a virus, because they match the electric-field line distribution between a pair of electrodes (see Figure 2c), which maximizes the impedance signal upon virus binding. This effect explains the low detection limit for viruses. Finally, dielectric peptide nanotubes can be aligned easily between electrodes by dielectrophoresis; in this way a wide variety of virus-sensing probes can be assembled on chips. These features make peptide-nanotube-based devices exceptionally sensitive and versatile sensors.

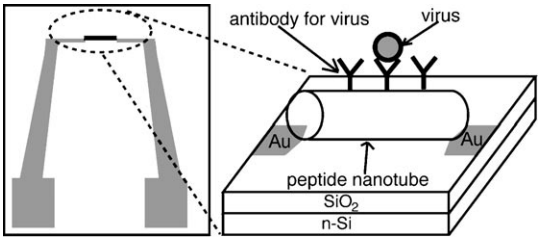

Figure 1. Design of the pathogen-sensor platform assembled from peptide nanotubes. The peptide nanotube incorporates virus-recognition elements on the surface. n-Si = n-type silicon.

The on-chip pathogen-sensing platform (Figure 1) consists of a pair of electrodes separated by a micrometric gap that is bridged by peptide nanotubes. In this platform configuration, when a sample was injected onto the chip, the binding event between the virus in the sample and its antibody on the peptide nanotube was detected by a capacitance change between the electrodes. Capacitance and impedance measurements were used previously to detect micron-sized cells,[14,15] and it was also demonstrated that a nanoscale capacitance probe could be used to characterize the composition of polymers and semiconductors.[16–19] However, capacitance measurements have not yet been used extensively in the development of pathogen nanosensors. Typically, a direct-current (DC) conductive probe has been used to detect small biological molecules and viruses on semiconductor nanowire-bridged sensing platforms.[20–23] However, in this study we applied an alternating-current (AC) capacitance probe for virus detection owing to the nonconductive nature of the nanotubes. With the AC probe, the contact between the peptide nanotube and the electrodes is not as influential to the signal as with the conventional DC conductive probe; therefore, the accuracy of detection was expected to be higher.

The peptide nanotubes used for sensor-chip fabrication were prepared by self-assembly from bolaamphiphilic peptide monomers and then coated with antibodies in a simple incubation process.[24–26] To assemble them onto the device platform shown in Figure 1, the peptide nanotubes were directed to the gap between a pair of electrodes by positive dielectrophoresis (Figure 2a,b).[27–29] Pathogen detection in this device configuration takes advantage of the difference in the dielectric properties of viral particles and water molecules. It is well established that viral particles have lower dielectric constants than water, in accordance with their core-shell structure.[30] Hence, the binding of viruses to the peptide

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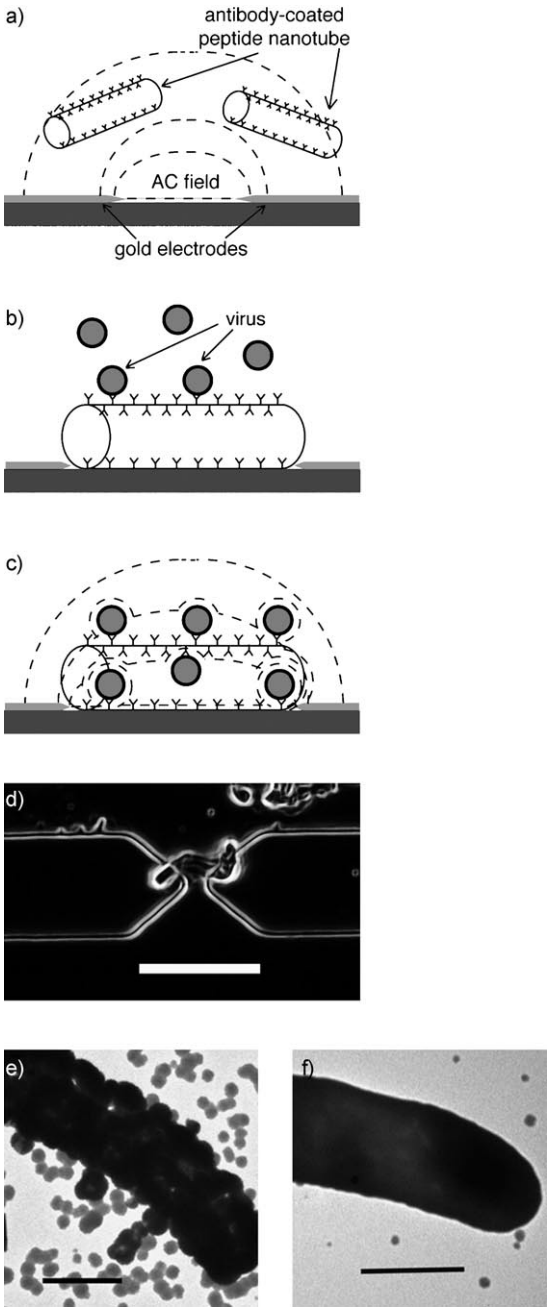

Figure 2. Fabrication of the peptide-nanotube sensor platform and its label-free electric detection of viruses. a) Peptide nanotubes are coated with an antibody against the targeted virus and injected onto the electrode-patterned platform while an AC field is applied; the peptide nanotubes are trapped in the gap between adjacent electrodes by positive dielectrophoresis. b) Peptide nanotubes bridging the electrodes bind viruses through biomolecular recognition. c) The presence of dielectric bioparticles in the region in which the electric field has the maximum strength results in a decrease of the capacitance between the electrodes. d) Optical image of a peptide nanotube located at the gap between electrodes as a result of positive dielectrophoresis (10 Hz, AC peak-to-peak potential: 5 V); scale bar: 10 μm. e) TEM image of the anti-HSV-coated peptide nanotube after incubation in the solution containing HSV-2; scale bar: 500 nm. f) TEM image of the peptide nanotube coated with mouse IgG after incubation in the solution containing HSV-2; scale bar: 500 nm.

nanotubes is expected to decrease the permittivity of the medium surrounding the nanotube and consequently decrease the capacitance between the electrodes (Figure 2c). We were able to detect the binding of nanoscale viral particles to the nanotube on the basis of the capacitance change, as the peptide nanotube was placed at the gap between the electrodes where the path of the currents was shortest and the electric field was strongest. In this device configuration, the major role of the nanotubes is to concentrate targeted viruses selectively by molecular recognition at this location. At this position, impedimetric detection with the electrodes is most sensitive. The very good match between the dimensions of the peptide nanotubes and the electric-field line distribution enhances the capacitance signals. To demonstrate the proof-of-concept of this fabrication process and detection scheme, we examined the label-free detection of herpes simplex virus type 2 (HSV-2) by the sensor assembled from peptide nanotubes.

Figure 2a–c illustrates the processes of platform fabrication with peptide nanotubes and virus detection. In steps a and b, the peptide nanotubes are directed to the gap between adjacent gold electrodes by positive dielectrophoresis. (The procedure for electrode fabrication is described in the Supporting Information). Dielectrophoresis refers to the force on polarizable particles in a spatially nonuniform electric field; when particles are pulled towards points of electric-field maxima, the phenomenon is termed positive dielectrophoresis.[31,32] Figure 2d shows a representative image of the electrodes on the platform after an AC field with a frequency of 10 Hz and a peak-to-peak potential of 5 V was applied in the presence of a solution of the peptide nanotubes. In this image, a peptide nanotube is located in the gap between the electrodes at the position at which the electric field has the maximum strength, as expected from the process of positive dielectrophoresis. This entrapment of the peptide nanotube took place at low frequency, in good agreement with previous reports on the dielectrophoretic focusing of other dielectric materials, such as cells, and indicates that an interfacial polarization process is responsible for this phenomenon.[33,34]

In step b in Figure 2, HSV-2 is bound to the anti-HSV-2 antibodies on the nanotube. Figure 2e shows the coated nanotube that results from incubation with a solution of HSV-2. The viral particles were packed closely on the nanotube through biomolecular recognition. In a control experiment, mouse immunoglobulin G (IgG) was coated on the nanotube instead of anti-HSV-2. No interaction between the IgG-coated nanotube and HSV-2 particles was observed (Figure 2f), as mouse IgG does not specifically recognize HSV-2. This control experiment indicates that the nanotube coated with anti-HSV-2 is capable of anchoring HSV-2 selectively without noticeable nonspecific binding. Later, this mouse IgG-coated nanotube was used as a blank to rectify the net capacitance values of HSV-2. The binding of HSV-2 to the peptide nanotube also confirms that the nanotube was coated fully with anti-HSV-2 upon incubation overnight.[35]

After the peptide-nanotube sensors had been immersed in a bovine serum albumin (BSA)–phosphate-buffered saline (PBS) solution for 1 h to prevent nonspecific adsorption of

the viruses to the chip surface, impedance spectra for HSV-2 were recorded with each peptide-nanotube immunosensor in glycine buffer by applying an AC field with a peak-to-peak potential of 10 mV at frequencies from 10 KHz to 1 MHz. Figure 3a shows the impedance spectra obtained for the sensor with the peptide nanotube coated with anti-HSV-2 before and after incubation with HSV-2 (10^4 pfu mL^{-1}; pfu = plaque-forming unit). The inset shows a simplified circuit model for the nanotube-based sensor in aqueous solution. This model was used to obtain capacitance values from measured impedance data (details for this circuit model are described in Supporting Information). At high frequencies, the value of the impedance modulus (circles) depended on the frequency, and the phase angle (triangles) was close to −90°. Thus, the system showed the typical behavior of a capacitor. The recognition of HSV-2 on the peptide nanotube increased the impedance in this region, as expected from a decrease in capacitance due to the inclusion of low-permittivity materials at the gap between the electrodes. This outcome is consistent with the proposed transduction mechanism of the peptide-nanotube-modified electrodes and shows the potential to apply the nanotube platform as a virus sensor.

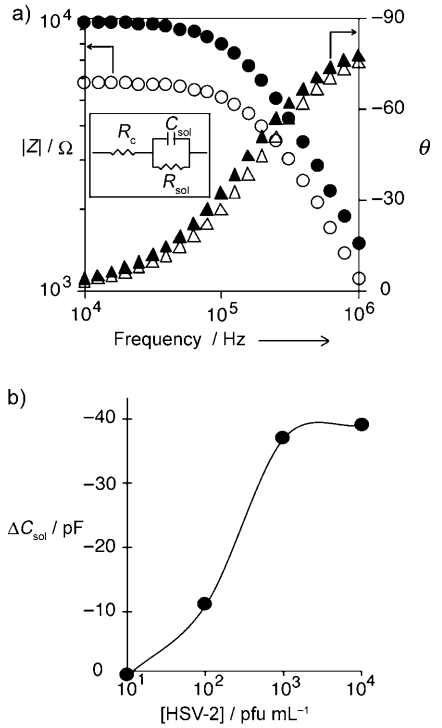

Figure 3. a) Impedance spectra of a sensing platform assembled from peptide nanotubes. ○: impedance-modulus values before incubation with HSV-2; ●: impedance-modulus values after incubation with HSV-2; △: phase angles before incubation with HSV-2; ▲: phase angles after incubation with HSV-2. Impedance data was fitted to the electric circuit in the inset. R_c is the resistance of the contacts, R_{sol} is the resistance of the solution, and C_{sol} is the capacitance of the solution, which is proportional to the permittivity of the solution. b) Correlation between capacitance and the concentration of HSV-2 in the sample solution. ΔC_{sol} is the corrected capacitance value after subtraction of the blank measurement.

To evaluate this peptide-nanotube platform for HSV-2 detection, we plotted the capacitance against the concentration of HSV-2 (Figure 3b). The capacitance value for each viral concentration was obtained by fitting the impedance data to the circuit shown in the inset of Figure 3a with the Zview2 software (for details on the equivalent circuit and the impedimetric analysis, see the Supporting Information).[36] For each point on the response curve of the sensor, the capacitance value was corrected by subtracting the value measured with the control nanotube platform, in which the nanotube was coated with mouse IgG and did not bind HSV-2 (Figure 2f). This correction is also useful for reducing the device-to-device variation in capacitance measurements of viruses. The differential capacitance values ΔC are negative, as HSV-2 binding to the antibody on the peptide nanotube results in a net decrease in capacitance (that is, a net increase in impedance) with respect to the blank measurement. This result is in accordance with the proposed transduction mechanism, which predicts a decrease in the permittivity of the solution due to the confinement of the virus in the region of the electric-field maximum induced by the interaction with the peptide nanotube. The capacitance change was detectable in the HSV-2 concentration range of 10^2–10^4 pfu mL^{-1} (Figure 3b). The peptide-nanotube platform integrated with electrical transducers has a better detection limit than other label-free detection approaches based on optical transducers for similar viral strains.[37] Our results support our proposition that peptide-nanotube-based biosensors are well suited for the highly sensitive label-free detection of viruses.

In conclusion, the peptide-nanotube sensor platform, which contains a pair of electrodes bridged by antibody-coated peptide nanotubes, was developed by combining top-down fabrication (photolithography of the electrodes) and bottom-up fabrication (dielectrophoretic self-assembly of the nanotubes). The binding of the virus to its antibody on the nanotube was detected by a capacitance change between the electrodes. This method for label-free electrical detection on a peptide-nanotube platform could be used to detect HSV-2 at a concentration of 10^2 pfu mL^{-1} within 1 h. The detection process could be scaled up readily by increasing the number of nanotubes and electrodes on the chip. In this way, the multiplexed detection of viruses should be possible, if peptide nanotubes coated with multiple antibodies are present on the array platform. The specificity of virus detection by this method is derived from antibody recognition. Furthermore, capacitance values measured for the viruses by the peptide-nanotube sensor could be used to verify and identify virus binding. Recently, the difference between the capacitance spectra of different viruses was shown to be large enough to distinguish virus strains.[35] Thus, the capacitance values measured for viruses with our sensor chip could also potentially be used to eliminate false positive signals in complex samples. This hypothesis is currently under investigation with a chip configuration for multiplex detection.

Experimental Section

The peptide nanotubes were prepared by self-assembly from the bolaamphiphilic peptide monomer bis(*N*-α-amidoglycylglycine)-1,7-heptane dicarboxylate and used as templates for the surface immobilization of antibodies.[38,39] They were coated with sheep polyclonal anti-HSV-2 (0.1 mg mL^{-1}, Abcam) by a method described previously for engineering the surface of peptide nanotubes to bind biomolecules through hydrogen bonding.[12] After incubation of the sample overnight with anti-HSV-2, BSA (10 μL, 40 mg mL^{-1}) in PBS buffer was added to block uncoated sites on the nanotube surface and prevent the nonspecific adsorption of viruses. A drop (0.3 μL) of the solution of the peptide nanotube was spotted onto the electrodes, and an AC field at a frequency of 10 Hz with a peak-to-peak potential of 5 V was applied to trap the nanotubes between the electrodes. To evaluate the performance of the system for the detection of the virus, a solution containing HSV-2 (10^1–10^4 pfu mL^{-1}) was incubated with the sensor platform for 1 h, and then impedance spectra were recorded in an AC field with a peak-to-peak potential of 10 mV in glycine buffer (250 mM) at frequencies from 10 kHz to 1 MHz with an SI 1260 Solartron impedance analyzer (for more-detailed procedures, see the Supporting Information).

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