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Bioaffinity Detection of Pathogens on Surfaces

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Abstract

The demand for improved technologies capable of rapidly detecting pathogens with high sensitivity and selectivity in complex environments continues to be a significant challenge that helps drive the development of new analytical techniques. Surface-based detection platforms are particularly attractive as multiple bioaffinity interactions between different targets and corresponding probe molecules can be monitored simultaneously in a single measurement. Furthermore, the possibilities for developing new signal transduction mechanisms alongside novel signal amplification strategies are much more varied. In this article, we describe some of the latest advances in the use of surface bioaffinity detection of pathogens. Three major sections will be discussed: (i) a brief overview on the choice of probe molecules such as antibodies, proteins and aptamers specific to pathogens and surface attachment chemistries to immobilize those probes onto various substrates, (ii) highlighting examples among the current generation of surface biosensors, and (iii) exploring emerging technologies that are highly promising and likely to form the basis of the next generation of pathogenic sensors.

Key words: Pathogen Detection, Surface Biosensors, Label-free Detection, Nanomaterials, Lab-on-a-chip

Introduction

Rapid and simple detection of pathogenic species continues to be an important research objective of immense significance for human health, prosperity and security. For example, fungal plant pathogens can result in large crop losses and the clandestine production and use of toxins produced by such pathogens is considered an ever present biothreat. This requires the monitoring of crops, food and water supplies as well as airborne contamination. Despite significant progress made in recent years in the environmental surveillance of harmful agents, the potential to inflict enormous damage to a nation's economy and security remains a strong driving force to develop robust, fieldable biosensors which shorten the time span between sample collection and results without compromising accuracy or sensitivity.

There are a number of well-established laboratory-based approaches for pathogen detection. Culture and colony counting[1] is the oldest bacterial analysis technique and continues to be a standard method that provides unambiguous results. However, culturing often requires up to several days depending on the microbe type thus making other techniques necessary. Molecular identification approaches are primarily based on the detection of specific nucleotide sequences within the pathogen genome or the detection of pathogen-specific protein molecules or epitopes on the cell surface using antibodies. The most widely used methods are based on various polymerase chain reaction (PCR) formats or enzyme-linked immunosorbent assays (ELISA). Even though both these solution-based approaches can be highly specific and sensitive, especially PCR, they often require the time-consuming extraction and purification of nucleic acids or antigens from samples. Other issues include contamination control and discriminating false positive and negative results. In addition, these assays are label-dependent involving the use

of reporter molecules conjugated to either an enzyme or fluorescent probe as well as having very limited capabilities for the simultaneous investigation of multiple targets in a single sample aliquot.

These limitations, among others, have promoted the exploration of a large variety of alternative biosensing technologies in recent years (see Figure 1). Of particular interest are surface bioaffinity sensors capable of direct detection. By direct we mean the detection of species without the need for prior chemical and/or enzymatic manipulation of the target sample prior to analysis. This can be achieved in a “label-free” format where the specific binding of a target onto a surface immobilized probe molecule is usually measured via a change in properties such as refractive index or mass. For targets with more than one epitope, a second labeled bioaffinity probe can be subsequently introduced to further amplify the detection signal. The challenge is to create pathogen sensing methodologies that efficient, highly robust and can be integrated into portable devices for on-site analysis without compromising sensitivity or specificity. Furthermore, the ability to handle complex samples with minimum or no preparation required beforehand is also highly desirable.

It is not the intention of this review to comprehensively discuss each reported approach that has emerged in recent years. Instead, our main focus is the manner in which the presence of the target is transduced (i.e. optical, electronic and mechanical) and the associated detection platform. However, an overview would not be complete without at least a brief discussion on surface chemistry. The remainder of the article is then composed of a further two sections with the first

discussing examples among the current generation of surface biosensors before highlighting new and emerging trends that are likely to form the next generation of pathogenic sensing technologies.

Choice of Surface Bioaffinity Ligands

The crucial first step to consider when applying conventional bioaffinity sensing formats as well as emerging technologies, including nanotechnology, is the design and surface immobilization of ligands that have a high specificity towards a particular target species. While initial approaches were typically limited to identifying single pathogens, there is an increasing demand for multiplexed detection. DNA microarrays have emerged as a key high throughput technology for bacterial and viral typing and detection where hundreds to thousands of individual DNA sequences can be compared in a single experiment. Such analyses first involve the successful extraction and PCR amplification of cleaned-up samples which typically restricts their use to within the laboratory. The utilization of DNA microarrays and fluorescence imaging for pathogen analysis have been nicely reviewed elsewhere and are not discussed further[2].

At present, antibodies remain the most established ligand for the rapid surface bioaffinity detection of pathogens, as demonstrated by the examples discussed in following sections. The surface immobilization of antibodies (and proteins in general) in a stable, high-density and reproducible manner without subsequent loss of bioactivity has proven to be significantly more challenging than that for DNA. However, a variety of chemistries have been developed in recent years allowing immobilisation onto a wide range of sensor surfaces (e.g. glass, silicon, gold,

plastic microwells, hydrogels, etc.)[3]. Surface attachment can occur via physiosorption onto polymer coated surfaces as well as covalent coupling through a cross linker via thiol, maleimide or amino groups and using affinity binding such as biotin/streptavidin[4]. Another issue is the considerable effort required to obtain antibodies that are highly specific towards a single target. A large number of protein and carbohydrate binding sites (epitopes) are common to various different species. The rational production of antibodies involves first identifying pathogen-specific surface proteins[5]. These are then cloned and expressed in order to be used as antigens for antibody production. This involves a great deal of work and consequently many proof-of-principle demonstrations of new sensing technologies focus on a relatively small number of antibody-pathogen interactions.

These challenges have encouraged researchers to explore alternative biomolecular ligands (or probes) whose protein binding properties are similar to or better than those of antibodies.

Particularly promising are nucleic acid aptamers, which are short, single-stranded RNA or DNA sequences (~20-30 bases long) that selectively bind to non-nucleic targets such as proteins as well as a large variety of other targets that include toxins, cells and tissues. Binding occurs via interaction of the target with particular 3-D loop structures formed by the nucleic acids.

Aptamers are typically selected from large libraries using a reiterative selection and amplification process known as SELEX[6]. This process combined with well-established methods for creating nucleic acids, their robustness and the opportunity to introduce additional chemical functionalities offer several advantages over antibodies. Consequently, a growing number of aptamers targeting specific pathogens have started to emerge in recent years[7]. In

another example, Sreevatsan *et al.* utilized DNA aptamers specific to Salmonella enteric serovars in PCR-based detection[8].

Surface-based Pathogen Sensors in Use Today

The new opportunities that a surface-based sensing approach provides such as greater multiplexing capability, enabling coupling with multi-step amplification schemes as well as interfacing with a variety of signal transduction mechanisms has resulted in the development of a wide range of surface-sensitive detection platforms. Figure 2 provides a schematic overview of various sensing methodologies representing the current generation of surface bioaffinity pathogen detection platforms and are discussed in more detail below. A more comprehensive list is provided in Table 1 which also includes typical examples of each technique along with the surface probe used and the reported detection limit.

Fluorescence microscopy

One of the most consistent challenges in performing quantitative biosensing is the requirement of a label to enable signal generation. This can involve directly attaching the label to the target pathogen itself or to a secondary or even tertiary recognition element. In a sandwich assay (see Figure 2a), immobilized antibodies capture the unlabeled target followed by the binding of a second fluorescently labeled antibody specific to a different site on the pathogen[9-12]. Recent examples include prototype fluorescence sensors that can measure intrinsic cellular fluorescence focusing on bacterial cells using hemin (ferriprotoporphyrin IX) tethered on a disposable chip [13], fluorescence array sensors utilizing the evanescent wave for excitation of fluorescently

tagged reporters in pathogen detection[14] as well as miniaturized real-time total internal reflection fluorescence (TIRF) array biosensors using planar waveguides created on different surfaces (glass, silica and polystyrene)[15]. Fluorescence-based analysis remains one of the most popular detection methodologies, however issues such as the additional expense of labeling, dye photobleaching and instrumentation costs have driven the emergence of alternative label-free methods. In particular, techniques with the potential for achieving the necessary sensitivity as well as being low-cost and portable are of especial interest to researchers developing new pathogen detection methods.

Surface plasmon resonance (SPR)

SPR is currently the leading technology for label-free pathogen detection. As shown in Figure 2b, a low power light source is coupled to the interface of a thin gold film via total internal reflection where propagating surface plasmon modes are excited depending on the photon frequency and incidence angle. The plasmon resonance is highly sensitive to local changes in refractive index within the evanescent field at the gold/ sample solution interface and thus does not require the use of a label. When pathogens specifically bind to antibodies immobilized onto the gold surface, changes in the intensity of light reflected off the surface are measured by the detector. The popularity of SPR is indicated by the growing number of companies offering either lab-based or portable SPR instrumentation such as GE Healthcare, IBIS Technologies, GWC Technologies and KMAC. Currently, most measurements are still restricted to one or two pathogen species per sample surface although larger array-type measurements have been performed for nucleic acid and proteomic studies[16]. Typically between one and eight flow

channels are used to deliver samples and controls to the surface and the associated signal changes compared. For example, Irudayaraj *et al.* utilized SPR immunosensors for the detection of various pathogens in conjunction with the modulation of sensor surface using a mixed monolayer of polyethylene glycol (PEG) terminated alkane thiols[17-18]. Homola *et al.* reported an eight-channel SPR sensor based on wavelength division multiplexing for the simultaneous detection of four foodborne pathogens using target specific antibodies[19]. Another recent example is the highly sensitive and selective SPR detection of *Bacillus anthracis* in the presence of other related *Bacillus* spores via the use of a mouse monoclonal antibody designated 8G3 raised against the target spores[20].

Higher degrees of multiplexing and sample detection throughput can be achieved using an SPR imaging platform where multiple ligands are immobilized in an array format with each element individually addressable (see Figure 3). As can be seen from the example in the Figure 3b inset, 16 S ribosomal RNA from *Escherichia coli* (*E coli*) could easily be screened using SPR imaging in conjunction with DNA microarrays fabricated on a self-assembled monolayer of the amine-terminated alkanethiol modified gold surface[21]. Homola *et al.* also employed a SPR imaging platform for the detection of foodborn pathogens[22] utilizing an array of sensing channels prepared by microspotting various thiolated DNA probe solutions complementary to specific 16 S ribosomal RNAs sequences of selected pathogen targets. Furthermore, SPR sensors are readily amenable to incorporation into portable and/or sensing platforms for pathogen taking advantage of the developments in microfluidics and the fabrication of miniaturized optics[23-24]. An extensive list of label -free SPR based detection of pathogens can be found in Table 1.

Quartz crystal microbalance (QCM)

An alternative label-free approach to pathogen detection is the use of a quartz crystal resonator sandwiched between two thin gold electrodes (see Figure 2c) which can be used in a liquid environment for biosensing. The quartz crystal oscillates at a particular resonance frequency and when a change in mass occurs such as due to adsorption of molecules onto the gold surface, the frequency decreases. Changes in oscillation damping can also be used to provide structural information about the thin molecular layers formed. Although QC resonators have been used for many years to control the vapor-phase growth of thin films it is only more recently that this technology has emerged as a competitor to SPR with companies such as Qsense and KSV Instruments providing products targeted at the biosensing market. Immunoassays can be created by immobilizing antibodies onto the gold surface however, unlike for SPR, measurements are restricted to a single target per electrode surface. In an early example, Guilbault *et al.* developed a QCM sensor for the detection of *Listeria monocytogenes*[25-26]. Wong *et al.* were able to detect low levels of salmonella as well as distinguish between different strains[27]. Here, the gold electrode layer was replaced with silver which was coated with a polyethylenimine (PEI) layer and crosslinked via glutaraldehyde which could be activated prior to the immobilization of antibodies. The use of a PEI polymer layer for antibody immobilization on a gold electrode has also been applied to detect rapidly detect the dengue virus from blood samples with a 100-fold better sensitivity than previous methods[28].

Optical waveguide sensors

Optical waveguide structures are another choice for measuring affinity binding (see Fig. 2d). As in the case of SPR, measurements are based on changes in refractive-index within an evanescent

field region, however the design of the waveguide structure can vary greatly. One approach becoming prominent for biosensing, including pathogen detection, is the resonant mirror design supporting different resonant angles for both transverse electric (TE) and transverse magnetic (TM) modes[29-32]. For example, Pazos *et al.* utilized a resonant mirror biosensor for the detection of yessotoxin (YTX) by measuring the changes in the refractive index occurring when the YTX interacted with the ligand, phosphodiesterase, immobilized on glutaraldehyde activated aminosaline surface [32]. Another design example is the metal clad leaky waveguide (MCLW) which has a much longer penetration depth into the sample medium above the detector surface than the resonant mirror. In conjunction with a disposable absorbing cladding material, the LW biosensor could be employed to detect *Bacillus subtilis var. niger* (BG) bacterial spores at 10^4 spores/ml as well as the sensing surface could be tailored to give a maximum extension of the evanescent field greater than the size of the bacteria captured by surface immobilized antibodies[33].

Atomic force microscopy (AFM)

Although AFM was initially developed in the mid-1980's, it is only after around 1995 that its use as a biological research tool began to flourish with improvements in instrumentation[34]. It is based on a very sharp probe tip, only a few nanometers thick, mounted on the end of a cantilever (see Figure 2e). When brought close to a surface a number of different forces can interact with the tip and are measured using a laser spot reflected from the top surface of the cantilever. Both quantitative and qualitative detection and characterization of pathogens adsorbed onto a surface has been achieved using tapping mode AFM which minimizes lateral or dragging force applied by the tip. A description of the different materials (typically glass, mica and silanized silica)

along with various surface chemistries for imaging the surface ultrastructure of various pathogens in real time has been nicely reviewed elsewhere[35]. Prominent examples include single virus particles such as the herpes simplex virus-1 and wild-type moloney murine leukemia, membrane proteins, and the rapid visualization of capsid and DNA deposited on a mica surface using extra oxide-sharpened silicon nitride nanotips in tapping mode[36,37]. Though there are still some sensing limitations associated with mechanical complexity, tip contamination, slow scan speed and instrument size, prominent developments and modifications addressing these issues are underway ensuring AFM will feature among the next generation of biosensing platforms.

Interferometer-based sensing

Interferometry is a well-established optical technique that has been successfully adapted for label-free measurements of bioaffinity interactions. Typically, changes measured in an interference pattern created when a polarized light source split into a reference beam and an analysis beam is reflected off a surface. Changes in refractive index at the surface create differences in phase which is converted in an amplitude change. Among the various types of interferometers, Mach-Zehnder, Young's, Hartman and backscattering configurations have been utilized for the detection of biomolecules including pathogens[38-40]. Ghadiri *et al.* demonstrated an approach utilizing biofunctionalized thin films of porous silicon to detect small molecular targets including 16-nucleotide DNA oligomers and proteins at concentrations in the picomolar to femtomolar range[41]. Also, in a novel approach based on a Young interferometer, Kanger *et al.* designed a chip consisting of four parallel channel waveguides connected at one end via a common light entry point[42]. The light emitted from the four channels at the opposite

end results in an interference pattern which is sensitive to refractive index changes in any one of the channels. A detection limit of 850 particles/mL was reported for the herpes simplex virus (HSV) type-1 where one of the channel surfaces was coated with a specific antibody against HSV with the technique easily adaptable to other targets.

Emerging Technologies for Pathogen Detection

The surface bioaffinity sensing schemes discussed in the previous section typically lack the sensitivity necessary for routine measurements at concentrations below the nanomolar level. The continual development of new probes and their surface immobilization is important to improve specificity, however, will not improve sensitivity by orders of magnitude. Recently, the implementation of nanomaterials possessing novel optical and electrical properties has begun to open up many new opportunities for pathogen sensing. In this section, we highlight recent efforts aimed at novel signal amplification strategies based on (i) the use of biofunctionalized nanomaterials as an amplification tool, (ii) designing new optical and electrical based detection methodologies, and (iii) developing miniaturized on-chip detection systems which also integrate sample processing.

Gold nanoparticles whose size and shape support the optical excitation of surface plasmons have been the most widely explored for use in various optical detection platforms[43]. Using well-established thiol-based surface attachment chemistries, the sensitivity of the first generation optical sensors discussed previously can be significantly improved via a secondary amplification step utilizing a specific nanoparticle/probe bioconjugate. In this typical sandwich assay design

the presence of the nanoparticle induces large changes in the localized refractive index and if the operational wavelength of the sensor overlaps the nanoparticle plasmon resonance then additional sensitivities can be achieved[44]. Often, sensitivities greater than conventional SPR imaging can easily be obtained[45].

Another approach involving gold nanoparticles is the design of simple colorimetric assays based on the controlled aggregation of a colloidal solution in the presence of a specific target. For example, galactose stabilized gold particles have been applied for the sensitive detection of ricin[46]. The use of the relatively small galactose probe promotes greater sensitivity than when larger probes (such as antibodies) are attached to nanoparticles which result in greater particle separation. In addition, nanorod shaped particles which exhibit both a transverse and longitudinal surface plasmon resonance with the latter extending into the visible and near-infrared have been shown to be more sensitive to local changes in refractive index than spheres. Irudayaraj *et al.* devised an assay for the multiplexed detection of several pathogens using several nanorod sizes each functionalized with an antibody specific to a different target. Both *E. coli* and *S. Typhimurium* were successfully detected by measuring shifts in the extinction spectrum in the regions associated with the longitudinal plasmon resonance[47].

Beyond the use of simple adsorption/scattering based measurements, the enhanced electric field properties of specially designed metallic nanostructures have encouraged the exploration of a number of different spectroscopies for pathogen detection. Surface enhanced Raman spectroscopy (SERS) offers opportunities for ultrasensitive detection with the advantage of enabling chemical analysis and pathogen fingerprinting. However, the development of robust

methods for integrating SERS into reliable sensing methodologies has continued to prove challenging. One approach is to use reporter molecules with distinct spectra to label pathogens of interest. Porter *et al.* designed a sandwich immunoassay chip format for the detection of feline calicivirus[48]. Antibodies specific to the virus were immobilized on gold nanoparticles along with an extrinsic Raman label 5,5-dithiobis(succinimidyl-2-nitrobenzoate) and were able to detect less than 100 virus surface binding events.

It is also possible to detect pathogens via SERS without using labels. Using a Ag nanorod array platform (see Figure 4A), Tripp *et al.* were able to uniquely identify different viruses as well as differentiate between strains of the same virus[49]. Detection of the respiratory syncytial virus (RSV) in small (0.5-1.0 μ l) volumes was identified by measuring the binding of the spike like glycoprotein projections on the membrane envelope through an amino group, which give rise to a strong band due to the C-N stretching mode. Different viral strains were characterized by differences in the relative intensities of peaks in the spectra due to unique pathogen surface proteins binding on the SERS substrate (see Figure 4B). As a result, a reference library of fingerprints could be established for various viruses and strains.

Another example of a novel optical biosensing technology benefiting from plasmonic nanostructures is the development of nanoparticle-enhanced diffraction gratings (NEDG)[44, 50]. Here, the coupling of the optical properties of both planar surface plasmons generated on gold diffraction gratings and adsorbed gold nanoparticle bioconjugates are employed (see Figure 5). In a first demonstration, locked nucleic acid probe molecules were immobilized on the grating to which target microRNA sequences were first hybridized followed by a polymerase A reaction to

create the surface poly A tails on the grating surface. When polythymine coated gold nanoparticles specifically hybridized to the polyadenosine tail, the diffraction efficiency was measured at the first order spot, which proportionally increased as a function of the target concentration. The NEDG sensors initially demonstrated the detection of microRNA and DNA at concentrations as low as 10 fM ranges[44, 50]. We envision that NEDG sensors possess hold significant potential for a wide range of applications including the highly sensitive detection of pathogens as well as other biological molecules.

In addition to surface sensitive optical sensors involving nanomaterials, electrochemical detection is an excellent alternative since electrical signals can be greatly amplified and multi-nano electrode platforms have the potential for high throughput detection. For example, electrochemical immunoassays using interdigitated nanostructured silicon transducers have been demonstrated for the rapid discrimination of live pathogens versus harmless dead ones[51]. This was achieved using a pair of interdigitated polycrystalline silicon electrodes whose surface was coated by thin layer of native silicon oxide upon which antibody probes were immobilized. Impedance spectra were measured at a high frequency range along with changes in the capacitance caused by the interaction of the live bacteria with supporting AFM measurements used to image bacterial cells. In another example, lithographically fabricated silicon nanowire transistors featuring 50 nm polysilicon nanowires connected with gold electrodes spaced 150 nm apart and a 200-500 nm polyimide insulating layer (see Figure 6a) have been developed for the ultra sensitive detection of the bacterial toxin, staphylococcus aureus enterotoxin B (SEB), at femtomolar concentrations[52]. As shown in Figure 6, the charge transfer resistances associated

with the mass transfer of SEB complexed with the anti-SEB on the nanowire electrode proportionally increased with the SEB concentration. The In_2O_3 nanowire FETs sensing platform was further developed for the detection of severe acute respiratory syndrome (SARS) virus by means of measuring the interaction of a biomarker associated with the SARS corona virus with the antibody mimic proteins immobilized on the nanowire surface[53].

Other types of nanomaterials besides metallic structures have also been applied to pathogen sensing. For example, peptide nanotubes were designed for the detection of viruses such as herpes simplex virus type 2, adenovirus, vaccinia and influenza type B at attomolar sensitivities[54]. The peptide nanotubes were functionalized with antibodies at their ends and fluorescent signaling units on their sidewalls. When viral pathogens were mixed with these antibody-coated nanotubes, the nanotubes rapidly aggregated around the viruses to form a networked structure. The size of the aggregates increased with the concentration of viruses and detected via flow cytometry by measuring forward light scattering intensity and fluorescence intensity of aggregated dye-loaded antibody nanotubes around viruses.

Finally, another emerging area we want to highlight is the promise of portable, rapid and highly sensitive diagnostic microchip based devices incorporating microfluidics alongside miniaturized electronics, optical elements, fluid handling components and data acquisition software[55]. The “lab-on-a-chip” concept involving a fully integrated system offers several advantages such as requiring very small amounts of sample, high precision, shortened analysis times and good reproducibility. With respect to pathogen detection most recent advances in this area have

focused on the challenge of incorporating conventional PCR based technologies[56]; for example, an integrated PCR–capillary electrophoresis chip[57]. One highlight is the combined use of the optothermal properties of gold nanorods in a microfluidic chip device along with a one-step real-time PCR system[58]. The longitudinal resonance of gold nanorods was used to transform near infrared energy into thermal energy resulting in effective on-chip pathogen lysis. The DNA extracted out of the *E. coli* BL21 cell body was transferred to a real-time PCR chamber for amplified detection without additional sample processing. Another interesting approach worth mentioning is the design of a complete integrated chip-based system (see Figure 7a) for the ultra-sensitive detection of avian influenza virus H5N1[59]. The sample viral RNA is first isolated, purified and preconcentrated using silica-coated superparamagnetic particles before undergoing RT-PCR on a miniaturized thermocycler. As depicted in Figure 7b, each step was performed in droplets on a Teflon coated disc manipulated under a controlled magnetic field. In particular, PCR was achieved within 30 minutes by moving the droplet clockwise over different temperature zones which was monitored in real time with fluorescence-based detection utilizing an integrated optical system. In addition to on-chip PCR devices, microchannels filled with a photopolymerized crosslinked polyacrylamide gel were developed to achieve improved electrophoretic separation of the antibody-analyte complex from excess antibody. The ratio of bound versus unbound immune-complexes in the microchannel was then measured by laser-induced fluorescence. The rapid detection of SEB, shiga toxin I and ricin at picomolar concentrations using minimal sample volumes (<10 μ l) was achieved in 20 minutes[60].

Conclusions/Outlook

Of the various sensing methodologies described here, optical-based surface-sensitive techniques currently remain the most promising for ultrasensitive high throughput pathogen detection. The introduction of new nanomaterials and lab-on-a-chip concepts has been shown to significantly advance current biosensing technologies in terms of sensitivity, disposability and cost-effectiveness. The ideal technique will be able to simultaneously monitor multiple targets directly in complex environments with minimum or no prior sample preparation and have a large measurement dynamic range from subfemtomolar to nanomolar concentrations. Furthermore, new sensing technologies should be capable of not only detecting the presence of a particular pathogen but also assessing its bioactivity to determine whether the pathogen is in an active state. This requires continual advances in both the design and availability of biomolecular probes specific to a wide range of targets along with improvements in surface chemistries which eliminate issues such as non-specific adsorption irrespective of sample type or complexity. Due to the continual demand for improved pathogen sensing, this field will remain at the forefront of development as one of the first applications with which to assess the performance of next generation sensors. We envision that development of new optical sensing techniques utilizing nanomaterials integrated within complete lab-on-a-chip platforms will feature prominently in the near future as this exciting area of research continues to rapidly develop.

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Figure Captions

Figure 1. Timeline describing the development of pathogen detection methods.

Figure 2. Schematic overview of surface-based optical detection platforms for pathogen detection; (a) fluorescence microscopy, (b) surface plasmon resonance (SPR), (c) quartz crystal microbalance (QCM), (d) optical waveguide sensors and (e) atomic force microscopy (AFM).

Figure 3. Schematic of SPR imaging set-up with the inset showing a representative SPR difference image showing the hybridization adsorption of 2 nM 16S rRNA from *E. coli* onto a three-component DNA array. The array element C is the 25mer DNA probe sequence complementary to *E. coli* RNA. Inset data is adapted with permission from ref. 21.

Figure 4. (A) Schematic diagram showing the detection of respiratory virus based on surface enhanced Raman scattering (SERS) using silver nanorod array substrates. (B) SERS spectra showing a) uninfected vero cell lysate, b) RSV-infected cell lysate and c) purified RSV.

Distinctive spectral bands assigned at 1066 cm^{-1} (C-N stretch), 835 cm^{-1} (tyrosine), and a doublet at 545 cm^{-1} and 523 cm^{-1} (S-S) appear in the RSV infected cell lysate samples but not in the uninfected cell lysates. Adapted with permission from ref. 49.

Figure 5. Schematic of nanoparticle enhanced diffraction grating setup for the detection of pathogens using various probes including aptamers, antibodies and short oligomers immobilized on gold line grating surfaces. Biofunctionalized gold nanoparticles (incl. nanorods) can be

utilized to enhance the diffraction signal in a sandwich format where the surface probe interacts with the target pathogen followed by the recognition of biomolecules coated on the nanomaterials. Briefly, p-polarized white light through a narrowband pass filter is impinged onto a prism/grating chip/flow cell assembly at a fixed incidence angle. Next, either the +1, 0 and -1 orders can then be imaged on a CCD camera or the +1 diffraction beam can be passed through a lens and detected using an avalanche photodiode (APD). The left bottom inset is the 3D image of the +1, 0, -1 orders. The right inset is a representative TEM image of gold nanorods with peak maxima at 510 nm and 720 nm.

Figure 6. (a) Schematic depicting an In_2O_3 nanowire device for SARS virus detection. The nanowire was functionalized with Fn probes which can specifically bind the target N protein. Bovine serum albumin (BSA) was used to prevent any nonspecific binding events. (b) Response curve for the N protein interacting with the Fn probe molecules immobilized on the surface of nanowire device. The arrows are the times when a given concentration of N protein solution was injected. The inset on the left side is to show the plateau and the definition of response time. Adapted with permission from ref. 53.

Figure 7. Schematics showing (a) the arrangement of droplets on a PCB printed circuit board, and (b) droplet manipulation using magnetic forces through a series of processes on a perfluorinated surface. G is the perfluorinated glass substrate, M is the permanent magnet, T is the miniaturized thermocycler indicating one of four donut-shaped circles, Sa is the raw sample solution spiked with *in vitro* transcribed HPAI H5N1 RNA including lysis/binding/enhancer

solution and silica particles, W1 and W2 are the washing solution 1 and 2, R is the RT-PCR mixture covered by mineral oil. Adapted with permission from ref. 59.

References

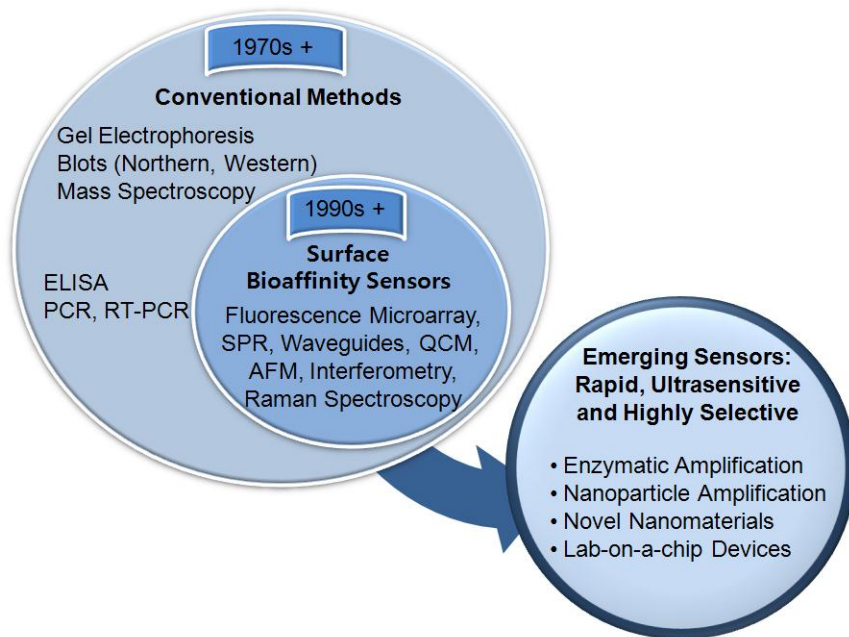
- [1] T. M. Strauba, D. P. Chandler, *J. Microbiol. Methods* 53, (2003) 185.
- [2] J. R. Epstein, I. Biran, D. R. Walt, *Anal. Chim. Acta* 469, (2002) 3.
- [3] H. Zhu, M. Snyder, *Curr. Opin. Chem. Biol.* 7, (2003) 55.
- [4] M. Uttamchandani, J. Wang, S. Q. Yao, *Mol. BioSyst.* 2, (2006) 58.
- [5] P. Leonard, S. Hearty, J. Brennan, L. Dunne, J. Quinn, T. Chakraborty, R. O'Kennedy, *Enzyme Microb. Technol.* 32, (2003) 3.
- [6] A. D. Ellington, J. W. Szostak, *Nature* 346, (1990) 818.
- [7] N. O. Fischer, T. M. Tarasow, J. B-H. Tok, *Curr. Opin. Chem. Biol.* 11, (2007) 316.
- [8] R. Joshi, H. Janagama, H. P. Dwivedi, T. M. A. S. Kumar, L. Jaykus, J. Schefers, S. Sreevatsan, *Mol. Cell Probes* 23, (2009) 20.
- [9] R. I. Stefan, J. F. Staden, H. Y. Aboul-Enein, *Fresenius J. Anal. Chem.* 366, (2000) 659.
- [10] C. A. R. Taitt, J. P. Golden, M. J. Feldstein, J. J. Cras, K. E. Hoffman, F. S. Ligler, *Biosens. Bioelectron.* 14, (2000) 785.
- [11] C. A. R. Taitt, Y. S. Shubin, R. Angel, F. S. Ligler, *Appl. Environ. Microbiol.* 70, (2004) 152.
- [12] K. E. Sapsford, M. M. Ngundi, M. H. Moore, M. E. Lassman, L. C. Shriver-Lake, C. R. Taitt, F. S. Ligler, *Sens. Actuators B* 113, (2006) 599.
- [13] H. Mason, C. Lloyd, M. Dice, R. Sinclair, W. Ellis, L. Powers, *Biosens. Bioelectron.* 18, (2003) 521.
- [14] C. R. Taitt, G. P. Anderson, F. S. Ligler, *Biosens. Bioelectron.* 20, (2005) 2470.
- [15] K. E. Sapsford, Y. S. Shubin, J. B. Delehanty, J.P. Golden, C.R. Taitt, L.C. Shriver-Lake, F. S. Ligler, *J. Appl. Microbiol.* 96, (2004) 47.
- [16] S. J. Oh, B. J. Hong, K. Y. Choi, J. W. Park, *OMICS: J. Integr. Biol.* 10, (2006) 327.
- [17] A. Subramanian, J. Irudayaraj, T. Ryan, *Biosens. Bioelectron.* 21, (2006) 998.
- [18] A. Subramanian, J. Irudayaraj, T. Ryan, *Sens. Actuators B* 114, (2006) 192.
- [19] A. D. Taylor, J. Ladd, Q. Yu, S. Chen, J. Homola, S. Jiang, *Biosens. Bioelectron.* 22, (2006) 752.
- [20] D-B. Wang, L-J. Bi, Z-P. Zhang, Y-Y. Chen, R-F. Yang, H-P. Wei, Y-F. Zhou, X-E. Zhang, *Analyst* 134, (2009) 738.
- [21] B. P. Nelson, T. E. Grimsrud, M. R. Liles, R. M. Goodman, R. M. Corn, *Anal. Chem.* 73, (2001) 1.
- [22] M. Piliarik, L. Párová, J. Homola, *Biosens. Bioelectron.* 24, (2009) 1399.
- [23] A. Dahlin, M. Zäch, T. Rindzevicius, M. Käll, D.S. Sutherland, F. Höök, *J. Am. Chem. Soc.* 127, (2005) 5043.
- [24] J. Ferreira, M. J. L. Santos, M. M. Rahman, A. G. Brolo, R. Gordon, D. Sinton, E. M. Girotto, *J. Am. Chem. Soc.* 131, (2009) 436.
- [25] M. Minunni, M. Mascini, R. M. Carter, M.B. Jacobs, G.J. Lubrano, G.G. Guilbault, *Anal. Chim. Acta* 325, (1996) 169.
- [26] R. D. Vaughan, C. K. O'Sullivan, G. G. Guilbault, *Enzyme Microb. Technol.* 29, (2001) 635.
- [27] Y. Y. Wong, S.P. Ng, M. H. Ng, S. H. Si, S. Z. Yao, Y. S. Fung, *Biosens. Bioelectron.* 17, (2002) 676.
- [28] C-C. Su, T-Z. Wu, L-K. Chen, H-H. Yang, D-F. Tai, *Anal. Chim. Acta* 479, (2003) 117.
- [29] H. J. Watts, C. R. Lowe, D. V. Pollard-Knight, *Anal. Chem.* 66, (1994) 2465.
- [30] N. J. Goddard, K. Singh, R. J. Holmes, B. Bastani, *Sens. Actuators B* 51, (1998) 131.

- [31] N. J. Goddard, K. Singh, J. P. Hulme, C. Malines, R. J. Holmes, *Sens. Actuators A* 100, (2002) 1.
- [32] M-J. Pazos, A. Alfonso, M. R. Vieytes, T. Yasumoto, J. M. Vieites, L. M. Botana, *Anal. Biochem.* 335, (2004) 112.
- [33] M. Zourob, S. Mohr, B. J. T. Brown, P. R. Fielden, M. McDonnell, N. J. Goddard, *Sens. Actuators B* 90, (2003) 296.
- [34] P. Parot, Y. F. Dufrene, P. Hinterdorfer, C. L. Grimellec, D. Navajas, J-L. Pellequer, S. Scheuring, *J. Mol. Recognit.* 20, (2007) 418.
- [35] Y. F. Dufrene, *J. Bacteriol.* 184, (2002) 5205.
- [36] M. Plomp, M. K. Rice, E. K. Wagner, A. McPherson, A. J. Malkin, *Am. J. Pathol.* 160, (2002) 1959.
- [37] Y. G. Kuznestov, A. Low, H. Fan, A. McPherson, *Virology* 323, (2004) 189.
- [38] R. G. Heideman, R. P. H. Kooyman, J. Greve, *Sens. Actuators B* 10, (1993) 209.
- [39] M. Weisser, G. Tovar, S. Mittler-Neher, W. Knoll, F. Brosinger, H. Freimuth, M. Lacher, W. Ehrfeld, *Biosens. Bioelectron.* 14, (1999) 405.
- [40] K. H. Seo, R. E. Brackett, N. F. Hartman, D. P. Campbell, *J. Food Prot.* 62, (1999) 431.
- [41] V. S-Y. Lin, K. Motesharei, K-P. S. Dancil, M. J. Sailor, M. R. Ghadiri, *Science* 278, (1997) 840.
- [42] A. Ymeti, J. Greve, P. V. Lambeck, T. Wink, S. W. F. M. Hövell, T. A. M Beumer, R. R. Wijn, R. G. Heideman, V. Subramaniam, J. S. Kanger, *Nano Lett.* 7, (2007) 394.
- [43] Z. Wang, L. Ma, *Coord. Chem. Rev.* 253, (2009) 1607.
- [44] A. W. Wark, H. J. Lee, A. J. Qavi, R. M. Corn, *Anal. Chem.* 79, (2007) 6697.
- [45] M. Ito, F. Nakamura, A. Baba, K. Tamada, H. Ushijima, K. H. A. Lau, A. Manna, W. Knoll, *J. Phys. Chem. C* 111, (2007) 11653.
- [46] C. L. Schofield, B. Mukhopadhyay, S. M. Hardy, M. B. McDonnell, R. A. Field, D. A. Russell, *Analyst* 133, (2008) 626.
- [47] C. Wang, J. Irudayaraj, *Small* 4, (2008) 2204.
- [48] J. D. Driskell, K. M. Kwarta, R. J. Lipert, J. D. Neill, J. F. Ridpath, M. D. Porter, *Anal. Chem.* 77, (2005) 6147.
- [49] S. Shanmukh, L. Jones, J. Driskell, Y. Zhao, R. Dluhy, R. A. Tripp, *Nano Lett.* 6, (2006) 2630.
- [50] H. J. Lee, A. W. Wark, R. M. Corn, *Analyst* 133, (2008) 596.
- [51] R. Rica, A. Baldi, C. Fernández-Sánchez, H. Matsui, *Anal. Chem.* 81, (2009) 3830.
- [52] N. N. Mishra, W. C. Maki, E. Cameron, R. Nelson, P. Winterrowd, S. K. Rastogi, B. Filanoski, G. K. Maki, *Lab Chip* 8, (2008) 868.
- [53] F. N. Ishikawa, H-K. Chang, M. Curreli, H. Liao, C. A. Olson, P-C. Chen, R. Zhang, R. W. Roberts, R. Sun, R. J. Cote, M. E. Thompson, C. Zhou, *ACS Nano* 3, (2009) 1219.
- [54] R. I. MacCuspie, I. A. Banerjee, C. Pejoux, S. Gummalla, H. S. Mostowski, P. R. Krauske, H. Matsui, *Soft Matter* 4, (2008) 833.
- [55] F. B. Myers, L. P. Lee, *Lab Chip* 8, (2008) 2015.
- [56] A. Lauri, P. O. Mariani, *Genes Nutr.* 4, (2009) 1.
- [57] A. R. Prakash, C. D. Rosa, J. D. Fox, K. V. I. S. Kaler, *Microfluid Nanofluid* 4, (2008) 451.
- [58] K. H. Cheong, D. K. Yi, J-G. Lee, J-M. Park, M. J. Kim, J. B. Edel, C. Ko, *Lab Chip* 8, (2008) 810.
- [59] J. Pipper, M. Inoue, L. F-P. Ng, P. Neuzil, Y. Zhang, L. Novak, *Nature Medicine* 13, (2007) 1259.
- [60] R. J. Meagher, A. V. Hatch, R. F. Renzi, A. K. Singh, *Lab Chip* 8, (2008) 2046.
- [61] Y. Zhang, J. T. Bahns, Q. Jin, R. Divan, L. Chen, *Anal. Biochem.* 356, (2006) 161.
- [62] F. Zezza, M. Pascale, G. Mule, A. Visconti, *J. Microbiol. Methods* 66, (2006) 529.
- [63] K-B. Lee, E-Y. Kim, C. A. Mirkin, S. M. Wolinsky, *Nano Lett.* 4, (2004) 1869.

Table 1. Summary of various surface bioaffinity sensing methods applied to a wide range of pathogens along with the type of surface probe used and the reported detection limit.

Technology Platform	Target Pathogens	Surface Probes	Detection Limit	Ref.
Fluorescence microscopy	Staphylococcal enterotoxin B	Antibody	10^3 - 10^6 cfu/mL	[10]
	<i>Campylobacter jejuni</i>	Antibody	500-3780 cfu/mL	[12]
	<i>Salmonella typhi</i>	Porphyrin	10^2 cells	[13]
	Dengue	Antibody	15 pmol/L	[61]
SPR	<i>Escherichia coli</i> (<i>E. coli</i>) O157:H7	Antibody	10^3 cfu/mL	[17]
	<i>Bacillus anthracis</i>	Antibody	10^4 cfu/mL	[20]
	<i>Fusarium culmorum</i>	Oligonucleotide	0.06 pg/30 ng	[62]
	<i>E. coli</i>	Oligonucleotide	2 nM	[21]
	<i>Brucella abortus</i> , <i>E. coli</i> , <i>Staphylococcus aureus</i>	Oligonucleotide	100 pM	[22]
QCM	<i>Listeria monocitogenes</i>	Antibody	10^7 cells/mL	[26]
	<i>Salmonella</i>	Antibody	10^4 cells/mL	[27]
	Dengue	Antibody	5 μ g/mL	[28]
Optical waveguide	<i>Staphylococcus aureus</i>	Protein	4×10^3 - 1.6×10^6 cells/mL	[29]
	Yessotoxin	Enzyme	3.85 μ M	[32]
	<i>Bacillus globigii</i>	Antibody	10^7 spores/mL	[33]
AFM	Herpes simplex virus	--	Single virus particle	[36]
	Moloney murine leukemia virus	Antibody	11-14 cells	[37]
	Human immunodeficiency virus-type 1	Antibody	25 fg/mL	[63]
Interferometer	<i>Salmonella typhimurium</i>	Antibody	1×10^4 cfu/mL	[40]
	Herpes simplex virus type 1	Antibody	850 particles/mL	[42]
SERS	Feline calicivirus	Antibody	1×10^6 viruses/mL	[48]
	Respiratory syncytial virus	--	100 pfu/mL	[49]

Figure 1



* In 1957, First Discovery of *Pandemic of Asian Flu* and *Spongiform Encephalopathy (kuru)*

Figure 2

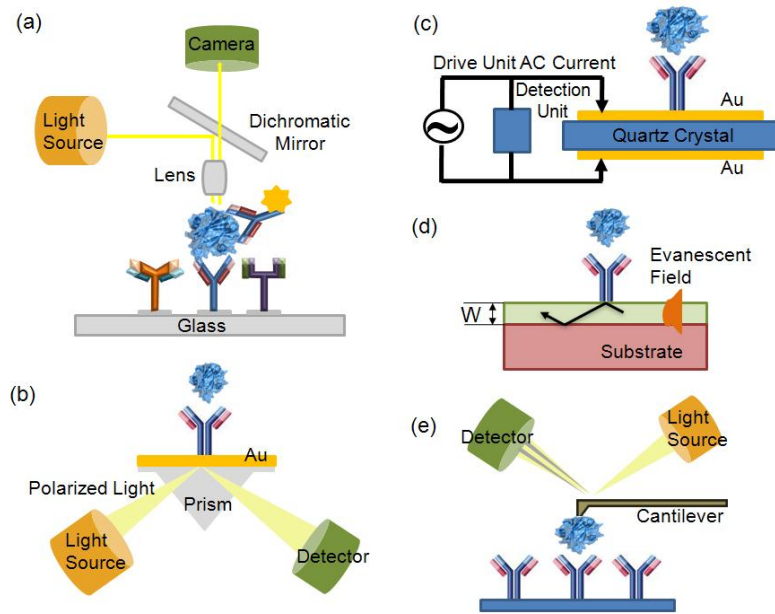


Figure 3

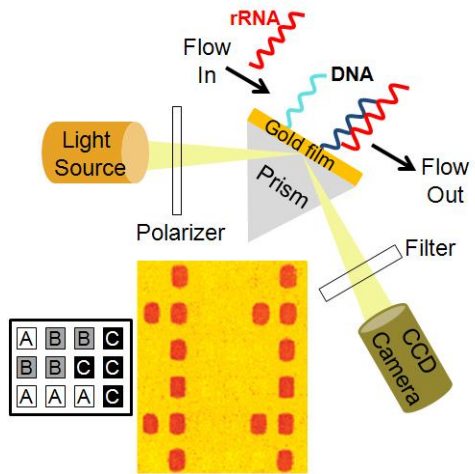


Figure 4

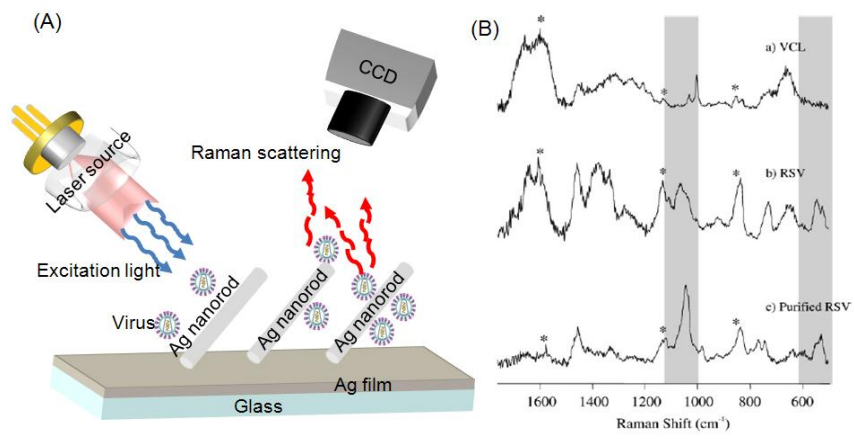


Figure 5

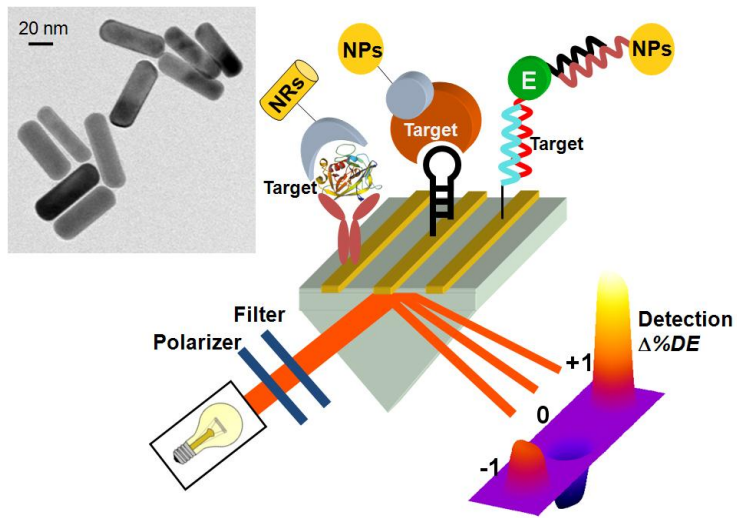


Figure 6

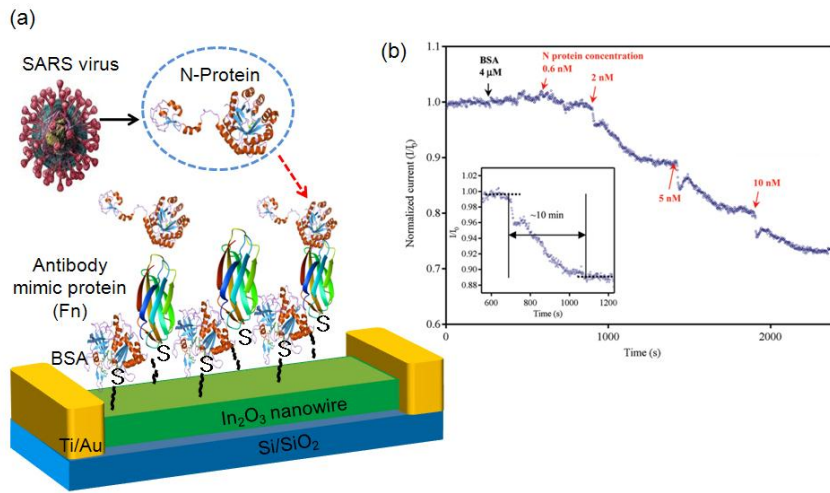


Figure 7

