Effect of electrode configuration on the sensitivity of nucleic acid detection in a non-planar, flow-through, porous interdigitated electrode

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Abstract

Electrical impedance spectroscopy (EIS) sensors though rapid, and cost-effective often suffer from poor sensitivity. EIS sensors modified with carbon-based transducers show a higher conductance, thereby increasing the sensitivity of the sensor towards biomolecules like DNA. However, the EIS spectra are compromised by the parasitic capacitance of the electric double layer (EDL). Here, a new shear-enhanced, flow-through nonporous, non-planar interdigitated microelectrode sensor has been fabricated that shifts the EDL capacitor to high frequencies. Enhanced convective transport in this sensor disrupts the diffusion dynamics of the EDL, shifting its EIS spectra to high frequency. Concomitantly, the DNA detection signal shifts to high frequency, making the sensor very sensitive, rapid with high signal to noise ratio. The device consists of a microfluidic channel sandwiched between two sets of top and bottom interdigitated microelectrodes. One of the sets of microelectrodes is packed with carbon-based transducer material like carboxylated single-walled carbon nanotube (SWCNT). Multiple parametric studies of three different electrode configurations of the sensor along with different carbon-based transducer materials are undertaken to understand the fundamental physics and electrochemistry. Sensors packed with SWCNT show femtomolar detection sensitivity from all the different electrode configurations, for a short target-DNA. A 20-fold jump in the signal is noticed from the unique working electrode configuration in contrast to the other electrode configurations. This demonstrates the potential of the sensor for a significant increase in sensitivity of detection of DNA and other biomolecules.

Introduction

Deoxyribonucleic acid/ribonucleic acid (DNA/RNA) are fundamental biomarkers for identification of specific disease ¹⁻³. For example, in a liquid biopsy, detecting and long-term tracking of circulating tumor DNA (ctDNA) in human blood are critical for early-stage cancer detection, treatment, and during remission ⁴. Analysis of ctDNA is challenging due to its extremely low concentrations in human blood (<1% in many cases) ⁵. Hence, sensitive detection of ctDNA is one of the major issues in this field. PCR-based Amplification technologies are well developed with sensitivities of less than 50 ng ⁶⁻⁹. However, most PCR-based methods need complex, expensive, and precise chemical procedures to acquire a reliable result. Besides, PCR amplification technologies pass through a series of reactions to increase the ctDNA concentration. Hence, the final reading often does not reflect the actual ctDNA concentration in the human body. Hybridization of target ctDNA molecule to short single-stranded DNA (ssDNA) called oligonucleotide is the preferred method of detection of ctDNA ¹⁰.

Electrochemical biosensors use faradaic techniques like cyclic voltammetry ¹¹, pulse voltammetry ¹² or non-faradaic techniques like electrical impedance spectroscopy (EIS) ¹³. Electrochemical biosensors provide automatic tracking and detection, a user-friendly platform, and low cost ¹⁰. Generally, the design of a biosensor consists of an analyte (with the target molecule), a bioreceptor (the capture molecule), a transducer, electronic components, and a display ¹⁴. The ssDNA oligonucleotide used for detection of ctDNA has a 5' modification designed to bind to the transducer. Electrochemical sensors covert the hybridization signal (binding of a ctDNA to an oligo) to an equivalent electrical/electrochemical signal. Currently, the detection sensitivity of electrochemical sensors for ctDNA is around ten picomolar without expensive electrode modifications or extensive protocols ¹⁵. Hence, there is a demand for simple electrochemical sensors

capable of detecting low amounts of ctDNA.

Among electrochemical biosensors, EIS-based biosensors have been used for detecting different biomarkers, such as proteins¹⁶, antibodies¹⁷ or DNA^{1, 12, 13}. EIS based sensors detect changes in the electric current from the electrode surface on the application of a sinusoidal electric voltage (AC). The changes are caused by the immobilization of the target analyte (DNA, antigen, among others) to receptors/molecule receptors on the electrode surface. Device sensitivity determines whether one can detect the physical or chemical changes on the electrode surface due to target binding. These electrochemical signal increases with usage of a nanomaterial as a transducer as has been shown elsewhere¹⁸⁻²⁰. Carbon nanotubes (CNTs) are one of many common materials used as a transducer in electrochemical biosensors²¹. Due to its high electrical conductivity and high surface area to volume ratios, CNTs make extraordinary transducer material. The surface of the CNT can also be easily modified and bonded to ssDNA oligonucleotide by standard coupling and functionalization reactions^{22, 23}.

The problem with EIS based sensors is the electrical double layer (EDL). EDL is a parasitic capacitor that reduces the sensor's sensitivity and increases the noise. Our group has introduced a novel electrochemical platform that uses shear-enhanced, flow-through, nanoporous, non-planar interdigitated μ IDE (NP- μ IDE) that allows for a significant reduction of the noise from the EDL. In this work, we show the DNA sensitivity of (NP- μ IDE) that packs carbon-based transducer materials between the bottom and top microelectrode. As has been shown earlier and here, our fabrication and device integration process is simple, easy to set up, cost-effective, and requires minimal operator handling/expertise²⁴. NP- μ IDE with packed carbon materials increases convective transport, disrupting the diffusive dynamics that govern the EDL. Hence,

the EDL capacitor signal moves to higher frequencies ^{24, 25}. These allow the measurement of the EIS sensor signal at significantly higher frequencies reducing the noise and making the system rapid. Different types of carbon transducer materials such as single-wall carbon nanotube (SWCNT), aligned multi-wall carbon nanotube (AMWCNT), single-wall carbon nanotube with carboxyl groups (SWCNT-COOH), carbon nanofiber (CNF) are tested in multiple electrode configurations with different voltages and potassium chloride (KCl) concentrations. ssDNA oligo probes are functionalized to the carboxylate CNT using standard EDC-NHS coupling. The device is tested with short target DNA. The detection limit is femtomolar. Further, the EIS sensor showed that among different electrode configurations, DNA detection sensitivity is higher for certain electrode configurations.

Methods and Materials:

Reagents and Instruments: Standard glass slides (1304G) with ground edges, 90° corners, and size 25x75x1 mm is ordered from Globe Scientific Inc. (USA). The DI water used in the experiments is obtained from a Milli Q Direct 8 Water Purification System with a conductivity of 7.9 $\mu\text{S}/\text{cm}$ W. Double-sided polypropylene (PP) tape (90880) with SR-26 silicone pressure-sensitive adhesives on both sides is obtained from ARcare, USA. The thickness of the tape is 142 μm including the PP layer and the two adhesives. Potassium Chloride (KCl), ACS grade is acquired from British Drug Houses (BDH). 4294A Precision Impedance Analyzer from Keysight Technologies is used for all the EIS measurements. Different transducer materials like carbon nanofiber (CNF 95%+), short single-wall carbon nanotube (SWCNT 98%+), carboxylic acid functionalized short single-walled carbon nanotube (C-SWCNT, 98%+) and aligned multi-wall carbon nanotube (MWCNT, 95%+), are all acquired from US Research

Nanomaterials Inc. Fabrication of the top and bottom microelectrode glass slide is carried out in the Nano-fabrication facility at CUNY Advanced Science Research Centre.

Device Fabrication: Cleaning steps for the standard glass slides, involve, (1) Two 10 minute 1:3 Piranha washes for removal of organic contaminants, (2) An AMD wash protocol for removal of any residual contaminants from the Piranha Wash. The AMD wash involves consecutively rinsing the glass slide by acetone, isopropanol, methanol, and DI water. Following the AMD wash, the glass slide is dried using a Nitrogen gun. (3) The glass slide is heated to 130°C for 30 minutes to eliminate any surface-bound moisture. Hexamethyldisilazane (HMDS) is spun onto the glass slides in two spins of 400 rpm for 15s and 1000 rpm for 45s with a ramping rate of 200 rpm/s using Brewer Science Cee's spin coater to promote photoresist adhesion. A positive tone photoresist AZ 1512 is spun onto the glass slide using two spins of 500 rpm for 10s, 3000 rpm for 45s at a ramping rate of 200 rpm/s and then soft-baked at 110°C for 60 seconds for a conformal coat of 1.39 μm . For contact lithography, an EV Group's EVG620 mask aligner is used to expose the glass slides with photoresist to an electrode mask. The UV exposure dosage is 350 J/m². Post-exposure, the slides are developed in fresh AZ 300 MIF Developer for 35s, followed by a deionized water wash and nitrogen drying. The pattern is checked for accuracy under a microscope. An AJA Orion 8E Evaporator System for electron beam (e-beam) is used to deposit 10 nm of Titanium followed by 100 nm of gold (both at 2 Å/s), on the patterned glass slides. The electrode geometry is developed post-deposition using lift-off by immersing in acetone bath after one day. The microchannel is constructed out of ARcare® 90880 polypropylene double-sided tape. The microfluidic channel is cut to dimension using the Cricut®. The dimensions of the channel are 48mm (L) x 500 μm (W) x 142 μm (H). The final device consists of

a microchannel made from the double-sided tape sandwiched between a top and bottom patterned microelectrode. Inlet and outlet holes for fluid flow are drilled by a 1mm diamond drill bit onto the top microelectrode glass slide. The adhesive on each side of the tape is attached to the bottom and the top microelectrode glass slide to form the NP- μ IDE.

Transducer Packing and Device Integration: The packing of the channel with transducer material is done using the following protocol. The liner from the bottom side of the double-sided tape is removed to expose the silicon adhesive. The channel is cut into the double-sided tape using Cricut machine. The cut tape is first aligned to the bottom microelectrode glass slide. Post alignment, the double-sided tape is fixed to the bottom microelectrode glass slide using the exposed silicon adhesive. The transducer material is mixed with 1X PBS to form a slug. The slug is loaded into the channel to form a tight packing with the top liner still on. The top liner is then removed to expose the silicon adhesive. The top microelectrode slide is aligned to the channel containing the transducer material (CNF, SWCNT, C-SWCNT, or MWCNT). The alignment is carried out under a microscope to ensure the inter-digitation of the non-planar electrodes.

KCL Run Protocol: The device is packed with CNF, SWCNT, C-SWCNT, or MWCNT using the transducer packing protocol as described before. The KCl is diluted with DI water to get the required KCl concentrations from 1M to 1 μ M. These devices are connected to the Impedance analyzer, Agilent 4294A for EIS readings using standard electrical connections (**figure 1**). The devices are first washed with DI water for 1 hour. After the wash procedure, KCl flows through the device at 1 μ l/min. The EIS signal for a KCl concentration is taken after 1 hour.

CNT Functionalization of SWCNT: Probe-DNA and Target-DNA oligo with the

sequence of 5'-/5AmMC6/CGTCCAAGCGGGCTGACTCATCAAG-3' and 5'-CTTGATGAGTCAGCCCGCTTGGACG-3' respectively are acquired from Integrated DNA Technologies (IDT). A10807 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDAC), and A10312 N-Hydroxysuccinimide (NHS) are acquired from Alfa Aesar with 98%+ purity. 2-(N-Morpholino) ethanesulfonic acid hydrate (MES) is acquired from Sigma-Aldrich with 99.5%+ purity. Phosphate Buffered Saline (PBS) Tablets is acquired from VWR. 30 mg of the C-SWCNT are vortexed/washed in the 0.1M MES buffer and centrifuged in a 1.7ml Eppendorf at 6000RPM (GeneMate Minifuge) thrice. During the wash, the suspension is replaced with fresh MES. The supernatant is taken out and activated with EDC-NHS coupling reaction with Prob-DNA oligo in 1X PBS solvent overnight. The DNA-functionalized C-SWCNT is washed in 1 X PBS (pH 7.4) and preserved in the refrigerator.

DNA Run Protocol: The DNA-functionalized C-SWCNT device is connected to the Impedance analyzer using standard electrical connections, as shown in **figure 1**. Target-DNA oligo concentrations are diluted to 1 μ M, 1nM, 10pM, 1pM, 0.1pM and 1fM with 1X PBS (pH 7.4). The DNA oligo sensor protocol involves the following steps. A NE-300 syringe pump from New Era Pump Systems, Inc. (USA) is connected to the inlet of the device. The device is first washed with 1X (pH 7.4) PBS solution at a flow rate of 1 μ l/min for 2 hours or until the EIS signal from the device stabilizes. The stabilization of the EIS signal is interpreted as two EIS measurements that do not change when taken 5 minutes apart. This stabilized EIS signal taken in 1X PBS is treated as the baseline measurement. Post-stabilization, the 1X PBS solution is switched to the 1 M Ethanolamine (99%+ pure from Alfa Aesar) in 1X PBS for 90 mins. Ethanolamine blocks non-reacted carboxy groups, which will reduce the non-specific adsorption of the target-DNA in the device [27-29]. Post-blocking, the device is washed

with 1X PBS for 2 hours to remove any remaining Ethanolamine. Target-DNA oligos flow into the system at 1 μ l/min for 2 hours. Post-Target-DNA oligo passage, the solution is switched back to 1X (pH 7.4) PBS solution (washing solution). The device is rinsed with 1X (pH 7.4) PBS solution at a flow rate of 1 μ l/min for 3 hours and 30 minutes to remove any unspecific bound Target-DNA in the device. This difference between the EIS signal from the pre and post-PBS wash is interpreted as the Target-DNA oligo capture signal.

Results and Discussions

Figure 1 shows the working of the sensor device. It is worthwhile to note that the sensor has a 2×2 configuration, i.e., two NP- μ IDE sets are placed in a single channel. One of the NP- μ IDE set is packed with transducer material. The other NP- μ IDE is empty. Flow passes through both of these NP- μ IDE sets, one after another. EIS data is recorded by different electrode configurations that are made using these two sets of NP- μ IDE. A material electrode configuration (ME) is the EIS signal from the first set of NP- μ IDE packed with transducer material (**Figure 1A**). The Clear electrode configuration (CE) is the EIS signal from the second set of NP- μ IDE that does not have any transducer material (**Figure 1B**). Finally, the working electrode configuration (WE) is a unique take on the electrodes. The first set of NP- μ IDE with packing are shorted together to form a single electrode (working). The second set of NP- μ IDE is also shorted to form a single electrode (counter). The EIS signal is now taken between the working and the counter electrode (**Figure 1C**). WE configuration allows us to observe any changes that take place in the packed NP- μ IDE set and contrast it against the empty NP- μ IDE set of electrode.

Each electrode configuration (ME, CE, and WE) are examined in details to

understand what electrochemical properties are measured by what electrode configuration. **Figure** (2A) shows the Nyquist curve for the EIS signal from SWCNT ME in 0.01 M KCl concentration at different voltages. It is worthwhile to note that the EIS signal shows a series LR circuit. Further, the packed electrode behaves like a pure ohmic resistor at low frequency due to the high electronic conductivity of SWCNT. It is worthwhile to note that SWCNT behaves as a pure conductor in the experimental voltage range as has been seen elsewhere ²⁶. Hence capacitive behavior of the electrode is absent. At higher frequency, the Nyquist spectra show up as an inductor. The packing of the conducting SWCNT in the channel is similar to that of twisted conducting wire. Like a twisted conducting wire in an applied electric field, the packed SWCNT behaves like an inductor at high frequency. It is interesting to note that the voltage does not have any effect on the Nyquist curve.

CE shows an atypical Randles circuit electrode semi-circle in the Nyquist plot (**figure** 2B). The electrode response between the NP- μ IDE shows a similar diffusion dominated behavior to the EIS response as seen from interdigitated gold planar electrodes ²⁷. The same diffusion layer is responsible for the Warburg impedance seen at the lower frequency. The NP- μ IDE geometry results in a finite diffusion layer. That results in a non-ideal Warburg that is more capacitive as has been seen elsewhere ²⁸. Further, as expected with an increase in voltage, the diffusion layer increases, which results in a progressively less capacitive Warburg resistor ²⁸. The WE electrode combination combines the above two configurations to result in a parallel RC circuit, as shown in **figure** (2C). However, the RC of the EIS spectra from the WE shows a significantly high R. It is hypothesized that this large R is due to the significant distance between the two sets of NP- μ IDE (~10 mm). We speculate that at lower voltages and low frequency, the scatter in the system is due to increased drift current as has been

seen elsewhere with high resistance.

A critical element for an EIS sensor is the response to electrolytes, specifically the conductivity of the electrolyte. **Figure** (3A) shows that for SWCNT ME, the EIS spectra is still an LR circuit in series at low KCl concentrations while at higher concentrations, a small RC circuit starts to appear. This RC at the higher KCl concentrations can be attributed to the increased capacitance of the EDL. The charge relaxation frequency ($1/RC$) decreases substantively at higher values of C, which allows the RC to dominate at the lower frequencies (L, the inductor is weak at low frequency). It is important to note that there are two current paths that help us to understand the origin of the C at higher concentrations and low frequencies. The first current path is directly through the packed SWCNT that leads to a purely resistive EIS signal at low frequency. The second current path is the ionic current in the electrolyte surrounding the SWCNT and the electrodes. At low concentrations, the electronic conductivity of the electrolyte is significantly lower in comparison to the packed SWCNT, and hence the current chiefly passes through the SWCNT. However, at higher concentrations, the ionic current is also significant. These ionic currents can lead to a charge accumulation near the electrodes/SWCNT. It is interesting to note that this is similar to the EIS signal observed from gold electrodes albeit shifted by frequency due to the L present across most of the frequency range. From **Figure** 4A, the only material that does not show this RC behavior at low frequency is the Aligned MWCNT. It can be hypothesized to the much higher conductivity of the MWCNT (> 1250 s/cm) in comparison to the other materials. Further, the SWCNT-COOH group shows the largest RC signature among all the different materials at high concentrations (**Figure** 3A). This is due to the presence of charged moieties (-COOH) on its surface that leads to an enhanced capacitor²⁹. This dominates the LR circuit even more than the other materials at the

high concentrations. Hence SWCNT-COOH crosses the resistance axis at a lower value than the MWCNT even though it has a lower conductivity than the MWCNT (as can be seen at the lower concentrations)

The CE (**figure 3B**) for SWCNT follows a traditional Randles circuit, with the charge transfer resistance (R_{ct}) decreasing with increase in KCl concentration. The WE (**figure 3C, 4B**) also follows a similar Randles circuit though with a much higher R_{ct} than the CE. The increase in R_{ct} can be attributed to the distance between two sets of NP- μ IDE. For the WE, the EIS spectra are more scattered at the lower concentrations and the lower frequencies (**figure 3C, 4B**). The problem of noise at the lower frequencies is due to the drift of the current at high R_{ct} and low frequency. Hence, this scatter is more pronounced in the lowest KCl concentrations, which has the highest R_{ct} in WE. In this manuscript, however, we are not too worried about the noise in the WE electrode as for DNA detection experiments, the electrolyte will have a high ionic concentration. DNA is much more stable at high ionic concentrations³⁰. Our electrolyte is 1X PBS media with a conductivity of 13,500 – 17,000 μ S/cm, which is equivalent to 0.1M KCl (12880 μ S/cm). Hence, EIS spectra taken in 1X PBS should not have any scatter at the low frequency.

DNA hybridization experiments are carried out using a probe-DNA and a target-DNA that is perfectly matched. The DNA's and the device protocol is described in details in the methods and material section. The initial EIS spectra in 1X PBS (SWCNT with probe-DNA only) and 1 X PBS washing post-target-DNA passage is acquired. The difference in the R_{ct} in the two EIS spectra is taken as the DNA hybridization signal. The change in the EIS spectra for two different electrode configurations, WE and ME are shown in **figure 5**. As can be seen from **figure 5**, the difference between the impedance circuit clearly shows the binding of the target-DNA oligo to the probe-DNA.

It is interesting to note that with the attachment of target-DNA, the R_{ct} is decreasing (**figure 5**). For SWCNT, carbon hybridizes into sp, sp² configurations with narrow gaps between their 2s and 2p electron shells. During the detection of DNA, as a π electron orbital is formed during the interaction of the double-stranded DNA with the SWCNT, specifically at the sp, sp² hybridization site. This π electron is often shared between the SWCNT and the double-stranded DNA ³¹. This manifests as an increased surface area of the transducer wherein the double-stranded DNA acts as an extension of the SWCNT. This should lead to an increased ionic current, which translated to a decrease in R_{ct} .

However, to get a clear picture of the sensitivity of the different electrode configurations, the change in R_{ct} is normalized to the initial R_{ct} value of the device. This is also as the packing can change across devices. Even though the device loading is approximately the same, however, human error can lead to small variance during loading. Our earlier papers show that the EIS spectra of the device are dependent on the device structure ²⁴. Hence a normalization would allow us to reduce/remove the variation due to the packing. **Figure 6** shows that post-target-DNA hybridization, the ME shows a much smaller change in comparison to the WE (20 times). This is expected due to the current path in the two electrode configurations. Indeed as the ME is chiefly resistive, a small proportion of the current is carried by the ions. Hence any changes in the overall current due to changes in the ionic current from the binding of the target-DNA is limited. The electronic current through the SWCNT is unaffected by DNA binding as the SWCNT is already very conductive. For the WE, the ionic current is the only way that the two sets of interdigitated electrodes can talk. Hence changes in the SWCNT from the binding of the target-DNA is registered at significantly higher levels for WE.

Interestingly though qualitatively the WE gives a very high sensitivity of 1

femtomolar, yet the WE gives poor quantitative results. We speculate that this could be due to the large distance between the two pairs of NP- μ IDE electrodes. It is possible that only the ions interacting with the tail end of the packed set of NP- μ IDE reach the clear NP- μ IDE set. Hence, the ionic current between the two sets of electrode only carries a fraction of the target-DNA binding information. Alternatively, the number of carboxy groups could be low, and the DNA at the lower concentration is saturated. Nevertheless, the sensitivity of the WE clearly shows that post electrode structure optimization (reducing the distance, anti-parallel orientation, among others) the sensitivity can be increased with significantly higher signal to noise ratio.

Conclusion

To conclude, we demonstrated here that NP- μ IDE could be used in several different electrode configurations, WE, CE, and ME to measure the binding of target DNA to probe DNA. The electrodes can be packed with different transducer material. The packed NP- μ IDE electrode current has two current paths for ME. At low ionic strength and with conductive transducer materials like MWCNT, the current is chiefly electronic and can miss a contribution from the electrode-electrolyte interface. For conductive materials with charged moieties, like C-SWCNT, the ionic current from the electrolyte-electrode interface is as strong as the electronic current at high ionic strength. The WE, on the other hand, will pick up any changes in the packed transducer material. However, due to the distance between the two pairs of NP- μ IDE in the WE configuration, scattering noise can dominate at low ionic strength. DNA sensitivity from both WE and ME configuration show very high sensitivity (1 femtomolar). Further, the change in the R_{ct} from the binding of the target-DNA to the probe-DNA is 20 times larger for the WE configuration in contrast to the ME.

Supplementary Material

See supplementary material for the EIS spectra from different Target-DNA attachment to SWCNT with probe-DNA for the WE configuration. The Target-DNA concentrations are (A) 10^{-2} nM, (B) 10^{-3} nM, (C) 10^{-4} nM, (D) 10^{-6} nM.

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List of Figures

Figure 1: Diagram of the experiential setup. The microfluidic devices are connected to an EIS machine (Agilent 4294A) from NP- μ IDE device. The solvent is controlled using a NE-300 syringe pump with a flow rate of $1\mu\text{l}/\text{min}$. (A) Material electrode (ME) configuration. (B) Clear electrode (CE) configuration. (C) Working electrode (WE) configuration.

Figure 2: EIS spectra of SWCNT packed NP- μ IDE devices in 0.01 M KCL for different configurations and voltages (A) ME electrode configuration. (B) CE electrode configuration. (C) WE electrode configuration.

Figure 3: Here the effect of KCL concentration on NP- μ IDE with single-wall carbon nanotube (SWCNT) is shown for: (A) ME configuration, (B) CE configuration and (C) WE configuration.

Figure 4: Here the effect of KCL concentration on NP- μ IDE with different packing material, namely, single-wall carbon nanotube (SWCNT), aligned multi-wall carbon nanotube (AMWCNT), single-wall carbon nanotube with carboxyl groups (SWCNT-COOH), carbon nanofiber (CNF) is shown for : (A) ME configuration at 1M, 1mM and $1\mu\text{M}$ KCl concentration and (B) WE electrode at 1M, 1mM and $1\mu\text{M}$ KCl concentration.

Figure 5: EIS spectra from target-DNA attachment to SWCNT with probe-DNA is shown for ME, and WE configuration for the target-DNA concentration of 1 femto Molar.

Figure 6: The change in the normalized value of R_{ct} with changes in DNA concentration is plotted for the SWCNT electrode used in figure 5 for (A) ME and (B) WE configuration.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Bibliography

1. H. Ilkhani and S. Farhad, *Anal Biochem* **557**, 151-155 (2018).
2. R. Sharma, S. E. Deacon, D. Nowak, S. E. George, M. P. Szymonik, A. A. S. Tang, D. C. Tomlinson, A. G. Davies, M. J. McPherson and C. Walti, *Biosens Bioelectron* **80**, 607-613 (2016).
3. Y. Liu, M. Wang, E. M. Marcora, B. Zhang and A. M. Goate, *Neurosci Lett* **711**, 134403 (2019).
4. X. Li, M. Ye, W. Zhang, D. Tan, N. Jaffrezic-Renault, X. Yang and Z. Guo, *Biosens Bioelectron* **126**, 596-607 (2019).
5. L. Gorgannezhad, M. Umer, M. N. Islam, N. T. Nguyen and M. J. A. Shiddiky, *Lab Chip* **18** (8), 1174-1196 (2018).
6. L. Xi, T. H. Pham, E. C. Payabyab, R. M. Sherry, S. A. Rosenberg and M. Raffeld, *Clin Cancer Res* **22** (22), 5480-5486 (2016).
7. K. L. Spindler, N. Pallisgaard, R. F. Andersen and A. Jakobsen, *Int J Cancer* **135** (9), 2215-2222 (2014).
8. L. A. Diaz, Jr., R. T. Williams, J. Wu, I. Kinde, J. R. Hecht, J. Berlin, B. Allen, I. Bozic, J. G. Reiter, M. A. Nowak, K. W. Kinzler, K. S. Oliner and B. Vogelstein, *Nature* **486** (7404), 537-540 (2012).
9. A. Stahlberg, P. M. Krzyzanowski, M. Egyud, S. Filges, L. Stein and T. E. Godfrey, *Nat Protoc* **12** (4), 664-682 (2017).
10. A. Tadimety, A. Closson, C. Li, S. Yi, T. Shen and J. X. J. Zhang, *Crit Rev Clin Lab Sci* **55** (3), 140-162 (2018).
11. J.-S. Do, Y.-H. Chang and M.-L. Tsai, *Materials Chemistry and Physics* **219**, 1-12 (2018).
12. E. O. Blair and D. K. Corrigan, *Biosens Bioelectron* **134**, 57-67 (2019).
13. G. Luka, A. Ahmadi, H. Najjaran, E. Alocilja, M. DeRosa, K. Wolthers, A. Malki, H. Aziz, A. Althani and M. Hoorfar, *Sensors (Basel)* **15** (12), 30011-30031 (2015).
14. N. Bhalla, P. Jolly, N. Formisano and P. Estrela, *Essays Biochem* **60** (1), 1-8 (2016).
15. S. Xu, J. Zhan, B. Man, S. Jiang, W. Yue, S. Gao, C. Guo, H. Liu, Z. Li, J. Wang and Y. Zhou, *Nat Commun* **8**, 14902 (2017).
16. Y. Huang, A. Hara, C. Terashima, A. Fujishima and M. Takai, *Carbon* **152**, 354-362 (2019).
17. G. Cabral-Miranda, A. R. Cardoso, L. C. S. Ferreira, M. G. F. Sales and M. F. Bachmann, *Biosens Bioelectron* **113**, 101-107 (2018).
18. J. E. Weber, S. Pillai, M. K. Ram, A. Kumar and S. R. Singh, *Materials Science and Engineering: C* **31** (5), 821-825 (2011).
19. S. Ding, S. R. Das, B. J. Brownlee, K. Parate, T. M. Davis, L. R. Stromberg, E. K. L. Chan, J. Katz, B. D. Iverson and J. C. Claussen, *Biosens Bioelectron* **117**, 68-74 (2018).
20. K. Awasthi, D. P. Singh, S. K. Singh, D. Dash and O. N. Srivastava, *New Carbon Materials* **24** (4), 301-306 (2009).
21. K. Nagaraju, R. Reddy and N. Reddy, *J Appl Biomater Funct Mater* **13** (4), e301-312 (2015).
22. A. Mirzapoor, A. P. F. Turner, A. Tiwari and B. Ranjbar, *Materials Science and Engineering: C* **104** (2019).
23. H. Wang, L. Zhang, Y. Jiang, L. Chen, Z. Duan, X. Lv and S. Zhu, *Sensors and Actuators B: Chemical* **261**, 441-450 (2018).
24. Y.-H. C. Zhenglong Li, Lixin Feng, De Dios Felix, Jacob Neil, Reis Moura Pedro Antoni, Maryom Rahman, Juliana Yang, Samar Azizighannad, Somenath Mitra, Sagnik Basuray, *Journal of the Electrochemical Society (Under Review)* (2019).
25. S. Basuray, S. Senapati, A. Aijian, A. R. Mahon and H. C. Chang, *ACS nano* **3** (7), 1823-

1830 (2009).

26. Z. El-Moussawi, A. Nourdine, H. Medlej, T. Hamieh, P. Chenevier and L. Flandin, Carbon **153**, 337-346 (2019).

27. E. Katz and I. Willner, Electroanalysis **15** (11), 913-947 (2003).

28. G. Barbero and I. Lelidis, Physical Chemistry Chemical Physics **19** (36), 24934-24944 (2017).

29. M. A. Atieh, O. Y. Bakather, B. Tawabini, B. Al-Tawbini, A. A. Bukhari, F. A. Abuilaiwi and M. B. Fettouhi, Bioinorg Chem Appl **2010**, 603978 (2010).

30. Z. J. Tan and S. J. Chen, Biophys J **90** (4), 1175-1190 (2006).

31. G. Lu, P. Maragakis and E. Kaxiras, Nano letters **5** (5), 897-900 (2005).

Different Electrode Configurations

**(A) Material electrode
ME**

**(B) Clear electrode
CE**

**(C) Working Electrode
WE**