Real - Time Electronic Detection of Water/Food Borne Pathogens

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ABSTRACT

BiSen Tech LLC is developing a true real time test for water-borne pathogens. The basic sensor element is an Orthogonal (off-set) Gate Field Effect Transistor (OGFET). The gate insulator surface is functionalized with appropriate molecular probes. Designed as an array, a target immobilized on any sensor element will cause the particular transistor to turn on. Vacant sensor elements remain off. Controlling certain sensor parameters creates a sensor system that is self-regenerating. No target preparation is required. Therefore the presence of pathogenic microbes is detected in 1 – 2 seconds.

Keywords: biosensor, pathogens, water-borne, real-time, detection

BACKGROUND

There is a critical need to be able to detect water-borne, food-borne, and air-borne pathogens as soon as they are present and as close as possible to the actual source. Unfortunately, existing detection methods take too long, are typically costly, and require laboratory analysis. For example, DNA analysis requires obtaining a sample, lysing cells, cell debris filtering, restriction enzyme processing, target amplification via polymerase chain reaction (PCR), labeling such as with fluorophores, electrophoretic separation, fluorophore excitation means, photographic detection, and comparison to a data library for identification; all requiring skilled technicians and costly laboratory equipment. Some claim the ability to perform PCR based detections within a few hours. Unfortunately, pathogens can be widely dispersed in those few hours. The traditional technique of collecting a sample and process amplification by incubation on a test matrix can take from several days to weeks. BiSen Tech LLC is developing a method to detect pathogenic microbes in 1 – 2 seconds. The patent protected biosensor [1] is an Orthogonal Gate Field Effect Transistor (OGFET). This new biosensor methodology is designed to continuously monitor critical locations without the need for human intervention.

TECHNOLOGY

BiSen Tech LLC has disclosed a new pathogen detection technology that can detect target pathogens within 1 – 2 seconds, provides continuous remote monitoring, is sensitive to a single capture event, and does not require PCR, or culturing, or reagents, or target preparation, or human intervention. The technology design is flexible, allowing for sensors to detect protozoa, bacteria, and viruses.

BiSen's technology is an array of specially designed Orthogonal (off-set) Gate Field Effect Transistors (OGFET) that exploits the bio-chemical, physical, and electrical properties of a target pathogen. The key property is the extraordinary electric field induced polarization of microorganisms: a very large dipole moment leading to a frequency dependent relative permittivity greater than 10^5.

A technical report by John H. Miller, Jr. and James R. Claycomb [2] describes the strong electric field induced polarization and presents data of the relative permittivity (ε) of *Escherichia coli* as a function of test frequency. The origin of the strong dipole moment of a microbe is a function of surface ion drift, within cell ion drift, macro-molecule polarization, and water molecule polarization. The very high relative permittivity falls off rapidly with increasing frequency. For example, at 10Hz measurement frequency, the relative permittivity is approximately 800,000. At 10kHz the value is approximately 80, which is the value of water.

BiSen's technology will have several hundred thousand individual sensor elements on an array chip of approximately 2cm x 2cm for protozoa, up to a million sensor elements for bacteria, and several million sensor elements for viruses. Realizing the relative permittivity frequency dependence, BiSen has defined a sensor array interrogation partitioning scheme that provides an electric field bias of 10ms to 100ms for each sensor element and yet the entire array is scanned every 1 – 2 seconds.

The sensor array is fabricated using standard integrated circuit manufacturing. For protozoa and bacteria 200nm lithography is more than sufficient. The sensor array chip will include all the supporting array circuitry including row-

column drivers, clock circuits, sense amplifiers, output drivers, etc. The OGFET gate insulator will be functionalized with molecular probes, such as oligonucleotides, olicopeptides, antibodies, etc. that are specific to the intended target species. The molecular probes are attached to the gate insulator. The actual metal gate is at a right angle to the plane of the transistor element (orthogonal) and spaced away from the channel region. Furthermore, the orthogonal metal gate does not overlap either the source or the drain of the sensor element transistor. If a sensor element remains vacant, the electric field from the interrogation gate bias is insufficient to form an inversion channel between the source and drain of the OGFET device and the transistor remain off (Figure 1).

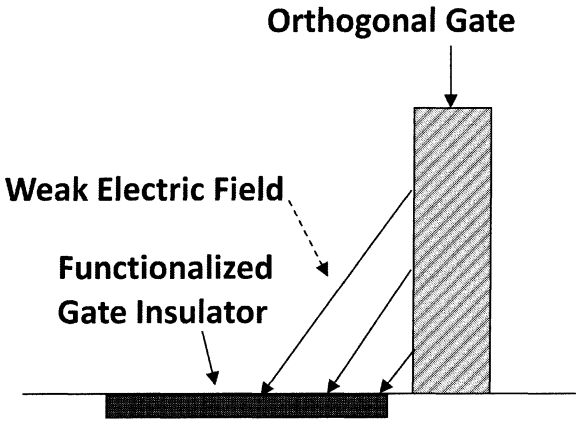

Figure 1. No inversion channel for a vacant sensor element

Next consider the case where a target pathogenic microbe has been immobilized by molecular probe – pathogen hybridization capture. Figure 2 shows the same perspective view as Figure 1, but with a target capture event.

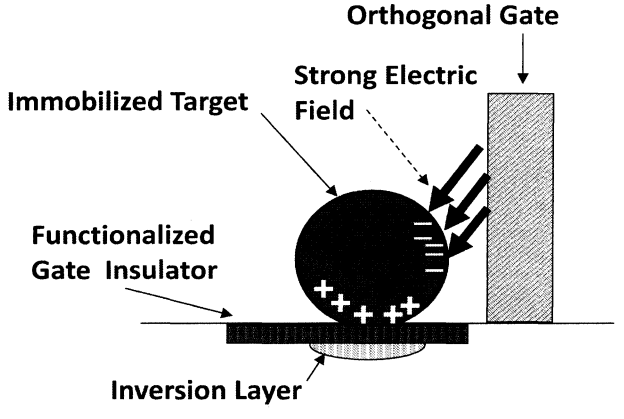

Figure 2. Inversion channel for an occupied sensor element

With the strong electric field polarization of the target pathogen, the pathogen effectively acts like an electric field shunt. Therefore, with the same gate bias as before, an inversion channel is formed, the transistor turns on, and a current is detected, indicating the presence of the target pathogen. The intensified arrows are meant as an indication of a strong electric field, sufficient to form an inversion channel, which is established using the interrogation gate bias. Note, for simplicity, the drain – substrate space charge region is not included in Figure 3.

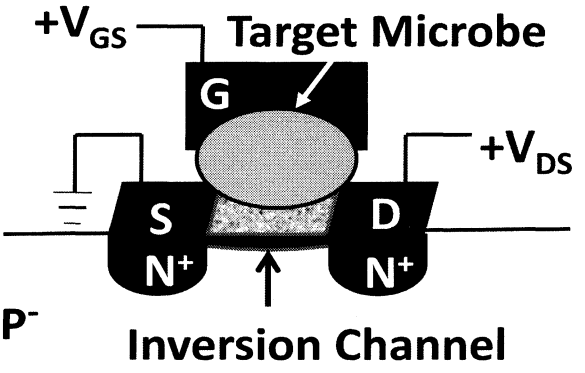

Figure 3. Occupied sensor element shows an inversion channel between source and drain.

The physical size of the target microbe dictates the geometrical dimensions of the sensor transistor element. The microbe needs to be reasonably close to the source region and the edge of the drain/substrate space charge region. Therefore, attention is given to the channel length. A very important point is the fact that the entire distance from the orthogonal gate and the channel region does not need to be occupied by the captured microbe. It only needs to be sufficient to shunt enough of the field to allow the formation of an inversion channel. Therefore, design flexibility exists for the channel width and the separation distance between the orthogonal gate and the functionalized channel region. This fact is a favorable forgiving factor when the target is not of simple spherical shape and allows a given design to be highly effective in detecting a rod shaped bacterium, for example.

Figure 4 is a calculated plot of the log of the drain current versus the applied orthogonal gate bias for a protozoan or a bacterium. The curve on the left shows a low threshold voltage for a sensor element with an immobilized target and the curve on the right show a rather high threshold voltage for a vacant sensor element.

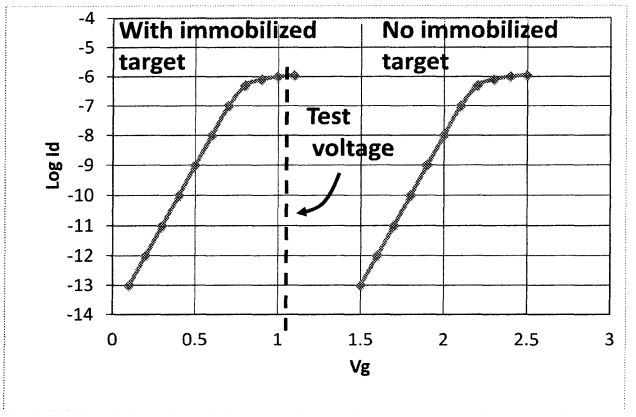

Figure 4. Log Id vs Vg showing large signal to noise ratio

It is obvious from the plots that the signal to noise ratio would only be limited by the room temperature thermally generated electron hole pairs. This means the current level will actually not be less than about 10^{-14} amp. Even so, that yields a signal to noise ratio of eight orders of magnitude.

As the target pathogens get smaller, such as viruses, the dimensions of the sensor element transistor get smaller. This leads to the threshold voltage difference between an occupied sensor element and a vacant sensor element to decrease, which causes a reduction in the signal to noise ratio. Of course, a signal to noise ratio of one or two orders of magnitude is more than sufficient. For extremely small viruses, such as the pathogen that causes foot and mouth disease in cattle, there are two design parameters that can be exploited to maximize the signal to noise ratio. Since it is advantageous to have the distance from the orthogonal gate to the functionalized channel be more dominating than the gate insulator thickness, this insulator can be reduced in thickness, but only to a limited degree. A more efficient way to increase the separation of threshold voltages is to apply a substrate (well) bias relative to the transistor source. This is commonly referred to as the body effect. For a uniformly doped channel region the threshold voltage increases with substrate bias as a function of the square root of the channel region doping level and directly proportional to the insulator thickness.

Figure 5 is a generalized representation of the so-called body effect and indicates how the signal to noise ratio can be increased for extremely small pathogenic target species.

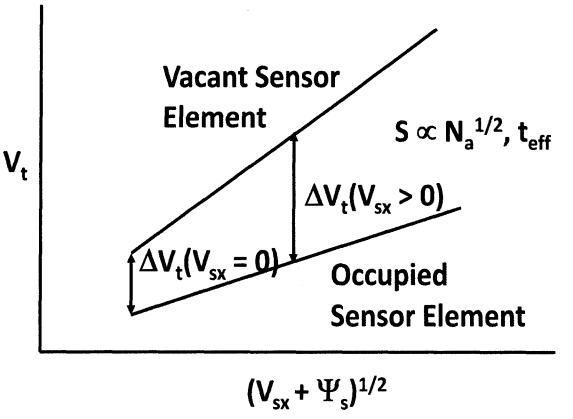

Figure 5. Sensitivity enhancement from a well to source applied voltage

A paper by Gossett A. Campbell, Raj Mutharasan [3] shows results using a piezoelectric cantilever beam functionalized to capture *Cryptosporidium parvum* oocyst and a measured reduction in resonant frequency of the beam as more and more targets were immobilized. One of their figures shows a particularly interesting phenomenon. With a constant flow rate of the target in a flow cell, after about 25 minutes the frequency shift saturated, that is, leveled out. Their experiment used four concentrations of the target, 0, 10^2, 10^3, & 10^4 oocysts per ml. Each test used the same flow rate. However, the time to saturation of frequency shift is nearly concentration independent. Since the test beams were identical, this effect cannot be due to target loading. For a given probe to target binding energy, or probe to sensor binding energy, apparently the average immobilization for their experiment was about 25 minutes: the likely explanation for the data. BiSen, therefore, will control how long a target microbe remains immobilized. Hence, if the target is eliminated from the test material through remediation, the sensor will self-clean. For extreme contamination levels whereby the sensor array itself becomes loaded with immobilization events, an array module can be simply removed and replaced with a clean array. The loaded array module can be placed in a DI water cleaning cell and thus be completely renewed.

As further indication of self-cleaning, a PhD thesis by Jean-Paul McGovern [4] used a similar cantilever piezoelectric beam. However, for his work he held the target concentration constant and varied the flow rate in his test flow cell. Consistent with [3], frequency shift saturation was observed. In his experiment, the rate of target loss

showed the expected relation to flow rate. In reviewing his data, it is important to note that for the case of zero flow, the immobilization rate is diffusion limited.

From the evidence of both [3] and [4], there are at least two parameters available for BiSen to control the average target pathogen immobilization retention time, namely binding energies and flow rates.

SUMMARY

BiSen Tech LLC is developing the only true real time biosensor capable of detecting water-borne pathogens in one to two seconds after they are presented to a sensor array. A novel semiconductor device detects a single microbe without any preparation of the target. Monitoring is continuous and the microbe is detected as is: no lysing of cells, no cell debris filtering, no chemical treatments, no target amplification, no fluorophores, no electrophoresis, no excitation means, no photographic means, and no waiting for analysis. BiSen sensor technology will become the new gold standard for ensuring safe food and clean water worldwide.

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