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Virus assay using antibody-functionalized peptide nanotubes†

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Abstract

Robust trace-level detection of viruses is crucial to meet urgent needs in fighting the spread of disease or detecting bioterrorism events. We report a new method for rapid and highly sensitive detection of viruses utilizing fluorescent antibody nanotubes. When viral pathogens were mixed with these antibody nanotubes, the nanotubes rapidly aggregated around the viruses to form a networking structure. Trace quantities of viruses such as herpes simplex virus type 2, adenovirus, vaccinia and influenza type B were detected on attomolar order by changes in fluorescence and light scattering intensities associated with aggregation of dye-loaded antibody nanotubes around viruses. High specificity of each antibody nanotube toward its targeted virus was demonstrated by quantifying concentrations of two different viruses in mixtures. This antibody nanotube assay detects targeted pathogens within 30 minutes after incubation with antibody nanotubes. This antibody nanotube assay could fill a pressing need to detect and quantify viruses both rapidly and sensitively.

Introduction

Robust trace-level detection of viruses is crucial to meet urgent needs in fighting the spread of disease or detecting bioterrorism events.¹ Improvement of pathogen detection could also contribute to public health by identifying (or assuring the absence of) contamination of regulated products, such as food, blood, or vaccines. Here, we report a new method for rapid and highly sensitive detection of viruses utilizing fluorescent antibody nanotubes. While various nano-materials and polymers have been used in development of pathogen-detection assays,^{2–9} we developed multi-functional nanotubes incorporating recognition units with antibodies at their ends and fluorescent signaling units at their sidewalls. Those recognition units and signaling units were immobilized onto template nanotubes, self-assembled from bolaamphiphile peptide monomer, bis(*N*- α -amidoglycylglycine)-1,7-heptane dicarboxylate.^{10–13} When viral pathogens were mixed with these antibody nanotubes in solution, the nanotubes rapidly aggregated around the viruses to form a networking structure (Fig. 1) and the

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size of the aggregates increased with the concentration of viruses as detected by light scattering. Trace amounts of viruses such as herpes simplex virus type 2 (HSV-2), vaccinia, adenovirus and influenza type B were detected on attomolar order by changes in fluorescence and light scattering intensities of the aggregated dye-loaded antibody nanotubes around viruses using flow cytometry, which also allowed us to simultaneously separate signals from unbound nanotubes (which otherwise were sources of background interference). As a comparison, the detection limit for viruses with antibodies directly tagged with fluorescent dyes is several orders of magnitude higher than that of this antibody nanotube assay.¹⁴ The light scattering and fluorescence changes caused by the nano-tube–virus aggregation could also be used to determine the concentrations of those viruses. After subtraction of background fluorescence emissions due to unbound nanotubes, fluorescence emissions from the nanotube–virus aggregates were linearly proportional to the added virus concentrations on a log scale. Thus, the integrated fluorescence intensities of the nanotube–virus aggregates in samples containing viruses could be used to assay samples containing these viruses. High specificity of each antibody nanotube toward the targeted virus was demonstrated by quantifying concentrations of two viruses in mixtures.

Current methods of virus detection such as electron microscopy, immunological assays, and virus culture assays each have strengths and weaknesses. Electron microscopy can be time-consuming and requires concentrations of virus on the order of magnitude of 10^6 particles ml^{-1} or higher while it only gives structural information.¹⁵ Virus culture assays, while capable of detecting just one infectious unit, need careful sample handling considerations to maintain the viability of the pathogens in order to prevent false negative results and also take days before results are obtained. Polymerase chain reaction (PCR)-based testing is capable of detecting a single copy of viral nucleic acids in as little as one hour with small sample volumes, however it can be subject to sample contamination that may become a source of false-positive results. PCR is also not capable of directly detecting complete virus particles, instead detecting viruses indirectly by amplifying small portions of their genomic DNAs or RNAs. Immunostaining has been used together with flow cytometry to detect virus-infected cells,¹⁶ however, this technique requires complex procedures with large sample volumes, >1 mL, and more than 10^5 copies of virus are necessary to detect virus particles directly.^{17–19} More recently, the use of quantum-dot-stained antibodies to detect virus-infected cells has been reported; this approach can have diagnostic utility, but may not be as useful in detecting extracellular virus.²⁰ Thus, a niche exists for a new assay that can detect viruses directly and rapidly with high sensitivity and low sample volumes, <1 mL.

This antibody nanotube assay has an advantage in its signal-to-noise ratio and sensitivity for pathogen detection. For example, the sidewalls of nanotubes can carry dye molecules on the order of 10^5 labels per nanotube if each molecule binds each peptide monomer on the assembled nanotube surface in a one-to-one ratio,^{21,22} and therefore the fluorescence signal can potentially be amplified to levels 10^5 times greater than traditional antibody–dye conjugation systems where one antibody is conjugated with a single dye molecule. Another advantage of this nanotube assay is its robustness of virus detection. This antibody–nanotube assay enables one to optically detect targeted pathogens in the trace levels of 10^3 pfu mL^{-1} within 30 minutes after incubation with antibody nanotubes. Antibodies robustly bind to various pathogen markers such as proteins and peptides,²³ carbohydrates,²⁴ or lipids,²⁵ and

the template nano-tubes can incorporate different antibodies, which would enable one to detect a variety of pathogens with this antibody nanotube assay. This new antibody nanotube-based assay has the potential to overcome some of existing pathogen assays' weaknesses by achieving rapid screening of viruses with low detection limits and high sensitivities.

Materials and methods

Synthesis of peptide nanotubes

Bis(*N*- α -amidoglycylglycine)-1,7-heptane dicarboxylate was prepared as previously described.²⁶ These bolaamphiphile peptide monomers (10 mg mL^{-1}) were dissolved in 50 mM sodium hydroxide overnight, and the pH of the resulting solution was adjusted to 4.5 with citric acid. After two weeks, the peptide nanotubes saturated the solution. These nanotubes were assembled by three-dimensional hydrogen bonds between amide and carboxyl groups of these monomers.²⁶ Then the nano-tubes were separated by size-separation columns,²⁷ resulting in a suspension of nanotubes whose average dimension was 120 nm (diameter) $\times$ 1 μm (length). The resulting nanotube solution was washed with deionized water and centrifuged three times at 14 000 RPM before functionalizing them with antibodies and dyes.

Antibody and dye immobilizations on the peptide nanotubes

The template nanotubes self-assembled from the bolaamphiphile peptide monomers previously immobilized antibodies on their surfaces *via* hydrogen-bonding to amide and carboxyl groups of those monomers that do not involve the three-dimensional hydrogen bonding of monomers for the nanotube formation.^{11,12} For this experiment, antibodies were immobilized at the ends of peptide nanotubes using Au nanoparticles as protective masks on the sidewalls of nanotubes.¹⁰ Polyclonal antibodies used in this nanotube assay were anti-HSV-2, anti-influenza, anti-adeno-virus, and anti-vaccinia, purchased from Abcam (Cambridge, MA). While the Au nanoparticle-masked nanotubes adsorbed antibodies on their entire surfaces, the chemical etching of the Au nanoparticles masks removed antibody molecules from the sidewalls but antibodies at the nanotube ends remained bound. To thiolate the sidewalls of nanotubes for the Au nanoparticle adsorption, 1.5 mL of nanotube solution was reacted with 15 mM *N*-hydroxysuccinimide (50 μL) and 75 mM 1-[3-(dimethyl-amino)propyl]-3-ethylcarbodiimide hydrochloride (50 μL) at room temperature for 3 hours to convert carboxyl on the side-walls to thiol. Both chemicals were purchased from Sigma and used as received. After the sample was washed and centrifuged, the nanotubes were reacted with 30 μL of 2-mercaptoethylamine (5 mM) (Sigma) for 12 hours at 4 $^{\circ}\text{C}$ to complete the sidewall thiolation. After another wash, 40 μL of Au nanoparticles (50 nm) (Ted Pella) were added for 6 hours to mask the sidewalls of nanotubes. After unbound Au nanoparticles were washed out, 0.25 mL of the complementary antibody ($10 \mu\text{g mL}^{-1}$) (Abcam) was incubated with the nanotubes overnight. The Au nano-particle masks were then removed by breaking Au-S bonds with 40 mL of an iodine solution (1 g KI and 0.3 g I_2 in 1 mL aqueous solution). After the resulting solution was washed 4 times, thiol groups that remained along the sidewalls of nano-tubes were used to covalently bind maleimide-functionalized Alexa fluorescent dye molecules. For this immobilization, first

those nanotubes were re-suspended in 10 μ M tris-(2-carboxyethyl)phosphine, hydrochloride (TCEP) to reduce disulfide bonds on the nanotubes immediately, and then C₅-Maleimide-Alexa-Fluor 488 or 633 dye (Molecular Probes) was added to these solutions for 2 hours at room temperature. The resulting nanotube solutions were washed thoroughly to eliminate the potential background interference from unbound fluorescent dyes left over in the solution. The nanotube vials were then wrapped in Al foil to prevent photobleaching of these dyes before use.

Viruses

Herpes simplex virus type 2 (HSV-2) strain 333 was grown in Vero cells as described.²⁸ Adenovirus Type 3 (ATCC# VR-847) was used as received from the American Type Culture Collection (Manassas, VA). Titered stocks of influenza vaccine type B, simian virus 40 (SV40), and vaccinia were obtained from Dr Zhiping Ye, Dr Andrew Lewis, and Dr Michael Merchlinsky (all at FDA/CBER), respectively. To create a series of spiked samples for analysis, 10-fold serial dilutions in 1X PBS were performed from virus stocks with known concentrations.

Flow cytometry and transmission electron microscopy (TEM)

All flow cytometry experiments were performed by a FACS-Aria (BD Biosciences) using a 100 micron tip in the low sort mode. Those tips were washed with buffers prior to every sampling. A 488 nm laser illumination source was used for the forward light scattering (FSC). The 488 nm FSC, the 516 nm Alexa-Fluor 488 peak fluorescence emission (FL-1), and 650 nm Alexa-Fluor 633 peak fluorescence emission (FL-2) were detected at 90°. Flow cytometry allows one to measure the FSC scattering and the fluorescence emission on each aggregate individually. The FSC and the fluorescence data were analyzed using the CellQuest Pro software package (BD Biosciences). For the sample preparation for TEM, a drop (5 μ L) of the sample solution was evaporated on a formvar-carbon 200 mesh copper TEM grid at room temperature. During the drying process, this grid was floated upside down on a drop of the solution, and then it was fixed with gluteraldehyde. The nanotube-virus aggregates were imaged by a TEM (Zeiss) at the FDA with an acceleration voltage of 100 kV.

For each batch, 0.5 mL of the prepared nanotube solution was analyzed by flow cytometry to determine the number of nano-tubes per mL, based on the raw number of fluorescence events observed in that entire sample. Typically, the concentrations of stock nanotube solutions were found to be between 3.3×10^7 and 8.6×10^7 nanotubes mL⁻¹. All solutions were diluted to yield a working concentration of 5.0×10^6 nanotubes mL⁻¹ in the final 500 μ L sample volume for each set of assay. After virus samples were added to 100 μ L of the antibody nanotube solutions, final concentrations of virus were adjusted between 10¹ and 10⁶ pfu mL⁻¹ with deionized water in the 1.0 mL sample solutions. For every virus-spiked sample, 0.5 mL of the virus solution was analyzed.

Results and discussion

When viruses were mixed with antibody-coated nanotubes in solution, these antibody nanotubes aggregated with viruses and formed a networking structure, as shown in Fig. 1. This TEM image shows that these nanotubes bound the roughly spherical virions, appearing as black spheres, in a “hub and spoke” fashion, consistent with the antibodies at both ends of nanotubes binding virions. We hypothesized that the size of virus–nanotube aggregates would increase as the concentration of virus increases; The increased size with more fluorescently-labeled nanotubes contained in aggregates would lead to an increase in fluorescence intensity. To test this hypothesis, the sizes and the fluorescence intensities of virus–nanotube aggregations were studied by light scattering and fluorometry.

Forward light scattering (FSC) of particles in flow cytometry increases as their size and refractive index increases, while side light scattering (SSC) provides information about the surface roughness of particles.²⁹ Since all our antibody nanotubes had similar refractive index, changes in the size of aggregates are the major contributor to changes in FSC intensity. Therefore, we used FSC to detect the nanotube–virus aggregates and also used this information to identify unbound antibody nanotubes in solution. Spherical aggregations of virus–nanotube networks possessed distinct size and shape that could be detected by their characteristic FCS and fluorescence intensities. Fig. 2a shows the distribution of the fluorescence intensity (*x*-axis) and the FSC intensity (*y*-axis) of anti-HSV-coated nanotubes in solution without HSV-2. Since flow cytometry evaluates each particle individually, each dot in Fig. 2 represents the FSC and fluorescence intensities for an individual aggregate or nanotube. In this measurement, the fluorescence intensity of the FL-1 dye on the nanotube was detected. Thus, neat antibody nanotubes mostly exhibited low fluorescence and FSC intensities. However, when HSV-2 was added to this solution, the intensity distribution of the fluorescence and the FSC changed. Fig. 2b shows the distribution of the fluorescence intensity and FSC intensity of anti-HSV nanotubes after addition of 500 μL of HSV-2 solution at 10^3 pfu mL^{-1} , and Fig. 2c shows the FL-FSC intensity distribution when HSV-2 solution was included with the antibody nanotube solution at a concentration of 10^6 pfu mL^{-1} . Both figures show that the FL-FSC distributions were spread toward higher intensities as the concentration of HSV-2 increased, indicating that the sizes of the virus–nanotube aggregates increased. The same trend in aggregation size change as a function of virus concentration was also observed for influenza B (see Fig. 4b). In Fig. 2a, a gate was set to exclude the signal contribution of unbound nanotubes from the signal of virus–nanotube aggregates. No fluorescence intensity was observed in the gate of Fig. 2a (as shown in Fig. 2d), because the gate excludes unbound antibody nanotubes. Therefore, dots outside the gate in the FL-FSC plot represent unbound antibody nanotubes and dots inside the gate represent antibody nanotubes binding HSV-2. Fig. 2e and 2f, respectively, show the integrated fluorescence intensities contributed from the antibody nanotube-HSV-2 aggregates inside the gates in Fig. 2b and 2c. This result is consistent with our hypothesis that integrated fluorescence intensities of the nanotube–virus aggregates increases as the virus concentration and the resulting aggregation size increase. The dot plots for other viruses are shown in ESI†. It should be noted that neat PBS buffer and neat HSV-2 stocks did not show

any FL intensities, indicating that the buffer and the neat virus did not contribute to the integrated fluorescence intensities in Fig. 2e and 2f.

When the integrated fluorescence intensities of nanotubes inside the gate were plotted as a function of HSV-2 concentration, there was a linear correlation between the fluorescence intensity and the virus concentration on a log scale, as shown in Fig. 3a. For each point in this plot, we ran 9 different batches and the error ranges are within 10%. This plot shows that the detection limit of the antibody nanotube assay for HSV-2 is 10^3 pfu mL⁻¹ when the 3σ level is applied. The size of the virus–nanotube aggregate increases as the virus concentration increases, likely since the larger aggregates contain more dye-labeled nanotubes. Fig. 3b shows that the integrated fluorescence intensity of anti-influenza nanotubes is proportional to the concentration of influenza-B on a log scale and the detection limit of this system (also applying 3σ limits) is $10^{3.5}$ pfu mL⁻¹. The gradient of the integrated fluorescence plot for adenovirus (Fig. 3c) was smaller as compared to other viruses, and the detection limit for adenovirus was higher ($10^{4.5}$ pfu mL⁻¹) than that for HSV-2 and influenza virus. The integrated fluorescence intensity of anti-vaccinia nanotubes increased as a function of the vaccinia concentration, as shown in Fig. 3d, however the detection limit for vaccinia was higher, $10^{4.5}$ pfu mL⁻¹.

For each assay, we identified a two part curve—a zero-slope threshold represented by the y-intercept and a linear portion with positive slope that varied among the assays as shown in Fig. 3. The theoretical best detection limit for each assay is represented by the inflection point between the zero-slope threshold and the positive-slope linear portion of the curve; for HSV, adenovirus and influenza this occurred around 100 viral particles, while for vaccinia this occurred at about 10 000 viral particles. The ability to distinguish the fluorescence intensity from background thus depended upon the actual threshold value, the slope of the increasing part of the curve, and the errors associated with making these comparisons. We hypothesize that the inflection point and the slope at which fluorescence intensity increases with the log of viral concentration depends upon the efficiency of virus–nanotube aggregation, which may differ based upon the relative sizes of virus and nanotubes due to the difference in the maximum number of nanotubes that could bind a single virus particle. It is reasonable to hypothesize that the efficiency of the aggregation has an influence on the inflection point, the slope, and the resulting detection limit since it represents how rapidly the size of virus–nanotube aggregates changes as a function of viral concentration.

To further understand the aggregation mechanism between the nanotube and viruses and its correlation with the detection limit, we examined four viruses whose sizes were different and we plotted each virus with the fluorescence and light scattering intensities. Fig. 4 shows the dot distributions in the FL-scattering plots of these viruses at the high viral concentration. The dot distributions of HSV-2 and influenza-B, whose size was 120 nm, were very similar (Fig. 4a and 4b); both dot distributions spread diagonally and the number of dots increased inside the gate as the virus concentrations increased. This diagonal spreading pattern indicates that the size of aggregates between viruses and nanotubes was amplified efficiently as the viral concentration increased, which appeared as steeper slopes

[†]Electronic supplementary information (ESI) available: Figures S1–S5. See DOI: [10.1039/b714470a](https://doi.org/10.1039/b714470a)

and lower detection limits in Fig. 3a and 3b. However, the dot distributions looked different when the nanotube aggregated with much larger or smaller virus. In Fig. 4c, for the smaller adeno-virus (70 nm), a much larger number of dots remained outside the gate as compared with HSV-2 and influenza B, which suggests that the binding between adenovirus and the antibody nanotube was not as efficient as that of other viruses. Thus, the change of integrated fluorescence intensity in Fig. 3c was much smaller as the adenovirus concentration increased and the gradient of the integrated fluorescence intensity plots as a function of viral concentration became smaller, which contributed the higher detection limit as compared to the size-matched HSV-2 and influenza B. In the case of the dot distribution of the much larger vaccinia (320 nm), the fluorescence intensity increased as the concentration of vaccinia increased but the light scattering intensity showed smaller change (Fig. 4d). The resulting horizontal spreading of dots indicates that the size change of the vaccinia–nanotube aggregates was small, and thus the inflection point of the vaccinia plot was shifted to higher concentration, which increased the detection limit. Therefore, these results suggest that there is some correlation between the size of virus and the detection limit of the nanotube assay.

The difference in dot distributions among viruses of different sizes reflects the virus–nanotube aggregation mechanism. While HSV-2 and influenza B have sizes similar to the diameter of antibody-immobilized nanotubes, vaccinia is much larger and adenovirus is smaller. When the antibody nanotubes target much larger viruses, many smaller nanotubes could stick around a single large virus, making it difficult to form large networking structures because larger and heavier virus particles cannot be anchored by the smaller nanotubes as efficiently as light and small virus particles; the small nanotubes cannot easily recruit another large virus particles to increase the size of the virus–nanotube aggregates. Since the nanotubes initially tend to be consumed by attaching to a larger virus rather than forming the aggregates, more nanotubes are required to form the aggregates with larger virus particles. In the case of smaller viruses such as adenovirus, the overall size of antibody–virus aggregates becomes smaller and the number of the larger networks decreases because the smaller surface areas of those virus particles limits multiple nanotube bindings. The larger vaccinia particles indeed did not show increasing size of the virus–nano-tube aggregates, as shown by the horizontal flat dot distribution in Fig. 4d when the vaccinia concentration was increased. In this plot, the fluorescence intensity increases as more nanotubes are attaching on a vaccinia particle while the light scattering intensity change is small due to the small size change of the nano-tube–virus aggregates. This size effect is also responsible for the shift of the inflection point in the vaccinia plot, such that the slope does not increase until the vaccinia concentration reaches 10^4 pfu mL⁻¹ (Fig. 4d); the large vaccinia particle accumulates small antibody nanotubes on its surface up to a certain number until these antibody nanotubes start forming the networking structure, and therefore the inflection point of the vaccinia plot shifts to the higher concentration (Fig. 3d).

In the case of the smaller adenovirus, fewer adenovirus particles bind antibody nanotubes to form the virus–nanotube networks, which decreases the number of dots inside the gate in Fig. 4c. The inefficient binding of nanotube with adenovirus is responsible for the small change in fluorescence intensities as a function of viral concentration in Fig. 3c. This aggregation mechanism is also consistent with our findings with SV-40, whose size is even

smaller than adenovirus; the dot pattern for SV-40 with a diameter of 50 nm matches the pattern for adeno-virus (see ESI†). These results support our hypothesis that the virus size influences the virus–nanotube aggregation mechanism and that further optimization of the size ratio could further enhance sensitivity to detect viruses.

To further examine the selectivity of our antibody nanotube-based assay, one type of antibody nanotube was used to quantify concentrations of its complementary virus in the mixture of two different viruses. In this experiment, anti-HSV-2-conjugated nanotubes, labeled with the FL-1 dye, were mixed with samples containing both HSV-2 and SV-40 in known concentrations. If these antibody nanotubes are very selective for virus-binding events and do not possess cross-reactivity, the degree of anti-HSV-2 nanotube aggregation should only depend on the HSV-2 concentration, thereby demonstrating the selectivity of this antibody nanotube. Fig. 5a shows the correlation between the concentration of HSV-2 and the integrated fluorescence intensity of anti-HSV-2 nanotubes when a 1 : 1 mixture of these two types of antibody nanotubes was incubated with a mixed viral sample, [HSV-2] = 10^2 – 10^6 pfu mL⁻¹. This correlation was examined by varying the added SV-40 concentrations from 0 to 10^6 pfu mL⁻¹ (each SV-40 concentration is shown in a different color in the ESI†). As shown in Fig. 5a, all of these plots can be overlaid in a straight line. We also examined additional SV-40 concentrations below 10^6 pfu mL⁻¹ with the same result (ESI†). Therefore, these plots indicate that the HSV-2 concentration is proportional to the fluorescence intensity of anti-HSV-2 nanotube-HSV-2 aggregates even in the presence of SV-40 background in the concentration range of 0– 10^6 pfu mL⁻¹. When Fig. 5a is re-plotted with the concentration of SV-40 as the *x*-axis and the integrated fluorescence intensity of anti-HSV-2 nanotubes as the *y*-axis, SV-40 does not affect the fluorescence intensity of anti-HSV-2 nanotube-HSV-2 aggregates at any concentration level as shown in Fig. 5b. This result also indicates that the degree of the anti-HSV-2 nanotubes aggregation only depends on the HSV-2 concentration and is independent of the concentration of SV-40. These results confirm the absence of non-specific cross-reactivity between the antibody nanotubes and the non-targeted virus. Thus, this antibody nanotube assay system is highly specific.

Conclusions

When viruses were mixed with antibody nanotubes in solution, we observed that these nanotubes rapidly aggregated around the viruses to form a networking structure. Trace amounts of these viruses were detected on attomolar order when the size of the aggregates changed as a function of the virus concentrations. Characteristic distributions of fluorescence and light scattering intensities of the aggregated dye-loaded antibody nanotubes around viruses could be used to eliminate signals from unbound nanotubes, which otherwise were sources of background interference. The best limit of detection for this assay was determined to be on the order of 10^3 pfu mL⁻¹ by creating standard curves between the integrated fluorescence intensities of the nano-tube–virus aggregates and the virus concentrations. These standard curves also allowed us to quantify viral concentrations based on their fluorescence intensities. The increased size of the nanotube–virus aggregates resulted in an increase of their fluorescence intensities since more antibody nanotubes were contained in the larger aggregates.

Our observation that the best detection limits are achieved when the viruses and nanotubes are comparable in size could be used to further improve confidence in assay specificity. Since the size matching between the diameter of nanotube and viruses can be recognized by the dot distribution in the fluorescence intensity – scattering plot as shown in Fig. 4, viruses whose sizes do not match with the diameter of nanotube can be identified and eliminated by the dot plots. If the antibody nanotube's diameter is selected to match with the targeted virus and the dot distribution of the nanotube–virus aggregates does not spread diagonally, the fluorescence signal of the aggregation is false-positive. Therefore, size matching identification *via* the dot distribution could be used as a criterion in addition to the antibody selection of the nanotube to detect and assay targeted viruses.

High specificity of anti-HSV-2 nanotubes toward the targeted virus was also demonstrated by quantifying the concentration of HSV-2 in mixed solutions of HSV-2 and SV-40. We have not yet tested other potential sources of interference, such as those that may be present in “dirty” samples containing complex compounds for the applications in the prevention of food poisoning and bioterrorism. Typically, we obtained the fluorescence data through flow cytometry within five minutes, and with standard curves previously prepared, the overall process takes only 30 minutes after sample solutions are prepared with antibody nanotubes. If the gating protocols for the fluorescence-FCS intensity distribution and standard curves between integrated fluorescence intensities of the nanotube–virus aggregates and virus concentrations are prepared for viruses as a library in advance, this nanotube assay can be rapidly applied to determine viral concentrations in unknown samples. Our current nanotube assay system likely detects both infectious and non-infectious viruses due to limitations in antibody recognitions. However, it seems unlikely that highly defective virus particles could function in the networking structures we observed, since fairly intact virions would be required for formation of networking structures. The development of a better read-out system such as multi-color coincidence detection will further improve the multiplex detections of viruses.⁷

This antibody nanotube assay could complement other existing viral assays such as PCR, which detects nucleic acids rather than virus structures, increasing the versatility of the antibody assay in the detection of a variety of viruses.

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Fig. 1. TEM image of networking aggregates between adenovirus (dark spheres) and anti-adenovirus nanotubes (rod) in a “hub and spoke” fashion. The concentration of HSV-2 was 10^6 pfu mL^{-1} , and the acceleration voltage was 100 kV. Scale bar represents 500 nm.

Fig. 2.

Distribution of forward light scattering (FSC) intensity and fluorescence intensity of (a) neat anti-HSV nanotubes (b) anti-HSV nanotubes with 10^3 pfu mL^{-1} HSV-2 in solution (c) anti-HSV nanotubes with 10^6 pfu mL^{-1} HSV-2 in solution. Integrated fluorescence intensities inside the gates in (a), (b), and (c) are normalized in (d), (e), and (f)

Fig. 3.

Linear correlations and fittings between the integrated fluorescence intensities of the antibody nanotube–virus aggregates and the concentrations on a log scale of (a) HSV-2 ($R^2 = 0.9010$), (b) influenza B ($R^2 = 0.9842$), (c) adenovirus ($R^2 = 0.9612$), and (d) vaccinia ($R^2 = 0.9503$). The fluorescence intensities those viruses are normalized.

Fig. 4.

Distribution of forward light scattering (FSC) intensity and fluorescence intensity of (a) anti-HSV nanotubes with 10^6 pfu mL⁻¹ HSV-2 in solution, (b) anti-influenza nanotubes with 10^6 pfu mL⁻¹ influenza-B in solution, (c) anti-adenovirus nanotubes with 10^6 pfu mL⁻¹ adenovirus in solution, and (d) anti-vaccinia nanotubes with 10^6 pfu mL⁻¹ vaccinia in solution.

Fig. 5.

Quantification of the concentration of HSV-2 in mixed solutions of HSV-2 and SV-40, probed by anti-HSV-2 nanotubes. (a) Correlation between the HSV-2 concentration and integrated fluorescence intensity of the anti-HSV nanotube-HSV-2 aggregates under known concentrations of the SV-40 addition (10^2 , 10^4 , 10^6 pfu mL $^{-1}$, shown in different colours). (b) Correlation between the SV-40 concentration and integrated fluorescence intensity of the anti-HSV nanotube-HSV-2 aggregates under known concentrations of the HSV-2 addition (10^2 , 10^4 , 10^6 pfu mL $^{-1}$).