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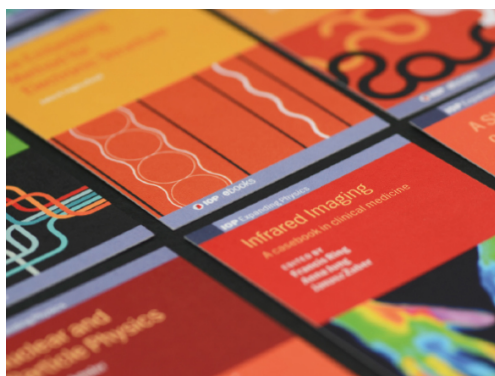
Advancing PoC Devices for Early Disease Detection using Graphene-based Sensors

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Advancing PoC Devices for Early Disease Detection using Graphene-based Sensors

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Abstract. Early detection of diseases is key to better disease management and higher survival rates. It aims at discovering conditions that have already produced biochemical changes in body fluids, but have not yet reached a stage of apparent physical symptoms or medical emergency. Therefore, early disease detection relies majorly on biochemical testing of biological fluids such as serum, in the body. The laboratories for these tests require biochemical-based instrumentations that are bulky and not commonly available especially in developing countries. Moreover, the tests are expensive and require trained personnel to conduct and interpret results. On the other hand, Lab-on-a-Chip (LOC) biosensors have a potential to miniaturize the entire biochemical/laboratory methods of diagnostics into versatile, inexpensive and portable devices with great potential for low-cost Point-of-Care (POC) applications. They are capable of providing accurate and precise information on the measured health indices for sub-clinical level of diseases. Nanotechnology-inspired biosensors have further advantages of low limit of detection (required for early diagnosis), real-time analysis and lesser sample volume requirement. Of all other nanomaterials, graphene is said to be the most promising, suitable for biosensing due to its biocompatibility and consistent signal amplification even under the conditions of harsh ionic solutions found in the human body. This paper reviews the potentials, fundamental concepts and related works in using Graphene-based Field Effect Transistors (GFETs) as biosensors for early disease diagnosis. This paper also highlights a low-cost patterning mechanism for preparing SiO_2/Si substrate for metal deposition (of the source and drain electrodes of FETs).

Keywords: Biosensors, Early Detection, Graphene, GFET, Lithography, Nanotechnology, POC, Shadow mask

1. Introduction

According to the United Nations Development Programme (UNDP), good health and well-being (SDG3) is critical for the attainment of the remaining 16 sustainable development goals (SDGs) and consequently the UN 2030 Agenda [1]. Significant research progress has been made against several leading causes of diseases and deaths worldwide, even though this progress varies widely within individual countries and also among countries. However, the world is still generally off-track in achieving the health-related SDGs. In addressing the global health challenges, a lot of emphasis is currently being placed on prevention and early stage detection of diseases as one of the ways forward [2], [3]. Early disease detection relies heavily on biochemical testing of biological samples in the

appropriate laboratories. Most of these tests depend on optical imaging using a fluorescent material, such as in Enzyme-Linked Immunosorbent Assay (ELISA) or the chromatographic technique called High Performance Liquid Chromatography (HPLC) that rely on the interaction of various components of an analyte with the solid adsorbent material within its column. Although these methods have high sensitivity and specificity, they require complex, bulky, and expensive instrumentation that are not commonly available in primary laboratories especially in developing countries. They also require highly professional knowledge and techniques to conduct experiments and interpret results. These challenges severely impede frequent health monitoring, prompt interventions that may succeed early diagnosis of diseases, point-of-care monitoring of treatment, monitoring of disease regression/ progression and others. Therefore, a lot of research is directed towards the development of test devices that can be used conveniently and reliably at the Point-of-Care (POC) [4]–[7]. It is noteworthy that the World Health Organization (WHO) already identified the place of such methods in diagnostics and have set out the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) criteria to be used as a benchmark for developing the most suited test method in resource-constrained situations [8]. Although developed by the Sexually Transmitted Diseases Diagnostics Initiative (SDI) of WHO [8], the ASSURED criteria is beginning to find a wider relevance as it is being applied to varied diseases. Biosensors have the potential to satisfy most/all of these criteria. They are capable of providing accurate/ precise information on the desired measured health indices as well as sub-clinical level of diseases [9], [10]. Biosensors based on label-free electrical detection methods, especially Field Effect Transistors (FETs) based ones, offer the most feasible POC test setup due to their cost-effectiveness, simplicity, fast response and low power requirement while offering high sensitivity and selectivity. Achieving more compactness facilitated by miniaturization of biosensors, is enabled by nanotechnology. This offers additional benefits including: low limit of detection (required for early diagnosis), real-time analysis and lesser sample volume requirement [4], [11]. The defining principle of nanotechnology is that as the size of materials reduce towards this nanoscale, the number of atoms at the surface of the material significantly increases compared to the bulk of the material. This phenomenon results in a large surface area to volume ratio. Most of the desirable properties of sensors are due to this large surface area to volume ratio.

Since the first exfoliation of a single atomic layer of graphene in 2004 by Geim and Novoselov [12], of all other nanomaterials, it is said to be the most promising nanostructured carbon material suitable for biosensing and has been under intense research for over a decade [11], [13]. Graphene is biocompatible and still produces signal amplification even under the conditions of harsh ionic solutions found in the human body [14]. Unlike the conventional silicon-based Metal Oxide Semiconductor FET (MOSFET), using graphene as a channel is increasingly being explored as a new category of nanoelectronic neuro- and bio-sensors [15], [16]. These sensors mostly rely on charge detection – where the analyte and sensor interaction changes the charge density in the vicinity of the nanomaterial. This in turn produces an electrically measurable signal through charge transfer between the biomolecules and the nanomaterial. This is possible because the charge carrier density of the nanomaterial can be varied by doping [17]; therefore, the adsorption of the analytes while appropriately modulating the gate voltage, produces the change in charge density. This paper details the fundamentals in using graphene in FETs for biosensing and also presents a low cost patterning mechanism for preparing SiO_2/Si substrate for metal deposition (of the source and drain electrodes of FETs).
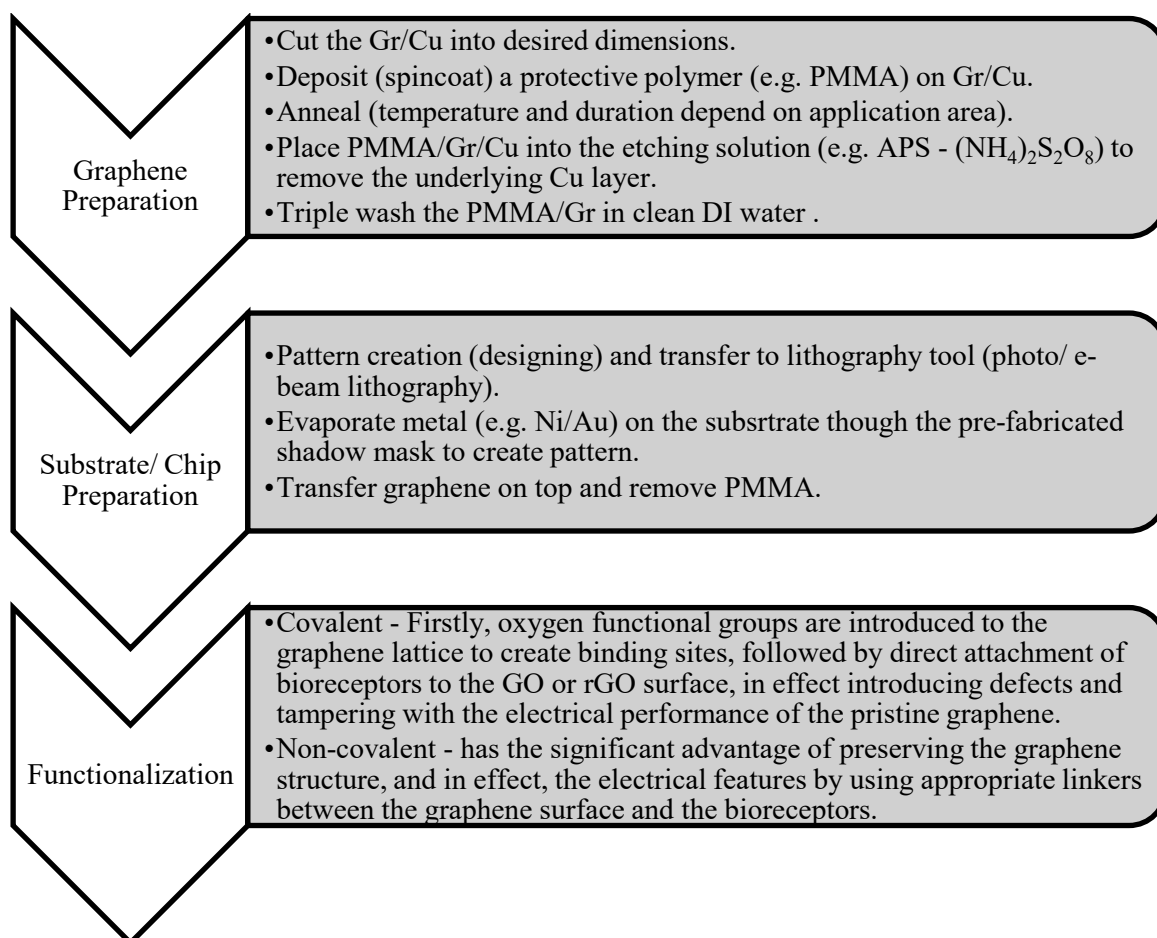
2. Graphene

Graphene is an atomically thin carbon based nanomaterial [18]. The main derivatives of graphene are: graphene oxide, reduced graphene oxide and graphene-based quantum dots. Graphene is a sheet made up of carbon atoms arranged in a honeycomb-shaped lattice of sp^2-hybridized carbon [19]. The geometry of graphene presents a very high surface to volume ratio, since it is only one atom thick [20], [21], therefore, exposing a significant portion of the surface area for detection of analytes (an advantage over other nanostructures in FET-based sensor designs). Graphene has no band gap and offers notable advantages of: carrier mobility ($\sim$200,000cm^2/V/s), electrical conductivity ($\sim$10^4 S/cm), optical transmittance and Young's modulus of $\sim$1 TPa [22]. It also exhibits ambipolar electric field effect such that it is able to detect both positive and negatively charged biomolecules [23].

Graphene synthesis can be categorized into: top-down and bottom-up approaches [24]. The focus of top-down approaches are aimed at reducing bulky and layered graphite compounds into mono and few-layer graphene since the compounds are made up of single-layer graphene with weak van der Waals interactions between adjacent layers. The focus of the bottom-up approach is to grow the required number of graphene layers. Several variants based on these two broad categories have been demonstrated; however, the four most used methods are: mechanical exfoliation of graphite, Chemical Vapour Deposition (CVD), graphitization of Silicon Carbide (SiC) surface and thermal reduction of graphite oxide [25], [26]. Graphene is now widely commercially available in form of graphene on copper foil (Gr/Cu) most of which are synthesized using CVD.

3. Fabricating Graphene Biosensors

Fabrication of graphene-based biosensors follow three major steps as seen in figure 1:

- Step 1 – graphene preparation for use in the biosensor (assuming graphene is purchased as Gr/Cu). This represents the process of preparing graphene for transfer from the copper foil to the desired substrate.
- Step 2 – substrate preparation. In this step, the resulting graphene from step 1 is transferred onto the resulting chip from step 2 before functionalization.
- Step 3 – functionalization of the resulting graphene transistor from step 2. This simply refers to the steps taken to attach the bio-receptor (antibodies, aptamers, nucleic acids etc.) specific to the target analyte to the graphene layer. It can be done covalently or non-covalently, each with its pros and cons.

Graphene Preparation

- Cut the Gr/Cu into desired dimensions.
- Deposit (spincoat) a protective polymer (e.g. PMMA) on Gr/Cu.
- Anneal (temperature and duration depend on application area).
- Place PMMA/Gr/Cu into the etching solution (e.g. APS - $(NH_4)_2S_2O_8$) to remove the underlying Cu layer.
- Triple wash the PMMA/Gr in clean DI water .

Substrate/ Chip Preparation

- Pattern creation (designing) and transfer to lithography tool (photo/ e-beam lithography).
- Evaporate metal (e.g. Ni/Au) on the subsrtrate though the pre-fabricated shadow mask to create pattern.
- Transfer graphene on top and remove PMMA.

Functionalization

- Covalent - Firstly, oxygen functional groups are introduced to the graphene lattice to create binding sites, followed by direct attachment of bioreceptors to the GO or rGO surface, in effect introducing defects and tampering with the electrical performance of the pristine graphene.
- Non-covalent - has the significant advantage of preserving the graphene structure, and in effect, the electrical features by using appropriate linkers between the graphene surface and the bioreceptors.

Figure 1. Graphene biosensor fabrication process

Functionalization essentially "gives life" to the biosensor. It defines the application of the biosensor where biomarkers specific to a condition are targeted and immobilized on the sensor using the appropriate chemicals and biomolecules. Table 1 presents several graphene-based biosensors reported in the literature; showing different substrate types, linkers, measurement setups, different forms of graphene and their respective performances (SE – sensitivity, SP – specificity, DL – detection limit, R – range & RT – response time). After fabrication, measurements are carried out. FET-based measurement setups (as shown in figures 2a-d) can either be carried out in vacuum using the back-gated, top-gated, dual-gated configurations, or modifications of these three as presented in literature or in an electrolyte commonly referred to as liquid-gated configuration.
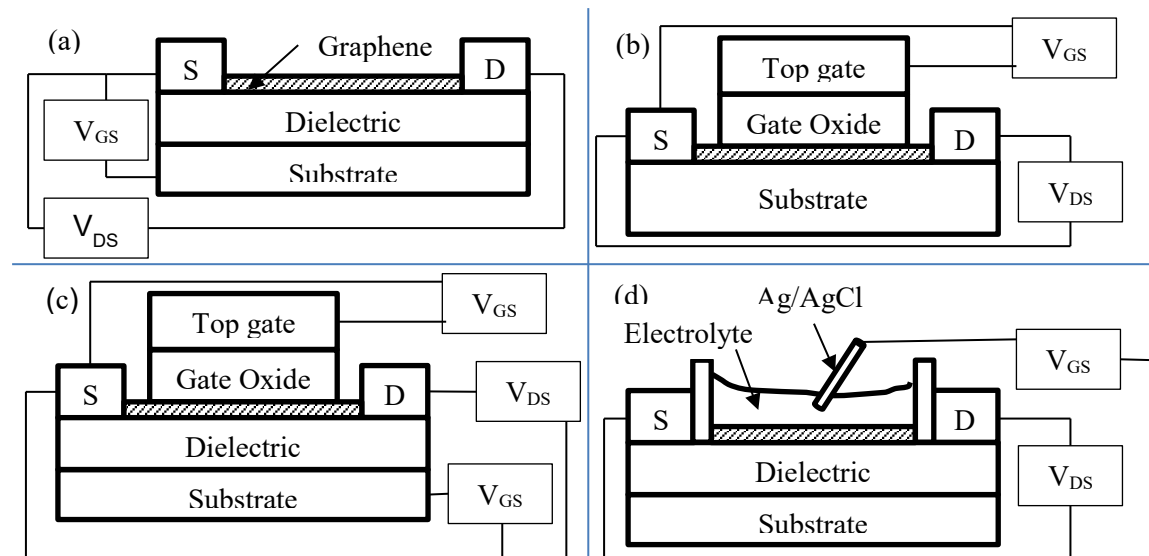

Figure 2. The GFET measurement setup in (a) represents a back-gated GFET; (b) represents a top-gated GFET; (c) represents a dual-gated FET, where the top-gate can also be a liquid-gated FET like in (d); (d) represents a liquid or electrolyte-gated FET

3.1. Low-cost patterning procedure for shadow masks
Lithography is a state-of-the-art method required to transfer desired patterns to a desired substrate using a mask. There are several types of lithography but the most common ones are ultraviolet (UV) lithography and electron beam (e-beam) lithography. Lithography is a complex procedure and highly error-prone; therefore, care must be taken in following through its requirements to obtain workable results. While UV and especially e-beam lithography can provide extremely good and reproducible patterns down to 1 μm and 1 nm respectively, they are also time consuming and expensive. Presented here is an alternative route to lithography and a simple approach to preparing shadow masks that can be used to cover a SiO$_2$/Si wafer in preparation for metal deposition of source and drain contact electrodes.

3.1.1. Methodology. The method is based on off-the-shelf commercially available mechanical plotter, Silhouette Cameo that is able to cut through a set of polymers or papers with reliably good feature sizes, down to 100 μm. To start with, an array of InterDigitated Electrode (IDE) structure was designed and arranged specifically to cover and align to crystallographic planes of a 4-inch silicon wafer diameter using the Silhouette Studio software as seen in figure 3a. The paper was then stuck to the Silhouette Cameo printing mat and tuned to the appropriate settings to print the desired pattern. After printing with the dice from the Cameo plotter, the mask was manually removed with the aid of a tweezer. Figure 3b shows the product of the lacerations made with the Cameo plotter while in the process of exposing the IDE-structured mask.
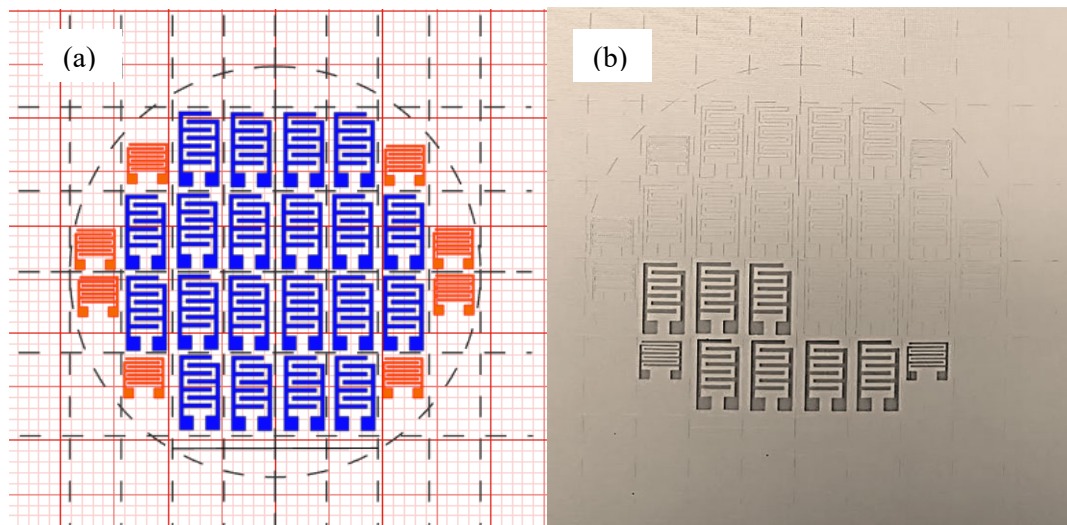
Figure 3. The Silhouette Studio layout of the IDE-structured mask is seen in (a); (b) shows the Silhouette Cameo plotter-printed mask with some of the IDE structure exposed.

After exposing the IDE structures representing individual transistors throughout the shadow mask, it is then held in place on the substrate (SiO$_2$/Si wafer) using kapton tape. The procedure for metal deposition using the CHA Evaporator is followed through to deposit a thin layer of Ni (10 nm) and Au (90 nm). Nickel is deposited first to serve as an adhesion layer, while gold is the metal contact serving as source and drain for the transistor.

3.1.2. Results and Discussion. Figure 4 shows some of the transistors realized from this process after breaking up the wafer into the individual IDE-structured transistors using a diamond scribe.
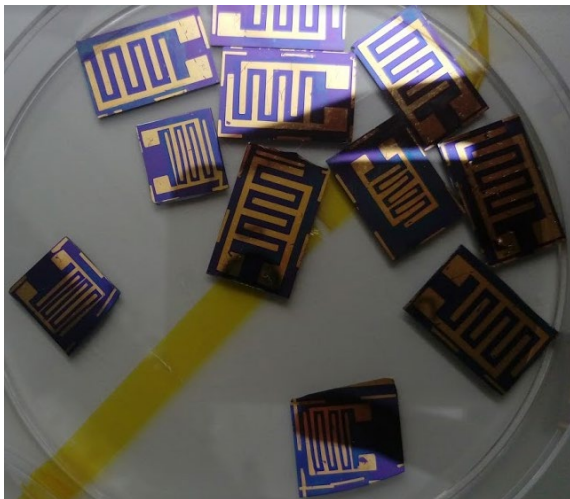
Figure 4. IDE-structured FETs fabricated using the low-cost mask patterning method.

We further tested the performance of these devices based on a liquid-gated GFET setup shown in figure 2d, using 0.01X PBS (~1.5mM ionic strength) as the electrolyte and a Keithley 2602A Source Measure Unit (SMU) coupled with a probe station. Figure 5 depicts the response of one of the devices showing the ambipolar nature of graphene and a p-type device.
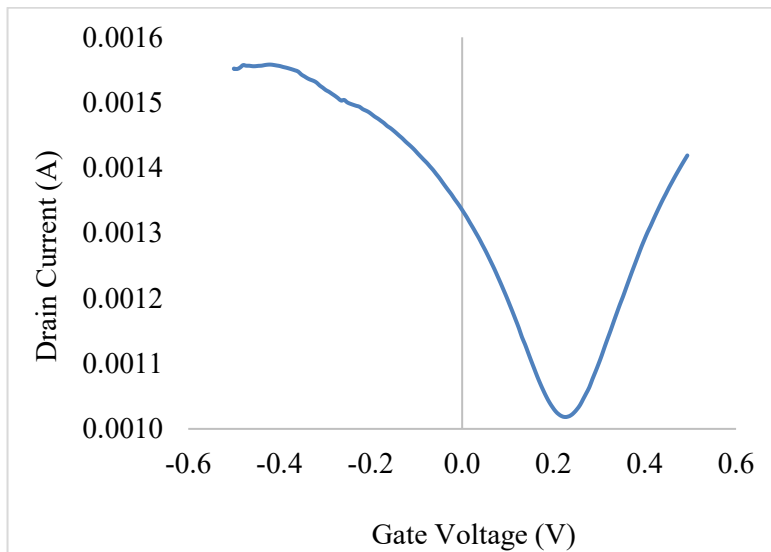
Figure 5. GFET Characterization

4. The Electrical Double Layer (EDL) of GFETs

Conventional graphene-based back gates FETs operate in the atmosphere and the field effect is introduced via application of potential through bulk silicon and accumulation of charges through the substrate dielectric (typically SiO_2). To build a reliable GFET biosensor, the gate oxide layer is replaced by an electrolyte in which the sensing takes place (see figure 2d). When a potential is applied between graphene channel and liquid gate (through electrolyte), an electrical double layer is formed at the interface according to the Helmoltz-Gouy-Chapman-Stern theories [27]–[30]. This setup is similar to the dielectric material for capacitors and the gate oxide for MOSFETs. This interface between the channel and the electrolyte is called the Electrical Double Layer (EDL), and it is understood and computed as a parallel-plate capacitor with a capacitance of C_{EDL}. C_{EDL} is then connected in series with the air-gap capacitance ($C_{air-gap}$, occurs due to the hydrophobic nature of graphene) and the graphene's inherent quantum capacitance (C_q) to obtain the total gate capacitance of the device as shown in figure 6 [31]. As a result, modulation of the graphene channel potential through the gate electrode occurs through capacitive processes, and the amount of voltage required to operate the device is typically within 1V which constitutes a very low power operation. The EDL is therefore the distance from the graphene surface, with a thickness equal to the Debye length (λ_D) which is defined by the electrolyte's molarity (M), as seen in equations (1) and (2).

$$C_{EDL} = \epsilon_0 \epsilon_r (\lambda_D)^{-1} \tag{1}$$

$$\lambda_D = 0.304(\sqrt{M})^{-1} \, [nm] \tag{2}$$
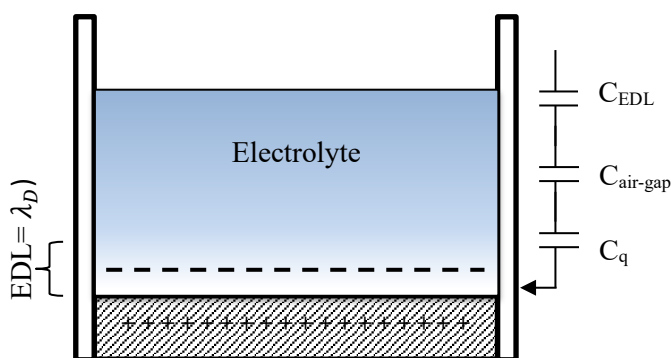
Figure 6. A liquid-based GFET biosensor highlighting the EDL from the graphene channel upwards.

Upon immobilization of the sample (the electrolyte) containing the desired analytes to bind with their bioreceptors on the graphene layer, there is a change in surface charge. Electronic changes resulting from binding interactions will only occur within the Debye length. Therefore, the target analytes must be sufficiently (as defined by λ_D) close to the sensor surface in order to trigger a response [32]. This is what brings some difficulty in liquid-based GFETs because the process to functionalize the GFET incurs some height, where typical heights of antibodies is 5 – 10nm and 10 – 15nm [5]. Also, from equation (2), it can be deciphered that the higher the molarity, the shorter the Debye length. For example, the electrolyte solution Phosphate Buffered Saline (PBS) of approximate molarities 150mM, 15mM and 1.5mM correspond to Debye lengths of 0.7nm, 2.3nm and 7.3nm respectively [5], [15], [33]. Therefore, there must be significant consideration in materials used to functionalize the graphene surface as well as the electrolyte's molarity to avoid electrostatic charge screening of the analytes.

5. Considerations for POC Testing

Graphene has great potentials in satisfying the ASSURED criteria of diagnostic devices. However, the measurement setup will largely influence the possibility of POC applications. With respect to the literature reported in table 1, there are three categories of sensors. References [34]–[41] detail liquid-gated FET configurations, [42], [43] report in-situ analyte measurement (liquid-based) using back-gated FET while other works reported dry (vacuum/ ambient conditions-based) measurements. In liquid-based FET setup, measurement is carried out while the sample containing the analyte is dispensed on the sensor surface, while in the third category, the sample containing the analyte is incubated on the sensor surface to allow antigen-receptor binding for a given amount of time (typically 30 minutes – 1 hour), followed by rinsing and drying of the chips and then measurements. In effect, biosensors based on the liquid-gated configuration are better positioned to fulfill the ASSURED criteria, specifically in User-friendliness and Equipment-free criteria. They also have lower energy requirement as mentioned earlier. On the other hand, "dry" measurements circumvent electrostatic charge screening due to Debye length/EDL, since the graphene channel potential is modulated through the substrate and not electrolyte.

Table 1. Graphene-based Sensors

Sensor type	Condition	Antigen	Detection Mechanism	Detection Limit	Response & Time	Ref
Liquid-gated GFETs	Heart failure	BNP	SiO_2/Si + RGO + PtNPs + anti-BNP (a silver wire reference electrode was used to realize a liquid-gated FET)	100fM	10 secs	[34]
	Prostate cancer	PSA-ACT	Glass + RGO + PASE + PSA monoclonal antibody (a platinum reference electrode was used to realize a liquid-gated FET)	100fg/mL	up to 10ng/mL and 100ng/mL	[35]
	Lead	Pb2+	SiO_2/Si + G + PASE + 8–17 DNAzyme (a gold wire was used to realize a liquid-gated FET)	37.5ng/L	–	[36]
	DNA	DNA	SiO_2/Si + RGO + PASE + PNA (a silver wire reference electrode was used to realize a liquid-gated FET)	100fM	–	[37]
	Cancer	miRNA	SiO_2/Si + RGO + AuNPs + PNA (a silver wire	10fM	–	[38]

		reference electrode was used to realize a liquid-gated FET)			
	DNA Hybridiza-tion	DNA	SiO$_2$/Si + SLG + PASE + probe DNA (Electrolyte-gated)	25aM	SE – 24mV/dec	[39]
	Diabetes	GOx	Glass + SLG + (GOx-CHIT/Nafion/PtNPs/graphene electrode)	0.5µM	0.5µM to 1mM	[40]
	Zika Virus	Zika NS1	SiO$_2$/Si + SLG + anti-Zika NS1 monoclonal antibody	450pM	–	[41]
Liquid drop, back-gated GFETs	EVD	EGP of the Zaire strain	SiO$_2$/Si + RGO + AuNPs + anti-Ebola probes	1ng/mL	a few secs SE – 6.5% and 14% for 1ng/mL to 444ng/mL respectively	[42]
	E.coli bacteria	E.coli	SiO$_2$/Si + TRMGO on AET-modified Au (source & drain) electrodes + AuNPs + anti-E.coli antibodies	10cfu/mL	up to 10^{3}cfu/mL	[43]
	Pregnancy	hCG	Si/C + MEG + APTES + anti-hCG (EDAC + NHS + anti-hCG)	0.62ng/mL	up to 5.62ng/mL SE – 142Ω/ng/mL	[44]
Dry, back-gated GFETs	Cancer	hCG	SiO$_2$/Si + MG + PASE + anti-hCG	~0.1pg/mL	SE – ~(61.8 ± 16.7)%/decade of hCG	[45]
	Genetics	DNA	SiO$_2$/Si + MG + PASE-NHS + probe DNA (22, 40, 60-mer)	1fM (for 60-mer)	–	[46]
	Immunity	IgG	SiO$_2$/Si + TRGO + AuNPs + anti-IgG	2ng/mL	up to 0.02mg/mL SP – 68%	[47]
	Immunity	IgG	SiO$_2$/Si + PECVD-VG + AuNPs + anti-IgG	2ng/mL	SP – 9.8% SE – up to 15%	[48]
	Immunity	IgG	SiO$_2$/Si + APTES + RGO in flake form + PtNPs + (CS2 + Protein G)	Drain current decreased one order of magnitude on introduction of target analyte		[49]
	E.coli bacteria	E.coli	SiO$_2$/Si + FLG + PASE + anti-E.coli antibodies	5x10^{3}cfu/mL	up to 5x10^{5}cfu/mL	[33]

6. Conclusion

The foregoing shows that significant research progress has been made so far towards diagnostics for POC settings. However, there remains a huge gap in going beyond proof-of-concepts to commercially viable devices i.e. building holistic sensors. Attention must also be given to ensuring accuracy of results and not just sensitivity, specificity, detection limit and other performance metrics.

Acknowledgement

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