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Molecular Analysis of Blood with Micro/Nano Scale Field Effect Transistor Biosensors

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Abstract

Rapid and accurate molecular blood analysis is essential for disease diagnosis and management. Field Effect Transistor (FET) biosensors are a type of device that promise to advance blood point-of-care testing by offering desirable characteristics such as portability, high sensitivity, brief detection time, low manufacturing cost, multiplexing, and label-free detection. By controlling device parameters, desired FET biosensor performance is obtained. This review focuses on the effects of sensing environment, micro/nanoscale device structure, operation mode, and surface functionalization on device performance and long-term stability.

Keywords

Biosensors; Blood analysis; Lab-on-a-chip devices; Molecular recognition; Transistors

1. Introduction

Rapid and accurate molecular analysis of blood is important in disease diagnosis and management. However, current methods of clinical molecular analysis of blood involve multiple healthcare workers in the processes of sample collection, analyte measurement, and result reporting with a total turn-around time that may take hours or more.^[1] A portable device that can measure molecular analytes in blood will rapidly deliver diagnostic information into the hands of the caregiver. Portable instruments called point-of-care testing (POCT) devices have found their way into clinical practice in a few areas of emergency medicine,^[2] surgery,^[3] and primary care,^[4,5] but widespread use of POCT devices in clinical practice has not yet occurred due to issues such as reliability, cost, and ease of use.^[4,6] The Field Effect Transistor (FET) biosensor is a type of device that promises to advance POCT of blood by offering desirable characteristics such as portability, high sensitivity, high specificity, low sample volume, brief detection time, low manufacturing cost, low power consumption, multiplexing, and label-free detection.^[7-10] In this review, FET biosensor theoretical operation is first discussed followed by an evaluation of the current literature of blood molecular analysis by FET biosensors. The evaluated topics include sensing environment, FET channel structure, device operation, surface functionalization, and long-term stability.

Many parameters are of utmost importance when designing a device to perform molecular analysis of blood. High sensitivity is necessary when the molecular analyte concentration is low. For instance, a normal level of the cancer biomarker prostate specific antigen (PSA) is less than 4.0 ng mL^{-1} ^[11] and the inflammatory marker IL-6 is less than 10 pg mL^{-1} .^[2] Additionally, highly specific molecular analyte sensing is important due to the vast quantity of molecular components in blood. Considering only proteins, tens of thousands to tens of millions of different protein structures exist within the blood.^[12,13] Another essential parameter is short detection time to enable rapid delivery of test results. During septic shock, measurements of blood inflammatory markers aid in timely and appropriate antibiotic treatment decisions that improve survival rate with early administration.^[2] Inexpensive fabrication and operation are also important device requirements. For population screening tests, low cost is essential.^[14] An additional necessity is multiplexing (the simultaneous detection of multiple analytes). In cancer diagnosis, accuracy increases when detecting multiple cancer biomarkers in comparison to a single cancer biomarker.^[14,15] Dynamic range, the ratio of the greatest to the least detectable analyte concentration,^[16] is also an important factor. Clinical laboratories currently measure 10 orders of magnitude of blood protein concentrations from the tens of mg mL^{-1} range for albumin to the pg mL^{-1} range for inflammatory proteins.^[12] In designing a useful POCT device, one must optimize the parameters of sensitivity, specificity, detection time, cost, multiplexing capability, and dynamic range for the particular clinical application.

Despite the use of POCT in a few segments of healthcare, widespread implementation has not yet occurred due to limitations in device reliability, durability, ease of use, and stringent medical regulatory requirements.^[6,17,18] One technology that promises to meet the demands of POCT of molecular blood analytes is biosensing with FET blood biosensors. In its most general form, a biosensor is a device that selectively binds an analyte using a biomolecular recognition mechanism and produces a detectable signal.^[19] A FET achieves the goal of biosensing by transducing the binding of an analyte at the FET surface to a change in current-voltage relationships among its electrodes. Demonstrated molecular blood analyte FET biosensors have shown rapid, label-free, inexpensive, portable, sensitive, specific, and multiplexing detection with low power consumption in microliter sample volumes.^[7-10]

FET biosensor operation is diagrammed in **Figure 1A**. The charge carriers, represented as circles in Figure 1A, are present in the substrate as a result of atomic doping during fabrication. In the case of Figure 1A, the negative charge carriers are the minority carriers in the p-type substrate. When analyte molecules present in the solution bind to receptors on the gate insulator, the electric field of the positively charged analyte molecules attracts negative charge carriers from the substrate to the channel. As the density of negative charge carriers in the channel increases, the electrical conductance between the source and drain electrodes also increases. The result is a quantifiable rise in drain current (I_D) corresponding to analyte concentration. Alternatively, a negatively charged analyte will decrease I_D in the FET biosensor shown in Figure 1A. To detect uncharged analytes, an indirect sensing method is employed. An enzyme is immobilized at the gate surface, catalyzes a chemical reaction with the analyte as the substrate, and causes a change in local pH.^[20] An increase (or decrease) in local H^+ ion concentration increases (or decreases) positive charge at the gate, modulates channel charge carrier concentration, and changes I_D .

The use of the FET in biosensing began in 1970 with the invention of the ion sensitive field effect transistor (ISFET) that was first used to measure NaCl concentration in an aqueous solution.^[21] The ISFET is a modification of the Metal Oxide Semiconductor FET (MOSFET) (see Figure 1B). The gate electrode is removed and the gate oxide is exposed to the solution. The ions in solution create an electric double layer at the gate oxide, modulate the concentration of channel charge carriers, and change I_D .^[21] The next important FET

biosensor advance added detection specificity by including a chemical membrane at the gate to selectively measure potassium concentration.^[22] Since its inception, FET biosensing has progressed to the detection of a wide variety of analytes by immobilizing receptors and enzymes to the gate surface as shown schematically in Figure 1A.

When the FET biosensor is operated in the linear region ($V_{GS} > V_t$ and $V_{DS} < V_{GS} - V_t$ for an n-channel device), the drain current obeys the equation: $I_D = C\mu(W/L)[(V_{GS} - V_t)V_{DS} - 0.5V_{DS}^2]$. V_{GS} is the gate to source voltage drop, V_{DS} is the drain to source voltage drop, V_t is the threshold voltage of the device, C is the capacitance per unit area at the insulator, μ is the charge carrier mobility, W is the channel width, and L is the channel length. For a FET biosensor, the binding of a charged analyte at the gate insulator modulates I_D by altering the surface charge and changing V_t of the device.^[23]

2. Current Research in the Field of Blood Molecular Analyte FET Biosensors

FET biosensor designers must control device parameters that influence detection time, sensitivity, specificity, stability (consistent operation through time), and power consumption. Four important controllable parameters include sensing environment, channel structure, operation mode, and gate/channel surface functionalization. By controlling these parameters certain aspects of device performance are improved over others. The following sections detail the effects of varying these parameters.

2.1 Sensing Environment

2.1.1. Ionic Concentration, pH, and Solution Heterogeneity—When detecting an analyte in blood or a buffer solution, three solution properties are critically important. The first property is ionic concentration. Ions in electrolyte solutions cause ionic charge screening by clustering near an analyte molecule of opposite charge.^[8] As ionic concentration increases, charge screening decreases the electric field at the channel caused by a charged analyte near the sensor surface.^[24] At a particular distance, the charges on the analyte are effectively screened by the ions in solution.^[25] This distance is the Debye length of the solution. For reference, a 1xPBS (phosphate buffered saline) solution (used to model physiological conditions) has a Debye length of only 0.7 nm.^[25] The second solution property is pH. The FET channel conductance change that occurs when an analyte binds at the gate is a function of solution pH and the pI of the analyte.^[8] Device sensitivity increases as the pH moves away from the pI of the analyte.^[24] This is due to charge accumulation on the analyte through protonation or deprotonation. With a greater magnitude of charge on an analyte near the sensor surface, an increased electric field reaches the channel. The third solution property is heterogeneity. The vast number of different molecules in blood requires a detecting mechanism at the gate of the FET that is highly specific. Without specificity, the device may respond to any number of analytes and interfere with the measurement of the target analyte.

2.1.2. FET Biosensors Detecting Analytes in Blood Products

To meet the requirements of detecting molecular blood analytes, FET biosensor designers must account for ionic concentration, pH, and solution heterogeneity. To compare the performance of blood FET biosensors, characteristics such as sensitivity, specificity, and long-term stability are worth quantifying. **Table 1** summarizes the performance of the devices in this review that measure analytes within blood products.

2.1.2.1. Controlling Ionic Concentration and pH of Blood Product Samples—To avoid the problems of uncontrolled pH and ionic concentration, researchers have developed

methods to detect blood analytes in controlled solutions. One method is to dilute the blood sample with a buffer solution that is optimized for analyte detection.^[11,26,27] However, a tradeoff exists for the dilution approach. While investigators gain control of the sensing environment, the analyte concentration is decreased, and the device sensitivity requirement is raised. A second approach to controlling ionic concentration is to desalt the serum.^[7,28] A lower salt concentration increases device sensitivity toward the analyte by decreasing ionic charge screening. However, the desalting process adds additional time and complexity to the testing procedure. There is also a possibility of losing the analyte during the desalting process.^[29] A third method to control pH and ionic concentration of the sensing environment is to first bind the analyte in the sample solution at the device surface and then to flush the system with a reference buffer of controlled composition.^[29,30] One study used multiple antibody binding and flushing steps to control the composition of the reference buffer.^[30] This system had a low limit of detection (LOD) and high specificity (see Table 1). Another study also used a flushing method to circumvent the constraints of a short Debye length in physiological solutions.^[29] The serum analyte was bound via antibodies at the gate, and the device was flushed with a reference buffer. The conductance change from before serum administration to after flushing determined the analyte concentration in the serum. A disadvantage of the flushing technique is an increase in the detection time due to multiple processing steps. One test lasted 20 minutes^[30] and the other lasted 17 minutes^[29] which are long compared to other blood FET biosensors (see Table 1). A fourth method to address the difficulty of sensing in physiological pH and ionic concentration is to first perform an enzyme-linked immunosorbent assay (ELISA) on the blood sample and then transport the catalytic products to the FET biosensor for measurement.^[31,32] The benefits of using an ELISA with a FET sensor are the high sensitivity and specificity of the ELISA without the need for optical read-out. However, both of these studies required overnight incubation of the enzyme-bound secondary antibody.

2.1.2.2. Specificity Testing with Blood Product Samples—Despite the need for high specificity in blood FET biosensors, the range of specificity testing is quite variable across studies. Since albumin is the most abundant protein in human blood, many studies tested specificity of the device to bovine serum albumin (BSA). Specificity was verified by demonstrating no change in device output when comparing device exposure to a pure buffer solution and device exposure to a buffer solution containing BSA.^[10,28,33,34] More rigorous specificity verification involved testing the device with various known concentrations of the analyte dissolved in blood products.^[11,32,35,36] Specificity testing with blood products exposed the devices to a wider variety of blood components than BSA buffer testing. If one of these blood components was able to bind to the FET receptor, the specificity testing would have revealed this through an unexpected device output. Even more rigorous specificity testing of the FET blood biosensors used a molecule with a structure similar to the analyte.^[31] A similarly structured molecule was the most likely candidate for nonspecific device operation and thus could confirm device specificity.

2.1.2.3. Long-term Stability Testing of Blood FET Biosensors—In addition to controlling the characteristics of the sensing solution and increasing device specificity, long-term device stability is critical for FET blood biosensors to achieve clinical implementation. Due to the tendency for surface receptors or enzymes to degrade through time, researchers must demonstrate consistent device performance following extended storage or repeated use. Table 1 reveals that a few studies performed rigorous long-term stability tests. One study demonstrated a decrease in device sensitivity by about 34% after 5 days of storage.^[33] Another investigation showed a device that was reusable at least 50 times after its reduced gate film was oxidized back to its original state.^[32] The duration of these 50 trials was not specified, but the large number of device recycling iterations is promising. Of the blood FET

biosensors reviewed, the most stable of all showed a decrease in device response by 20% after 40 days of daily use and negligible device degradation after 46 days in storage.^[36]

2.2 FET Channel Structure

2.2.1. Planar Versus Nanostructure Channels—In addition to controlling the sensing environment, FET biosensor designers experiment with a variety of FET device architectures. The use of different structures results in a variety of device characteristics. The two primary classifications of FET biosensor structures are planar and nanostructure FETs based on the size of the device channel. Both have their accompanying advantages and disadvantages.

One of the major differences between planar and nanostructure FET biosensors is the device sensitivity. The traditional FET biosensor structure includes a planar channel architecture consisting of a flat channel region underlying a flat gate insulator. Since the channel is much larger than the charged analyte molecules, many molecules must bind at the gate to change the surface charge.^[8] The result is an inherently low sensitivity of planar channel devices. In contrast, FET biosensors with nanostructure channel architecture have an inherently high sensitivity.^[38] In 2001 Cui et al. demonstrated that a silicon nanowire (NW) channel between a source and drain electrode is extremely sensitive to analyte binding due to the large surface area to volume ratio of the NW.^[39] When a charged or polar analyte binds to a NW, the result is a change in the conductance of the bulk of the NW compared to only a shallow surface depth as with a planar channel device.^[40,41] Nanostructure sensors have an LOD three to four orders of magnitude better than planar sensors for a given detection time.^[42]

Another device parameter that is greatly different between planar and nano FETs is the detection time. When considering diffusion-limited transport of target analyte to the sensor surface, nanostructure sensors have a linear relationship between density of bound analyte and time, but the density of analytes on planar structured sensors is proportional to time to the one-half power.^[42] An environmental parameter that affects the detection time is the ionic concentration of the sensing solution. However, planar and nanostructure devices are affected differently. Increasing the ionic concentration causes an increase in detection time for both nanostructure and planar FET biosensors, but the increase in detection time for planar sensors is an order of magnitude greater than that for nanostructure biosensors for a given increase in ionic concentration.^[43] The result is a slower response of planar devices compared to nanostructure devices.

Despite the apparent inferiority of planar channel FET biosensors compared to their nanostructure counterparts based on sensitivity and detection time, other factors are also important to consider when designing a biosensor for a particular application. The nominal concentration of the analyte may not require a sensor that approaches the technological frontiers of LOD and detection time. In this case, device fabrication costs, ease of implementation, and reliability may have greater impact on the suitability of one device structure over another. Due to the vast combinations of device parameters and applications, neither planar nor nanostructure devices are inherently superior to the other.

2.2.2. Optimizing the Sensitivity of Planar Channel FET Biosensors—Despite the low sensitivity of planar FET biosensors, various methods are used to increase sensitivity in planar devices. One planar device with a high sensitivity is the AlGaIn/GaN heterojunction FET.^[26] A two dimensional electron gas (2DEG) is formed at the interface between the AlGaIn and the underlying GaN layers in the AlGaIn/GaN high electron mobility transistor (HEMT).^[9] The high sensitivity of the 2DEG to charges on the AlGaIn gate surface makes it ideal for biosensor applications.^[9] Studies have shown that AlGaIn/

GaN HEMT biosensors respond to an analyte within 25 seconds^[26,44-48] and can achieve a LOD down to 10 pg mL⁻¹.^[44] AlGaIn/GaN HFETs also have a far lower electrical drift through time in physiological buffer solutions than silicon-based FETs.^[49] In addition, GaN is a biocompatible material.^[50] The long-term stabilities of the AlGaIn/GaN HEMT devices covered in this review were not evaluated. However, the devices with antibody gate receptors were probably more stable since they did not depend on enzyme function.^[26,44,46,47]

Another way to increase the sensitivity of planar channel FETs is to use nanoparticles (NPs) to increase the concentration of receptors at the gate^[33,51,52] or to enhance the transduction of analyte concentration to changes in I_D .^[53,54] One study showed antibody-conjugated gold NPs embedded in a polymer on an extended gate (an electrode wired to the gate) FET.^[33] The gold NPs gave a large surface area for antibody-analyte interactions, and the device operated in whole blood. A different device used NPs conjugated with enzymes to detect triglycerides in PBS.^[52] Very high sensitivity was achieved by another planar channel transistor by using secondary anti-PSA antibodies bound to gold NPs to bind PSA at the gate.^[54] The gold NPs were reported to amplify source to drain conductivity, and the device achieved an LOD of 1 pg mL⁻¹ of PSA. Another study used MnO₂ NPs within a polymer layer at the gate to enhance activity of an enzyme bound to the polymer surface.^[53] Of these planar FETs with NPs, three gave rapid results within 2 minutes,^[33,52,53] whereas one device required an hour for analyte and secondary antibody processing.^[54] Long-term stability ranged from 1 month,^[53] to 2 weeks,^[52] to 5 days.^[33]

2.2.3. Types of Nanostructure Channel FET Biosensors

2.2.3.1. Silicon Nanowire Channel FET Biosensors: Si NWs commonly compose the channel of nanostructure FET biosensors. Despite the sensitivity advantage of NW FETs, the primary drawback is the poorly controlled placement of prefabricated silicon NWs onto device substrates.^[8,55,56] In 2007, this challenge was confronted with a Si NW patterned directly on a device substrate using an ultrathin silicon-on-insulator wafer to define the channel height and anisotropic etching to define the channel width.^[57] The result was a Si NW fabrication process that overcame minimum width constraints of lithographic technology and did not require the difficult task of positioning prefabricated NWs on the device. Additionally, Si NWs patterned directly on a substrate result in NWs with uniform dimensions.^[58] However, many groups continue to develop NW FETs with prefabricated NWs due to the disadvantages of NWs patterned directly on the surface. These disadvantages include time-consuming fabrication methods and NWs limited to larger cross-sectional dimensions.^[58] Based on the comparisons summarized in **Table 2**, the LOD of Si NWs patterned directly on the device substrate is better overall than the LOD of prefabricated Si NW devices. The prefabricated NW devices detected down to the range of tens of fg mL⁻¹ and hundreds of fM while the devices with the NWs patterned directly on the surface detected down to ones of fg mL⁻¹ and ones of fM. The detection times of both types of devices were mostly in the hundreds of seconds range. The extremely fast 5 second response for one of the devices^[59] was possibly due to the unique sensing mechanism of the analyte cleaving a surface molecule rather than binding to a surface receptor. The testing conditions of these devices were widely variable from μ M electrolyte concentrations to much more rigorous testing in serum. Verified long-term stability ranged from six days,^[60] to three days.^[61]

An additional advantage of NW FETs is the small size enables multiple devices to exist on a single substrate. Multiplexing occurs when each device detects a different analyte simultaneously. Progress in FET biosensor multiplexing has occurred, but has yet to attain its full potential. One device detected three analytes simultaneously but had great

multiplexing potential since it had 100 Si NW FETs on a single substrate.^[10] Another device detected a single analyte but had five NW FETs.^[7] A third device detected three analytes simultaneously,^[28] and a fourth used five NW FETs on one substrate to measure the same analyte redundantly.^[62]

2.2.3.2. Carbon Nanotube Channel FET Biosensors: Besides NWs, carbon nanotubes (CNTs) are also used to form FET channel nanostructures. When an analyte binds at the surface of a CNT FET, the CNT conduction is modulated by the electrostatic effects of a charged analyte (as with NW FETs) and also by a shift in metal work function at the contacts.^[67] Passivation of metal contacts is necessary to remove the variable response caused by the shift in metal work function upon analyte binding.^[67] Advantages of CNT FETs are biocompatibility^[68] and high sensitivity since all atoms of CNTs are on the surface.^[69] However, CNT electrical properties are more difficult to control than semiconductor NWs because comparable doping methods do not exist for CNTs.^[27,40]

CNT FETs come in two varieties. The first type is networked CNT FETs where a disorganized group of CNTs form the channel between the source and drain. The second type involves one or more CNTs forming the channel without contacting one another. One study compared the two types directly and showed that the aligned CNT FET had a three times greater sensitivity.^[70] In contrast, another study claimed that networked NT FETs have greater sensitivity due to more receptors on the network of NTs.^[34] Interestingly, the results from the literature presented in **Table 3** agree more with the latter argument. The networked devices had an LOD down to the ones of pg mL^{-1} and the ones of pM range whereas the aligned devices had an LOD only down to the tens of pg mL^{-1} and hundreds of pM range. Both types of devices had detection times from the tens to hundreds of seconds with two exceptions. The hour detection time was due to an additional washing and drying procedure,^[71] and the two hour detection time was due to performing an ELISA on the device surface.^[72] The long-term stability was reported as three days for one device^[73] but not reported for the other devices in Table 3.

2.2.3.3. Other Nanostructure Channel FET Biosensors: Another approach to fabricate nanostructure channels is by forming polymer nanotubes (NTs). Polypyrrole is a biocompatible conducting polymer^[81] used in polymer NT FET biosensors.^[34,81,82] One device detected 50 nM thrombin in a solution of 140 mM NaCl.^[34] Another device detected 400 fM VEGF in 10 mM PBS and was washed, dried and reused three times with minimal sensitivity degradation.^[82] The same study compared the sensitivity between FETs with NT diameters of 200 nm and 120 nm. Similar to NW FETs, the smaller diameter NT FET had a greater sensitivity. Long-term stability was not reported for these polymer NT FETs.

Other unique methods of increasing FET biosensor sensitivity with nanostructures exist. One method is forming NWs with materials besides silicon, such as indium oxide.^[74,83,84] Due to the absence of native oxide formation on the In_2O_3 surface, analytes are able to bind closer to the In_2O_3 NW channel.^[85] Another unique method of increasing sensitivity is by increasing the local analyte concentration with electric fields near the FET channel.^[86,87] One study achieved an LOD of 10 aM of PSA in 10 μM PBS by increasing the PSA concentration at the Si NW with nearby electrodes to attract proteins to the Si NW by dielectrophoresis.^[86] The detection time was within one second. Another group created a FET with a 790 nm gap between the source and drain electrodes without a physical channel between the source and drain.^[87] An AC electric field attracted ions to the channel gap and increased the local conductance between the electrodes. The device limitation was that it could only detect an analyte indirectly by measuring changes in ionic concentration as a result of an enzymatic reaction in the solution.

2.2.4. Nanostructure FET Biosensor Modeling and Theoretical Limitations—

With nanostructure FET biosensors achieving an LOD in the fM range, computational studies have modeled FET biosensor operation and analyzed the physical limits of biosensor capability. One computational modeling study used the diffusion-capture model and the Poisson-Boltzmann equation to verify several important relationships between sensitivity and the sensing environment when the NW FET is operated in accumulation mode.^[43] The applicable range of analyte concentrations is located approximately in the fM to nM range. In this concentration range, the normalized channel conductance increases linearly with pH, increases with the natural logarithm of analyte concentration and time, and decreases with the natural logarithm of electrolyte concentration.^[43] The linear-log dependence on time is only valid before the sensor probes are saturated with analyte molecules.^[43] These four relationships between device sensitivity and sensing environment were shown to correspond accurately with experimental data. An additional insight gleaned from the computational model is that an increase in the ionic concentration also increases detection time.^[43]

Of significant importance to FET biosensor designers are the effects of nanostructure channel dimensions on biosensor sensitivity. According to Nair and Alam,^[43] the sensitivity of a NW FET biosensor is given by the equation: $sensitivity = 4\sigma/(qdN_D)$. The term σ is the charge density induced in the NW, q is the charge of an electron, d is the NW diameter, and N_D is the impurity doping concentration in the NW. Decreasing NW diameter and decreasing doping concentration both increase the sensitivity by resulting in a decreasing number of channel charge carriers.^[24,55,62] With fewer charge carriers, a charged analyte at the surface of the nanostructure is able to deplete a greater proportion of the channel carriers.^[55,62] The result is a greater relative change in channel conductance for each bound analyte and thus a greater sensitivity. However, limitations to decreasing NW diameter and doping concentrations exist. Channel width is limited by NW fabrication techniques.^[55] The doping concentration lower limit is caused by the random location of dopant atoms in a nanostructure when the dopant atom population becomes low and causes unpredictable device sensitivity.^[24] With NW diameters less than 20 nm, quantum mechanics are necessary to model charge transport through the NW.^[88]

Related to the goal of improving the LOD of FET biosensors through decreasing the size of channel structures is achieving single molecule detection. One computational study modeled a Si NW FET with a single receptor (single-stranded DNA) bound to the NW surface.^[88] The simulation tested NWs with diameters of 10, 12, and 14 nm with the expected result of decreased sensitivity for increasing NW diameter. According to the results of this study, single molecule detection is possible with a FET biosensor even in a pH 7 solution containing 10 mM NaCl. Clearly, this study supports the promise of label-free single molecule detection in physiological solutions. However, a primary limitation to real-life application is the placement of a single receptor on the NW surface.^[88]

Another important parameter to model for FET biosensors is the detection time. Before a target analyte molecule can bind at the sensor surface and electrostatically modulate the channel conductance, the molecule must diffuse from the bulk solution to the sensor surface. This diffusion process takes time and sets lower limits on achievable detection times at a given analyte concentration. A mass-transport computational model verified the theoretical plausibility of femtomolar detection limits with detection times on the order of minutes for nanoscale sensors.^[89] Two adjustable parameters that influence the rate of analyte adsorption onto the sensor surface are sensor shape and dimensions. The same study also showed that the adsorption rate is a linear function of NW length but only proportional to NW radius to the one-fourth power or less.^[89] However, decreasing NW diameter also decreases the minimum number of analyte molecules required to bind to the surface for detection.^[42] A lower number of required molecules aids in decreasing detection time, and

the LOD is improved due to the proportional relationship of LOD to NW radius to the second power.^[42] Also, single molecule detection time increases more slowly with decreasing analyte concentration than when multiple molecules are required for detection.^[90] To further improve detection time beyond those available by device scaling, specialized fluidic injection methods are necessary due the temporal requirements of analyte diffusion.^[58] However, even the benefit of fluidic delivery of target analytes decreases rapidly as the dimensions of the nanostructure decrease.^[89]

Device scaling not only influences the average detection times, but more surprising results occur due to the statistical variability of analyte arrival time as NW diameter decreases.^[42] Go and Alam showed that this variability is especially true at low analyte concentrations, and results in discrepancies between the average detection times (predicted by the diffusion-capture model) and experimental detection times.^[90] As the sensitivity of a nanostructure sensor is increased, the minimum detection time decreases more rapidly than the average detection time.^[90] This means that the variability in analyte arrival time continues to increase with smaller device dimensions. In addition, the study presented the example that when detecting three molecules or less, the minimum detection times are two to three orders of magnitude less than the average response times.^[90] This statistical model gives an explanation for experimental LODs in the fM range when theoretical LODs are in the pM range for detection times on the order of 100 seconds.^[90]

2.3 Device Operation

Another approach to device optimization is in the operational mode of the FET biosensor. When V_{GS} is slightly less than V_t (for an n-channel device), a transistor is operating in subthreshold mode. In this mode, the drain current (I_D) is quite low and is an exponential function of V_{GS} .^[91] This contrasts with accumulation mode where I_D is a linear function of V_{GS} . When operating a FET biosensor in subthreshold, the device operates with a much lower drain current.^[91-93] In addition, the screening length for channel charge carriers is longer in subthreshold than in accumulation mode.^[94] This means a charged analyte at the surface of a NW in the subthreshold region will deplete more of the cross sectional area of the NW than in accumulation mode.^[94] The result is an increased sensitivity in subthreshold mode. The greater sensitivity was demonstrated when the LOD for a particular device was 0.75 pM when operated in accumulation mode and improved to 1.5 fM when operated in subthreshold.^[94] The low drain current and high sensitivity of subthreshold operation are ideal for portable diagnostic sensors. Low current increases battery life and high sensitivity improves the LOD.

The processing of sensor output is another area available for biosensor optimization. To decrease the variability across NW FET biosensors, one study demonstrated a unique calibration method that measured the change in channel current when the analyte bound and divided this current change by the derivative of the channel current with respect to the gate voltage (V_G).^[95] The result was a calibrated sensor response equal to $\Delta I_{DS}/(dI_{DS}/dV_G)$. The coefficient of variance was decreased from 25% when using the relative change in channel current as sensor response to 16% when using the calibrated response. The decreased variability of the calibrated response resulted from eliminating the dependence of response on the highly variable threshold voltage of NW FETs.^[95] The improved consistency of sensor response by the calibration technique adds further evidence for the viability of multiplexing NW FETs despite differences that may arise between each FET during fabrication.^[95]

2.4 Gate/Channel Surface Functionalization

2.4.1. Common Surface Linker Functionalization Procedures—Besides sensing environment, channel structure, and operation mode, surface functionalization of the FET biosensor gate insulator or channel nanostructure with organic molecules is another important parameter in biosensor design. The organic layer formed by surface functionalization gives the FET both sensitivity and specificity to analyte detection.^[50] Before analyte receptors or enzymes are bound at the gate or channel, the surface is usually functionalized with linker molecules that provide binding sites for the receptors and form an organic interface at the device surface that protects receptors from degradation.^[50] The functionalization layer may chemically passivate the sensor surface by inhibiting reactions, such as oxidation, or electrically passivate the surface by decreasing surface charges present at sensor surface atoms.^[96] Additionally, tightly packed monolayers of linker molecules decrease the interaction of ions or nonspecific analytes with the sensor surface.^[97] As shown in **Table 4**, some of the most common linker functionalization procedures for FET biosensors are hydrosilylation, forming a gold to sulfur bond, and bonding 1-pyrenebutanoic acid succinimidyl ester to CNTs. Occasionally, a linker is avoided by adsorbing the receptor or enzyme directly to the surface^[75,76] or using a gold gate electrode as the sensing surface.^[31] Exclusion of a linker is also possible due to the ability of peptides to bind directly to semiconductor and insulating surfaces.^[98]

2.4.2. Other Surface Linker Functionalization Procedures—As well as the common linker functionalization procedures shown in Table 4, other functionalization procedures also exist. One procedure forms polymer layers on the gate insulator or channel nanostructure. Examples of polymer layers include enzymes embedded within a polymer,^[36,110] enzymes bound to the surface of layered polymers,^[73] antibodies bound to a polymer covering a CNT,^[71] and antibodies bound to gold NPs embedded in a polymer.^[33] A second functionalization procedure results in a molecular oxidation/reduction (redox) layer on the gate surface. During analyte detection, an enzymatic reaction changes the oxidation state of the redox layer covering the gate which changes the surface charge and thus modulates the channel current. The enzymes that initiate the oxidation/reduction reaction are bound to the redox layer,^[111] added to the sensing solution,^[35] or in a separate ELISA that produces the molecule that reacts with the redox layer.^[32] These surfaces are reset to their initial state with electrical^[111] or chemical methods^[32] for device reuse. Additional functionalization procedures used less common linkers such as cyanuric-chloride to link an enzyme to an Al₂O₃ gate insulator^[112] and a hydrophobic linker, CDI-Tween 20, to bind antibodies to CNTs.^[79]

2.4.3. Surface Receptors—In addition to linker functionalization, receptor surface density and type of receptor are also important parameters for sensor gate/channel functionalization. In FET biosensors the receptor molecule gives the device specificity to the target analyte. Since the binding of a charged analyte to the receptor is responsible for modulating device electrical characteristics, the signal to noise ratio of the biosensor is determined by the receptor density.^[113] A computational model is available that aids in determining the optimal duration of the receptor functionalization procedure.^[113] This same model also verified detection specificity of 1 part per billion for a receptor density of greater than $2 \times 10^{12} \text{ cm}^{-2}$.^[113]

A majority of the FET biosensors used enzymes or antibodies for analyte detection, but a few studies utilized more unique receptors to address some issues with enzymes and antibodies. Enzyme detection requires the analyte to serve as a substrate for an enzymatic reaction that produces a charged species, and antibody detection requires the binding of a charged molecule within the Debye length of the solution. Two studies detected an

uncharged analyte with a surface protein receptor that contained a charged reporter molecule that was released upon analyte binding.^[51,66] Another study used only the antigen binding fragment of an antibody as the receptor.^[77] Since the antibody fragment was smaller than the entire antibody, the analyte bound closer to the gate surface and thus modulated the channel carrier concentration more greatly. The LOD improved from 100 ng mL⁻¹ with an intact antibody to 1 pg mL⁻¹ with the antibody fragment. Besides protein-bound reporters and antibody fragments, other studies used aptamers (oligonucleotide receptors) to bind analytes.^[27,34,78] Aptamers offer greater stability and analyte binding affinity than antibodies.^[27] The greater binding affinity causes more analytes to bind receptors and thus improves the sensitivity.^[78]

However, with greater binding affinity, the analyte will not disassociate as easily so the sensor will have less capability to detect fluctuating analyte concentrations.

2.4.4. The Effects of Surface Functionalization on FET Biosensor Performance

—As previously described, many functionalization procedures are available to modify the gate surface of FET biosensors for analyte detection. A few studies described the effects of surface functionalization on device performance. One study functionalized the gate surface with a combination of linker molecules and spacer molecules.^[11] With only linkers, the surface receptors were so dense that the bound analyte could not approach the surface to modulate the channel current. By adding spacer molecules to decrease the surface density of receptors, the device sensitivity increased due to the analyte approaching closer to the surface. Another study tested device sensitivity versus enzyme concentration within the gate polymer and found maximum sensitivity at 3% enzyme to polymer.^[36] Another group tested dynamic range as a function of surface functionalization.^[53] The result was that three layers of polymer, NPs, and enzyme gave the widest dynamic range in comparison to one, two, and four layers. In a study involving thiol functionalization of a gold gate electrode, the optimal duration of the functionalization procedure was determined by the time that gave the largest device signal strength.^[107] Lastly, the dependence of device stability and gate coverage on functionalization procedure was also studied.^[104] Using a silane linker molecule as the first functionalization layer increased surface enzyme lifetime and increased enzyme coverage in comparison to omitting the silane functionalization step.

2.5 Sensor Long-term Stability

For implementation of FET blood biosensors in clinical POCT devices, the FET biosensor performance must not degrade following extended storage or repeated use. Due to the importance of device stability (consistent operation through time), long-term stability testing of FET biosensors is often performed. Of the devices analyzed for long-term stability, two types of tests were conducted. One test measured sensitivity change after storage, and the other measured sensitivity change through repeated use over an extended time.

Several studies tested changes in device sensitivity following storage with a variety of storage conditions and durations (see **Table 5**). Storage durations ranged from 1 month,^[104] to 2 weeks,^[52,106] to 5 days.^[33] Variations in storage conditions included ambient air,^[104] 4°C in air,^[33] 2°C in PBS,^[106] and 4°C in unspecified conditions.^[52]

In addition to measuring sensitivity changes following storage, several studies measured FET biosensor sensitivity changes throughout repeated use over an extended time (see Table 5). Frequency of repeated use ranged from daily,^[36,61,73] to weekly.^[53] The device with the greatest long-term stability had a polymer functionalized gate/channel. This FET biosensor maintained a constant sensitivity for 1 month of weekly testing.^[53] Other biosensors with a polymer functionalization suffered sensitivity degradation after 3 days^[73] and 12 days^[111] into testing. The devices functionalized with an enzyme receptor and silane linker had

sensitivity degradation at day 3^[61] and day 6^[60]. One biosensor study tested for long-term stability through storage and repeated use.^[36] The biosensor was functionalized with an enzyme in a polymer. One of these devices was tested daily for 40 days with a sensitivity decrease of 20%. However, an identical device stored for 46 days operated like new. Ideally, optimized device designs could extend the long-term stability to even greater lengths of time.

3. Summary and Outlook

As described in this review, many FET biosensor design parameters have considerable effects on device performance. Through optimization of these parameters, designers can meet the needs of rapid and label-free molecular analysis of blood. Research areas that can especially benefit from further development are the expansion of FET biosensor multiplexing capabilities, increased long-term device stability, and comparison with other label-free sensing technologies. Highly multiplexed sensor systems will provide a platform for thorough blood analysis with rapid delivery of results and minimal blood volume requirements. Additionally, long-term device stability is necessary for FET blood biosensors to have a reasonable shelf-life or to operate continuously in any *in vivo* applications. An important relationship for further investigation is the effect of surface functionalization on device stability. Since the molecular environment at the FET surface influences receptor lifetime,^[50] surface functionalization is certainly a significant factor in long-term stability. In addition to multiplexing and long-term stability, another important area for investigation is comparing FET biosensor operation and feasibility with other label-free detection technologies such as nanoparticle-based systems. Studies of the advantages and disadvantages of these various systems alone and/or integrated with FET biosensors will aid researchers in determining which systems have the best characteristics for a given application. Throughout all further endeavors to design and improve FET blood biosensors, computational models are available to serve as a guide for device development. Theoretical predictions can direct experimentation so that researchers can achieve better results with less expenditure of time and resources. Through careful optimization of device parameters, and insightful application of new technologies, sensor designers will continue to expand the limits of biosensor capabilities.

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Biography

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Figure 1.

A) Operation of a FET biosensor in solution. B) Cross-section of a MOSFET

Blood FET Biosensors

Table 1

Sample	Sample Volume	Multiplexed?	Analyte	Receptor	LOD (range)	Detection Time	FET Architecture	Long-term Stability tested?	Ref.
Whole blood	20 μ L	Yes	PSA	Antibody	2.5 ng mL ⁻¹	< 1200 seconds	Si nanoribbon	No	[30]
Serum	-	Yes (in buffer)	CA15.3	Antibody	30 U mL ⁻¹	~ 500 seconds	Si nanowire	No	[10]
1% serum in buffer	> 10 μ L	No	PSA	Antibody	0.9 pg mL ⁻¹	10 seconds	AlGaIn/GaN HEMT	No	[26]
Serum	-	No	Vitellogenin	Antibody	3.95 μ g mL ⁻¹	~ 400 seconds	Polymer NT	No	[34]
Plasma	100 μ L	No	Thrombin	Aptamer	166 mM	Overnight	Planar gold extended gate	No	[31]
Whole blood	10 μ L	No	IL-1 β	None	1 pg mL ⁻¹	60 seconds	Planar polymer extended gate	Yes	[33]
Blood in buffer	10 μ L	No	Hemoglobin	Antibody	(125-197 μ g mL ⁻¹)	~ 780 seconds	Si NW	No	[27]
Desalted serum	20 μ L	No	Thrombin	Aptamer	-	< 200 seconds	Si NW	No	[7]
Serum in buffer	5 μ L	No	cTNT	Antibody	30 fg mL ⁻¹	~ 100 seconds	CNT	No	[11]
Serum	-	Yes	PSA	Antibody	1 ng mL ⁻¹	< 75 seconds	Si NW	No	[28]
Serum	2.5 μ L	No	cTNT	Antibody	100 fg mL ⁻¹	-	Planar gold extended gate	No	[35]
Plasma	100 μ L	No	CK-MM	SAM	100 fg mL ⁻¹	Overnight	Planar gold extended gate	Yes	[32]
10% serum in buffer	1.5 mL	No	Cholesterol	SAM	(0.33-2.33 mg mL ⁻¹)	< 600 seconds	Planar polymer	Yes	[36]
Serum	-	Yes	IL-1 β	Enzyme	-	1000 seconds	Si NW	No	[29]
Serum	-	No	Urea	Antibody	(0.2-114 ng mL ⁻¹)	~ 50 seconds	Si NW	No	[37]
Serum	-	No	CEA	Antibody	1 ng mL ⁻¹	~ 50 seconds	Si NW	No	[37]
Serum	-	No	PSA	Antibody	1 ng mL ⁻¹	~ 50 seconds	Si NW	No	[37]
Serum	-	No	AFP	Antibody	1 ng mL ⁻¹	~ 50 seconds	Si NW	No	[37]
Serum	-	No	CRP	Antibody	1 ng mL ⁻¹	~ 50 seconds	Si NW	No	[37]

α -fetoprotein (AFP), C-Reactive Protein (CRP), Carbon Nanotube (CNT), Cardiac Troponin-T (cTNT), Carcinoembryonic Antigen (CEA), Creatine Kinase MB (CK-MB), Creatine Kinase MM (CK-MM), Interleukin-1 β (IL-1 β), Limit of Detection (LOD), Nanowire (NW), Prostate Specific Antigen (PSA), Self-Assembled Monolayer (SAM)

Table 2

Silicon Nanowire FET Biosensors

Prefab NWs?	Analyte	LOD	Detection Time	Sensing Solution	Ref.
Yes	PSA	75 fg mL ⁻¹	500 seconds	1 mM PBS, 2 mM KCl, pH 7.4	[10]
Yes	Thrombin	330 pM	780 seconds	Acetate buffer, pH 4.5	[27]
Yes	CRP	1 ng mL ⁻¹	50 seconds	Serum	[37]
Yes	VEGF	104 pM	100 seconds	PBS, pH 5.4	[63]
Yes	MMP-9	300 nM	5 seconds	-	[59]
Yes	PSA	150 fM	1000 seconds	100 μ M PBS, 100 μ M KCl, pH 7.4	[64]
No	cTnT	1 fg mL ⁻¹	200 seconds	0.1 mM PBS	[7]
No	cTnT	1 fg mL ⁻¹	75 seconds	PBS pH 7.4	[28]
No	PSA	1 pg mL ⁻¹	250 seconds	1 μ M PBS, 2 μ M KCl, pH 7.6	[65]
No	19-NA	1.3 fM	500 seconds	0.1 mM Tris buffer, pH 7.5	[66]
No	PSA	1 ng mL ⁻¹	400 seconds	10 μ M PBS, 20 μ M KCl, pH 7.6	[55]
No	PSA	1 fg mL ⁻¹	250 seconds	1 μ M PBS, 2 μ M KCl, pH 7.6	[62]
No	Dopamine	1 fM	-	10 mM MES buffer, pH 6.0	[56]

19-norandrostendione (19-NA), Cardiac Troponin-T (cTnT), C-Reactive Protein (CRP), Interleukin-2 (IL-2), Limit of Detection (LOD), Low Density Lipoprotein (LDL), Matrix Metalloproteinase-9 (MMP-9), Prefabricated (Prefab), Phosphate Buffered Saline (PBS), Prostate Specific Antigen (PSA), Vascular Endothelial Growth Factor (VEGF)

Table 3

Carbon Nanotube FET Biosensors

Networked/Aligned	Analyte	LOD	Detection Time	Sensing Solution	Ref.
Networked	PSA	1.0 ng mL ⁻¹	100 seconds	10 mM PBS, pH 7	[11]
Networked	PSA	50 ng mL ⁻¹	500 seconds	PBS	[74]
Networked	ACh	1 pM	15 seconds	DI Water	[73]
Networked	IgG	1 pM	100 seconds	10 mM PBS, pH 7.4	[75]
Networked	IgG	500 ng mL ⁻¹	600 seconds	15 mM PBS, pH 7.4	[76]
Networked	IgG	1 pg mL ⁻¹	25 seconds	10 mM PBS, pH 7.4	[77]
Networked	IgG	10 µg mL ⁻¹	1 hour	PBS	[71]
Networked	IgG	400 pg mL ⁻¹	2 hours	-	[72]
Aligned	IgE	250 pM	1200 seconds	10 mM PBS	[78]
Aligned	PSA	100 pM ⁻¹	300 seconds	PBS with 1 % Tween 20	[70]
Aligned	Anti-HA Ab	50 pg mL ⁻¹	-	-	[69]
Aligned	CEA	54 pg mL ⁻¹	50 seconds	1 mM PBS, pH 6.5	[79]
Aligned	S-100B	1 ng mL ⁻¹	300 seconds	-	[80]

Acetylcholine (ACh), Anti-hemagglutinin Antibody (Anti-HA Ab), Carcinoembryonic Antigen (CEA), Deionized Water (DI Water), Immunoglobulin G (IgG), Immunoglobulin E (IgE), Immunglobulin G (IgG), Phosphate Buffered Saline (PBS), Prostate Specific Antigen (PSA)

Table 4

Common FET Biosensor Surface Linker Functionalization Procedures

Functionalization	Gate Material	Ref.
Hydrosilylation	Si or SiO ₂	[7,10,25,27-29,37,51,55,56,59,62,63,65,66,86,99]
	Al ₂ O ₃	[60,61,100]
	Si ₃ N ₄	[101,102]
	Ta ₂ O ₅	[103,104]
	CNT	[69]
	TiO ₂	[105]
	ZnMgO	[106]
Thiol	Au	[26,32,35,44,46,47,107-109]
1-Pyrenebutanoic acid succinimidyl ester	CNT	[70,77,78,80]
No Linker	Au	[31]
	CNT	[75,76]
	SiO ₂	[87]
	ZnO	[45,48]

Table 5**Long-term FET Biosensor Stability and Functionalization**

Storage or Reuse	Functionalization	Storage/Testing Conditions	Result	Ref.
Storage	Antibody receptor, polymer linker	4°C in air for 5 days	Sensitivity degrades 34%	[33]
Storage	Enzyme receptor, silane linker	2°C in PBS for 2 weeks	No performance degradation	[106]
Storage	Enzyme receptor, silane linker	4°C for 2 weeks	No performance degradation	[52]
Storage	Enzyme receptor, silane linker	4°C in Tris buffer for 1 month	No performance degradation	[104]
		Ambient air for 10 days	No performance degradation	
Reuse	Redox polymer	Reused 50 times	No performance degradation	[32]
Reuse	Polymer, embedded CNTs and SiO ₂ NPs, surface enzyme	Tested daily for 9 days	Sensitivity degrades at day 3	[73]
Reuse	Redox polymer, surface enzyme	Tested on days 1,5,6, 12	Sensitivity stable after day 6, but degrades by day 12	[111]
Reuse	Enzyme receptor, silane linker	Tested daily	Sensitivity degrades at day 3	[61]
Reuse	Enzyme receptor, silane linker	Tested on days 1,3,6	Sensitivity degrades at day 6	[60]
Reuse	Polymer, embedded MnO ₂ NPs, surface enzyme	Tested weekly for 2 months	Sensitivity stable after 1 month, but degrades 11% by 2 months	[53]
Both	Polymer, embedded enzyme	Tested daily for 40 days Stored for 46 days	Sensitivity degrades 20% No performance degradation	[36]