Rapid pathogen detection using an organic field effect transistor

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A pentacene field effect transistor (FET) for the detection of DNA from pathogenic organisms is fabricated. The pentacene FET is an excellent candidate for disposable sensor applications because of its low-cost fabrication process and fast detection. A viral (λ-phage) genomic DNA was chosen as a model organism and its presence was successfully detected by probe DNA hybridisation on the pentacene layer. The process produced a dramatic change in the channel current and field-effect mobility of the devices. This result demonstrates the feasibility of our device as a disposable sensor for DNA hybridisation and can lead to the development of a biosensor for rapid pathogen detection.

1. Introduction: In the post-human-genome sequencing era, the detection and quantification of DNA are of great importance to many applications, such as medical diagnostics, forensic investigation, genotyping and pathogen detection [1–7]. In particular, detection of pathogen, such as bacteria, virus, fungi and so on, is of utmost importance primarily for health and safety reasons and involves isolation of DNA from pathogen cells, followed by traditional methods of DNA detection. These traditional methods mainly focus on chromatophore or radioactive labelling; optical detection using fluorochrome-tagged oligonucleotides [8, 9] or polymerase chain reaction-based amplification of the target DNA [10]. However, these methods have limitations because of the complications in sample preparation as well as the necessary usage of complex and expensive optical, electrochemical or thermocycling systems, along with specialised analysis. Compared with these techniques, a pathogen sensor using the organic field effect transistor (OFET) is an excellent candidate for the application as disposable sensors owing to their potentially low cost and convenient fabrication process and quicker detection [11, 12]. Moreover, it is easier to integrate OFETs with biological systems because of the biocompatibility and flexibility of an organic semiconductor material. For these reasons, we have fabricated pentacene FETs in the present work for the detection of pathogen. Pentacene was the choice for the organic semiconductor material because of its excellent electrical properties and ease in immobilisation of DNA over it.

The DNA molecules were immobilised on the hydrophobic pentacene surface by physical adsorption and hydrophobic interactions. These molecules also have negatively charged phosphate groups on their backbone, which affects the electrical performance of the pentacene FETs. When the DNA molecules are immobilised on the pentacene surface by physical adsorption and hydrophobic interactions.

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I dramatic difference in the channel current (on the pentacene FETs is shown in Fig. 1. 

3. Results and discussion: The immobilisation of DNA on pentacene FET was confirmed through fluorescence microscopy. The images of fluorochrome labelled DNA were obtained with a fluorescence microscope (Olympus BX50, Japan) using excitation and emission wavelengths as 510–490 and 590 nm, respectively. Ethidium bromide (EtBr) was used as fluorochrome to intercalate the DNA. Fig. 1a shows the fluorescence image of a control device, in which the pentacene channel was labelled with EtBr without DNA immobilisation. As expected, the control device did not show fluorescence on exposure. Another device was immobilised with ds-DNA (Poly A + T) with the usual immobilisation procedure and further interacted with EtBr. As EtBr is an intercalating dye and binds only to the ds-DNA, therefore the device with immobilised ds-DNA (Poly A + T) showed red fluorescence (Fig. 1b), which confirms the immobilisation and hybridisation of DNA on a pentacene FET. The sensing mechanism of DNA on the pentacene FETs is shown in Fig. 1c. In this study, we found a dramatic difference in the channel current ($I_{DS}$) and changes in the field effect mobility ($\mu_{FEF}$) pattern on exposure to either 50-mer, 200 pmol ss-DNA or ds-DNA on the same pentacene FET. Fig. 2 shows the difference in the sensor output and transfer characteristics from the original pentacene FETs (without immobilised DNA) compared to the pentacene FETs with immobilised probe DNA (ss-DNA) or target DNA (ds-DNA after hybridisation). After immobilising ds-DNA, the channel current ($I_{DS}$) of the device reduced approximately to 87.67% compared to that of the original device (at $V_{DS} = -30$ V, $V_{GS} = -30$ V) which was more than that for ss-DNA (80.35%). In addition, the field effect mobility ($\mu_{FEF}$) reduced from 2.286 cm$^2$/Vs to 0.473 and 0.334 cm$^2$/Vs for ss-DNA and ds-DNA, respectively. The reduction in the channel current ($I_{DS}$) and field effect mobility ($\mu_{FEF}$) following DNA hybridisation was dramatic and in contradiction with previous reports [13, 14], wherein the researchers have demonstrated an increase in channel current ($I_{DS}$) because of hybridisation of DNA on the pentacene surface. Such difference could be owing to the use of a buffer in these reports compared to double-distilled water that we used in our present work in order to avoid the effect of charges carried by buffer components. It can be safely regarded as more authentic to use plain distilled water to determine the change in channel current than by using buffer components that carries charge in itself. Furthermore, the reduction in channel current and $\mu_{FEF}$ because of DNA hybridisation have been demonstrated previously by our group [15], wherein we reported our feasibility study for sensing DNA molecules through hybridisation on the pentacene surface. The DNA should ideally attract holes after attachment to pentacene and that shall decrease the lifetime of these holes from the channel region, thereby reducing the channel current level and field effect mobility.

Keeping up with this trend, the ds-DNA reduced the channel current ($I_{DS}$) and field effect mobility ($\mu_{FEF}$) even to a greater extent when compared with that by the ss-DNA, since the ds-DNA carry more net negative charge. This enabled the direct electrical detection of the target DNA through the measurement.
of channel current ($I_{DS}$) and field effect mobility ($\mu_{FET}$) for the pentacene FETs. The net difference in the channel current ($I_{DS}$) and field effect mobility ($\mu_{FET}$) on the pentacene FETs because of the single- or double-stranded DNA was the basis for the analysis of the pathogenic DNA through probe hybridisation.

Further, to derive the correlation between device response and DNA concentration, the output and transfer characteristics of the devices were measured with varying concentrations of the ss-DNA on the pentacene surface (Fig. 3). As DNA concentration was increased, the channel current ($I_{DS}$) also decreased and so a decrease in the field effect mobility ($\mu_{FET}$) was observed ranging from 2.286 cm$^2$/Vs to 1.323, 1.081 and 0.473 cm$^2$/Vs for 50, 100 and 200 pmol ss-DNA, respectively. Such a reduction in the channel current ($I_{DS}$) and field effect mobility ($\mu_{FET}$) was owing to the increase in the immobilised DNA concentration on the pentacene surface, which collectively attracts more holes from the channel region. The sensitivity of our device was found to be 0.282 $\mu$A/pmol and 0.00836 cm$^2$ V$^{-1}$ s$^{-1}$/pmol of ss-DNA with respect to reduction in channel current and field effect mobility, respectively. These results indicate the possibility of dynamic response from devices having low concentrations of hybridised targeted DNA.

Finally, as a proof of concept, we attempted to detect a model organism bacteriophage lambda through hybridisation of its genomic DNA over a probe-immobilised pentacene FET device. For this purpose, a 20-mer ss-probe 5’-GCA-AGT-ATC-GTT-TCC-ACC-GT-3’ was immobilised on a pentacene surface and was characterised (Fig. 4). Subsequently, the $\lambda$-phage DNA digest (with restriction enzyme BstII) was preheated at 100°C for 30 min to separate its strands and 1 µl of this sample (containing 1 µg DNA) was applied on the probe-immobilised FET surface. Restriction digestion of $\lambda$-phage DNA ensured five smaller fragments of ds-DNA for ease in separating the strands and subsequent hybridisation. The devices were air dried, washed with DI water and used in further characterisation as before. As seen from Fig. 4, the device showed significant change in electrical properties in response to complementary DNA from $\lambda$-phage. After hybridisation of $\lambda$-phage DNA, the channel current ($I_{DS}$) of the device reduced approximately to 44.88% compared to devices with only probe DNA (at $V_{DS} = -30$ V, $V_{GS} = -30$ V). In addition, the field effect mobility ($\mu_{FET}$) also reduced approximately to 40.67%. On the other hand, during a control experiment, the pentacene FET device did not show significant change in electrical properties when the same concentration of bacterium Escherichia coli (E. coli) DNA was applied over a 20-mer probe 5’-GCA-AGT-ATC-GTT-TCC-ACC-GT-3’ hybridised device. In this device, E. coli DNA did not immobilise on the pentacene channel layer as the 20-mer probe was already immobilised on this surface. In addition, as expected, E. coli DNA did not hybridise to 20-mer probe as it was specifically designed against its target from $\lambda$-phage. This result indicates the possibility of selective response from devices having specific probe DNA molecules immobilised on the pentacene. Moreover, the response time for these sensors was about 65 min, which mainly included drying time for ds-DNA on the pentacene surface following DNA hybridisation. The sensor was stable up to 30 days when stored at room temperature in a non-humid condition. The sensor having ss-DNA immobilised produced up to 95% response as compared to the original device when stored under such condition. These results suggest a feasible
DNA hybridisation sensor with high sensitivity, selectivity and relatively shorter response time when compared with existing methods involving DNA hybridisation.

4. Conclusion: Therefore, in conclusion, the single-stranded λ-phage probe DNA was immobilised on the surface of the pentacene layer and hybridised with its double-stranded target DNA, thereby producing a significant change in the performance of the pentacene FETs. It was attributable to the net negative charges on the DNA molecules having the ability to attract holes from the channel region. The electrical characterisation of the pentacene FETs with the immobilised ds-DNA produced a lower current output ($I_{DS}$) and field effect mobility ($\mu_{FET}$) since the ds-DNA carry more net negative charge. It was possible to selectively quantify the target DNA from the organism. With these results, we propose a ‘label-free’ detection technique for pathogen with high sensitivity and selectivity. This method shall enable the possibility for a portable and disposable pathogen sensor and can also find use in diverse applications such as medical diagnostics, forensic investigations, genotyping as well as combinatorial synthesis.

5 References