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The Use of Nanoarrays for Highly Sensitive and Selective Detection of Human Immunodeficiency Virus Type 1 in Plasma

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ABSTRACT

Arrays of antibodies with well-defined feature size and spacing are necessary for developing highly sensitive and selective immunoassays to detect macromolecules in complex solutions. Here we report the application of nanometer-scale antibody array-based analysis to determine the presence of the human immunodeficiency virus type 1 (HIV-1) in blood samples. Dip-pen nanolithography (DPN) was used to generate nanoscale patterns of antibodies against the HIV-1 p24 antigen on a gold surface. Feature sizes were less than 100-nanometers, and the activity of the antibody was preserved. HIV-1 p24 antigen in plasma obtained directly from HIV-1-infected patients was hybridized to the antibody array in situ, and the bound protein was hybridized to a gold antibody-functionalized nanoparticle probe for signal enhancement. The nanoarray features in the three-component sandwich assay were confirmed by atomic force microscopy (AFM). Demonstration of measurable amounts of HIV-1 p24 antigen in plasma obtained from men with less than 50 copies of RNA per ml of plasma (corresponding to 0.025 pg per ml) illustrates that the nanoarray-based assay can exceed the limit of detection of conventional enzyme-linked immunosorbent assay (ELISA)-based immunoassays (5 pg per ml of plasma) by more than 1000-fold.

Arrays of proteins with well-defined feature size and spacing are important for studying surface-cellular interactions[1,2] and detecting specific biomacromolecules.[3−5] Recently, there have been significant developments in the use of nanolithography techniques for patterning surfaces with proteins on the submicrometer length scale.[6−13] Dip-pen nanolithography (DPN) is one technique that has shown particular promise in this area, allowing one to prepare standardized multicomponent arrays of biomolecules that can retain their biorecognition properties once transferred to a surface.[14−22] A key issue pertains to the potential of such nanostructures in medical diagnostics, and at present it is unclear what advantages such structures will offer for clinical applications.[23] In principle, one can use smaller sample volumes and achieve higher sensitivity due to the small size of the entire array and the individual features that comprise the array. Such improved detection systems would enable the diagnosis of infection with HIV-1 in the setting of mother-to-child transmission, for example, where small sample volumes and the presence of immune complexes consisting of passively transferred maternal antibodies and HIV-1 antigen present diagnostic challenges. Polymerase chain reaction (PCR) and other forms of target amplification have enabled the development of powerful tools for detecting and quantifying HIV-1 nucleic acid targets for clinical diagnosis and prognosis. Though simpler to perform, conventional immunoassays for HIV-1 Gag p24 cannot achieve this level of sensitivity. Herein, we show how DPN-fabricated nanoarrays of modified monoclonal antibodies against HIV-1 p24 can be used to detect the protein in plasma samples using gold nanoparticles modified with polyclonal anti-p24 IgG as probes in a three-component sandwich (Scheme 1). These data illustrate our capability to detect and measure HIV-1 p24 antigen by a nanoarray-based assay that exceeds the limit of detection of conventional ELISA-based immunoassays and provides a level of sensitivity comparable to a PCR-based assay without target amplification.

In a typical experiment, a nanoarray for the HIV-1 immunoassay was fabricated by initially patterning 16-mercaptohexadecanoic acid (MHA) into dot features as small as 60 nm (10 × 10 spot array) on a gold thin film using DPN. The large spacing between features improved our ability to locate the original pattern after reaction with biomolecules or gold nanoparticle probes. At pH 7.4, the

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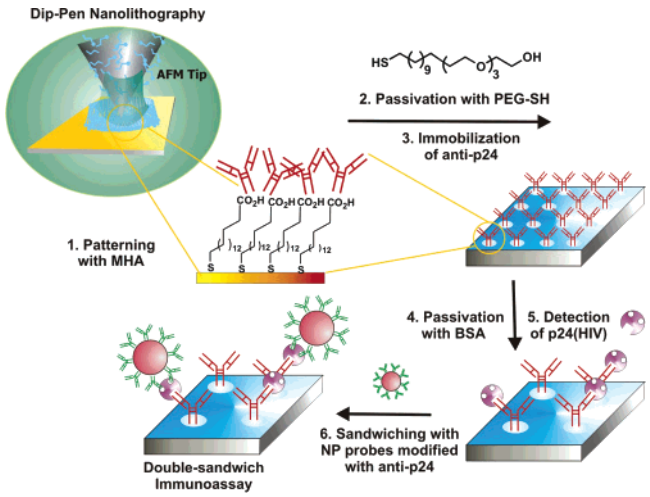
Scheme 1. Schematic Representation of the Immunoassay Format Used to Detect HIV-1 p24 Antigen with anti-p24 Antibody Nanoarray



MHA is deprotonated[24] and therefore the nanofeatures are negatively charged. To minimize nonspecific binding of proteins on the inactive portions of the array, the areas surrounding the MHA patterned features were passivated with PEG-alkythiol (11- mercaptoundecyl-tri(ethylene glycol)) by placing a droplet of a 1 mM ethanolic solution of the surfactant on the patterned area for 2 h followed by copious rinsing with ethanol and, then, Nanopure water. After passivation, mouse monoclonal antibodies to the HIV-1 p24 antigen (anti-p24, 200 μg/mL, 10 mM PBS (phosphate buffered saline), Abcam, Cambridge, UK) were immobilized on the patterned MHA dot features by immersing the template in a solution containing the anti-p24 IgG for 1 h. The substrate was then vigorously rinsed with 10 mM PBS and Tween-20 solution (0.05%). It is well known that IgG will adhere to the negatively charged deprotonated MHA surface features through electrostatic interactions (Scheme 1 and Figure 1A), thus retaining its biological activity toward its target antigen.[25,26] Unmodified MHA features were passivated with BSA (10% solution in 10 mM PBS) to prevent unwanted binding from plasma samples.

In a typical assay, an anti-p24 nanoarray was immersed in a plasma sample containing HIV-1 p24 from the AIDS Clinical Trials Group Virology Laboratories Quality Assurance Program (VQA, serially diluted to yield from 200 pg/mL to 0.2 pg/mL, in 0.5% Triton X-100 in RPMI 1640 media) for 1 h. The binding of the protein to the nanoarray of anti-p24 IgG was confirmed by AFM, which showed a modest height increase (2.3 ± 0.6 nm ($n = 10$)) for each of the features within the array (Figure 1B). To amplify the signal associated with p24 antigen binding to the array, gold nanoparticle probes, which were heavily functionalized with polyclonal antibodies to the p24 antigen, were reacted with the nanoarray by soaking the array in a solution containing anti-p24 IgG coated gold nanoparticles (20 nm, 10 nM in 10 mM PBS) for 1 h. We used AFM to interrogate the antibody chips by measuring height differences between features that have reacted with the HIV-1 p24 and gold nanoparticles and features that have not. A significant topography change in (20.3 ± 1.9 nm ($n = 10$)) accompanied the nanoparticle binding to the captured p24 molecules (Figure 1C). This increase in height was consistent with a 1:1:1 reaction between monoclonal anti-p24 IgG absorbed onto the MHA features on the substrate, HIV-1 p24, and polyclonal anti-p24 IgG functionalized gold nanoparticles. To validate selectivity, anti-p24 nanoarrays incubated in the presence of plasma samples without HIV-1 p24 and then queried with the anti-p24 IgG gold nanoparticle probes showed no height increase for the anti-p24 features.

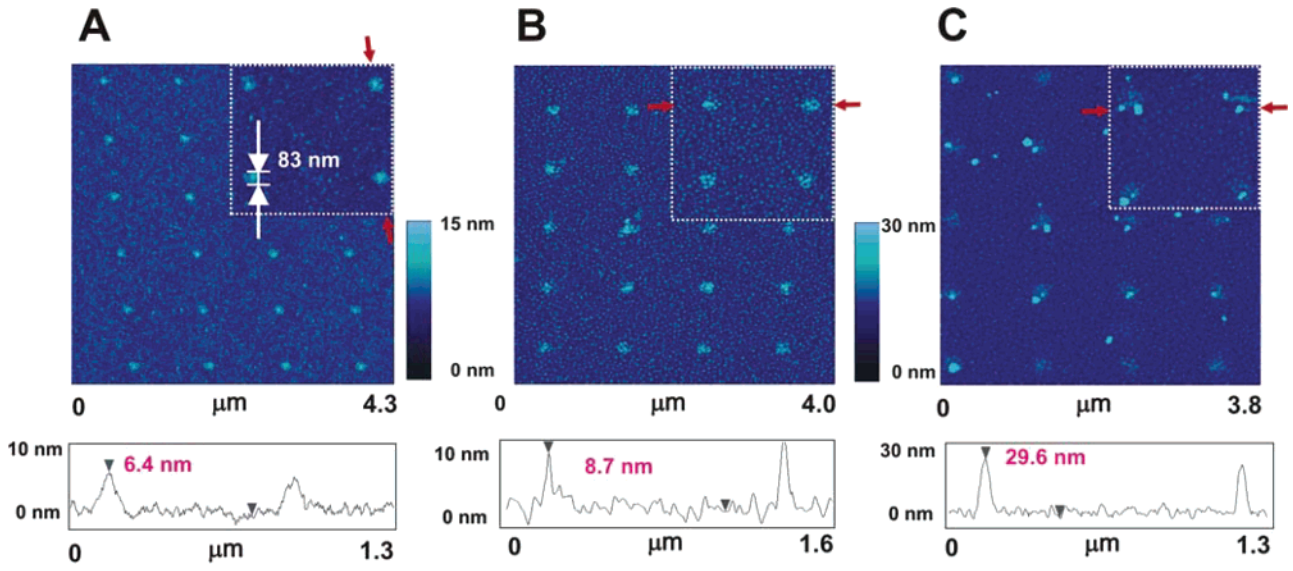


Figure 1. HIV-1 p24 antigen (0.2 pg/mL) detection with a nanoarray. (A) Anti-p24 IgG protein nanoarray. Topography trace of adsorbed anti-p24 IgG (6.4 ± 0.9 nm ($n = 10$)) on MHA, showing a height profile consistent with a monolayer of anti-p24 IgG. (B) After p24 binding to anti-p24 IgG, an average height increase of 2.3 ± 0.6 nm ($n = 10$) for the IgG features is observed. (C) p24 detection after amplification with anti-p24 IgG coated gold nanoparticles (20 nm). An average topographic change of 20.3 ± 1.9 nm ($n = 10$) is observed.

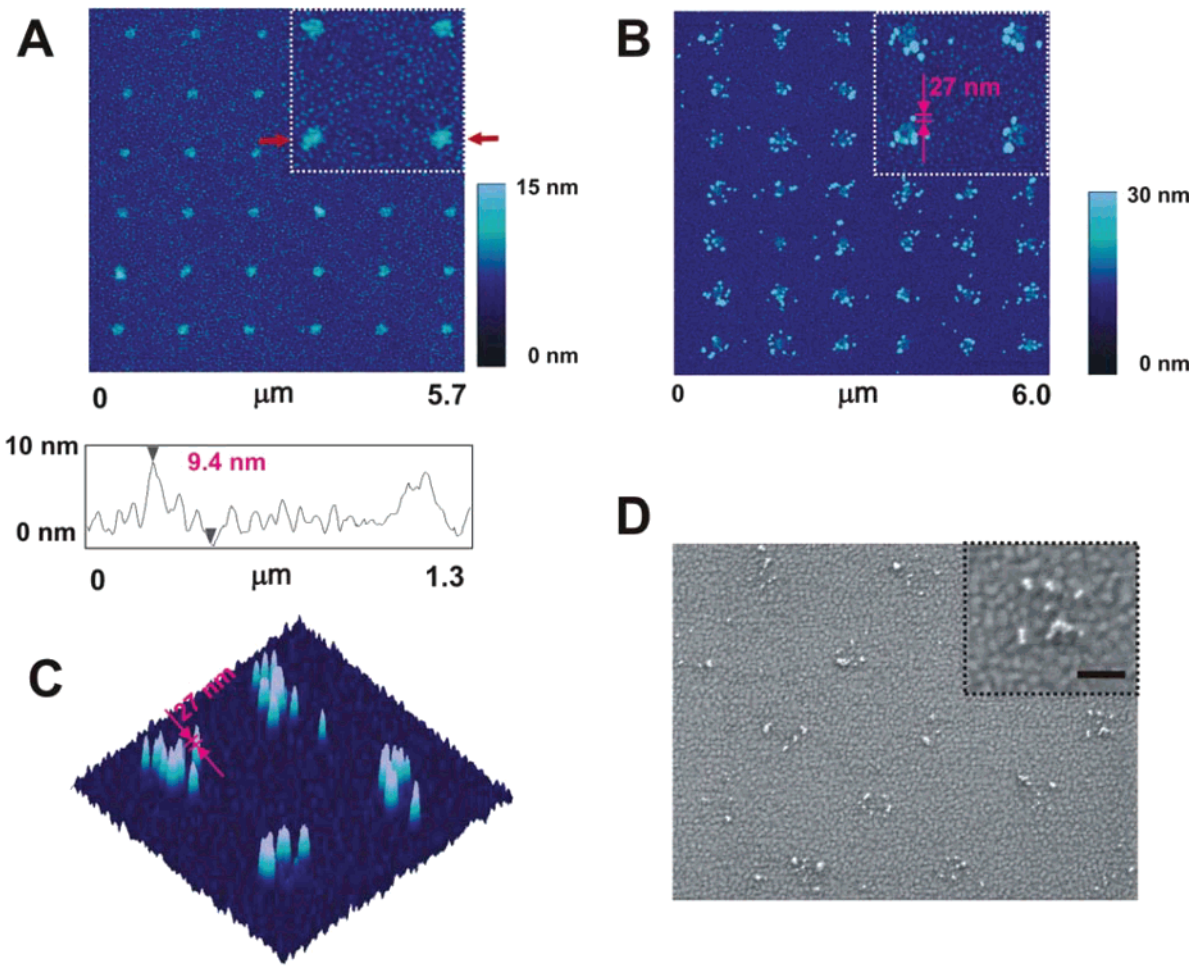


Figure 2. Detection of HIV-1 p24 antigen in plasma obtained from a study subject with less than 50 copies of plasma HIV-1 RNA using the nanoarray. (A) After p24 binding to anti-p24 IgG feature area. A height profile verifies the p24 binding to anti-p24 IgG features. (B) p24 detection after signal amplification with anti-p24 IgG coated gold nanoparticles. Each nanoparticle probe can be resolved with an apparent average diameter of 27.0 ± 1.5 nm (n = 10). The AFM measured diameter is bigger than the actual nanoparticle probe diameter (20 nm) due to tip convolution. (C) A high resolution, three-dimensional image of a smaller section of the area represented by image (B). (D) A FE SEM image of a portion of the chip used to obtain the AFM image in (B). Scale bar = 150 nm.

To assess the applicability of this new approach, the nanoarray-based immunoassay was used to screen plasma samples from HIV-1-infected (n = 8) and -uninfected (n = 10) men enrolled in the Chicago component of the Multicenter AIDS Cohort Study (MACS). The study participants infected with HIV-1 were selected based on their levels of HIV-1 RNA in plasma measured by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The levels of plasma HIV-1 RNA ranged between 3,500 copies per ml of plasma to less than 50 copies per ml of plasma. Because the HIV-1 p24 Gag antigen is bound by p24-specific antibodies soon after primary infection and becomes undetectable in the majority of people with disease, we treated 90 μL of each sample with glycine hydrochloride (90 μL, 1.5 M, pH = 2.4, PerkinElmer, Inc.) to dissociate the immune complex followed by neutralization with TRIS-hydrochloric acid (1.5 M, pH = 8.3, PerkinElmer, Inc.) and permit detection of the protein with the nanoarray.[27] A topographic signal, consistent with nanoparticle binding, for more than 60 of the 100 spots was considered positive for HIV-1 p24, and an array that showed less than 10% binding (10 of 100 spots) was considered nonspecific and therefore negative.

After immune-complex dissociation to disrupt the HIV-1 antigen−antibody complexes, we could detect HIV-1 p24 antigen in a 1 μL sample of plasma from all eight men infected with HIV-1 and none of the ten uninfected controls in less than six hours. Duplicate assays showed reproducible results. HIV-1 p24 was not detected in samples from the uninfected controls or from men infected with HIV-1 who had less than 50 copies of RNA per ml of plasma by a conventional ELISA-based immunoassay (Supporting Information). The height profile in AFM topography verified p24 Gag protein binding to anti-p24 IgG features and the anti-p24 IgG functionalized gold nanoparticles (Figure 2 A, B, and C). Because gold nanoparticles are good electron microscopy labels, we imaged them by field-emission scanning electron microscopy (FE SEM) to verify their selective complexation to the captured target protein (Figure 2D). Measurable amounts of HIV-1 p24 found in plasma from men with less than 50 copies of RNA per mL (corresponding to 0.025 pg per mL) show that the nanoarray-based assay exceeds the limits of detection of conventional ELISA-based immunoassays (5 pg per mL of plasma).[27,28] Thus, the nanoarray-based assay successfully achieved highly

sensitive and selective detection of HIV-1 in microliter-scale volumes of plasma.

The availability of sensitive and specific detection methods to identify HIV-1 in clinical specimens is highly desirable. This is the first example of a clinical application of a nanoarray in biodetection with real patient samples. In its present format, the assay is qualitative, with a level of sensitivity that rivals the current generation of RT-PCR-based assays for detection of HIV-1 in plasma.[23] Furthermore, the assay only requires one microliter of input sample to get the reported results, a particular advantage for repeated testing with the blood volumes obtained from infants and children. Although these data show proof-of-concept, we expect that this platform can be extended to the detection of other pathogenic microbes in microliter-scale volumes of clinical sample in parallel. The nanoarray-based assay has the desired sensitivity, whereas antibodies of matched avidity and affinity will achieve the desired biological analyte selectivity. When coupled to a simple detection method with a high probe-to-target ratio, the application of an immunoassay using nanotechnology to detect HIV-1 p24 antigen in small volumes of peripheral blood from people at-risk for or infected with HIV-1 could have significant impact as a medical diagnostic.

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Supporting Information Available: Table of immunologic data for the eight men infected with HIV-1, the HIV-1 patients' sample information, and the method to prepare antibody-functionalized Au nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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