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Review Article

Nanotechnology tools for single-virus particle detection

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Abstract

The development and potential application of nanotechnology tools for single-virus particle detection by emergent nanotechnology is likely to revolutionise diagnosis and determining treatment endpoints for life threatening virus infections. Direct detection of biological macromolecules using semiconducting nanowires or carbon nanotubes for electrical field change measurements is a milestone application in this field. The promise of selective detection at a single particle level (stochastic sensing) with nanowire or nanotube field-effect transistor-based devices is a major breakthrough for outbreak situations, where a rapid and specific detection of the viral agent allows intervention at public health level. The same technology would be eminently suitable for bedside diagnosis and therapeutic intervention.

Key words: *Nanotechnology, outbreak, virus*

Introduction

Nanomedicine is an avenue for synchronous exponential fruition of advances in chemical and physical sciences and their adaptation to the field of medicine. This article talks about the development and potential application of nanotechnology tools for single-virus particle detection as gleaned from the state-of-the-art articles that have been published in the last four to five years. The developing technologies are highly innovative and their potential application in the field of infectious disease diagnosis, in particular viral infections, is absolutely fascinating.

Scope

Medical diagnostics, especially in the field of clinical virology, significantly depends on detection of viral antigen, virus particle or genomic material in samples from infected individuals. Antibody detection is not very useful in the concurrent diagnosis of infections except those infections with prolonged incubation period and chronic infection status established as in the case of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). Even so, the serological profile in the infected individual has little bearing on therapy. Antibody detection of the IgM class with highly sensitive assays is also not useful to help the treating physician intervene with effective antiviral. In contrast, techniques that detect viral genome or virus specific target sequences have enhanced sensitivity and achieve diagnosis early in the acute phase of illness allowing for specific intervention. The currently available technology targeting the viral genome are polymerase chain reaction (PCR) based and have in general a detection limit of 50–200 copies per mL of plasma or other body fluids using the real time PCR format assays. Presently, the new frontier of nanotechnology that has emerged has researchers developing cutting edge technology for medical intervention and diagnosis.

The advance towards single virus particle detection by emergent nanotechnology is likely to revolutionise diagnosis and determining treatment endpoints for life threatening virus infections. One promising approach for the direct detection of biological macromolecules uses semiconducting nanowires or carbon nanotubes using electrical field change measurements. These are configured as field-effect transistors, wherein conductance changes on binding of macromolecules (intrinsically charged) to receptors that are anchored on the surface of the devices. Recent work indicates selective detection at a single particle level (stochastic sensing) with nanowire or nanotube field-effect transistors. The important scientific advantages that accrue are selective detection by the affinity of the receptor and the potential to analyze single particle bound/unbound kinetics, which provides information on binding times in virus–receptor interactions. Furthermore, single particle sensitivity enables simple charge-based detection of macromolecules. In outbreak situations, a rapid and specific detection of the viral agent requires biosensors able to generate a quantitative signal from individual viral particles.

Principle of Single Virus Particle Detection

The application of nanowire field effect transistors has shown promise for direct real-time electrical detection of single virus particles with high selectivity. In a series of experiments with influenza virus and antibody it was shown

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that nanowire arrays coated with antibodies could enable measurement of discrete conductance changes characteristic of specific binding and unbinding of influenza A (InfA) but not paramyxovirus or adenovirus. The specificity was documented by simultaneous electrical and optical measurements using fluorescently labelled influenza A. The conductance changes corresponded to binding/unbinding of single viruses at the surface of the coated nanowire. The detection mechanism was facilitated by a field effect as determined by varying the pH conditions in the experiment. Also, the nanowire sensing devices rapidly determine isoelectric points and variations in receptor-virus binding kinetics at different conditions. Further, multiple viruses were shown to be specifically detected in parallel studies of nanowire devices modified with antibodies specific for either influenza or adenovirus. The system is dynamic enough and versatile to allow integration of these nanowire devices enabling the potential for simultaneous detection of multiple distinct viruses at the single virus level. This is especially useful if packaged for etiological diagnosis of viral syndromes or in bio-warfare threats.

Developments in the Field

The first significant development in the area of single virus particle detection was achieved when silicon nanowire device arrays were synthesised by chemical vapour deposition with 20 nm gold nanoclusters as catalysts, silane as reactant, and diborane as p-type dopant (with a B/Si ratio of 1:4,000, Tirmethylboron (TMB) p-type dopant (an impurity element added to a crystal lattice in low concentrations in order to alter the optical/electrical properties of the crystal) for the vapour-liquid-solid growth of boron-doped silicon nanowires. Arrays of silicon nanowire devices were engineered by photolithography with Ni metal contacts on silicon substrates with a 600 nm-thick oxide layer. The deposition of about 50 nm thick silicon nitride ($Si_3N_{4)}$ coating isolates the nanowires from the metal contacts, a 2 μm spacing is maintained between source-drain electrodes (active sensor area). A two-step procedure is used to covalently link antibody receptors to the surfaces of the silicon nanowire devices. The device was tested with different concentration of virus solutions (influenza type A, 10^9 to 10^{10} particles per ml). The nanowire device arrays were fed the virus samples at flow rate of 0.15 mL/hr in buffered solution through fluidic channels formed by 0.1 mm-thick glass cover slip for combined electrical/optical measurements. Electrical measurements were made between 17 and 79 Hz, inclusive and shown to be independent of frequency within this range. The modulation amplitude was set at 30 mV and the dc source-drain potential zeroed to avoid electrochemical reactions. *Optical Imaging was carried out by* laser scanning confocal microscope with $DiIC_{18}$ dye excited at 532 nm. This method enables detection of Influenza A virus particle (label-free) binding of single virions producing definitive changes in the resonance frequency/wavelength of a whispering-gallery mode excited in a micro spherical cavity. The magnitude of the discrete wavelength-shift signal was found significantly enhanced by reducing the microsphere size. The size and mass of about 5.2 x 10(-16) g of a bound virus particle are determined directly from the optimal resonance shift.[1]

A recent report demonstrates discrete changes in frequency of whispering gallery modes (WGMs). WGMs refer to the measurable resonances produced by light bouncing around the object's circumference and occurs inside round objects (spheres, cylinders, disks) where the resonance is restrained by total internal reflection and stays inside the object. Light resonance is the measurable change in the wave length of incident light as a function of the nanoparticle. WGMs are useful analytic tools as resonant frequencies depend strongly on the refractive index, and the frequencies correspond to an integral number of wavelengths in one trip around the circumference. Thus, the properties of an unknown analyte dissolved in a droplet of fluid can be inferred by analyzing the resonant frequencies of the droplet's whispering-gallery modes. The technique is especially attractive for bio sensing in small volume of samples because the volume of droplets typically measured is in the range of 10-100 pico litres. It has been elegantly shown with the Influenza A virions that when they bind to a microsphere cavity, a "reactive" perturbation of the resonant photon state is achieved and this is confirmed in measurements. It has been shown that by reducing the microsphere radius to just < 40 μm and light wavelength near 760 nm is optimal for documenting clear binding of single InfA virion with favourable signal-to-noise ratio. The analysis derives distinct changes in resonance wavelength related to the size and mass of the adsorbed virion. WMG appears superior to quantify virion size and mass compared to nanofibres and interferometric approaches based on light scattering. WGM resonances are perturbed by longer wavelength as particles with polarization higher to water molecules adsorb to a microsphere cavity and individual binding events produce discrete steps in the time-trace of the wavelength shift signal. A distributed feedback laser (DFB) (≈1,311 nm nominal wavelength) excites WGMs and the spectrum is analyzed by a photo detector that measures transmission through the fibre. The microsphere-on-a-stem is mounted on the sample cell and immersed in PBS solution. A small box encloses the sample cell to control air flow and humidity level. The transmission spectrum is determined every ≈20 ms and the resonance wavelength shift is estimated.[2]

In a first of its kind, gold particles with diameters between 2.5 and 4.5 nm were introduced into the inner cavity of an icosahedral brome mosaic virus. The optical properties of single gold-marked virions were tested *in vitro* with respect to the characteristic plasmon polariton resonance. Plasmons are collective oscillations at optical

frequency of the free electron gas density; whereas polaritons are quasiparticles formed from strong coupling of electromagnetic waves with an electric excitation, an expression of the common quantum phenomenon known as level repulsion. Polaritons describe the crossing of the light wave dispersion caused by interacting resonance. When plasmons are confined to surfaces and interact strongly with light resulting in a polariton, it is called surface plasmons. This effect occurs at the interface of a vacuum or material with a positive dielectric constant and a negative dielectric constant. They play a role in Surface Enhanced Raman Spectroscopy (SERS**).** This sensitive technique results in 10^{14}-10^{15} enhancement of the Raman scattering by molecules adsorbed on rough metal surfaces and allows for detection of single molecules. This effect can be used to study the mechanisms and kinetics of ligands binding to receptors.

The shift in the plasmon polariton resonance of a single Au (gold) particle encapsulated in a virus with respect to a free particle in solution indicates a close interaction between the basic residues on the inner wall of the capsid and the negative surface charge of the particle. The authors suggested that it will be possible to use encapsulated Au particles to track changes in the viral capsid volume in a physiological environment, a development useful in unravelling virus infection events.[3]

An earlier report indicated a successful model of *in vitro* self-assembly conditions that generated infectious virions with brome virus capsid proteins around negatively charged gold nanoparticles cores. The optical properties (elastic light scattering) and the influence of the core size of the resulting virus-like particle were described. The authors indicated the formation of a closed shell as opposed to an amorphous protein coat using different coatings on the nanoparticle core. This approach could lead to real-time monitoring of viral traffic of single virus particle and chemical sensing along the intracellular and intercellular viral pathways. This could contribute to a better understanding of the virus transport and cellular compartmentalization.[4]

A recent study applied a circulating-flow quartz crystal microbalance (QCM) bio sensing method combined with oligonucleotide-functionalized gold nanoparticles (i.e. AuNP probes) for detection of dengue virus (DENV). Two kinds of specific AuNP probes were linked by the target sequences onto the QCM chip to amplify the detection signal, i.e. oscillatory frequency change (DeltaF) of the QCM sensor in the DNA-QCM method. The amplified target sequences from the DENV genome acted as a bridge for the layer-by-layer AuNP probes' hybridisation in the method combining the amplification and detection. On AuNP size based evaluation on the layer-by-layer hybridisation, it was found that 13 nm AuNPs showed the best hybridization efficiency. The DNA-QCM bio sensing method was able to detect 2 PFU mL (-1) of dengue virus.[5]

More recently, a method for simultaneous recording of high-resolution topography and cell surface fluorescence in a single scan has been developed. This approach has been defined by the scientists as scanning surface confocal microscopy. Imaging of individual fluorescent particles in the nanometre range on fixed or live cells has been possible by the resolution the system allows. This technique records the interaction of single virus-like particles with the cell surface and demonstrated that single particles sink into the membrane in invaginations akin to ingestion by caveola or pinocytic vesicles. This method has great applications as it provides a technique for elucidating the interaction of individual viruses and other nanoparticles with cells. Gene therapy vector interaction with target cells could be investigated.[6]

An electrochemical Deoxyribonucleic acid (DNA) biosensor (label-free) based hybridisation using 4, 4'-diaminoazobenzene (4, 4'-DAAB) and multi-walled carbon nanotube (MWNT)-modified glassy carbon electrode (GCE) has been developed for short DNA sequences of hepatitis B virus (HBV). The hybridisation was measured by differential pulse voltammetry (DPV). DPV is a form of electrochemical measurement. A series of regular voltage pulses are superimposed and the current is measured before each potential change. The current difference is plotted as the readout. A decrease in the peak current of 4, 4'-DAAB indicated hybridization of probe with the target. This electrochemical approach is sequence specific. This sensor system had a dynamic range between 7.94 x 10(-8) M and 1.58 x 10(-6) M, with HBV DNA sequence detection limit of 1.1 x 10(-8) M.[7]

Synthetic polypeptides (2-5 nm in size, less than 10 kDa) that bind to their target analytes with high affinity and specificity are called antibody mimic proteins (AMPs). These agents behave like conventional antibodies but are much smaller. Recently, AMP use in the field of nanobiosensors has been reported. Nanowire based biosensors coated with AMP (Fibronectin, Fn) was first used to detect nucleocapsid (N) protein of the coronavirus (SARS-CoV) the cause of Severe Acute Respiratory Syndrome (SARS) in an innovative device. N protein was detected at subnanomolar concentration even in the presence of substantially large amounts of bovine serum albumin as background noise, i.e. high signal to noise ratio acquisition.[8]

Application in Infectious Disease Diagnosis

Viruses are important causes of human disease with high morbidity and some with significant mortality. In the evolving global scenario, viruses are worrisome as agents for biological warfare and terrorism. Early sensitive and specific detection methods of viruses are vital to the response in the face of naturally occurring or induced viral epidemics. Diagnosis of infectious diseases in the laboratory has taken advantage of various developments in the field of

immunology, biotechnology and now nanotechnology.[9] This is especially true for diagnosis of viral infections. The enzyme-linked immunosorbent assay (ELISA) was widely introduced in laboratories for the diagnosis of viral infections in the 1980s. The next huge stride was the development of the PCR and its modifications and their application in viral detection which is still the work horse in many clinical laboratories especially in developed countries. Traditional methods for viral detection like isolation of viruses, immunological assays, transmission electron microscopy and PCR-based testing of viral nucleic acids have certain individual limitations and are not very suitable to field or bed side use. Nanotechnology offers the opportunity to remove this constraint. Nanotechnology achieves detection at a single virus level.

In the understanding of viral diseases and therapy as of date, there are many issues related to virus load in tissues and blood. The detection of very low number of viral particles is relevant in certain infectious conditions, totally irrelevant in others and may depend on the body compartment for meaningful interpretation. In the case of blood borne viruses like HIV, HCV and HBV demonstration of even a single virus particle per unit volume of blood (mL) assumes significance for therapy endpoints and donated volunteer blood safety.[10-12] The early detection of a single particle of herpes simplex virus (HSV) in cerebrospinal fluid (CSF) of individuals with encephalitis will impact on successful therapy, patient survival and prognostication.[13]

The drive to develop single virus particle detection technology across the Board has to be tempered with a good understanding of viral disease processes. A case-in-point is the infection with cytomegalovirus (CMV) in immunosuppressed transplant recipients, wherein any virus present in the blood compartment has no bearing on the evolution of the disease process. The body's defences control the virus to a large extent and disease is usually seen associated with high loads of virus in the blood compartment.[14]

Today there is a widespread appreciation of the emerging and re-emerging arboviral infections because of climatic changes as a result of global warming. There was a major multi-country epidemic of Chikungunya virus in 2005-2006.[15] Earlier, there was the spread of the West Nile virus to North America in 2003-2004.[16] A rapid and sensitive detection method for the early diagnosis of infectious dengue virus urgently needs to be developed as this is at present a major public health problem at a global level.[17] A recent study applied a circulating-flow quartz crystal microbalance (QCM) towards this end; the technology holds great promise.[5] The current H1N1 Influenza virus outbreak in different parts of the globe is declared a global pandemic by the World Health Organisation (WHO),[18] surely warranting rapid devices for field use at international airports.

In summation, the exciting new developments in biosensors, combining immune-active reagents or bioactive peptides with electrical sensing and optical imaging, is likely to open a whole new dimension in viral detection. These devices will be field stable usable as well as bedside appliances that have the potential to revolutionize viral diagnosis for patient care and public health initiatives in the face of natural epidemics or bio-terror attacks.

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