Abstract

Using nanotechnology methods, a simple lab-on-chip nano-device can be formulated which will act as a point-of-care diagnostic tool. This research proposal suggests a diagnostic nano-device designed using a protein-gold 2D array bi-conjugate system. A protein-gold nanoparticle hybridized 2D array chip can incorporate a broad range of disease-specific antigen binding proteins, conjugated to 13nm gold nanoparticles. It would contain a micro-fluidics chamber where the whole sample would be sorted to the cell level using acoustic waves, thereby increasing the efficiency and specificity of detection. Multiple copies of a pathogenic antigen binding protein can be incorporated in a single device which can be detected through multiple Raman-encoded released dyes, providing multiplexing and high specificity. A simple lab-on-chip nano-sensor device can speed up the processing, increase diagnostic efficiency, and allow multiple disease detection in single device. It can also eliminate the need for extensive lab processing of samples, and fluid-samples can be loaded directly onto the device to detect the pathogen through the interaction of its membrane bound proteins with Au-conjugated proteins.

Introduction and Literature Review

The processing, analysis and detection of infectious diseases is time consuming, costly, and sometimes requires extensive lab testing. The diagnostic tests routinely followed in labs do not multiplex the results, making it impossible to detect multiple pathogens
while running a limited number of samples. Further, the lack of availability of automated diagnostic tools requires extensive knowledge of virology and microbiology to detect pathogens in samples obtained from patients. The whole process of lab-based diagnosis (e.g. PCR, plating and microbiology assays) is time consuming and costly, and results in direct exposure with pathogenic samples.

A simple lab-on-chip nano-device that can detect multiple types of diseases through a single test can relieve a significant burden on the healthcare industry. It can also increase the efficiency of diagnosis through multiplexing, making it more time-effective and lowering costs. It also eliminates direct contact with highly virulent pathogens. In case of multiple bacterial infections, such as pneumonia, a single "miracle" device would be able to detect and quantify the pathogenic load, making it easy for doctors to adjust drug dose. Further, a lab-on-chip nano device can eliminate the requirement for proper handling and extensive assays for sample processing.

The whole device will incorporate two nano-technology concepts: first, a micro-fluidics chamber synthesis to compartmentalize the cells to the single-cell level using acoustic waves. The running buffer in these channels would help to sort cells in the sample, prevent photo-bleaching, and decrease non-specific binding with detection protein in the second compartment (the detection chamber). The second chamber of the nano-chip device would consist of multiple protein-gold nanoparticles conjugated for disease specific binding (1). The optical sense would then be utilized to detect the fluorescence signal given off as a SERS dye signal in the chamber in the presence of a pathogenic agent in the sample (3).
Figure 1: The flow-sheet diagram of multiple infection nano-device assembly. Stage by stage development of nano-device, the corresponding characterization techniques along with its working principle is highlighted.

The Design of the Device

There would be two compartments in the working model of our micro-fluidics nanodevice. The first would be a single cell sorter micro-fluidics chamber designed to concentrate the running sample. Second would be a nano-sensor compartment which would provide high specificity and detection to the pathogens present in the sample.

Microfluidics Chamber

The working principle of this part of device is through generation of high acoustic pressure waves to separate different cells, proteins, and debris of fluid sample at a single cell level. When the pathogen enters the detection chamber, specificity and ease of detection could be increased.

- The chip is plated on a microfluidic compartment with flowing buffer which separates the cells at a single cell level prior to detection. The acoustic waves are the driving force behind the buffer flow in the microfluidics channels.
- The desired pattern can be made on a silicon wafer using standard photolithography processes to create micro-channels. Aluminum layers of
various thicknesses can then be deposited onto different glass wafers using an electron beam evaporator to create coating.

- A running buffer is prepared for micro-fluidic channel using Tris-borate EDTA (TBE) buffer. This buffer solution would further contain polyvinylpyrrolidone, surfactant, to prevent the adhesion of the specimen to the surface of the nano-channels. β-mercaptoethanol would be added to reduce photobleaching effect of buffer (6).

The 2D Protein-Gold Nanoparticles Array

The 2D protein gold nanoparticle array part of our device would be composed of a silicon substrate, the size of a glass slide, on which gold nanoparticles conjugated with the proteins can act as a pathogen detection chamber immediately following the single cell sorter chamber. The fluorescent emission as a measure of optical signals would be the working principle for this part of device.

- The Gold nanoparticles of size 13 nm can be carefully synthesized with thiol functionalized organic layer using the Brust method.
- The Thiol functionalized Au-NPs can then be used as building blocks, allowing the assembly of sulphur functionalized disease specific antigen binding proteins.
- This process would then create a 2D array hybrid of protein-gold conjugate on silicon substrate. The protein-gold hybrid can be allowed to fabricate on silicon-based substrate using alkane-thiol treatment on amino-propyl triethoxy saline monolayer, which causes the adsorption of hybrids on 2D array (3,5).
- The whole nano-sensor device can then be designed so the Au-Nps are conjugated with different disease-specific antigen binding protein in a single device linked with a different Raman's dye at each end (4).

Operation of the Nanosensor

Gold Nanoparticles (AuNP)-based nanosensor functions on the basis of the hybridization technique. Oligonucleotides used for the hybridization reaction will be specific for the detection of disease causing pathogen. Specific probes against genes coding for env protein of HIV-1 can be ligated to Thiol groups which, when binding to
its target, exhibit change in the surface Plasmon resonance of AuNPs. This is because
the bounded probe would exhibit different dimensions and size resulting in slightly
different SPR (7). Fluorescent probes can also be used to add into the properties of such
nanosensors. The two different probes (red and green fluorescence) used in this
particular array can be exhibited at the same excitation wavelength. In the presence of
target DNA (Env gene), green fluorescence probe will bind it which will be followed by
the binding to the surface of AuNPs.

While in the presence of another DNA, the oligonucleotide probe showing specificity to
target DNA (e.g. Botulinum toxin, a neurotoxic protein secreted by Clostridium
botulinum) will bind to AuNP assembly to exhibit fluorescence in the red wavelength
spectrum (7). Utilizing these specific probes (molecular beacons), a nanosensor can be
devised which can hypothetically detect any disease using this particular strategy
through incorporation of molecular beacons with the same absorption spectrum but
different emission spectrum.
Figure 2: The Diagrammatic depiction of synthesis, assembly and functionalization of 2D Au-protein array for multiple disease detection.

Characterization and Optimization of the Equipment

The characterization and optimization of the equipment will come in two stages: the characterization of the nanoparticles and the calibration of fluorescent nanosensors.

Characterization of Nanoparticles

Nanosensors’ optimization criteria is based on physical characterization of nanoparticles being utilized. Size, surface topography, and measurement of \( \zeta \)-potential are the gold standard for the nanoparticles’ characterization. These techniques will provide an overview on the surface charge density and its stability along with the polydisperse nature of nanoparticles. Atomic force Microscopy (AFM),
Scanning / Transmission Electron Microscopy (SEM, TEM), Florescence Spectrometry, X-ray Photoelectron spectrometry are few other techniques that will be taken into consideration for characterization of nanoparticles. Dynamic Light scattering provides an insight into the colloidal stability of Nanoparticle dispersion (9).

Calibration of Fluorescent Nanosensors

Chemically, pH sensitivity will be characterized by calibration of fluorescent nanoparticles towards hydrogen ions. Measurement of Fluorescence Intensity will be determined by a fluorescence intensity measurement before the dialysis and after performing the dialysis procedure. The dialysis procedure will allow us to evaluate the innate fluorescence potential of nanoparticles to be used as flurophores in the nanosensors (9). The need for the accurate detection, and the quantification of fluorescent signals in relation with the analyte, is a crucial step which requires accurate concentration along with the establishment of an operational range for the nanosensors (10).

Novel Features of our Design

A point-of-care gold 2D array nanodevice, when conjugated with a micro-fluidic system, can act as an automated system with multiplexing ability and innovative high throughput tool for disease detection because:

● Multiple disease protein gold hybrids fixed on a single 2D array can provide a multiplex assay
● In the presence of a specific pathogen's DNA within a processed sample, a highly specific (with minimum background noise and non-specific detection) Raman dye would light up in the device.,
● The whole device can be linked to a colorimetric-based computational system which can provide a quantitative analysis of pathogen load.
● This design provides the ability to detect multiple pathogens in a patient's samples simultaneously, such as in the case of multiple infections in a patient.
● Due to the cell sorter compartment, no pre-sample processing is required, leading to reduced cost and processing time for the infection detection.
Conclusion and Future Applications

Having a built-in sense to detect a disease will prove to be a lifesaving ability that will change the lives of millions of people every year. Until now, research has taught us to form habits and break others that alter our chances of having certain diseases. While this research has been helpful, there are genetic and environmental factors that we can’t necessarily change.

Many times, once we find out someone has a disease, it is already too late to incorporate an effective treatment. In these cases, medicine aims to alleviate symptoms. A sensor in our bodies that would tell us as soon as something goes wrong would almost eliminate this unfortunate circumstance that affects hundreds of thousands of families a year.

Often, patients that are immunocompromised can be afflicted with multiple ailments at the same time. Many of these different illnesses can be detected with specific tests, and it can take a lot of time to collect samples, and even more time to await results. Being able to sense multiple illnesses with one chip at the same time will allow for more time to initiate effective treatment even earlier.

Patients with diseases such as HIV and syphilis also stand to benefit with such diagnostic technology. A recent study in Rwanda described the use of a $34 USD lab-on-chip capable of detecting HIV and syphilis. In comparison, the usual ELISA test costs $18,000 USD. Experts believe that the early detection of syphilis could help reduce transmission and deaths by a factor of 10. They also believe the they could stop the transfer of HIV by such a large degree that it would eventually eliminate the disease (2). Not only would a lab-on-chip be life-saving and time-saving, but it would also be very cost-effective.

Another application of such a sense would be for detecting an infectious virus, such as the West Nile virus, which is spread by infected mosquitoes. It can cause symptoms such as headaches, fever, and body aches. In more serious cases, it can cause fatal
neuroinvasive disease such as aseptic meningitis, encephalitis, or acute flaccid paralysis. The Agricultural Research Service’s Center for Grain and Animal Health Research in Manhattan, Kansas, and the University of Wyoming are using surface-enhanced Raman scattering (SERS) with gold nanoparticles to design tests that quickly identify the virus.

As illustrated in Figure 2 above, SERS technology is based on the concept that molecules have their own unique Raman scattering signal. The wavelengths can be detected with a spectroscope. Molecules give off Raman spectra that have sharp peaks, making them more distinguishable than with fluorescence, where molecules emit broad peaks. Relating to human senses, this is similar to vision as the sensor would “see” the light. If a molecule is moved in close proximity to a metal like gold or silver, as with SERS technology, the signal is enhanced dramatically.

Using SERS, scientists have already designed a nucleic acid diagnostic assay that captures Raman dyes and brings them close to the gold nanoparticles in a compound solution. They also developed an immunological assay that rapidly detects antibody responses. The advantage of SERS is that it amplifies the signal without requiring an enzymatic reaction or long incubation. It can be done in a complex solution without multiple washing steps. (8)

References

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